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All the API Members,

I had been the Editor-in-Chief of this very prestigious Journal since March 2015. I am presenting to you my last issue of May 2022 as Editor-in-Chief. It’s been seven years that I have tried my best to serve the Physicians in India. I had a rich fulfilling experience during my tenure.

It was just because of my Mentor Late Dr. Siddharth N Shah that I could achieve something in my career in API. It was unfortunate we lost him suddenly a year back. I had a very strong support and guidance from Dr YP Munjal all throughout. I was supported by Dr. Shashank R Joshi especially during Covid times when things were difficult to get JAPI ready on time. Dr. Mangesh Tiwaskar and Dr. Agam Vora were besides me, especially in raising finances for the Journal. The entire Editorial Board and Referees have done wonderful job for the Journal over the years. I am grateful to all the Presidents and Governing Body and Faculty Council members of API and ICP during my tenure for their support and guidance. API staff led by Mrs. Sunita Shukla and JAPI staff under Mr. Narayan Murkar helped me all throughout my tenure.

In last seven years I was successful in introducing online submission for authors. Also, free access was given to all the articles in JAPI. ‘MyJAPI’ App on Android and IOS platform was started which gave access to all the articles at a click on your mobiles. During Covid times, research articles on Covid-19 were published on priority. We have Editorials related to Covid-19 almost for two years. This helped our Physicians to keep themselves updated with emerging Covid-19 infection.

I solely take all the responsibility of any shortcomings and mistakes that happened inadvertently. I hope you all will forgive me for that.

From next issue you will see JAPI in new format under leadership of new Editor-in-Chief of JAPI Dr. Mangesh Tiwaskar along with new Editorial Board. I wish him great success and surely he will take JAPI to new heights in coming years.
Chronic kidney disease (CKD) is defined as “abnormalities of kidney structure or function [as evidenced by estimated glomerular filtration rate (eGFR) of <60 mL/min/1.73 m²], the persistent presence of manifestations that are suggestive of kidney damage (one or more marker of kidney damage: histological abnormalities, active urine sediments, proteinuria, structural abnormalities, electrolyte and other abnormalities due to tubular disorders or a history of kidney transplantation), or both, present for more than 3 months”.1 End Stage Renal Disease (ESRD, also known as Kidney Failure) is defined as eGFR <15 mL/min/1.73 m².

CKD is increasingly being recognized as a global public health concern given its profound medical and economic implications. Medical implications of CKD include its multimorbid afflictions which range from increased risk of cardiovascular events, cognitive decline, and hospitalization, not to mention the high risk for all-cause and cardiovascular mortality. Of even greater concern is the progressive rise in CKD cases worldwide adding more and more to the global burden of CKD in the last 20 years. Various factors responsible for this burgeoning problem include the aging global population as well as the rising prevalence of diabetes mellitus and hypertension. Prevalence of CKD varies anywhere from 9% to 20%, in different areas of the world and equally affects developing and developed nations. Data from GBD 2017 has reported 697.5 million CKD cases with the global prevalence estimated as 9.1% (8.5 to 9.8).2 Mortality due to CKD was found to be around 1.2 million and the global mortality rate for CKD increased by 41.5% from 1990 to 2017. Currently, CKD is ranked as 12th leading cause of death.2 India contributed around 17% of the global population. Mortality due to CKD rose from 0.59 million (1990) to 1.18 million (2016) which was higher than other low- and middle-income nations with almost identical socio-demographic parameters, suggesting the need to improve current management practices to improve clinical outcomes.3

Even though CKD is a relentlessly progressive disease, there are differences in the patterns of progression in different patients or in the same patient at different time-points. Differences in the trajectories of renal function reflect heterogeneity in aetiology of CKD, supervening comorbidities, concomitant clinical interventions including nephrotoxic drugs and adverse environmental exposures.4 In view of this, strategies to retard disease progression in CKD will need to carefully address these issues as well as use valid measures of treatment benefit such as reducing mortality and need for dialysis along with preventing eGFR decline. Given the multiple risk factors for CKD, each of these factors represent a window of opportunity for prevention either through pharmacologic or non-pharmacologic means (Figure 1). Lifestyle factors such as diet, smoking, lack of physical activity, and sleep deprivation are well-known risk factors that are associated with the progression of CKD. Other contributory factors include hypertension, diabetes mellitus, gout, and cardiovascular diseases can also aggravate eGFR decline (Figure 1).5 Recently, atrial fibrillation (AF) has been demonstrated to be a contributor to the rapid decline in eGFR.6

Intrinsic factors related to kidneys such as GFR, proteinuria, patterns of glomerular injury, renal morphology such as glomerulosclerosis, tubular atrophy, extent of tubulointerstitial fibrosis and presence of obstructive nephropathy also play an important role in the deterioration of renal function.7 Polymorphisms in the genes involved in various pathways that are associated with inflammatory reactions, fibrosis, CKD worsening, and the renin-angiotensin-aldosterone system (RAAS) have been suggested to affect the progression of CKD.8 AKI is one of the most important risk factors for the rapid decline in eGFR and is associated with adverse renal outcomes. AKI is never self-limited, as it can lead to subsequent risk of AKI.
episodes, and potentially, to onset of CKD. Older age, delayed recovery from AKI, severity/frequency of AKI, etiology of AKI, comorbidities, and presence of proteinuria are all risk factors that have now been shown to trigger the progression of AKI to CKD.9

Aetiology of CKD also indicates patterns of renal disease progression. Diabetes mellitus is the most common cause of CKD/ESRD which involves complex and multifactorial pathogenesis. Clinical manifestations of diabetic kidney disease (DKD) include impaired renal function with proteinuria. Early-onset diabetes, hypertension, obesity, the severity of proteinuria, and smoking are the risk factors for origin and progression of DKD. Adequate glycaemic control along with RAAS blockade is the cornerstone of DKD management. Recently, sodium-glucose cotransporter-2 (SGLT2) inhibitors have increasingly been recognized as renoprotective agents in DKD as well as in non-diabetic kidney disease patients. Renoprotection effect of SGLT2 inhibitors facilitate by enhancing glycaemic control, improving cardiac functioning, and reducing body weight as well as restoring extra-renal/intra-renal hemodynamics including BP reduction, re-activating tubuloglomerular feedback, and promoting natriuresis.10 SGLT2 inhibitors also reverse PCT hypertrophy that facilitates reduced energy demand, and subsequently less oxidative stress, fibrosis, and growth factor expression.11

Adequate BP control (as defined by goal SBP <140 mmHg in CKD, and <130 mmHg in CKD and proteinuria) is linked to improved renal prognosis. Recently, KDIGO has recommended SBP targets to <120 mmHg in hypertensive patients with CKD.7 Apart from lifestyle changes including the DASH diet, salt restriction (<2 g per day of sodium), smoking cessation, weight loss, and adequate physical exercise (aiming for at least 30 minutes 5 times per week), pharmacotherapy can be effective with the use of renoprotective agents, including ACEI/ARBs.7 Novel emerging nonsteroidal mineralocorticoid receptor antagonists (MRAs) such as finerenone and esaxerenone have better anti-inflammatory and anti-fibrotic effects.12 Sacubitril and valsartan are also beneficial in reducing the severity of proteinuria in heart failure patients with a reduced ejection fraction.13 Other accepted strategies to prevent CKD progression include optimizing treatment of metabolic acidosis since it can increase in endothelin production, aggravate inflammatory responses, increased aldosterone secretion, and cause tubulointerstitial fibrosis. Using alaki therapy (e.g., NaHCO3 and Na-citrate) while targeting serum bicarbonate in the range of 22-26 meq/L has been shown to slow renal progression.14 Nutritional interventions in CKD is another rapidly evolving field in CKD prevention strategies. It has been shown that a high protein intake leads to glomerular hyperfiltration which leads to glomerulosclerosis culminating in deterioration of kidney function. Hence, dietary protein restriction has long been suggested as the cornerstone of nutritional therapies for patients with CKD. In general, recommended dietary allowance for proteins is 0.5-0.6 g/kg body weight/day for stage 3 to 5 CKD patients without diabetes, and 0.6-0.8 g/kg/d for those with diabetes while at any point of time avoiding protein intake higher than 1.3 g/kg/d in all stages of CKD. A complete dietary pattern in CKD patients should include all 8 essential amino acids in addition to histidine. When prescriing a low protein diet, at least 50-75% of the protein should have high biological value. Compared with animal protein, a vegetarian diet has less influence on glomerular hemodynamics.15 A vegetarian diet is associated with lower net acid production, lower plasma phosphate, and a decrease in FGF23 levels resulting in a lower risk of renal fibrosis, and slower progression of renal insufficiency. A low protein diet supplemented with ketoanalogues (“Keto-diet) has been shown to be effective in delaying the progression of CKD and reducing the risk of dialysis initiation in patients with or without advanced CKD.16 Regular physical activity during a low protein diet with ketoanalogues will not cause net protein catabolism. However, it will help with an improvement in nutrition status, inflammation, and muscle strength if energy supply is adequate.

Thus, in the broader context of research to retard CKD progression, the original study of Sengupta et al, assumes great importance.17 The authors in this study tested the hypothesis that oxidative stress, which is a known pathophysiological process driving CKD progression, can be mitigated using free radical scavengers such as N-acetylcysteine in combination with nutritional supplements such as pyridoxamine or taurine and can thus can potentially reverse the decline in eGFR. The study was a randomized single-centre, open labelled, 3 arm study, consisting of 69 pre-dialysis non-diabetic CKD patients who were simultaneously assigned to either placebo (n=22), fixed dose combination of taurine (500 mg)+ NAC (150 mg) and fixed dose combination of NAC (300mg) + pyridoxamine (50 mg) in addition to continuing with standard of care therapy which consisted of isocaloric (25-30 KCl/kg/d) and low protein(0.6 g/kg/d) diet. The primary endpoint was a change in estimated GFR at 6 months. The study used a unique statistical methodology called empirical distribution function (EDF) plots, which is often used in exploratory or preliminary studies. While it is beyond the scope of this commentary to discuss this methodology, the EDF basically measures a testing parameter (here change in eGFR) as a continuous plot of the percent change from baseline on the horizontal axis and the cumulative percent (percentile) of patients experiencing that change in parameter on the vertical axis. The Kolmogrov-Smirnov test is then used to test whether two or more empirical distributions, represented by different treatment arms are different. Using this technique, the authors showed that the arm taking NAC+pyridoxamine had a significant increase in eGFR at 6 months than control group or NAC+ taurine. The magnitude of benefit was noted to be greater in a sub-group of patients without metabolic acidosis as defined by serum bicarbonate <22mmol/L and in patients with baseline eGFR > 45 ml/min, thus showing that there was greater utility of this novel combination therapy in early CKD. Of note, no significant change in proteinuria was seen in either of the 3 arms.

Results of study by Sengupta et al, are likely to resurrect the extremely conflicting evidence-base for use of antioxidants in renal disease. Notwithstanding initial enthusiasm of using NAC to prevent acute kidney injury in setting of i.v. radiocontrast exposure, the large multi-centre randomized PRESERVE trial showed no benefit.18 This was disappointing given that experimental models of ischemia/
reperfusion showed mitigation of renal injury with this intervention. Similarly, evidence for benefits of NAC in CKD is conflicting with some improvement in proteinuria but minimal benefit on renal function. Simil

arly compared the use of dietary supplement taurine which has been shown to play an important role in renal cell cycle/apoptosis, tubular ion absorption, renal blood flow, and ischemic/reperfusion injury. However when tested in clinical studies, there was some or no benefit in proteinuria and minimal beneficial effects on renal functions in diabetic kidney disease. Pyridoxamine (a vitamin B6 derivative), which is a free oxygen radical scavenger and potent inhibitor of advanced glycation end products has been shown in experimental models to retard diabetic kidney disease. Clinical-translational studies are still sparse but, when available in diabetic CKD showed marginal benefit on renal function over a 52-week period only in a subset of patients with baseline Scr < 2.0 mg/dL. In this regard the study of Sengupta et al is unique as it has used a hitherto untried combination of NAC and pyridoxamine in a cohort of non-dia

betic kidney disease and showed a significant improvement in GFR in as early as 6 months, especially in a subset of early CKD.

While Sengupta et al, should be complemented on showing beneficial effects using a low-risk intervention on the trajectory of CKD, the results need to be interpreted cautiously. First, the method for estimated GFR is not mentioned. This is because there is considerable bias in eGFR equations with the CKD-EPI equation considered more precise and accurate than the MDRD equation. Second, study duration is too short to predict long term renal outcomes. Thirdly, it is unclear if change in estimated GFR over 6 months is a valid end point since GFR trajectories in CKD can be very erratic. Given the pivotal role of endpoints in accelerated drug discovery in CKD, the recently concluded workshop of the US-FDA and the National Kidney Foundation (NKF) recommended that the most valid measures of treatment effects are either albuminuria reduction of > 30% within 6 months or improvement in GFR slope by around 0.5 to 1.0 mL/min/1.73 m2 over 2 to 3 years. Using these yardsticks, the results of this study fall way short of expectations. Additionally, it is noted that the median change in GFR at 6 months in the NAC+pyridoxamine was statistically significant from the other two arms only because there was minimal change in eGFR at 6 months (in NAC+pyridoxamine arm) and not due an absolute increase in GFR. While this could be an early signal for renal benefits, there is no details of slope of GFR to give any suggestion that this might be the case. As further evidence of minimal clinical benefit, we see that changes in proteinuria at 6 months was not different in either of the groups. Finally, we have no information on how many of these patients were on Renin-angiotensin-aldosterone blockers or SGLT-2 inhibitors which are proven agents affecting GFR trajectory.

To conclude, the study of Sengupta et al, raises the exciting possibility of using agents to reduce oxidative stress and reverse the relentless march of CKD. However, conclusions reached in the study need verification in robust, multicentre studies that measure valid renal endpoints to justify their use in a population that is already burdened with multiple and expensive pharmacotherapies.

References

### Rich evidence in Indian patients with proven clinical superiority vs other combinations

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</table>

### Your trusted choice for managing Allergic Rhinitis


Reference:

1. Walekar A, Chodankar D, Naqvi M, Trivedi C: Assessment of Bioequivalence of Fexofenadine and Montelukast Fixed Dose Combination Tablet Versus Separate Formulations of the Individual Components at the Same Dose Levels. Indian journal of pharmaceutical sciences, 2016, 78(5), 65656
4. Concomitant bilastine and montelukast as additive therapy for seasonal allergic rhinoconjunctivitis and mild-to-moderate asthma. The SKY study, 2019

Comparative Study to Evaluate the Effect of Low-Protein Diet Supplementation with Taurine and N-Acetylcysteine, N-Acetylcysteine and Pyridoxamine Dihydrochloride in Preventing the Progression of Chronic Renal Failure in Patients with Non-Diabetic Kidney Disease

Pratim Sengupta1*, Sumanta Biswas2, Tapas Roy3

Abstract

Background: Chronic Kidney Disease (CKD) has multifactorial etiology and there are lots of grey zone in understanding its complex pathophysiology. There is no silver bullet for optimal care of CKD. Oxidative stress being well understood and considered as an important common progressive factor for CKD of different etiology. Several research studies focused on reducing oxidative stress and have shown diverse outcomes. In this randomized, open-label, three arms, controlled, single-center study we evaluated the role of N acetylcysteine which is a direct scavenger of free radical, in combination with taurine and pyridoxamine in retarding the progression of non-diabetic kidney disease.

Methods: 69 non-dialysis, non-diabetic patients diagnosed with chronic renal failure with GFR more than 15 ml/min/1.73m2 and less than 60ml/min/1.73m2 receiving standard of care were enrolled in the study, of which 22 were in the placebo arm, 23 treated with NT (500 mg Taurine + 150 mg NAC) arm and 24 in the NP (300mg NAC+ 50mg pyridoxamine di-hydrochloride) arm. The subjects in the treatment arm received the study drug twice a day along with low protein (0.6gm protein per Kg body weight) isocaloric diet with 25-30 Kcal/Kg/D and were evaluated monthly up to 6 months. Change in eGFR across 3 groups over 6 months were compared.

Result: Mean age of the subjects was 57 ± 13 years of 56.25% were male and 43.75% were female. 69 patients completed the study. The Empirical Distribution Function (EDF) of NP group was dominant over control and NT group indicating a positive effect of NT on non-diabetic CKD at 10% level of significance. In the subgroup analysis a significant effect was observed in the cases of patients receiving NP with baseline eGFR more than 45 ml/min. The mean increase in eGFR readings over six months was 8.15 units higher in the NP group than in the control group. The two-sided p-values of the t-test, the Wilcoxon test and the Kolmogorov-Smirnov test were 0.0496, 0.0316 and 0.0354, respectively. Thus, all the three tests reject the hypothesis of identical changes in eGFR at the 5% level. In subjects with bicarbonate more than 22 mg/dl, the mean increase in eGFR over six months was 10.86 units higher in the NP group than in the control group indicating NP has a positive effect on increasing eGFR over 6 months, in patients without the presence of any metabolic acidosis. The two-sided p-vales of the t-test, the Wilcoxon test and the Kolmogorov-Smirnov test were 0.0325, 0.0205 and 0.1495, respectively. Thus, two of the three tests reject the hypothesis of identical changes in eGFR at the 5% level which clearly indicates that NP had better efficacy than other groups.

Conclusion: N-acetylcysteine along with pyridoxine may be a useful intervention along with a low protein diet in retarding progression of CKD in the nondiabetic population in early CKD.

Introduction

Oxidative stress and tissue hypoxia plays the crucial role in development of renal fibrosis. There are several armamentariums to reduce oxidative stress and hypoxia to halt the progression of Chronic kidney disease (CKD). Apart from dietary protein restriction with low protein diet there are few other nutraceuticals that have been found to exhibit anti-oxidant property to reduce oxidative stress in damaged kidney. N-acetylcysteine (NAC) is an acetylated variant of amino acid L-cysteine, which is a direct scavenger of free radicals that can improve blood flow through nitric oxide-mediated vasodilation; it is also a precursor for glutathione synthesis that protects the kidney from injury induced by contrast media, ischemia, and toxins. Taurine (Tau) is a beta amino acid with antioxidant properties and has been proved to possess a renoprotective effect in rat models. Pyridoxamine (PM) is a structural analog of vitamin B6 that interferes with oxidative macromolecular damage via a number of different mechanisms and has been found to exhibit anti fibrotic activity in AKI patients. The

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Received: 22.04.2021; Revised: 25.11.2021; Accepted: 18.01.2022
present study aims to study the effect of N Acetylcysteine in combination with taurine and pyridoxamine dihydrochloride in retardation of chronic renal failure in non-diabetic kidney disease.

**Methods**

**Patient population**

The study was a randomized, open label, three arms, controlled, single-centre study to evaluate the effect of low-protein diet supplementation with taurine and N-acetylcysteine (Tab NT) and N-acetylcysteine and pyridoxamine dihydrochloride (Tab. NP) in preventing progression of chronic renal failure in patients with non-diabetic kidney disease.

75, non-dialysis, non-diabetic patients diagnosed with chronic renal failure with GFR more than 15 ml/min/1.73m2 and less than 60ml/min/1.73m2 receiving standard of care based on investigator’s discretion, were screened in this study.

**Study design**

The study comprised of 2 periods-

- Screening period: Day-15 to Day 0:
- Treatment and follow-up period: Day 1 to Day 180

During the 180 days of treatment and follow-up period subjects was evaluated every month during their study visits. Eligible patients were randomized, by using a random number table system into one of the three treatment arms in 1:1:1 ratio. The study arms included:

- **Study Arm-1**: Low-protein diet (0.6g/kg BW/day)
- **Study Arm-2**: Low-protein diet (0.6g/kg BW/day) supplemented with 500mg Taurine + 150 mg NAC; Dose: 1 tab NT twice daily
- **Study Arm-3**: Low-protein diet supplemented with 300 mg NAC+ 50 mg pyridoxamine di- hydrochloride; Dose: 1 tab NP twice daily

Patients were evaluated monthly up to 6 months. In addition to the low protein diet, the study medications were taken orally, twice a day for 6 months. The study did not restrict any subject from continuing their usual standard of care.

The study protocol was approved by Institutional Ethics Committee Belle Vue Clinic, Kolkata and informed consent was obtained from all study subjects.

**Selection of Study Population**

Subjects were screened based on the following inclusion and exclusion criteria:

**Inclusion criteria**

1. Non-diabetic patients
2. Males or females ≥18 or ≤ 80 years of age
3. Chronic renal failure patients with GFR more than 15 ml/min/1.73m2 and less than 60ml/min/1.73m2 receiving standard of care based on investigator’s discretion
4. Life expectancy greater than one year
5. Well controlled blood pressure (MAP<125mm of Hg)

**Exclusion criteria**

1. Patient incapable of following study requirement to control diet
2. Patient with ongoing acute inflammation or AKI
3. Patients maintained on dialysis.
4. Patient anticipating for kidney transplant within next 6 months, or those who had a kidney transplant.
5. Subjects taking any other dietary supplement or nutraceutical product apart from the study intervention
6. Gastrointestinal dysfunction requiring parental nutrition
7. Other serious disease (e.g., Cirrhosis, stage IV NYHA cardiac failure, stroke etc) within the last 3 months
8. Severe edema or serious cavity effusion
9. Drug abuse
10. Diagnosed malignancy or ongoing treatment for malignancy during 6 months prior to study inclusion
11. Ongoing treatment for chronic infection like Tuberculosis, HIV, hepatitis B or HCV
12. Receiving the long term systemic steroid hormone or immunosuppressive agents (e.g., Cyclophosphamide, Cyclosporine, Tacrolimus, Azathioprine) treatment
13. Proteinuria>10 gm/day
14. Hb<8g/dl or <16g/dl
15. BMI <18 or >30 kg/m2
16. Subject intolerance or known hypersensitivity to N-acetylcysteine, taurine or pyridoxamine dihydrochloride
17. Pregnant or nursing females; females of childbearing potential who are unwilling or unable to use an acceptable method of contraception as outlined in this protocol from Day 1 until the month 6 visit
18. Participation in other product clinical trial within 30 days prior to this trial
19. Any other systemic disease or any other abnormal laboratory values which as per investigator will interfere with patient’s participation in study

Drug compliance was checked using a drug accountability log sheet. Dietary compliance was monitored by using assessments like protein equivalent of nitrogen appearance (PNA) or protein catabolic rate (PCR), normalized protein catabolic rate (nPCR), malnutrition inflammation score (MIS) and monthly dietary recall dairy.

**Statistical analysis**

**General Statistical approach**

The change in eGFR in six months of 3 groups were qualitatively compared by Empirical Distribution Function (EDF). Three methods were used for testing the distributional equality of 3 groups: the student’s t-test, the Wilcoxon rank sum test and the Kolmogorov test. Statistical significance was considered at 5% and 10% level of significance. Two tailed t test was also applied wherever applicable.

**Determination of sample size**

Targeting Power of the two-sample t test being 0.8, alpha error 0.05, assumed standard deviation being 1, the minimum sample size in each group was estimated as 21. Considering 15% dropout the final sample size in each group considered minimum of 25, and thus a total of minimum 75 (n=25) must be screened.

**Result**

After screening 75 patients, a total of 69 subjects fulfilled the inclusion criteria and were enrolled in the study, of which 22 were in the placebo arm, 23 treated with NT (500 mg Taurine + 150 mg NAC) arm and 24 in the NP (300 mg NAC + 50 mg pyridoxamine di-hydrochloride) arm. The Consolidated Standards of Reporting Trials (CONSORT) subjects
Table 1: Demographic details

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<tr>
<td>Non Veg (n%)</td>
<td>72 (90%)</td>
<td>15 (18.75%)</td>
<td>23 (28.75%)</td>
<td>23 (28.75%)</td>
<td>0.703</td>
</tr>
<tr>
<td>Male (n%)</td>
<td>45 (56.25%)</td>
<td>15 (18.75%)</td>
<td>16 (20%)</td>
<td>14 (17.50%)</td>
<td></td>
</tr>
<tr>
<td>Female (n%)</td>
<td>35 (43.75%)</td>
<td>10 (12.50%)</td>
<td>9 (11.25%)</td>
<td>16 (20%)</td>
<td></td>
</tr>
</tbody>
</table>
0.1495, respectively. Thus, two of the three tests reject the hypothesis of identical changes in eGFR at the 5% level which clearly indicates that NP had better efficacy than other groups.

A future analysis with larger sample size may be able to establish significance of some of the marginal differences identified in this analysis.

**Change in proteinuria**

No significant difference was observed across the three groups in change in proteinuria over 6 months.

**Discussion**

While chronic inflammation and oxidative stress are the main pathophysiological processes in the development of chronic kidney disease, there are limited armamentarium available to reverse the detrimental effects of these processes. Among the few available drugs N-Acetyl cysteine (NAC) is an old player that has been found to protect the kidney from injury induced by contrast media, ischemia, and toxins. Dietary taurine supplementation ameliorates renal disease including models of refractory nephrotic syndrome and diabetic nephropathy in experimental animals. On the other hand, another potent molecule Pyridoxamine (a vitamin B6 derivative) which is known to be an effective scavenger of reactive oxygen species and a potent inhibitor of advanced glycation end products have been studied in retarding progression of diabetic nephropathy in few clinical trials. In our study we tested the effect of 2 drugs: NT (NAC+ pyridoxine hydrochloride) and NP (NAC+ taurine) with a controlled group in non-diabetic CKD population. All the 3 groups were put on low-protein isocaloric diet. In our study where we have put our patients on a low-protein diet along with our tested nutraceutical products, we have found a marginal increase in eGFR among NP group compared to NT and controlled group, but the difference was not statistically significant. To further stratify our observation, we divided the 3 groups based on eGFR. So every group was further subdivided into two subgroups, one with baseline eGFR more than 45 ml/min and other with eGFR less than 45 ml/min. The idea behind this was that the progression of CKD varies according to the stage of CKD, hence we targeted early CKD group in the analysis to see the effect of the tested drug. After adjusting all confounding variables, interestingly we have found a significant positive increase in eGFR with NP group over 6 months compared...
to other groups. This observation clearly indicated that N-Acetyl cysteine along with pyridoxine hydrochloride have some favourable effect in early stage of CKD, by reversing the impact of oxidative stress and inflammation, that by enlarge plays a major role in arresting CKD progression. The antioxidant N acetylcysteine (NAC) is a source of sulphhydryl groups in cells and, due to its interaction with ROS, is a scavenger of free radicals.\textsuperscript{10} It has also been found that NAC administration improves endothelial function while reducing inflammation and fibrosis. There was a significant increase in eGFR in NP group in populations with base line eGFR more than 45 ml/min indicating the potency of the drug in early CKD. Ours is the first study that have tested the impact of NAC and pyridoxine combination in non-diabetic CKD population. Metabolic acidosis is a common complication of chronic kidney disease. Evidence suggested that metabolic acidosis is a contributor of CKD progression. Among 1,781 participants in the MDRD study, with CKD stages 2–4, with serum bicarbonate levels ≤20 mEq/L were associated with a higher risk of kidney failure (hazard ratio (HR) 2.22 (95% CI, 1.83-2.68)) compared with bicarbonate level ≥26 mEq/L, after adjusting for demographic and cardiovascular disease factors, serum albumin, proteinuria, and cause of kidney disease.\textsuperscript{11} Hence, we have considered the presence of metabolic acidosis as one of confounding variables in our analysis. In the sub-group analysis with patients having serum bicarbonate more than 22 mEq/L had significant increase in eGFR in NP group compared to control and Nefrosave group. However, we did not find any significant changes in eGFR in other sub-group analysis like patients age, blood pressure control, dietary habit and dietary compliance. One limitation of our study was small sample size of the study population.

Conclusion

In conclusion the findings of this study indicated that N-acetyl cysteine along with pyridoxine may be a useful intervention along with a low-protein diet in retarding progression on CKD in the nondiabetic population. These combinational nutraceutical compounds have shown a significant positive impact on eGFR when used in early CKD. Ours is the first study that has studied the effect on these compounds in non-diabetic CKD population. However, this pilot study opens a new arena of research where a much more randomized clinical trial with a larger sample size must be considered to find the impact of these nutraceutical combinations in the non-diabetic CKD population.

References


Prevalence and Profile of Obstructive Sleep Apnea in Patients with Interstitial Lung Diseases of North India

Tome Kamgo\textsuperscript{1}, Sonam Spalgais\textsuperscript{2}, Raj Kumar\textsuperscript{3}

Abstract

Background: The OSA is commonly found in ILD with overall incidence varying from 17 to 88 %. The morbidity and mortality of OSA are high when it occurs with chronic respiratory diseases like ILD. There is lack of data on sleep breathing disorder in ILD patients from India. The present study aims is to assess the occurrence of OSA in ILD patients and its correlation with other parameters.

Method: Prospective observational study of 41 ILD patients of one year duration. All patients underwent detailed clinical examination, radiological, laboratory investigations and Type 1 diagnostic polysomnography according to AASM guideline. The severity of OSA was defined as per AHI and the correlation of OSA with other parameters were assessed.

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Received: 27.11.2020; Revised: 27.10.2021; Accepted: 15.11.2021
Introduction

Interstitial lung diseases (ILD) are a challenging and diverse group of >200 inflammatory and fibrotic lung disorders associated with substantial morbidity and mortality. \(^1,2\) Many of these diseases evolve from an initial inflammatory process involving the lung interstitium with or without involvement of lung vasculature and airways. Overtime inflammatory process may lead to advancing fibrosis, especially in cases where diagnosis and treatment are delayed. The progressive nature of the disease with marked dyspnoea renders these patients with limited mobility and leads to decline in physical activities. \(^3-4\)

The overall prevalence and incidence of total ILDs is varying according to the study population and geographic location with sarcoidosis and idiopathic interstitial fibrosis (IPF) being the common subtypes. \(^5-7\) As per Duchemann D et al the overall crude prevalence of ILD was 97.9/100000/year with the most prevalent diagnosis being sarcoidosis (42.6%). Similarly various studies from India also have shown variable prevalence of different subtypes. However the commonly seen ILDs are IPF, sarcoidosis and hypersensitivity pneumonitis (HP). \(^8-11\)

The Obstructive sleep apnea (OSA) is the most common type of sleep disordered breathing and is characterised by recurrent episodes of upper airway collapse during sleep, leading to frequent arousals, intermittent hypoxia, sleep fragmentation and thus poor quality of sleep. \(^12\) The morbidity and the mortality of OSA are high especially when it occurs concomitantly with respiratory diseases. \(^13\) The prevalence of OSA varies according to age, study population and definition of hypopnea. \(^14-15\) The estimated prevalence of symptomatic OSA is 3 and 8% in men and 1 and 5% in women. \(^16\) The OSA is associated with increased incidence of hypertension, diabietic mellitus, heart failure, coronary artery disease, stroke and death. \(^14,17\)

There is a lack of proper population prevalence study of OSA in India due to paucity of sleep related health care facilities. The various studies from India reported that the prevalence of OSA was nearly 3–4%. \(^18-19\)

The OSA is a heterogeneous disorder with different mechanism that leads to collapse of upper airway. There have been few studies describing OSA in patients with ILD. However small studies reported high variation in the prevalence of OSA in ILD ranging from 17 to 88%. \(^20-22\) It was back in 1985, Perez-padilla et al in a study of 11 ILD patients reported that patients had decreased REM sleep and significant sleep fragmentation. \(^23\)

The co-existence of both diseases have significant morbidity, mortality and treatment related complications. The sleep related breathing disorders are frequently seen in ILD patients. The timely diagnosis and treatment of OSA in ILD patients decreased symptoms and increases quality of life. However there is a lack of study on sleep abnormalities in ILD patients from India. The aim of this study is to assess the occurrence of OSA in patients of ILD and correlate the severity of OSA with clinical and lung function

### Table 1: The detail of various HRCT finding in ILD patients

<table>
<thead>
<tr>
<th>HRCT Findings</th>
<th>Sarcoidosis=10 n (%)</th>
<th>NSIP=5 n (%)</th>
<th>HP=39 n (%)</th>
<th>CTD=5 n (%)</th>
<th>ILD=41 n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground Glass</td>
<td>9(90)</td>
<td>5(100)</td>
<td>19(100)</td>
<td>0</td>
<td>1(50)</td>
</tr>
<tr>
<td>Opacity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>34(82.9)</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>9(90)</td>
<td>5(100)</td>
<td>6(31.6)</td>
<td>5(100)</td>
<td>2(100)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>27(65.9)</td>
</tr>
<tr>
<td>Traction</td>
<td>7(70)</td>
<td>3(60)</td>
<td>3(15.8)</td>
<td>5(100)</td>
<td>2(100)</td>
</tr>
<tr>
<td>Bronchiectasis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20(48.8)</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td>9(90)</td>
<td>2(40)</td>
<td>1(5.3%)</td>
<td>1(20)</td>
<td>1(50)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14(34.1)</td>
</tr>
<tr>
<td>Pleural Opacity</td>
<td>3(30)</td>
<td>2(40)</td>
<td>0</td>
<td>5(100)</td>
<td>1(50)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11(26.8)</td>
</tr>
<tr>
<td>Honeycomb</td>
<td>1(10)</td>
<td>0</td>
<td>0</td>
<td>4(80)</td>
<td>1(50)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6(14.6)</td>
</tr>
</tbody>
</table>

### Table 2: Details of Polysomnography findings in OSAs patients

<table>
<thead>
<tr>
<th>Polysomnography parameters</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sleep Efficiency (%)</td>
<td>73.92±10.68</td>
</tr>
<tr>
<td>Total Sleep Time (minute)</td>
<td>340.54±79.40</td>
</tr>
<tr>
<td>Sleep Latency (minute)</td>
<td>18.86±16.86</td>
</tr>
<tr>
<td>Stage 1(%)</td>
<td>13.81±0.07</td>
</tr>
<tr>
<td>Stage 2(%)</td>
<td>35.62±0.15</td>
</tr>
<tr>
<td>Stage 3(%)</td>
<td>31.92±0.13</td>
</tr>
<tr>
<td>REM (%)</td>
<td>17.37±0.12</td>
</tr>
<tr>
<td>Hypopnea (%)</td>
<td>6.11±5.66</td>
</tr>
<tr>
<td>Mean Oxygen Saturation (%)</td>
<td>14.49±32.80</td>
</tr>
<tr>
<td>Wake After Sleep Onset (minute)</td>
<td>108.08±56.82</td>
</tr>
</tbody>
</table>

### Table 3: Correlation of OSA with pulmonary function test BMI and ODI

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Polysomnography Correlation</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV1/FVC AHI</td>
<td>-0.417 P=0.007</td>
<td></td>
</tr>
<tr>
<td>FEVI AHI</td>
<td>-0.024 P=0.882</td>
<td></td>
</tr>
<tr>
<td>FVC AHI</td>
<td>0.096 P=0.550</td>
<td></td>
</tr>
<tr>
<td>DLCO AHI</td>
<td>-0.024 P=0.889</td>
<td></td>
</tr>
<tr>
<td>TLC AHI</td>
<td>-0.079 P=0.627</td>
<td></td>
</tr>
<tr>
<td>ODI AHI</td>
<td>0.725 P=0.000</td>
<td></td>
</tr>
<tr>
<td>BMI AHI</td>
<td>0.535 P=0.000</td>
<td></td>
</tr>
<tr>
<td>6MWT AHI</td>
<td>-0.061 P=0.704</td>
<td></td>
</tr>
</tbody>
</table>

### Result

The mean age was 55.5±10 years with 73% female. The mean BMI of 28.8±6.5 kg/m² with dyspnea and cough were the common symptoms. The most common type of ILD was HP (46.3%). The common HRCT findings were GGO (82.9%), and fibrosis (65.9%). The OSA was seen in 32(78%) patients with 24 females. The AH1 showed significant positive correlation with BMI (p=0.000, r= 0.535) and ODI (p=0.000, r=0.725), while negative correlation with FEV1/FVC% (p=0.007, r= -0.417).

### Conclusion

The prevalence of OSA is common in north Indian ILD patients. Polysomnography should be done in all the patients with ILD whenever feasible for early diagnosis and treatment of OSA, as the coexisting of both will further decrease quality of life and increase morbidity in this chronic progressive lung disease.

### Fig. 1: Details of frequency and severity of OSA in different types of ILDs
Methods

This study was a prospective observational study conducted at Vishwanathan Patel Chest hospital of Vallabhbhai Patel Chest Institute, University of Delhi from April 2018 to March 2019. The study was carried out after the approval of the institutional ethics committee. It was a study of one year duration in 41 confirmed ILD patients. All the enrolled patients underwent detailed clinical examination radiological and laboratory investigations (HRCT chest, pulmonary function test, serological investigations, fiberoptic bronchoscopy, and histological assessment). All the patients underwent OSA evaluation by Type 1 diagnostic polysomnography (PSG) according to American Academy of Sleep Medicine guideline. After diagnosis of OSA based on polysomnography, the severity was defined as per apnoea hypopnoea index (AHI). The severity was normal if (AHI < 5/hour), Mild OSA (5-15/hour), Moderate OSA (16-30/hour) and Severe OSA (>30/hour). Patient with prior OSA, psychiatric disorder, age less than 18 and cognitive impairment were excluded from study. We also calculated the correlation of OSA with various pulmonary function test parameters, body mass index (BMI), and oxygen desaturation index (ODI) using Pearson’s correlation method.

Type 1 Polysomnography: Polysomnography was done by comprising of at least 7 channels namely Electroencephalogram, Electrooculography, Electromyography, nasal transducer, pulse oximeter, and chest and abdominal effort leads. In PSG an apnoea is defined as the complete cessation of airflow for at least 10 seconds. Whereas hypopnoea is defined as a reduction in airflow (30%) that is followed by an arousal from sleep accompanied by decrease in oxygen saturation by 3%. The AHI is calculated by dividing the number of apnoea hypopnoea events by the number of hours of sleep. On the basis of AHI Obstructive sleep apnea hypopnoea syndrome is defined as apnoea hypopnoea index (AHI) >5 with excessive daytime sleepiness. 24

Statistical Analysis: The variables collected were entered in Microsoft Excel (Office 2007 or higher) and analysed using SPSS (version 19 or higher). There were no comparisons in the study. The continuous data is presented as mean with standard deviation and categorical data is presented as percentages. The correlation coefficient was calculated by Pearson’s correlation method. The P value <0.05 was considered as significant.

Results

The mean age of patients was 55.5±10years (30-70years) with majority of patient 30(73%) were female. Thirty (75%) patients were housewives by occupation, while remaining 11 (27%) were employed. Seven (17%) patients had history of smoking. The mean BMI was 28.8±6.5 kg/m² and 15(36.5%) patients had BMI of >30Kg/m². The most common presenting symptoms were dyspnea and cough in 100% followed by joint pain (17%), dysphagia (7%) and one each case of haemoptysis and skin symptoms. On examination digital clubbing was found in 8/41(44%) and chest auscultation revealed crepitation in 36/41(88%) and rhonchi in 2/41(5%) patients. The most common type of ILD was HP 19(46.3%) followed by Sarcoidosis 10 (24.4%), CTD-ILD 5 (12.2%), nonspecific interstitial pneumonia (NSIP) 5 (12.2%) and IPF 2 (4.9%) patients.

The common chest X ray finding was reticulo-nodular opacities in all the patients with involvement of all zones in nearly 60% cases. While common abnormalities on HRCT chest were ground glass opacities (83%) and fibrosis (66%). The details of HRCT finding with various types of ILD are shown in Table 1. All the patients were able to perform the spirometry. However one patient was not able to perform lung volumes measurement, while DLCO performed by 37 patients only. The mean FEV₁ % and FVC% predicted value was 63.6±15.0 and 64.3±12.6 with the mean FEV₁/FVC of 84.7±6.4. The mean TLC% predicted value was 70.2±16.0% and the mean DLCO% predicted value was 70.0±33.3%. All the patients completed the 6 minute walk test. Majority of patients 33(80%) completed the 6MWT without desaturation while desaturation of >4% was found in 8(19.5%) patients. The serological workup for ILD was done in all the patients. Rheumatoid factor was positive in 4(9.8%) followed by c-ANCA 2(4.9%), p-ANCA 2(4.9%) and ANA 1(2.4%) patient.

The overall OSA on PSG was seen in 32(78%) patients with 24female and 8males. The OSA was mild in 15(46.9%), moderate in 7(21.9%) and severe in 10(31.3%) cases. Six patients with OSA were smoker, while remaining 26(81%) non-smoker. Among the subtypes of ILD, the highest frequency of OSA was seen in HP (50%), followed by sarcoidosis (22%) and NSIP (15.6%). The detail of OSA severity in different type of ILD is shown in Figure 1. The details of other polysomnography parameters in ILD patients are shown in Table 2. The overall sleep duration was decreased with mean total duration

![Fig. 2: (a, b and c) Correlation of AHI with BMI, ODI and FEV1/FVC; (d) Correlation of BMI with ODI](image)
of 340±79.4 minute. The sleep latency was almost normal with mean of 18.86±16.86 minute. The sleep efficiency was decreased with mean of 73.9±10.6%. Over all there was slightly decreased REM sleep (17%) and increased non-REM sleep (83%). Among stages of non-REM sleep, there was increase in stage I and III (N1-14%, N3-32%) and decrease in stage II (N2-35%) sleep. The AHI showed statistically significant positive correlation with BMI (p=0.000, r=0.535) and ODI (p=0.000, r=0.725). Among various lung function parameters, AHI had statistically significant negative correlation with FEV1/FVC% (p=0.007, r=-0.417). However AHI showed no statistically significant correlation with other PFT parameters including FVC, FEV1, DLCO and TLC. There was also statistically significant positive correlation between BMI and ODI (p=0.008, r=0.406). The AHI showed significant correlation with 6MWT distance (r=-0.061, p=0.704). The detailed of AHI correlation with various parameters are shown in Table 3 and Figure 2.

**Discussion**

The occurrences of OSA in patients with ILD have been a growing field of research over the past few years and several studies have reported the association of OSA in ILD. Most of the studies in relation to OSA and ILD are from other countries and the data from India is scarce. This study was done to generate more evidence on the existing data of the occurrence of OSA in the patients of ILD from India. Timely diagnosis and management of sleep disorders in patients of ILD may help in better outcome of these patients and also improve the quality of life.

Various small studies from other parts of world found that the OSA is frequently seen in patients of ILD. Recently a review showed that the overall sleep breathing disorder was seen in 44-72% of ILD patients.23 Pihlil A et al in a study of OSA among different types ILD patients found the overall frequency of OSA was 68%. The frequency of OSA was highest in IPF compared to other ILDS.26 Mavroudi M et al in a study showed that the OSA was seen in 78% of IPF and 57% of sarcoidosis patients.27 Aydogdu M et al in another study of 37 ILD patients revealed that the prevalence of OSAS was 64.8%. They also found that the total sleep time, time spent in NREM sleep stage III and IV, and in REM sleep were decreased.20 Mermigkis C et al in a study of 18 IPF patients also showed OSA in 11(61%) patients and all patients showed a reduction in sleep efficiency, REM sleep, and slow wave sleep.21 We found overall prevalence of OSA in 78% patients in the present study. The moderate to severe OSA was seen in 17(53%) patients. The total sleep time, sleep efficiency, REM sleep and ODI were also decreased. So the overall frequency of OSA was slightly higher in present study. This high frequency of OSA may be due to different subtypes of ILDs as majority of our patient were of HP, while most of the published literature included IPF and sarcoidosis patients. The other possibilities may be due to different genetic, socio-economic, environmental and demographic factors as the present study is of Indian patients.

As per the severity of OSA, we found mild OSA in 15(46.9%), moderate in 7(21.9%) and severe in 10(31.3%) cases. The severity was mild in nearly half of the patients. Similarly Mavroudi M et al in a study shown that the overall severity of OSA was mild in IPF(68%) and sarcoidosis (52%).27 In present study within the subtypes of ILD, the occurrence of OSA was seen in 100% of IPF and NSIP patients followed by HP (84%), sarcoidosis (70%) and CTD-ILD(60%), but the maximum number of OSA patient was seen in HP 16cases followed by sarcoidosis (7), NSIP (5), CTD-ILD(3) and IPF (2). This higher number of HP subtype ILD patient is due to not normal distribution of enrolled patients as the study was designed to study the OSA among overall ILDs. Similarly Pihtili A et al in a study also found high frequency of OSA in IPF compared to other subtypes.26 While Lancaster LH et al found higher prevalence of OSA in IPF patients with more severe disease. This was explained by the patient’s characteristics as they included all IPF patients of older age, higher BMI with more co-morbidities.27 The two common types of ILDs with OSA in our study were HP and sarcoidosis. The higher number of patients with above two types was due to the higher overall enrolment of such as 29(71%) of patients belonged to these subtypes of ILDs. Even the previously published data on ILD subtyping from north India has shown that the common types of ILDs are sarcoidosis, IPF and HP.9,10,11,28,29 Also recent studies have shown that HP is being recognised as one of the common type of ILD even without occupational history in India.30,31

The various studies on OSA in ILD patients concluded that the OSA is common among ILD patients but which type of ILD is more prone to OSA is not yet clarified due to unequal distribution of the subtypes patients in the study population. So there is need for larger study with equal number of all the types of ILD patients for polysomnography to assess the frequency of OSA in different subtypes of ILD. The mechanism of OSA in ILD is not fully understood and consider as multi-factorial. However one of the reasons for high OSA is explained by small lung volumes in ILD leading to increase traction on the upper airway and hence increase collapsibility, which is exacerbated in sleep by reduced tone of the intercostals muscles, and hence further reduction in the functional residual capacity.30

There was statistically significant positive correlation of AHI with BMI and ODI. There was no significant correlation of AHI with FEV1, FVC, TLC and DLCO. The FEV1/FVC showed statistically significant negative correlation with BMI. Similar to our study Mermigkis C et al in a study of OSA among IPF patients found that the AHI was positively correlated with body mass index (p<0.0001, r = 0.80) and negatively correlated with FEV1 (p=0.04, r=-0.49) and FVC% (p=0.08, r=-0.42).31 Utpat K et al in a study found that the mean FVC and 6MWT distance were lower in SDB patients than ILD patients without SBD. The mean values of both parameters were further decreased with severity of OSA.33 Zang XL et al in a study of fibrotic ILD patients found that the all lung function parameter were lower in OSA patients compared to non-OSA patients but it was not statistically significant.32 While Lee JH et al in a study of 86 ILD patients from Korea found that the lung function parameters (FVC, FEV1, DLCO) and 6MWT distance were higher in OSA patient compared to non-OSA but not statistically significant.35 So there is a
need for larger study of various sleep breathing disorder in different types of ILD with correlation of various physiological parameters. This will help in early diagnosis of coexisting OSA in ILD and treatment for better quality of life in this non reversible chronic lung disease.

Among the limitations, the present study was of one year duration hence there was a limitation of time. So the types of ILDs enrolled were not uniform with one type with large number of patients and other with few. This may explain the high number of HP patients and 100% of IPF with OSA. Overall there is scarcity of study on OSA in ILD patients from our country. So even with small sample size, this study may be an eye opener for clinician and researcher for further research. This will also lead to early diagnosis of co-existent OSA and better treatment. The other limitation was that it was a single centre study with only 41 patients. So the population of study is low for prediction of OSA among ILD patients in India. Also the prevalence of ILD subtypes is different from other parts of India. So there is a need of larger multicentre study from India with uniform enrolment of various subtypes. About strength this is one of few studies from India on OSA in ILD patients. The study also highlight the important of early diagnosis and treatment of co-existence OSA in ILD patients for better symptom control and quality of life. We advised for screening sleep study in ILD patients whenever feasible.

**Conclusion**

The prevalence of OSA is common in north Indian ILD patients. Polysomnography should be done in all the patients with ILD to facilitate early diagnosis and treatment. The coexistence of OSA with ILD will further decrease quality of life and increases morbidity in this chronic non-reversible progressive lung disease.

**References**


In the management of Heart Failure and T2DM with multiple CV risk factors

EmilDap
Dapagliflozin 5mg/10mg Tablets
Empower Heart

In Hypertension associated with CHF - Post MI

METPURE®-TEL
S(−)Metoprolol PR 25 mg & Telmisartan 20/40 mg Tablets
Controls Hypertension, Ensures Cardiac PROTECTION

In Hypertension with Diabetes

Temsan®-AM
Telmisartan 40 mg & S(−)Amlodipine 2.5/5 mg Tablets
Swift BP Reduction, Assured Control
Physicians’ Knowledge, Attitudes, Practices and Perceived Needs Regarding Specialised Geriatric Healthcare: A Nation-wide Survey in India

Santosh Salagre1*, Amey Kundawar2, Abhishek Ukarde2, Akash Mantri2, Nikita Chandak2, Prarthna Srivastava2, Shaurya Jain2, Tejas Saha2, Vaibhav Karandekar2

Abstract
Background: The geriatric population in India is projected to increase from 8% to a staggering 20% by 2050. The combination of a population boom along with advances in medicine resulting has resulted in an increase in life span, leaving India with a potential geriatric healthcare crisis in its hands. India currently produces as few as 20 geriatricians per year due to limited PG seats and has only a handful of fully functioning Geriatric Departments in the public healthcare sector. Thus, there is a need to fully assess the knowledge, attitudes and current practices in geriatric healthcare among medical professionals and interns across the country.

Objective: To investigate the knowledge, attitudes and practices and perceived needs of physicians towards specialised geriatric healthcare and to assess the views towards geriatric medicine as a career option among medical interns in India.

Design: Cross-sectional, web-based survey by forwarding the link via social media platforms.

Setting: MBBS graduates undergoing their rotatory internship and residents/postgraduate doctors in specialties relevant to the care of older persons throughout India.

Participants: A total of 800 Indian medical interns and professionals.

Measurements: Demographic characteristics of medical professionals and interns included age, gender, branch of practice, working sector, availability of any geriatric facility in their workplace, etc. Responses were weighted to maintain nationwide representativeness. Knowledge, attitudes, current practices and perceived needs regarding specialised geriatric healthcare were the primary outcome measurements.

Results: Insufficient knowledge (48.5% of professionals) regarding any specialised branch of geriatric healthcare was found. Only 9.0% medical professionals performed ‘Comprehensive Geriatric Assessment’ and even the mean score of practice of CGA was low. 96% professionals and 92% interns felt the need for specialised geriatric services throughout India with majority feeling the need for specialised OPDs. 32.7% of Interns were willing to opt for post-graduation in Geriatrics, if given a choice and those with any specialised geriatric facility available at their institute were more willing. More than 85% professionals and interns had affirmative attitudes towards the possible benefits of specialised healthcare.

Conclusion and Implications: There is poor practice of specialised geriatric healthcare throughout India and also a high prevalence of perceived needs among professionals and interns regarding facilities like OPDs, wards and departments. Highly affirmative attitudes were observed among both interns and professionals indicating the acceptance of suggested strategies. Majority of interns were convinced of opting for post-graduation in geriatrics when incentivized, indicating the need for prioritizing interns for capacity building in the future.

Introduction
The elderly population in India that accounted for 8.6% in 2011 has been projected to increase to 19% by the year 2050.1-3 India has thus acquired the label of ‘An ageing nation’.4 It is estimated that it will take only 25-30 years for the ≥65 years age group to reach double the number of children under the age of 5. This implies that in the near future, more geriatricians will be required than pediatricians. Preparations therefore need to begin decades in advance.5,6

In India, the elderly people suffer from dual medical problems, i.e., both communicable as well as non-communicable diseases. This is further compounded by impairment of special sensory functions like vision and hearing. A decline in immunity as well as age-related physiological changes lead to an increased burden of communicable diseases in the elderly.6 However, current literature suggests that unsupportive attitudes with, limited awareness, lack of knowledge and non-acceptance of geriatrics as an established discipline result in...
The main issue in geriatric care is not merely concerned with the physiological phenomenon which is inevitable, but also with the medical health problems and diseases specifically afflicting an individual in old age warranting medical management in order to sustain comfortable and healthy aging. Thus geriatric care has to address two problems- first, basic health promotion to delay the rate of physiological aging and second, medically manage diseases and disorders incident to old age.6

At the tertiary care level, which comprises super specialty and medical college hospitals, there needs to be provision of geriatric wards and separate OPDs. A ‘multi-disciplinary team’ specifically trained to meet the requirements of the geriatric population needs to be created.4

The healthcare system in India needs to address ageism. Care for the elderly is fast emerging as a critical element of both public and private concern.7 The current scenario in India is witnessing a major sparsity of geriatricians which is a clear consequence of lack of education and development of Geriatrics as a branch of medicine.8 Geriatric healthcare thus requires unprecedented attention and rightly so, as the world is witnessing the phenomenon of global ageing.

This study aimed to investigate the knowledge, attitudes, practices and perceived needs of physicians towards specialised geriatric healthcare and to assess the views towards geriatric medicine as a career option among medical interns in India.

Materials and Methods

Study participants

We performed a cross-sectional, questionnaire-based study. The study was conducted in medical professionals and medical interns. 400 medical professionals and medical interns each were recruited for a total of 800 as the sample size for this study. Due to unavailability of any literature, maximum variability, which is equal to 50% (population proportion, p = 0.5), 95% confidence level with ± 5% precision was considered for sample size calculation. By formula for sample size calculation (n = Z²p (1-p)/e²; where Z is Standard error at 95% Confidence Interval, p is population proportion and e is precision) for sample size calculation, the required sample was 384.16 rounding off to 400. Hence a sample of 400 was considered for each subgroup.

Eligibility criteria were: MBBS graduates undergoing their compulsory rotatory internship or residents and postgraduate doctors who were practicing one of the following branches: Medicine, Surgery, Obstetrics and Gynaecology, Ophthalmology, ENT (Otorhinolaryngology), Psychiatry, Orthopaedics and Community medicine. We excluded participants practicing outside India.

Instrument and procedure

Two separate questionnaires were developed for two study groups viz. interns and medical professionals. The questionnaire items consisted of multiple choice questions and Yes/No answer type questions.

A focus group discussion among medical interns was conducted and medical professionals were subjected to unstructured interviews to gather information regarding the current practices with respect to geriatric healthcare and views on specialised geriatric healthcare and geriatric medicine as a career option. A structured questionnaire was drafted after analysing their responses and with the help of previous foreign studies.9,10 The validation of questionnaires was done by experts and a pilot study among both interns and medical professionals.

The study was approved by the Institutional Ethics Committee. Participants were recruited prospectively to the study, which took place between April 2019 and October 2019. Interns and medical professionals from various government and private institutes in various states throughout India were recruited by forwarding the link of Informed Consent Document to be digitally signed along with self-administered online questionnaires via social media platforms. The participation was anonymous.

Statistical analysis

Statistical analysis was done by using descriptive statistics. Data was collected in a predesigned Microsoft Excel sheet and analysed in SPSS 24.0. Continuous variables were presented as mean values ± standard deviation (SD), and categorical variables were presented as percentages. We quantified the practices, perceived needs and views in the form of proportions of interns and professionals. We assessed associations between type of institute, region of workplace, branch of medicine and practices, perceived needs and views. Chi-square test was applied for sub-group comparisons and checking the associations for proportions of categorical variables. All statistical analysis was performed at 95% Confidence Interval and P-value < 0.05 was considered significant.
Factors convincing them to opt for this branch: Less availability of MD seats (85.1%), Different interests (232.86%), Less pay (73.27%), Minimal scope for research (70.26%), Less job satisfaction (136.50%).

Reasons for opting for this branch: More job satisfaction (53.40%), Better scope for research (51.38%), Knowledge about any specialised branch of geriatric healthcare (177.44.25%), Knowledge about Comprehensive Geriatric Assessment (CGA) (168.42%).

The study was conducted to assess the willingness of medical professionals and interns to opt for geriatrics and the availability of specialisation in their institute. The study was carried out in 2022.

Table 2: Knowledge about Specialised Geriatric Healthcare

<table>
<thead>
<tr>
<th>Knowledge about any specialised branch of geriatric healthcare</th>
<th>Medical professionals (n = 400)</th>
<th>Interns (n = 400)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knowledge about Comprehensive Geriatric Assessment (CGA)</td>
<td>194 (48.50%)</td>
<td>229 (57.25%)</td>
</tr>
<tr>
<td>Knowledge about Affection towards elderly</td>
<td>177 (44.25%)</td>
<td>190 (47.50%)</td>
</tr>
<tr>
<td>Knowledge about More job satisfaction</td>
<td>168 (42%)</td>
<td>-</td>
</tr>
<tr>
<td>Knowledge about Better scope for research</td>
<td>158 (39.5%)</td>
<td>-</td>
</tr>
<tr>
<td>Knowledge about Different interests</td>
<td>132 (33%)</td>
<td>-</td>
</tr>
<tr>
<td>Knowledge about Less pay</td>
<td>117 (29%)</td>
<td>-</td>
</tr>
<tr>
<td>Knowledge about Minimal scope for research</td>
<td>108 (27%)</td>
<td>-</td>
</tr>
<tr>
<td>Knowledge about Less job satisfaction</td>
<td>100 (25%)</td>
<td>-</td>
</tr>
<tr>
<td>Knowledge about Less scope for practice in India</td>
<td>96 (24%)</td>
<td>-</td>
</tr>
<tr>
<td>Knowledge about Less availability of MD seats</td>
<td>88 (22%)</td>
<td>-</td>
</tr>
<tr>
<td>Knowledge about Factors convincing them to opt for this branch</td>
<td>80 (20%)</td>
<td>-</td>
</tr>
<tr>
<td>Knowledge about Increased pay</td>
<td>72 (18%)</td>
<td>-</td>
</tr>
<tr>
<td>Knowledge about More scope for research</td>
<td>66 (16%)</td>
<td>-</td>
</tr>
<tr>
<td>Knowledge about Increment in post-graduate (MD) seats</td>
<td>58 (14%)</td>
<td>-</td>
</tr>
<tr>
<td>Knowledge about Better infrastructure dedicated to geriatrics</td>
<td>56 (14%)</td>
<td>-</td>
</tr>
<tr>
<td>Knowledge about Increased scope for practice</td>
<td>52 (13%)</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3: Attitudes towards specialised geriatric healthcare

<table>
<thead>
<tr>
<th>Specialised geriatric healthcare would:</th>
<th>Medical professionals (n = 400)</th>
<th>Interns (n = 400)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Provide a holistic approach for treatment</td>
<td>373 (93.2%)</td>
<td>352 (88.0%)</td>
</tr>
<tr>
<td>Reduce polypharmacy and potentially inappropriate medications</td>
<td>365 (91.2%)</td>
<td>356 (89.0%)</td>
</tr>
<tr>
<td>Promote psychological wellbeing of patients</td>
<td>380 (95.0%)</td>
<td>356 (89.0%)</td>
</tr>
<tr>
<td>Improve quality of life in patients</td>
<td>361 (90.2%)</td>
<td>356 (89.0%)</td>
</tr>
<tr>
<td>Aid healthy ageing</td>
<td>353 (88.2%)</td>
<td>356 (89.0%)</td>
</tr>
<tr>
<td>Willing to acquire more knowledge and training in geriatric healthcare</td>
<td>378 (94.5%)</td>
<td>22 (5.5%)</td>
</tr>
</tbody>
</table>

Table 4: Attitudes towards geriatric medicine as a career option

<table>
<thead>
<tr>
<th>Willing to opt for post-graduation (MD) in geriatrics</th>
<th>Medical professionals (n = 400)</th>
<th>Interns (n = 400)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Willing to opt for post-graduation (MD) in geriatrics</td>
<td>131 (32.7%)</td>
<td>123 (30.7%)</td>
</tr>
<tr>
<td>Reasons for opting for this branch</td>
<td>131 (32.7%)</td>
<td>123 (30.7%)</td>
</tr>
<tr>
<td>Affection towards elderly</td>
<td>49 (37.4%)</td>
<td>41 (33.8%)</td>
</tr>
<tr>
<td>Better scope for research</td>
<td>53 (40.4%)</td>
<td>47 (38.8%)</td>
</tr>
<tr>
<td>More job satisfaction</td>
<td>51 (38.9%)</td>
<td>46 (38.3%)</td>
</tr>
<tr>
<td>Better scope for practice considering their increasing population</td>
<td>84 (64.1%)</td>
<td>76 (63.3%)</td>
</tr>
<tr>
<td>Unwilling to opt for post-graduation (MD) in geriatrics</td>
<td>269 (67.2%)</td>
<td>307 (79.3%)</td>
</tr>
<tr>
<td>Reasons for not opting for this branch</td>
<td>269 (67.2%)</td>
<td>307 (79.3%)</td>
</tr>
<tr>
<td>Different interests</td>
<td>232 (58.2%)</td>
<td>208 (52.0%)</td>
</tr>
<tr>
<td>Less pay</td>
<td>73 (27.1%)</td>
<td>68 (17.0%)</td>
</tr>
<tr>
<td>Minimal scope for research</td>
<td>70 (26.0%)</td>
<td>66 (16.5%)</td>
</tr>
<tr>
<td>Less job satisfaction</td>
<td>136 (49.3%)</td>
<td>120 (30.0%)</td>
</tr>
<tr>
<td>Less scope for practice in India</td>
<td>135 (50.1%)</td>
<td>123 (30.7%)</td>
</tr>
<tr>
<td>Less availability of MD seats</td>
<td>85 (35.3%)</td>
<td>78 (19.5%)</td>
</tr>
<tr>
<td>Factors convincing them to opt for this branch (n = 269)</td>
<td>85 (31.6%)</td>
<td>82 (20.5%)</td>
</tr>
<tr>
<td>Increased pay</td>
<td>85 (31.6%)</td>
<td>82 (20.5%)</td>
</tr>
<tr>
<td>More scope for research</td>
<td>66 (24.5%)</td>
<td>63 (16.0%)</td>
</tr>
<tr>
<td>Increment in post-graduate (MD) seats</td>
<td>183 (68.0%)</td>
<td>174 (44.0%)</td>
</tr>
<tr>
<td>Better infrastructure dedicated to geriatrics</td>
<td>218 (81.0%)</td>
<td>208 (52.0%)</td>
</tr>
<tr>
<td>Increased scope for practice</td>
<td>110 (40.9%)</td>
<td>87 (21.7%)</td>
</tr>
</tbody>
</table>

Table 5: Association between willingness to opt for geriatrics and availability of geriatric facility

<table>
<thead>
<tr>
<th>Availability of any specialised geriatric facility</th>
<th>Willing to opt for post-graduation (MD) in geriatrics</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>54 (42.18%)</td>
<td>74 (57.82%)</td>
</tr>
<tr>
<td>No</td>
<td>77 (28.30%)</td>
<td>195 (71.70%)</td>
</tr>
</tbody>
</table>

*p indicates a significant association

**Ethical considerations**

The study was carried out in compliance with the principles of the World Medical Association Declaration of Helsinki. The eligible participants were given an online-administered Informed Consent Document informing them that participation was anonymous and voluntary and that choosing not to participate would not affect their studies or future careers negatively. The ethical clearance was obtained from the Institutional Ethics Committee.

**Results**

**Demographic data**

We received a total of 800 responses with 400 medical professionals and interns each. The demographic details of Medical professionals and interns recruited in the study are summarized in Table 1.

**Knowledge about specialised geriatric healthcare**

Less than half of the medical professionals (48.5%) had knowledge about the existence of any specialised branch of geriatric healthcare, in contrast to the 57.25% Interns. Around 44% medical professionals knew about Comprehensive Geriatric Assessment (CGA). 42% Medical professionals had knowledge about physiotherapists and occupational therapists being a part of CGA. Only 17.75% medical professionals had knowledge about all the three aspects (Table 2).

**Attitudes towards specialised geriatric healthcare**

A majority (more than 85%) of the medical professionals and interns had an overall affirmative attitude towards possible benefits of specialised geriatric healthcare. 93.2% medical professionals believed that specialised geriatric healthcare would ‘provide a holistic approach for treatment’ which was significantly higher than interns (p = 0.03). 10% medical professionals believed that specialised geriatric healthcare would not ‘aid healthy ageing’ which was significantly higher than interns (p = 0.0003). More than 90% medical professionals and interns believed that specialised geriatric healthcare would ‘promote psychological well-being of patients’. 94.5% medical professionals were willing to acquire more knowledge and training in the field of geriatric healthcare (Table 3).

**Attitudes towards Geriatrics as a career option**

32.7% Interns were willing to opt for post-graduation (MD) in geriatrics, if given a choice. Interns with any specialised geriatric facility available at their institute were more willing to opt for this branch (42.18%) than those with no facility (p = 0.006) indicating a significant association (Tables 4, 5).

The common reason chosen by majority (64.1%) for willingness to opt for this branch was a ‘better scope for practice considering their increasing population’. While the leading common reason (86.2%) for not opting for this branch was that they had ‘different interests’, others were ‘less job satisfaction’ and ‘less scope for practice in India’ (50%). The commonest factors convincing them to opt for this branch were a ‘better infrastructure dedicated to geriatrics’ (81.0%) and an ‘increment in post-graduate (MD) seats’ (68.0%).

**Current Practices in specialised geriatric healthcare**

More than 85% of the medical professionals were facing difficulties while addressing geriatric patients. The
most common (98.0%) difficulties were ‘unrelated history’ and ‘requirement of more time’.

Assessment of practice of CGA was done considering 10 factors. Although 98.7% professionals claimed to have been checking allopathic medications and prescriptions, only 67.5% assess polypharmacy. Less than 50% medical professionals assess mental health of their geriatric patients. Only 9.0% medical professionals performed ‘Comprehensive Geriatric Assessment’. Only 12.7% referred their geriatric patients to physiotherapists and occupational therapists for CGA. The mean score of practice of CGA (out of 10) was 5.40 ± 2.35 (Table 6).

There was a significant association between branches of practice and CGA practice score by one-way ANOVA test. The highest mean CGA practice score was observed in psychiatrists (6.34 ± 2.73) and orthopedicians (6.19 ± 2.48) and lowest in ophthalmologists (3.58 ± 2.11) (Table 7).

**Table 7: Comparison and association between branches of practice and CGA practice score of medical professionals (n = 400)**

<table>
<thead>
<tr>
<th>Branch of practice</th>
<th>Mean CGA practice score</th>
<th>Significance (One-way ANOVA test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Community medicine</td>
<td>5.05 ± 2.24</td>
<td>F value = 7.669* p &lt; 0.0001* (indicates a significant association)</td>
</tr>
<tr>
<td>ENT</td>
<td>5.10 ± 1.63</td>
<td></td>
</tr>
<tr>
<td>Internal medicine</td>
<td>5.89 ± 1.84</td>
<td></td>
</tr>
<tr>
<td>Obstetrics and Gynecology</td>
<td>5.70 ± 1.96</td>
<td></td>
</tr>
<tr>
<td>Ophthalmology</td>
<td>3.58 ± 2.11</td>
<td></td>
</tr>
<tr>
<td>Orthopedics</td>
<td>6.19 ± 2.48</td>
<td></td>
</tr>
<tr>
<td>Psychiatry</td>
<td>6.34 ± 2.73</td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>5.08 ± 2.68</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>5.40 ± 2.33</td>
<td></td>
</tr>
</tbody>
</table>

**Perceived needs regarding geriatrics and specialised healthcare**

More than 80% medical professionals and interns felt the need for various specialised geriatric services throughout India with majority feeling the need for specialised OPDs (96% medical professionals and 92% interns). More medical professionals working in a public sector (97.18%) felt the need for specialised geriatric OPDs than those working in a private sector (94.76%) with a significant difference (p < 0.00001). More medical professionals working in a public sector (90.14%) felt the need for specialised geriatric departments (p = 0.015).

97.2% professionals felt the need for Comprehensive Geriatric Assessment (CGA) in every geriatric patient.

Nearly 80% medical professionals felt the need for an allied subject and clinical postings on geriatrics in PG courses. A contrasting observation was made between the felt needs for a short (allied) subject and clinical postings in UG courses among medical professionals and interns. More interns (74.50%) felt the need for a short (allied) subject on geriatrics than medical professionals (55.25%) with a significant difference (p < 0.00001). In contrast, more medical professionals (77.25%) felt the need for clinical postings in geriatrics than interns (29.50%) with a significant difference (p < 0.00001) (Tables 8, 9, 10).

**Discussion**

**Summary of the findings**

The data were collected from Interns and Medical professionals of eight specialties in India, with all levels of seniority of the doctors, types and levels of medical organizations, covering those working in both urban and rural areas. The data covered physicians from 23/29 states and 5 union territories of India, representing nearly all regions of India. Since the respondents are those with higher educational background and majority of them working in higher level hospital than average, their knowledge, attitudes, practices, and working in a public sector (90.14%) felt the need for specialised geriatric departments (p = 0.015).

97.2% professionals felt the need for Comprehensive Geriatric Assessment (CGA) in every geriatric patient.

Nearly 80% medical professionals felt the need for an allied subject and clinical postings on geriatrics in PG courses. A contrasting observation was made between the felt needs for a short (allied) subject and clinical postings in UG courses among medical professionals and interns. More interns (74.50%) felt the need for a short (allied) subject on geriatrics than medical professionals (55.25%) with a significant difference (p < 0.00001). In contrast, more medical professionals (77.25%) felt the need for clinical postings in geriatrics than interns (29.50%) with a significant difference (p < 0.00001) (Tables 8, 9, 10).

**Table 9: Comparison of perceived needs of professionals from various working sectors**

<table>
<thead>
<tr>
<th>Need for geriatric facilities</th>
<th>Working sector</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>specialised geriatric OPD</td>
<td>Public</td>
<td>138 (97.16)</td>
</tr>
<tr>
<td></td>
<td>Private</td>
<td>199 (94.76)</td>
</tr>
<tr>
<td></td>
<td>Both</td>
<td>36 (75.0)</td>
</tr>
<tr>
<td>p = 0.00001*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>specialised geriatric depart</td>
<td>Public</td>
<td>126 (90.14)</td>
</tr>
<tr>
<td></td>
<td>Private</td>
<td>187 (89.04)</td>
</tr>
<tr>
<td></td>
<td>Both</td>
<td>36 (75.0)</td>
</tr>
<tr>
<td>p = 0.015*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*indicates a significant association
regarding to specialised geriatric healthcare may be overestimated.

Insufficient knowledge (48.5% of medical professionals) regarding any specialised branch of geriatric healthcare was obvious especially among medical professionals as compared to interns.

Only 9.0% medical professionals performed ‘Comprehensive Geriatric Assessment’ and even the mean score of practice of CGA was low (5.40) indicating a poor practice of comprehensive assessment. More than 80% medical professionals and interns felt the need for various specialised geriatric services throughout India with majority feeling the need for specialised OPDs (96% medical professionals and 92% interns).

A significant proportion (32.7%) of Interns were willing to opt for post-graduation in geriatrics, if given a choice and those with any specialised geriatric facility available at their institute were more willing. A majority (more than 85%) of the medical professionals and interns had an overall affirmative attitude towards possible benefits of specialised geriatric healthcare and were willing to acquire more knowledge and training in the field of geriatric healthcare. We did not find any studies investigating the knowledge, attitudes and practices and perceived needs of doctors or interns towards specialised geriatric healthcare.

Strengths and limitations

To the best of our knowledge, this is the first study in India to assess physicians’ knowledge, attitudes and practices regarding specialised geriatric healthcare in India.

In our study, participants were recruited and the survey was administered via WhatsApp (one of the most commonly used social media in India). It is a convenient, economic and timesaving method to survey a diverse population with a wide reach. Our study covered 23 out of 29 states and 5 union territories in India. We recruited the population proportionately by correlating with size of target population from different states to maintain a nationally representative data. As many medical professionals in India work in private sectors, we had a significant proportion of our sample belonging to the private sectors.11

However, our study had a few limitations. Firstly, there is a possibility of a potential selection bias as our sample was not completely randomly selected. We used purposeful (non-probability) sampling technique to effectively recruit proportionately from various states to maintain representativeness. But for randomization, the first-round invitations were requested to send our links to their own clinical connections. Secondly, our study population constituted a low proportion of rural physicians since they are less likely to be on the groups with our clinical connection. General practitioners constitute a significant section of the healthcare providers for elderly in India but our study couldn’t cover this population. Thirdly, since the study considered snowball sampling, there is no way to calculate the number of people who saw the link but chose to ignore it.

Implications for doctors and policymakers

Our study showed an insufficient knowledge among physicians about existence of geriatrics and specialised geriatric healthcare. Less than half of the doctors had no knowledge about CGA and that occupational therapists and physiotherapists are a part of CGA. This was reflected as their poor practice of CGA including less referral to Occupational therapists and Physiotherapists. However, 95% doctors were willing to acquire more knowledge and training in specialised geriatric healthcare. This education can be promoted by organizing various seminars, workshops, CMEs etc. at the institutional, university and national levels. This can also be done by the introduction inUG and PG curriculum. Geriatrics in curriculum was more acceptable to interns in the form of a short subject and in the form of clinical postings for medical professionals. Considering the recently-implemented competency based curriculum, both a short subject and clinical postings are the need of the hour so that we can produce medical graduates who can practice principles of geriatrics irrespective of their specialty.12 The referral to OTs and PTs could also be a repercussion of their unavailability in many regions, demanding their increment for capacity building.13 Specialised geriatric healthcare and CGA are best practiced in specialised OPDs, wards and departments.14

Additionally, various difficulties faced by the doctors while addressing a geriatric patient in their practice can also be effectively tackled by specialised facilities. The overall affirmative attitude towards their possible benefits by a large proportion of the physicians reinforces their need. The National Program for Health Care of the Elderly (NPHCE) launched by the government in 2011 had sanctioned a number of such facilities but the implementation is still scarce.15 Our study showed a huge perceived need by physicians for these geriatric facilities. This compels that the implementation of geriatric OPDs, wards and departments should be given its due importance by the government.

India faces a huge dearth of geriatricians to meet the demands of the increasing geriatric population.8 However, one-third of the interns are willing to opt for becoming geriatricians if given a choice. The reasons for unwillingness to opt also included minimal scope for research and less scope for practice in India. But, this scope is ever increasing with an increasing population and hence opening avenues for development in this field. The medical undergraduates need to be made aware about the scope of this branch. Our study showed that a significant proportion of interns were willing to opt for this branch if provided with an increment in MD seats and better infrastructure. The association discovered between the availability of a specialised geriatric facility and willingness to opt for geriatrics implicate that development of infrastructure for specialised geriatric healthcare would further increase interest among the medical fraternity and strengthen geriatric healthcare.

Future research

The assessment especially of the perceived needs should be conducted in a larger sample for statistical accuracy. Studies involving assessment of practices and a need of Occupational therapists, Physiotherapists, general practitioners, nurses etc. should be undertaken. The attitudes of medical students regarding geriatrics as a career option and its inclusion in medical curriculum also need to be explored. Ultimately, interventional studies assessing the translation of change in the knowledge and attitudes into practice should be promoted for
analysing the effect of the initiatives.

Conclusion

This study concluded that currently, there is a poor practice of specialised geriatric healthcare throughout India but also showed a high prevalence of perceived needs among medical professionals and interns regarding specialised geriatric facilities like OPDs, wards and departments. An observation of highly affirmative attitudes was made among both the study groups indicating the acceptance of proposed strategies. A high number of interns were convinced of opting for post-graduation in geriatrics when provided with various incentives indicating the need for their provision to increase the number of geriatricians for capacity building in the near future.

Acknowledgement

Authors acknowledge Dr. Hemant Deshmukh, Dean Seth GS M C and KEMH Mumbai, Dr. Milind Nadkar, Professor and Head, Department of Medicine, Seth GS M C and KEMH Mumbai for their guidance and support. Authors are grateful to Dr. Nilakshi Sabnis, Assistant Professor, Department of Medicine KEMH and Dr. Munira Hirkani, Associate Professor, Department of Physiology KEMH for their valuable inputs in research formulation.

References

Evaluation of Teneligliptin a DPP4 Inhibitor in Terms of Efficacy and Safety with Respect to QT/QTc Prolongation in Patients with Type II Diabetes Mellitus (T2DM)

Deepak Bhosle1*, Bhakti Chandekar2, Shaikh Alimuddin2

Abstract

Introduction: Low risk of hypoglycemia and weight neutrality have increased the administration of dipeptidyl peptidase 4 (DPP-4) inhibitors in patients with T2DM in clinical practice. Currently Teneligliptin is prescribed as a second or third add on to the standard treatment with other classes of oral hypoglycemic agents (OHAs) to achieve targeted glycemic control in type 2 DM patients.

Methods: An open label, interventional, single arm, 12 weeks study was conducted on 160 patients with type 2 DM at MGM Medical College, Aurangabad with Teneligliptin 20 mg once a day as add on to the ongoing standard treatment with other classes of OHAs. Changes in glycemia parameters like FBS, PPBS HbA1C, body weight were assessed and twelve lead ECG was recorded with safety assessment at baseline and follow-up visits. The QTc was calculated by using the Bazett’s formula (QTc=QT/√RR). The study was conducted with an objective to assess efficacy and safety of Teneligliptin with respect to QT/QTc prolongation in patients with T2DM.

Results: A significant reduction was seen in the glycemic parameters like FBS, PPBS HbA1C from the baseline values (P<0.001) but no significant change in the QT interval (P=0.9563) and QTc interval (P=0.5594) from the baseline to the end of study at 12 weeks.

Conclusion: Tenelegliptin is a promising new drug to help to achieve targeted glycemic control in patients with T2DM without prolonging the QT/QTc interval.

Introduction

Diabetes mellitus, a heterogeneous group of metabolic syndromes is characterized by an elevation in blood glucose. A variety of pathogenic mechanisms involving insufficient insulin secretion, reduced responsiveness to endogenous or exogenous insulin, increased glucose production, and/or abnormalities in fat and protein metabolism have been recognized. The resulting prolonged hyperglycemia is the major cause of chronic long term microvascular complications of diabetes such as retinopathy, neuropathy, nephropathy, and macrovascular complications like cardiovascular diseases, cerebrovascular accidents and peripheral vascular diseases.

The worldwide prevalence of diabetes mellitus is increasing alarmingly and it is estimated to rise to 10.2% (578 million) by 2030. As per International Diabetes Federation (IDF) South-east Asia (SEA) estimates, DM is a growing challenge among Indian population with a prevalence of 8.9%.

The choice of glucose lowering agent must be made carefully, particularly when a diverse range of pharmacological agents (consisting of at least 12 drug classes) are available for the treatment of T2DM. Of these, biguanides, sulfonylureas (SUs), meglitinides, dipeptidyl peptidase - 4 inhibitors (DPP4i), thiazolidinediones - 4 inhibitors, alpha glucosidase inhibitors, and sodium glucose co-transporter 2 (SGLT2) inhibitors are the commonly used oral antidiabetic agents (OADs) both as mono and combination therapy in T2DM patients. According to the American Diabetes Association (ADA) and European Association for the Study of Diabetes (EASD) guidelines metformin has been suggested to be used as a first-line drug treatment along with lifestyle modifications (LSMs). Both agencies recommend an addition of a second drug if monotherapy with metformin along with LSMs fails to achieve glycemic control within the target levels as laid down by ADA. According to the latest ADA-EASD joint statement released in October 2018 use of the newer cardio-friendly drugs for treatment of T2DM has been highly recommended. Differing from the recommendations of EASD-ADA, Japanese Diabetes Society (JDS) emphasizes more on the pathophysiology of patients’ diabetes and recommends use of any antidiabetic drugs that are appropriate to it. Thus the incretin-based drugs especially DPP-4 inhibitors are considered to be the first choice therapy in Japanese type 2 diabetes patients according to the recommended guidelines.

Dipeptidyl peptidase 4 (DPP-4) inhibitors considered as a relatively new category, produce their effects by increasing the concentration of active forms of incretin, such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP). DPP-4 inhibitors show marked effects on glycemic control, particularly when used in combination with other oral hypoglycemic agents (OHAs). The presence of five consecutive rings of the molecule is a requirement for the DPP-4 inhibitors, which is absent in the naturally occurring GLP-1 and GIP. A novel DPP-4 inhibitor, Teneligliptin, produces a potent and long-lasting effect by virtue of its unique structure exhibiting five consecutive rings. Teneligliptin was...
teneligliptin, with respect to QTc
is available regarding the safety of cardiac safety of antidiabetic drugs prescribed in India. \[10\]
Currently DPP-4 inhibitors have been consistently prescribed in India. \[10\]
Since September 2012, teneligliptin has been commercially sold in Japan.

In the recent past all the gliptins were questioned for development of pancreatitis especially Sitagliptin and regarding cardiac safety in terms of QT/QTc prolongation especially teneligliptin. Efficacy and safety evaluation with respect to QT/QTc assessment studies associated with use of teneligliptin in patients with type 2 diabetes were conducted in Japan and other countries. \[12\]
The maximal dose of teneligliptin is considered to be 20 mg/day or 40 mg/day depending upon the glycemic status of the patient of teneligliptin, and no adverse events (AEs) related to QT prolongation were detected with these doses. \[12\]
But, during initial safety assessment studies, teneligliptin at 160 mg/day dose, was associated with changes in QT interval. \[12\]
As diabetic patients are more prone to develop cardiovascular diseases, cardiac safety of antidiabetic drugs must be ensured and demonstrated. In India not much published data is available regarding the safety of teneligliptin, with respect to QTc prolongation at therapeutic doses, in patients with type 2 DM. \[13\]

The present study was conducted to assess teneligliptin in terms of efficacy and safety with respect to QT/QTc prolongation in patients with T2DM.

**Material and Methods**

12 weeks prospective, open label, single center, single arm, interventional, clinical study, was conducted at MGM Medical College, Aurangabad in collaboration with Department of Medicine. Patients aged 18 to 65 years (N=160) who were either on mono therapy or on combination therapy of oral hypoglycemic agents (OHAs) other than DPP4 inhibitors for Type 2 Diabetes Mellitus (T2DM), T2DM patients of either sex (male or female) with HbA1c > 7.0% were included in the study. Newly diagnosed patients of T2DM, Type 1 DM, Gestational DM, Patient on insulin therapy, Patients with marked baseline prolongation of QT/QTc interval, having history of additional risk factors for Torsades de pointes (TdP), Patients with the use of concomitant medications that have the potential to prolong the QT/QTc interval, Patients with a history of seizures, history of stroke, and cardiovascular events, Patients with history of DKA, Patients with history of hepatic diseases and renal diseases were excluded.

All the patients participating in the study were explained clearly about the purpose and nature of the study in the language they can understand. They were included in the study only after obtaining a written informed consent form (ICF).

The study was commenced following the approval of the Institutional Ethics Committee. All information pertaining to the patient visiting out patient department, such as patient’s age, gender, occupation, relevant history, past history and drug therapy given will be recorded in a Case Record Form (CRF).

Details of the prescribed drugs for diabetes mellitus, and all other drugs used in the patient during treatment were recorded. They include the dose, duration, type of dosage form used, frequency of drug administration etc. and necessary information was recorded in a structured CRF.

Patients were given Teneligliptin 20 mg once a day as an add on to the standard treatment. Metformin was the most common drug prescribed as mono therapy and combination of Glimepiride and Metformin was the preferred combination as a dual therapy along with teneligliptin.

The mean QT interval at screening visit 1 (Day 0, baseline ECG) was 344.68 ± 20.07 milliseconds (msec), while at visit 2 (Day 1, 4 hours after Teneligliptin dosing) it was 344.48 ± 22.21 msec, at visit 3 (6 weeks) it was 344.50 ± 21.97 msec, and at visit 4 (12 weeks) it was 344.63 ± 22.13 msec. (table 1).

No significant difference was seen in the QT interval (P=0.9563) at the end of 12 weeks (Table 1). The mean QTc interval at baseline was 395.27 ± 25.09 msec, while at visit 2 it was 396.71 ± 25.39 msec, at visit 3 it was 395.26 ± 24.52 msec, and at visit 4 it was 396.93 ± 25.51 msec. (Table 2). There was no statistically significant difference in QTc interval from baseline to any subsequent follow-up visits. Therefore
A significant reduction was seen in glycemic parameters like fasting blood sugar (P<0.001), postprandial blood sugar (P<0.001), and HbA1c (P<0.001) at the end of 12 weeks, from the baseline values (Table 3). The average reduction of 32.8 mg% was marked in FBS, a reduction by 48.7 mg% was observed in PPBS and HbA1c was reduced by 1.03 % at the end of study duration (Table 3). The body weight was reduced by an average of 0.44 kg at the end of 12 weeks which was not significant (P= 0.5819) (Table 4).

**Discussion**

Type 2 diabetes mellitus (T2DM) is a chronic disease that develops as a result of defective insulin secretion and is frequently associated with obesity-related insulin resistance. Involvement of multiple physiological pathways and complex pathogenesis of diabetes explains the multifaceted morbidity noted in individuals with T2DM. The reduction in fasting and postprandial blood glucose levels by DPP-4 inhibitors is attributed to their effects on enteroinsular axis consisting of GLP and GIP which consequently increase the sensitivities of both β- and α- cells to glucose levels. Because of the low risk of hypoglycemia and being weight neutral the administration of DPP-4 inhibitors in patients with T2DM has been markedly increased in clinical practice.15

Teneligliptin, a DPP4 inhibitor, was approved for the management of type 2 diabetes mellitus in Japan (2012), in South Korea (2014), and in India (2015). In adults, usually teneligliptin is orally administered at a dosage of 20 mg once daily, which can be increased up to 40 mg per day depending upon the values of glycemia parameters. The elimination of metabolic products via renal and hepatic excretion, patients with renal impairment need no dose adjustment. Teneligliptin has a similar safety profile as compared with other available DPP-4 inhibitors. However, caution must be exercised while administering teneligliptin to patients who are prone to QT prolongation.12

To determine threshold pharmacologic effect of a drug on cardiac repolarization “thorough QT/QTc study” has been explained.16 The risk of development of Torsades de pointes is linked with the prolongation of QT interval. According to the USFDA level of regulatory concern for cardiac safety of any drug, drugs prolonging the mean QT/QTc interval by >20 ms are considered to be having proarrhythmic potential; and those which prolong the mean QT/QTc interval by around 5 ms or less are usually considered to be nonarrhythmogenic.16

According to the teneligliptin data submitted to PMDA, (Pharmaceuticals and Medical Devices Agency) Japan, based on the thorough QT/QTc study, clinically recommended doses (20 mg and 40 mg), of teneligliptin do not cause QTc prolongation. Patients taking teneligliptin along with drugs having known potential to cause QT prolongation on their own, should be carefully observed.17 Hypoglycemia considered as one of the strongest QTc prolongators, should also be watched for when a combination therapy with other hypoglycemic drugs is administered.19

In this study at week 12 the average change in the mean QT interval from baseline ECG, was 0.03msecs. The average change in the mean QTc interval from baseline ECG, was 1.652 msecs at the end of 12 weeks. No significant difference was seen in the QT as well as QTc interval at each visit subsequent to the baseline visit and there was no significant change in the QT interval (P=0.9563) and QTc interval (P=0.5594) at the end of study.

At the end of 12 weeks a significant reduction (P<0.001) was seen in glycemic parameters such as fasting blood sugar, postprandial blood sugar and Hba1c. The body weight was also reduced but it was not a significant reduction (P= 0.5819).

A significant reduction in glycemic parameters with teneligliptin daily 20 mg as a monotherapy was observed in a 3 months study by Kutoh et al which was done in 31 japanese patients with type 2 DM who had never received teneligliptin. TREAT INDIA study also observed similar significant reduction in parameters like FBS, PPBS and Hba1c at the end of 3 months from the baseline values with teneligliptin therapy.19 Q SET study performed over a period of 3 months by S Erande et al also concluded that Teneligliptin at a therapeutic dose of 20 mg/day or 40 mg/day improved glycemic parameters significantly and did not cause QTc interval prolongation.20

In our study patients were given Teneligliptin 20 mg once a day as an add on to the standard treatment with other OHAs such as biguanides, sulfonylureas, glitazones either as mono therapy or combination therapy. The drug was well tolerated by the patients except 14 patients in the study reported hypoglycemia but did not withdraw and completed the study. The main drawback of the study was it was an open label study and the study duration

---

**Table 1: Values of QT interval (in msec) at subsequent visits**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Baseline value Mean+ SD</th>
<th>At 12 weeks Mean+ SD</th>
<th>Mean difference</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>QT (msec)</td>
<td>395.27±25.09 (Visit 1)</td>
<td>396.93±25.39 (Visit 2)</td>
<td>1.441 0.6283</td>
<td>0.9563</td>
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</table>

**Table 2: Values of QTc interval (in msec) at subsequent visits**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Baseline value Mean+ SD</th>
<th>At 12 weeks Mean+ SD</th>
<th>Mean difference</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>QTc (msec)</td>
<td>396.48 ± 22.21 (Visit 1)</td>
<td>344.50 ± 21.97 (Visit 2)</td>
<td>1.18 0.817</td>
<td>0.5819</td>
</tr>
</tbody>
</table>

**Table 3: Values of glycemic parameter like FBS, PPBS, Hba1c**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Baseline value Mean+ SD</th>
<th>At 12 weeks Mean+ SD</th>
<th>Mean difference</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBS (mg%)</td>
<td>172.80±17.23 (Visit 1)</td>
<td>140.40±17.23 (Visit 4)</td>
<td>32.8 P&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>PPBS (mg%)</td>
<td>255.88±12.01 (Visit 1)</td>
<td>206.89±12.02 (Visit 4)</td>
<td>48.7 P&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>9.02±0.94 (Visit 1)</td>
<td>7.96±0.94 (Visit 4)</td>
<td>1.03 P=0.001</td>
<td></td>
</tr>
</tbody>
</table>

**Table 4: Values of body weight**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Baseline value Mean+ SD</th>
<th>At 12 weeks Mean+ SD</th>
<th>Mean difference</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight (Kg)</td>
<td>81.84±14.07</td>
<td>81.40±14.46</td>
<td>0.4281 P= 0.5819</td>
<td></td>
</tr>
</tbody>
</table>
Serum Magnesium Levels in Critically Ill Patients on Admission in ICU and its Correlation with Outcome

Jivabhai Solanki1, Kiran Runwal2, Nandkumar Beke2, Arun Bahulikar3, Deepak Phalgune4*

Abstract

Background: Many studies found that only hypomagnesemia, but not hypermagnesemia is linked with increased mortality. However, reports of mortality due to magnesium dysregulation in the critical care setting are controversial.

Objectives: To study serum magnesium levels in critically ill patients on admission in intensive care unit (ICU) and its correlation with patient’s need and duration for ventilator support, duration of ICU stay, incidence of cardiac arrhythmias and mortality.

Methods: Two hundred forty six critically ill patients admitted in ICU with Acute Physiology and Chronic Health Evaluation (APACHE) II scores>10, were included for this prospective observational study. Serum total magnesium level was measured at the time of admission to ICU. Primary outcome measure was ICU mortality whereas, secondary outcome measures were patient’s need and duration for ventilator support, duration of ICU stay, and incidence of cardiac arrhythmias. Categorical and continuous variables were tested using Chi-square/Fisher’s exact test and analysis of variance respectively. Multivariate logistic regression analysis was carried out to determine association of serum magnesium levels with ICU mortality.

Results: Incidence of ICU mortality was significantly higher in group of patients with hypomagnesemia compared to those with normal magnesium levels. Hypomagnesemia was associated with need and longer duration of ventilator support, longer duration of ICU stay, higher APACHE II score, QTc prolongation, higher incidence of cardiac arrhythmias compared to patients with normal magnesium levels. Hypomagnesemia was an independent and statistically significant determinant of ICU mortality.

Conclusions: Hypomagnesemia was associated with higher mortality rate, longer duration of ventilator support and ICU stay, and higher APACHE II score in critically ill patients.

References


Introduction

Magnesium is essential for human health, and ionized magnesium is involved in the interaction of more than 300 enzyme reactions and is important for electrolyte homeostasis, membrane stability, cell division, and generation of action potentials.\(^1\) Magnesium disturbance is a common problem in both critical care settings and in the general population. Magnesium dysregulation mainly impacts neuromuscular and cardiovascular functions.

The incidence of hypomagnesemia varies from 20% to 65% in intensive care unit (ICU) patients.\(^5\) Hypomagnesemia may present as tetany, vertigo, reversible psychiatric aberrations, seizures, cardiac arrhythmias, hypertension, muscular weakness, acute cerebral ischemia and asthma.\(^2\) The pathology of magnesium deficiencies is multifactorial including gastrointestinal disorders, renal loss, renal diseases, drug-induced loss, metabolic acidosis, and other causes.\(^3\) In addition, critically ill patients have several potential risks of magnesium dysregulation. It was significantly associated with increased and prolonged need for mechanical ventilation, difficulty to wean, prolonged ICU stay and increased mortality in critically ill patients.\(^4\) Hypermagnesemia is less common and mostly due to renal failure or iatrogenic. Prevalence of hypermagnesemia was reported to be 7.3%.\(^6\) It can lead to severe muscle weakness, respiratory depression, hypotension, cardiac arrhythmia and ultimately progress to cardiac arrest.\(^7\) Many studies found that only hypomagnesemia, but not hypermagnesemia is linked with increased mortality.\(^8\) However, reports of mortality due to magnesium dysregulation in the critical care setting are controversial.\(^9\) Also, it is unknown whether comorbidities of the study population has any effect on this association. Whether hypomagnesemia directly contributes to cellular alterations leading to increased mortality, morbidity and poor patient outcome in critically ill patients or it is just a marker of critical illness, is not clear.\(^10\) Hence, an attempt was made to study serum magnesium levels in critically ill patients on admission in ICU and its correlation with patient’s need and duration for ventilator support, duration of ICU stay, incidence of cardiac arrhythmias and mortality.

Material and Methods

This prospective observational study was conducted between July 2017 and October 2018. After approval from the scientific advisory committee (Letter No- RECH/SAC/2017-18/293) and institutional ethics committee (Letter No- RECH/EC/2017-18/368), written informed consent was obtained from all patients. Two hundred forty six critically ill patients admitted in ICU with APACHE II scores>10, and aged between 18 years and 80 years of both genders were included. Patients who received magnesium supplements, diuretics and aminoglycosides or blood transfusions prior to ICU admission, chronic alcoholics, pregnant women with eclamptic-seizures were excluded from the study.

Patients’ clinical history was taken. Clinical examination, a 12 lead ECG and relevant blood and urine investigations were performed as per pre-tested study proforma. Patients’ Acute Physiology, and Chronic Health Evaluation II (APACHE II) score was calculated. Blood was collected in a clean dry test tube and transported to the biochemistry laboratory. Serum total magnesium level was measured at the time of admission to ICU by methyl thymol blue method. (Machine/Instrument- Dimension EXL with LM, Company- SIEMENS). The normal reference range of serum magnesium level in our laboratory is 1.7-2.4 mg/dL.

The patients were divided into three groups based on serum magnesium levels:

- Group 1: The patients with Mg level with normal range 1.7-2.4 mg/dL (normal magnesium level).
- Group 2: The patients with Mg level less than 1.7 mg/dL (hypomagnesemia).
- Group 3: The patients with Mg level more than 2.4 mg/dL (hypermagnesemia).

Patients detected with hypomagnesemia/hypermagnesemia were treated as per the standard guidelines. Patients need and duration of ventilator support, cardiac arrhythmia, QTc interval, number of days of ICU stay, and ICU deaths were noted. The QTc was defined as prolonged if the duration was > 440 ms in men and >460 ms in women.\(^11\)

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### Table 1: Comparison of baseline and outcome variables

<table>
<thead>
<tr>
<th>Patient group</th>
<th>Hypomagnesemia (n=87)</th>
<th>Normal Mg levels (n=136)</th>
<th>Hypermagnesemia (n=23)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age in years ±SD</td>
<td>62.4±11.4</td>
<td>57.8±9.6</td>
<td>61.9±7.9</td>
<td>0.003*</td>
</tr>
<tr>
<td>Gender (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>46 (52.9)</td>
<td>80 (58.8)</td>
<td>19 (82.6)</td>
<td>0.036**</td>
</tr>
<tr>
<td>Female</td>
<td>41 (47.1)</td>
<td>56 (41.2)</td>
<td>4 (17.4)</td>
<td></td>
</tr>
<tr>
<td>Diabetes Mellitus (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>59 (67.8)</td>
<td>69 (50.7)</td>
<td>14 (60.9)</td>
<td>0.040***</td>
</tr>
<tr>
<td>No</td>
<td>28 (32.2)</td>
<td>67 (49.3)</td>
<td>9 (39.1)</td>
<td></td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>61 (70.1)</td>
<td>74 (54.4)</td>
<td>12 (52.2)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>26 (29.9)</td>
<td>62 (45.6)</td>
<td>11 (47.8)</td>
<td>0.049***</td>
</tr>
<tr>
<td>Mean APACHE II score ± SD</td>
<td>23.6±6.3</td>
<td>19.7±4.1</td>
<td>22.2±4.9</td>
<td>0.001*</td>
</tr>
<tr>
<td>Need of ventilator support (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>45 (51.7)</td>
<td>33 (24.3)</td>
<td>5 (21.7)</td>
<td>0.001**</td>
</tr>
<tr>
<td>No</td>
<td>42 (48.3)</td>
<td>103 (75.7)</td>
<td>18 (78.3)</td>
<td></td>
</tr>
<tr>
<td>Mean duration of ventilator support in days ± SD</td>
<td>4.9±1.8</td>
<td>3.9±1.7</td>
<td>4.5±1.2</td>
<td>0.047*</td>
</tr>
<tr>
<td>Mean ICU duration in days ± SD</td>
<td>6.2±2.3</td>
<td>4.5±1.7</td>
<td>4.8±2.1</td>
<td>0.001*</td>
</tr>
<tr>
<td>Mortality (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expired</td>
<td>44 (50.6)</td>
<td>34 (25.0)</td>
<td>3 (13.0)</td>
<td>0.016**</td>
</tr>
<tr>
<td>Survived</td>
<td>43 (49.4)</td>
<td>102 (75.0)</td>
<td>20 (87.0)</td>
<td></td>
</tr>
<tr>
<td>Mean QTc (ms) ± SD</td>
<td>469.4±28.6</td>
<td>449.9±21.5</td>
<td>455.9±26.3</td>
<td>0.001*</td>
</tr>
<tr>
<td>QTc</td>
<td>Abnormal</td>
<td>71 (81.6)</td>
<td>78 (57.4)</td>
<td>13 (56.5)</td>
</tr>
<tr>
<td>Normal</td>
<td>16 (18.4)</td>
<td>58 (42.6)</td>
<td>10 (43.5)</td>
<td></td>
</tr>
</tbody>
</table>

*ANOVA test was used; **Fisher’s exact test was used; ***Chi square test was used; Mg- Magnesium; SD-Standard deviation; ICU-Intensive care unit; APACHE II- Acute Physiology and Chronic Health Evaluation II
Table 2: Incidence of cardiac arrhythmia according to levels of serum magnesium

<table>
<thead>
<tr>
<th>Incidence of cardiac arrhythmia</th>
<th>Hypo-magnesemia (n=87)</th>
<th>Normal Mg level (n=136)</th>
<th>Hyper-magnesemia (n=23)</th>
<th>Total (n=246)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VT</td>
<td>Yes</td>
<td>10 (11.5)</td>
<td>3 (2.2)</td>
<td>1 (4.3)</td>
<td>14 (5.7)</td>
</tr>
<tr>
<td>No</td>
<td></td>
<td>77 (88.5)</td>
<td>133 (97.8)</td>
<td>22 (95.7)</td>
<td>232 (94.3)</td>
</tr>
<tr>
<td>VF</td>
<td>Yes</td>
<td>3 (3.4)</td>
<td>10 (0.7)</td>
<td>0 (0.0)</td>
<td>4 (1.6)</td>
</tr>
<tr>
<td>No</td>
<td></td>
<td>84 (96.6)</td>
<td>135 (99.3)</td>
<td>23 (100.0)</td>
<td>242 (98.4)</td>
</tr>
<tr>
<td>AF</td>
<td>Yes</td>
<td>6 (6.9)</td>
<td>5 (3.7)</td>
<td>0 (0.0)</td>
<td>11 (4.5)</td>
</tr>
<tr>
<td>No</td>
<td></td>
<td>81 (93.1)</td>
<td>131 (96.3)</td>
<td>23 (100.0)</td>
<td>235 (95.5)</td>
</tr>
<tr>
<td>SVT</td>
<td>Yes</td>
<td>4 (4.6)</td>
<td>1 (0.7)</td>
<td>0 (0.0)</td>
<td>5 (2.0)</td>
</tr>
<tr>
<td>No</td>
<td></td>
<td>83 (95.4)</td>
<td>135 (99.3)</td>
<td>23 (100.0)</td>
<td>241 (98.0)</td>
</tr>
<tr>
<td>VPCs</td>
<td>Yes</td>
<td>14(16.1)</td>
<td>4 (2.9)</td>
<td>0 (0.0)</td>
<td>18 (7.3)</td>
</tr>
<tr>
<td>No</td>
<td></td>
<td>73 (83.9)</td>
<td>132 (97.1)</td>
<td>23 (100.0)</td>
<td>228 (92.7)</td>
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<tr>
<td>VPCs to VT</td>
<td>Yes</td>
<td>5 (5.7)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>5 (2.0)</td>
</tr>
<tr>
<td>No</td>
<td></td>
<td>82 (94.3)</td>
<td>136 (100.0)</td>
<td>23 (100.0)</td>
<td>241 (98.0)</td>
</tr>
</tbody>
</table>

Fisher’s exact test was used: Mg- Magnesium; VT- Ventricular tachycardia; AF- Atrial fibrillation; SVT- Supra ventricular tachycardia; VPC- Ventricular premature complexes

Primary outcome measure was ICU mortality whereas, secondary outcome measures were patient’s need and duration for ventilator support, duration of ICU stay, QTc interval and incidence of cardiac arrhythmias. On the basis of a previously published study, a sample size of 246 patients was calculated by a formula with 80% power and 5% probability of Type I error to reject null hypothesis.

Data collected were entered in Excel 2007 and analysis of data was done using Statistical Package for Social Sciences (SPSS) for Windows, Version 20.0, (IBM Corporation, Armonk, NY, USA) for MS Windows. The data on categorical variables is shown as n (% of cases) and data on continuous variables is shown as mean ± standard deviation (SD). The inter-group statistical significance of difference of categorical variables was tested using Chi-square test or Fisher’s exact test. The statistical significance of inter-group difference of means of continuous variables was tested using analysis of variance (ANOVA). The underlying normality assumption was tested before subjecting the study variables to ANOVA. Multivariate logistic regression analysis was carried out to determine the independent association of serum magnesium abnormality with mortality, ICU duration, need for ventilation and incidence of ventricular premature complexes (VPCs). The confidence limit for significance was fixed at 95% level with p-value < 0.05.

Results

Of 246 cases studied, 87 (35.4%), 136 (55.3%), and 23 (9.3%) had hypomagnesemia, normal magnesium levels, and hypermagnesemia respectively. As depicted in Table 1, the mean age of the patients who had hypomagnesemia or hypermagnesemia was significantly higher than patients who had normal serum magnesium levels. Percentage of male patients was significantly higher in hypermagnesemia group. Prevalence of diabetes mellitus (DM) and hypertension was significantly higher in hypomagnesemia group. Mean APACHE II score of the patients who had hypermagnesemia or hypomagnesemia was significantly higher than patients who had normal serum magnesium levels. Percentage of patients who required ventilator support was significantly higher in hypomagnesemia group. Mean duration of ventilator support was significantly higher in hypomagnesemia and hypermagnesemia group. Mean duration of ICU stay, mean QTc, abnormal QTc, and incidence of mortality was significantly higher in hypomagnesemia group.

As depicted in Table 2, significantly higher proportion of patients who had hypomagnesemia had higher incidence of ventricular tachycardia (VT), and VPCs, whereas incidence of ventricular fibrillation (VF), atrial fibrillation (AF) and supra ventricular tachycardia (SVT) did not differ significantly across various serum magnesium levels in the study group. Incidence of conversion from VPCs to VT was significantly higher in patients who had hypomagnesemia (Table 2). We performed multivariate logistic regression analysis to obtain the independent determinants of mortality as a binary outcome variable.

The multivariate analysis revealed that hypomagnesemia was an independent and statistically significant determinant of incidence of mortality [Odds Ratio 2.46 (Confidence interval 1.22 – 4.17), p = 0.046]. There was no association found between hypermagnesemia and ICU mortality.

Discussion

Two hundred and forty six patients were enrolled in the present study and serum total magnesium levels were evaluated on admission. Patients were divided into three groups: normomagnesemic group (1.7-2.4 mg/dL), hypomagnesemic group (<1.7 mg/dL), and hypermagnesemic group (>2.4 mg/dL). The serum magnesium levels were correlated with patient’s outcome based on need and duration for ventilator support, duration of ICU stay, QTc interval, incidence of cardiac arrhythmias and mortality in ICU. Hypomagnesemia was significantly associated with increased ICU mortality, need for ventilator support, longer duration of ventilator support and longer stay in ICU. Higher APACHE II score, QTc prolongation, higher incidence of VPC and VT were observed in patients with hypomagnesemia.

In our study, of 246 cases studied, 87 (35.4%), 136 (55.3%), and 23 (9.3%) had hypomagnesemia, normal magnesium levels and hypermagnesemia respectively. Dabbagh OC et al., and Mousavi SAJ et al., reported prevalence of hypomagnesemia 39.4% and 33% respectively which is comparable to our study. In our study, 59/142 (41.5%) patients with DM, had hypomagnesemia. Prevalence of hypomagnesemia was significantly higher in patients who had DM. Hypomagnesemia has been known to be associated with DM attributing to increased renal losses of magnesium that accompany glycosuria. There is a strong relationship between hypomagnesemia and insulin resistance. Gupta SS et al., reported that hypomagnesemia was more common in DM patients. Studies by Ugaragol PG et al., Limaye CS et al., and Bharath MS et al., also reported similar findings. In the present study, the prevalence of hypomagnesemia was significantly higher among the patients who had hypertension. It has been postulated that hypomagnesemia contributes to the development of hypertension.
and cardiovascular disease (CVD). In experimental studies, magnesium has been shown to regulate vascular tone, endothelial function, vascular inflammation, and glucose and lipid metabolism. As a result, nutritional magnesium has both direct and indirect impacts on the regulation of blood pressure and therefore on the occurrence of hypertension.27 Kiran HS et al.,28 reported 53% of hypertensives had hypomagnesemia. Prasad G et al.,29 observed that patients admitted in ICU with critical illness with history of hypertension were associated with increased prevalence of hypomagnesemia (38.3%). However, Khan AM et al.,30 reported that there was no association between serum magnesium levels and the subsequent development of hypertension, CVD, or all-cause mortality.

In our study, mean APACHE II score ± SD in hypomagnesemic group, normal magnesium group and hypermagnesemic group was 23.59 ± 6.26, 19.65 ± 4.12, and 22.22 ± 4.90 respectively. Mean APACHE II score was significantly higher in patients who had hypomagnesemia/ hypermagnesemia compared to patients with normal magnesium levels. A prospective observational study conducted on 250 seriously ill patients by Gowda VK et al.,30 reported that patients with had hypomagnesemia mean APACHE II score 24.13 ± 4.14. Kiran et al.,30 observed that patients with hypomagnesemia compared with normomagnesemia had higher APACHE II score on admission (24.13 vs 22.47). In our study, of 87 cases that had hypomagnesemia, 45 (51.7%) needed ventilator support. The percentage of patients, who required ventilator support, was significantly higher among hypomagnesemic group compared to normomagnesemic group. Kiran HS et al.,30 observed that the patients with hypomagnesemia compared to patients with normomagnesemia needed ventilator support more frequently (35% vs. 17%).

Studies conducted by Bharath M.S. et al.,30 Ugaragol PG et al.,31 Limaye CS et al.,32 and Mousavi SAJ et al.,33 reported that hypomagnesemic group had longer stay in ICU 16.0 and 18.4 days respectively, whereas Soliman HM et al.,10 reported that there was no difference in the length of ICU stay between the groups. In the present study, mean duration ± SD of ICU stay was 6.2 ± 2.3 days, 4.5 ± 1.7 days and 4.8 ± 2.1 days in hypomagnesemic, normal magnesium, and hypermagnesemic group respectively. The mean duration of ICU stay was significantly higher in hypomagnesemic group of patients as compared to normomagnesemic and hypermagnesemic group of patients. Chen M et al.,34 and Mousavi SAJ et al.,33 reported that hypomagnesemic group had longer stay in ICU 16.0 and 18.4 days respectively, whereas Soliman HM et al.,10 reported that there was no difference in the length of ICU stay between the groups. In the present study, mean duration ± SD of QTc in hypomagnesemic group was 469.4 ± 28.6 ms. Mean QTc was significantly higher in patients with hypomagnesemia compared to normal magnesium levels patients. The percentage of patients who had abnormal QTc was higher among hypomagnesemic group compared to normomagnesemic group. Hypomagnesemia can cause cardiac arrhythmias including atrial and ventricular tachycardia, prolonged QT interval and torsades de pointes.32

In our study, significantly higher proportion of cases in hypomagnesemic group had incidence of VT and VPCs. Of 87 cases who had hypomagnesemia, 14 (16.1%) had VPCs and 5/14 (35.7%) were converted to VT. Magnesium deficiency reduces cardiac Na-K-ATPase, leading to higher levels of sodium and calcium and lower levels of magnesium and potassium in the heart. This increases vasoconstriction in the coronary arteries, which can induce coronary artery spams, myocardial infarction and arrhythmias.33 Magnesium increases the ventricular threshold for fibrillation. Sinus node refractoriness and conduction in the AV node are both prolonged. Khan AM et al.,34 observed that low serum magnesium was moderately associated with the development of AF in individuals without cardiovascular disease. Ceremuzynski L et al reported that serum magnesium levels were lower in patients with complex ventricular arrhythmias.35 Naksuk N et al.,36 observed no association between serum magnesium levels and QTc interval or sudden cardiac death. Markovits N et al.,37 reported that hypomagnesemia was associated with incident AF over prolonged but not short-term follow-up periods. A prospective observational study conducted by Gowda VK et al.,30 reported that there was no association of hypomagnesemia with arrhythmia.

In our study, incidence of mortality was significantly higher in hypomagnesemia patients compared with normal magnesium levels patients. Our study showed hypomagnesemia is the independent and statistically significant determinant of increase in ICU mortality, however there was no association found between hypomagnesemia and ICU mortality. Magnesium deficit has been discussed as a possible contributing factor to the development of cardiovascular diseases, ventricular arrhythmias and sudden death as it plays a critical role in modulating vascular smooth muscle tone, endothelial function and vascular excitability.38 Many studies have reported that patients with hypomagnesemia compared to patients with normomagnesemia, had higher mortality rate.39,12,28 Huiggen HJ et al.,36 and Escuela MP et al.,39 observed no correlation between hypomagnesemia and mortality, but noted a higher mortality rate among hypermagnesemic patients.30

Limitations

Apart from serum magnesium, confounding factors such as presence of other electrolyte imbalance like serum sodium, potassium, calcium and phosphorus, which are known to occur in critically ill patients can impact prolonged ICU stay, need of mechanical ventilation, increased ventilatory days, arrhythmias and mortality were not taken into account in this study. We did not study the effects of the changes in the magnesium levels during the course of ICU stay on the outcome.
Conclusions

Incidence of ICU mortality was significantly higher in group of patients with hypomagnesemia compared to those with normal magnesium levels. Hypomagnesemia was associated with a need and longer duration of ventilator support, longer duration of ICU stay, higher APACHE II score, QTc prolongation, higher incidence of VPCs and VT compared to patients with normal magnesium levels. Hypomagnesemia was an independent and statistically significant determinant of increase in ICU mortality.

References

Neuroborreliosis in India – A Diagnostic Challenge and a Great Mimicker: A Case Series

Bhawna Sharma¹, Madhuparna Paul²*, Ashok Panagariya³, Parul Dubey⁴

Abstract

Objectives: Neuroborreliosis is generally known to be a disease confined to the Western part of the globe. It is not commonly encountered in this part of the world. Interestingly, we recently came across a series of cases of Lyme’s disease with a plethora of neurological presentations. Most of the cases were a diagnostic dilemma, with poor response to immunotherapy and on subsequent evaluation all were found to have positive Borrelia antibodies.

Materials and methods: Eight cases were selected from the tertiary care hospital in North western India. Patients were suspected to have Neuroborreliosis whose neurological presentations were atypical for other classical neurological disorders, who had a progressive or relapsing clinical course and had responded poorly to the initial treatment given for the previous neurological diagnosis. Skin lesions were present in some cases. The patients underwent a detailed clinical assessment which comprised of an elaborate history including history of travel, any insect bite or skin rashes along with a complete systemic and neurological examination. All the required blood investigations, Magnetic Resonance Imaging (MRI) Brain, Computer Tomography Angiography (CT), Nerve conduction study (NCS) and Electromyographic (EMG) studies and Cerebrospinal fluid (CSF) studies were done as indicated in each case. Borrelia antibody titre was done in all the patients using immunoblot technique.

Results: Among the 8 patients, 6 were male and 2 were females. The age group was between 25-70 years. The clinical presentation was acute, subacute or chronic. One patient gave a clear history of tick bite. Two patients had skin lesions and one had the pathognomonic “eschar”. All the suspected 8 patients had either IgG or IgM or both IgG and IgM Borrelia antibodies positive. Almost all the patients had previously received either steroids or intravenous immunoglobulins, but had not adequately responded to immunotherapy. These patients were given a trial of injectable Ceftriaxone and oral Doxycycline. Most of them either showed partial or complete clinical improvement.

Conclusion: Lyme’s disease, a common disease of the west does exist in the Indian subcontinent as well. Because of increasing global travel and migration and change in vector habitat the disease seems to have percolated in the non endemic areas too. Proper history of travel or exposure to tick bite is important. We want to emphasize, Neuroborreliosis, a great mimicker may have diverse and varied neurological presentations and has a potential for reversibility with appropriate treatment even after a significant delay in diagnosis.

Introduction

Lyme disease is a zoonotic tick borne disease with approximately 10-15% patients developing neuroborreliosis.¹ It is caused by the spirochete Borrelia burgdorferi with majority of human infection being caused by the genotypes B. burgdorferi s.s., B. afzelii and B. garinii.²³ The disease is endemic in the United States (US) and Europe but due to global travel and change in vector habitat it is being seen in the Indian subcontinent as well.⁴

It is transmitted by the bite of infected ticks of Ixodes Ricinus complex family.¹,² The size of the nymph of Ixodes is very small and often the bite in humans goes unnoticed. Thus a clear history of tick bite is available in only 40% cases. The classical skin lesion following the bite of the tick is the “eschar”. Other skin lesions caused by the tick bite are erythema migrans, erythematous skin rash or secondary annular lesions present in two-third of cases.²⁵

Lyme disease is a multisystem disease. Apart from the skin, other systems either in isolation or combination, may also be involved, for eg. Rheumatological (migratory arthritis, chronic arthalgia or myalgia), Cardiac (myopericarditis or AV block), Lymphoreticular (fatigue, malaise and lymphadenopathy), Neurological (Neuroborreliosis) etc.²³

Neuroborreliosis can present as an early disseminated (symptoms of <6 months duration) or delayed (symptoms of > 6 months duration) forms. The characteristic features are different in either forms.

Lymphocytic meningitis, encephalomyelitis, acute painful radiculoneuropathy or cranial neuropathies may be seen in the acute phase. Meningitis and meningoencephalitis are the commonest acute presentations.³ Cranial neuropathies are seen in one third of the cases in early neuroborreliosis. VII cranial nerve is most commonly involved though multiple cranial nerves also may be simultaneously involved. Bilateral facial palsy is a
common presentation. Lymphocytic meningoradiculitis (Bannwarth syndrome or Garin-Bujadoux-Bannwarth syndrome) is seen in one third of the cases. Plexus, radicles or nerves may be involved either individually or in combination. Patients present with severe radicular pain in lumbosacral and cervical regions, asymmetric motor weakness and paresthesia which usually responds well to treatment.

On the other hand, chronic encephalomyelitis, encephalopathy or chronic radiculoneuropathy may be seen in the late phase. Patients may present with decreased concentration, memory impairment, insomnia or hypersomnolence. Psychiatric problems in the form of depression or psychosis may also be seen with a variable response to treatment.

The diagnosis is made on the basis of history of tick bite, epidemiology, clinical features and serological tests. The presence of eschar or erythema migrans is 100% specific and 57-86% sensitive in epidemic areas. Tissue diagnosis like biopsy, culture, microscopy are definitive diagnostic tests but usually not available. Because of fastidious growth it is not possible to grow the organism in culture. Polymerase Chain Reaction (PCR) in blood and Cerebrospinal fluid (CSF) has low sensitivity but is useful in synovial fluid. The serological test is commonly used for diagnosis.

Neuroborreliosis is treated with intravenous Ceftriaxone for a period of 14 days. Oral treatment is with Doxycycline, Amoxycillin or Cefuroxime for 4-6 weeks.

This study reports eight cases of Neuroborreliosis with varied clinical presentations and positive Lyme serology test.

### Material and Methods

There was a total of eight cases attending the outpatient department of a Superspeciality centre in North western India in last five years. These are the cases with diagnostic dilemma and Neuroborreliosis was investigated as diagnosis of exclusion. Six of them were males and two were females. All the patients were evaluated with a detailed history and clinical examination. The necessary investigations for example Electromyography (EMG), Nerve conduction study (NCS), Magnetic Resonance Imaging (MRI) Brain were done. CSF examination was also done. All patients underwent Lyme’s serology by Immunoblot assay. They were treated with injectable Ceftriaxone and oral Doxycycline for 14-21 days. Some had a partial response to treatment and some had a complete recovery (Table 1).

#### Case 1

A 58 years old man presented with three months history of erythematous maculopapular lesions in the trunk and bilateral lower limbs, (Figure 1 a, b) which was followed 1½ months later, by proximal muscle pain and weakness in all four limbs. Fifteen days prior to presentation, he had generalized fatigability (without any diurnal variation) and breathlessness on exertion without any history of difficulty in swallowing or chewing or any facial weakness, visual complaints, muscle wasting or twitching, bladder or bowel complaints, back pain or joint pain. There was a history of transient fever for 3-4 days, a week before the onset of skin rash. He had travelled to Germany and Poland one year back. On examination, an eschar was noted in the neck (Figure 2). He was conscious, oriented and cooperative, cranial nerve examination was normal including normal neck and facial muscles. Power in the proximal muscles of lower and upper limbs was 3/5, distal muscle power was normal with normal Deep tendon reflexes (DTR) and intact sensation. Plantar responses were flexor bilaterally. There was mild muscle tenderness. Serum creatine phosphokinase (CPK) was 3876 IU/L. EMG was myopathic pattern. Complete haemogram, liver and renal function tests, blood sugar, thyroid function tests, blood sugar, thyroid function test, viral markers, vasculitic profile, Immune 17 were normal. So, a clinical diagnosis of inflammatory myopathy was considered. He had been treated with steroids followed by intravenous immunoglobulins prior to admission, without any benefit. The patient refused muscle biopsy. The presence of eschar and history of travel to Europe, absence of immunological and vasculitic markers and a lack of response to immunotherapy led us to consider possibilities other than dermatomyositis, so before initiating further immunosuppressant, Borrelia serology was sent. Lyme IgG and IgM came back positive. He was started

<table>
<thead>
<tr>
<th>Name</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>Clinical features</th>
<th>Unique Lab findings</th>
<th>Duration of illness</th>
<th>Serum Lyme titre</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 SC</td>
<td>58</td>
<td>M</td>
<td>Cough, skin rash, proximal myopathy</td>
<td>Raised CPK EMG s/o myopathy</td>
<td>6 mnths</td>
<td>IgG+ Ig M+</td>
</tr>
<tr>
<td>2 LS</td>
<td>32</td>
<td>M</td>
<td>Progressive, upper limb distal onset, UMN +LMN Quadriparesis without bladder bowel involvement</td>
<td>EMG active denervation+ chronic reinervation s/o neurogenic affection</td>
<td>1 yr</td>
<td>IgG+</td>
</tr>
<tr>
<td>3 BKS</td>
<td>68</td>
<td>M</td>
<td>Progressive asymmetric LMN quadriparesis (prox+dist) with tremors</td>
<td>NCS s/o MMNCB</td>
<td>2 mnths</td>
<td>IgG+</td>
</tr>
<tr>
<td>4 VG</td>
<td>24</td>
<td>M</td>
<td>Recurrent paraparesis with bladder bowel involvement</td>
<td>MRI Dorsal spine-focal T2W hyperintensity at DIO-11 level</td>
<td>10 mnths</td>
<td>IgM+</td>
</tr>
<tr>
<td>5 ML</td>
<td>33</td>
<td>F</td>
<td>Radicular pain and asymmetric proximal weakness of b/l lower limbs with perineal numbness and urine and fecal incontinence with mild left facial paresis</td>
<td>MRI L/S spine and conus- Patchy enhancement of cauda and conus NCS asymmetric patchy demyelinating and axonal inv. Of lower limb nerves</td>
<td>4 days</td>
<td>IgM+</td>
</tr>
<tr>
<td>6 GS</td>
<td>34</td>
<td>M</td>
<td>Recurrent lower cranial nerves palsy</td>
<td>MRI Brain: asymmetric soft tissue swelling and enhancement of rt. cavernous</td>
<td>lyr</td>
<td>IgM+, IgG+</td>
</tr>
<tr>
<td>7 KS</td>
<td>45</td>
<td>F</td>
<td>B/L Bell’s palsy Prox + dist lower limb weakness</td>
<td>NCS: axonal affection of facial and lower limb nerves, CSF lymphocytic pleocytosis</td>
<td>20 days</td>
<td>IgM+</td>
</tr>
<tr>
<td>8 NK</td>
<td>57</td>
<td>M</td>
<td>CVA: Left hemiplegia with prior h/o cervical myelitis</td>
<td>MRI Brain :Rt. MCA territory infarct MRI spine: cervical myelitis</td>
<td>2 days</td>
<td>IgM+</td>
</tr>
</tbody>
</table>
on injection Ceftriaxone and tablet Doxycycline. He responded well and returned to work in 6 months.

**Case 2**

A 32 years old man from Dubai presented with one and a half year history of insidious onset progressive weakness of all four limbs, distal more than proximal. There was only a flicker of movement in the distal part of upper limbs while the proximal upper limb power was 3/5. In the lower limbs, distally the power was 1/5 and proximally it was 3/5. The DTRs were brisk in the upper limbs and normal in the lower limbs and plantar responses were flexor. There were extensive fasciculations and wasting without any bulbar, bladder, bowel or sensory involvement. His serum CPK was 1299U/L. NCS showed axonal affection of bilateral peroneal nerves. EMG showed active denervation with chronic reinnervation in all the four limbs without any spontaneous activity in the dorsal paraspinal muscles. Repetitive nerve stimulation was negative for decremental response. CSF examination was normal. Antinuclear antibody (ANA) profile, Anti Acetylcholine receptor antibody (AChR Ab) and Anti Muscle kinase antibody (Anti MuSK antibody, paraneoplastic profile, anti Ganglioside M1 (GM1) antibody were negative. Genetic tests for Motor Neuron Disease (MND) and Spinal Muscular atrophy (SMA) were negative. The patient was given a clinical diagnosis of Amyotrophic Lateral Sclerosis (ALS).

On further enquiry he recalled that he had a non specific low grade fever with myalgia at the onset of the illness along with a skin lesion one and a half year back (of which he had taken a photograph on his phone which he showed us) (Figure 3) which had lasted for about 10 days. This history guided us to send Lyme’s serology which came to be positive for IgG. He was started on Ceftriaxone and Doxycycline and showed partial recovery. In the follow up visit, 3 months later, the power showed improvement in lower limbs. The power was 3/5 distally and 4/5 proximally. The power in the upper limbs was 4/5 on follow up.

**Case 3**

A 68 years old man, known case of hypertension and Chronic Obstructive Pulmonary Disease (COPD) for 10 years and history of Percutaneous transluminal coronary angioplasty (PTCA) one year back presented with one and a half month history of difficulty in walking; initially involving the left lower limb and progressing within two months to involve the right lower limb also. There were tremors in both hands (left more than right) with history of significant weight loss in the last 2 months. There was no history of any insect bite or skin rash or travel abroad. On examination, he had a normal cognition and cranial nerve examination including face, neck, tongue was normal. He had a mild weakness and wasting of interossei of both hands. Power in the upper limbs was 5/5 with minimal grip weakness, 4/5 at hips, 5/5 at knees, 4/5 at ankles and trunk muscles were normal. DTRs were absent in the lower limbs and were 1 + in the upper limbs. Plantar responses were flexor. Sensory examination was normal. Routine blood investigations revealed a raised Erythrocyte sedimentation rate (ESR) and positive C-reactive protein (CRP). CSF showed normal protein with lymphocytic pleocytosis. Anti AchR and Anti MUSK antibodies, Antinuclear antibody (ANA) profile, viral markers, serum VDRL, anti Ganglioside and paraneoplastic antibodies were all negative. NCS showed a motor axonal neuropathy involving left lower limb in peroneal nerve distribution. Probable conduction block was noted in proximal segment of bilateral ulnar, median and left radial nerve above axilla. EMG was normal except poor recruitment in distal lower limb muscles. Repetitive Nerve Stimulation Test (RNST) was negative for decremental response. Based on the clinical and electrophysiological assessment a provisional diagnosis of Multifocal Motor Neuropathy with Conduction Block (MMNCB) was made elsewhere and he was started on Intravenous Immunoglobulin (IV Ig) with partial improvement. On review of his investigations further search for an infective cause was made in view of reactive CSF, and Borrelia serology was sent which came back positive for IgG and negative for IgM. Hence this case was considered a late manifestation of...

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**Fig. 1:** (a) Erythematous maculopapular skin lesions on trunk

**Fig. 1:** (b) Erythematous maculopapular skin lesions on legs

**Fig. 2:** Eschar on neck

**Fig. 3:** Erythema migrants
Case 4

A 24 years old male, was admitted with history of recurrent episodes of bilateral lower limb weakness and numbness with bladder and bowel involvement with three episodes within 10 months with partial recovery in between. There was no history of fever preceding the illness and no associated visual or any other history suggestive of cranial nerve involvement. There was no history of skin rashes, photosensitivity, bite or travel abroad. He received intravenous Methylprednisolone on every occasion with only partial relief. Routine investigations were normal. MRI Dorsal spine showed focal T2W hyperintensity within the cord at D10-D11 level centrally with subtle expansion of cord suggestive of non compressive myelopathy possibly demyelination (Figure 4). MRI Brain showed a developmental venous anomaly in right parietal lobe. VEP showed normal P100 latency. CT angiogram of the spine was normal. CSF showed 10 cells with lymphocytic pleocytosis, normal biochemistry, CSF IgG index and Oligoclonal band (OCB) were normal. Anti Neuromyelitis optica (NMO) and anti Myelin oligodendrocyte glycoprotein (MOG) antibodies, viral markers, ANA profile, serum lactate dehydrogenase (LDH), serum Veneral disease research laboratory (VDRL) test were negative. Serum copper level was normal. In view of recurrent history and partial response Lyme antibody was sent. Borrelia IgM antibody was positive. Injection Ceftriaxone and oral Doxycycline were given. He responded very well with gradual recovery with no further relapses till date.

Case 5

A 33 years old lady, a pharmaceutical professional, without any comorbidities, presented to the casualty with 4 days history of acute onset low back pain radiating to both lower limbs and asymmetric proximal weakness of both lower limbs with perineal numbness and urinary and fecal incontinence. She also had paresthesia in bilateral hands but no weakness in upper limbs and she had developed a mild left facial paresis just one day prior to presentation. She had travelled to Tamil Nadu (Tuticorin and Madurai) about three weeks prior to presentation and had gone to Maharashtra (Solapur) in the same week. She did not recall any bite or skin rash during the visit. She gave history of fever with chills and body aches two weeks prior to the onset of these symptoms which had lasted for 2 days.

On examination she had a LMN right facial paresis and an asymmetric (right > left) LMN paraparesis, with asymmetric saddle anaesthesia (in L5, S1, S2, S3, S4, S5 dermatomes). Clinically this was an acute conus cauda presentation with facial nerve involvement. MRI spine with contrast revealed a patchy enhancement in the region of conus and cauda (Figure 5), but no intramedullary hyperintensity. MRI brain was normal. NCS revealed anti neural oligoclonal band (OCB) and an asymmetric, patchy, mild, demyelinating and axonal involvement. CSF showed raised protein (125.87 mg/dl), 6 cells (all lymphocytes) with no atypical cells. Possibility of an infective cause was considered in view of just a marginal pleocytosis. CSF viral PCR came negative.

Routine blood tests were normal. ESR was 40 mm, serum CPK was 174 IU/L. Serology for malaria, Dengue and Leptospira were negative. Viral markers including HIV, HBsAg, anti HCV and serum VDRL were negative. Serum NMO antibody was negative. Serum Angiotensin converting enzyme (ACE), Vitamin D level, B12, folate, thyroid function, ANA and serum protein electrophoresis were normal. Borrelia serology was sent in view of the meningoradiculitis like presentation with facial nerve involvement (Bannworth syndrome). Borrelia IgM came positive, IgG was negative.

She was treated with intravenous Methylprednisolone for 5 days and Doxycycline for 3 weeks and she showed a complete recovery within a month and has never relapsed till the last follow up about three months back.

Case 6

A 34 years old male with history of chronic headache presented with acute onset diplopia and inability to move the right eye to the right side, facial deviation to the left side and paresthesia in the right side of forehead and cheek. There was a diffuse headache without any associated nausea or vomiting. On examination there were right sided V (V1, V2), VI, VII cranial nerve palsies. There was no papilloedema. Rest of the neurological examination was normal. MRI Brain and orbits showed
asymmetric soft tissue thickening with enhancement of right cavernous sinus probably inflammatory or infective in etiology (Figure 6). CSF showed raised protein and 4 cells (100% lymphocytes) suggestive of albumino-cytological dissociation. He was treated with tapering dose of oral steroids elsewhere and he completely recovered within 3 months. One year later he again developed left sided LMN VII nerve palsy. MRI Brain was advised this time but he refused. In view of recurrent lower cranial nerve palsy the second tier of investigations was advised. Serum ACE level, Lyme titre and Immune17 were sent. Lyme titre was positive (Lyme IgG and IgM positive). Patient was diagnosed to have Lyme’s disease. He was started on injectable Ceftriaxone and oral Doxycycline along with IvIg. She responded well.

Case 7

A 45 year old female presented with bilateral facial nerve palsy for 20 days and weakness of both lower limbs for 5-6 days prior to admission associated with severe radicular pain in the lower limbs without bulbar, bladder or bowel involvement. There was no history of preceding fever or backache. She had asymmetric LMN paraparesis with bilateral facial palsy. Routine blood investigations were normal. MRI brain and spine with contrast were normal. CSF showed lymphocytic pleocytosis. NCS showed demyelinating and axonal affection of upper and lower limb nerves. She was on IvIg but even after 3 days of treatment there was no significant improvement. ANA, HIV, HBs Ag, anti HCV, vitamin B12, VDRL, Brucella IgM negative, serum calcium, vitamin D, ACE enzyme levels were sent and all were normal. Lyme’s IgM Ab was positive surprisingly. She was treated with injectable Ceftriaxone and oral Doxycycline along with IvIg. She responded well.

Case 8

A 57 year old male, non smoker, non alcoholic, without any risk factors presented with sudden onset left hemiplegia with left upper motor neuron type facial palsy of 2 days duration. One month before admission, he was admitted elsewhere with acute onset quadriplearesis with bladder and bowel involvement with history of significant recovery with steroids. On interrogating he also revealed history of low grade fever, weight loss with polyarthalgia for the last 3 months. No skin rashes or any other skin lesions were noted. On examination he was conscious and oriented, had dysarthria, left UMN facial palsy, had spasticity in all 4 limbs. Power was 1/5 in left upper and lower limbs and 4/5 in right upper and lower limbs. DTRs were brisk in all four limbs and plantar responses were bilateral extensor. This patient had a stroke like presentation with left hemiparesis and recovering myelitis with a background of systemic involvement. MRI brain showed a right Middle Cerebral Artery (MCA) territory acute infarct and MRA showed occlusion of right MCA (Figure 7) and MRI spine showed C5-C7 T2W hyperintensity (Figure 8).

MRI Brain was advised this time for search for the commoner causes. One month before admission, he was admitted elsewhere with acute onset of systemic involvement. MRI brain showed a right Middle Cerebral Artery (MCA) territory acute infarct and MRA showed occlusion of right MCA (Figure 7) and MRI spine showed C5-C7 T2W hyperintensity (Figure 8). MRI brain and neck vessels, ECG, 2D Echocardiography, fasting blood sugar, HbA1c, lipid profile, renal, liver, thyroid function tests were all normal. Visual Evoked potential (VEP) was normal. CSF showed 25 cells (lymphocytes 80%, polymorphs 20%) with normal biochemistry. CSF OCB was negative. In view of recurrent neurological deficits with systemic involvement, the following were sent: CSF-TB PCR, CSF panfungal PCR, CSF cryptococcal antigen, VDRL, HIV, immune profile, SACE level, Anti NMO and MOG antibodies, Brucella serology IgG/IgM, Lyme serology IgG/IgM. Lyme IgM titre was strongly positive. Patient was treated with ceftriaxone, steroids and antiplatelets. Patient significantly improved and was able to walk with support within a fortnight during hospitalisation.

Discussion

The authors came across 8 cases of Neuroborreliosis with variable neurological presentations with both early and late forms highlighting the fact that it can be a great mimicker of various neurological diseases and must be considered in the appropriate clinical setting when a diagnostic dilemma prevails despite a detailed work up for search for the commoner causes. Among 8 patients 2 had skin lesions. One case had a history of travel abroad. Only one patient had history of tick bite. The demographic profile of the patients is shown in the table (Table 1).

Neuroborreliosis may have a plethora of clinical presentations and often goes undiagnosed, especially so in non endemic regions as ours. Lyme disease has been reported from Northern and Southern India with dermatological and ophthalmological presentations. In this case series we had only two cases with skin lesions and one of them had an “eschar” and only one case had a history of travel to Europe.

Case 1 mimicked dermatomyositis where the patient presented with proximal muscle pain and weakness. The musculoskeletal presentation of Lyme disease mimicking dermatomyositis has been reported in literature.

Case 2 A young man presented with features suggestive of MND.
but with some atypical associations (like the patient had a toxic look and there was a preceding history of fever). MND like presentation has been described as an unusual manifestation of Neuroborreliosis.11

Case 3 had a Multiple motor neuropathy like late presentation. A case of motor neuropathy presenting as Post Lyme Disease Syndrome (PLDS) has been reported in literature.12 It is a very rare presentation involving immune reactivation following acute Neuroborreliosis. In our case there was no past history suggesting acute Lyme’s disease, but serum IgG positivity indicates prior infection.

Lyme Neuroborreliosis can present with acute transverse myelitis, subacute transverse myelitis or recurrent myelitis.11,14 The patient here (Case 4) presented with recurrent myelitis without any specific cause and only with partial response to immunosuppressants. He showed good response with injection Ceftriaxone and oral Doxycycline without any further relapse.

Bilateral facial nerve palsies with painful LMN paraparesis is the well known Lyme associated lymphocytic meningoradiculitis (Bannwarth syndrome). One of the patient (Case 5) had Bannwarth like presentation. It is relatively rare but case reports are documented in literature.15

Multiple cranial nerve palsies is a common early manifestation of Neuroborreliosis. Case 6 in this series presented with recurrent lower cranial neuropathies. There are many case reports of multiple cranial neuropathies due to Lyme disease and which responds well to antibiotics.16

Neuroborreliosis can mimic GBS. Case 7 presented with an acute onset LMN quadruparesis with LMN facial palsy. Lyme Neuroborreliosis presenting as GBS has also been reported in literature.17

Cerebrovascular manifestations of Neuroborreliosis have been reported in literature.18,19 The commonest is ischemic stroke and TIA. Middle cerebral artery territory is most commonly involved. Here (Case 8) myelitis followed by stroke led to further investigation for secondary causes of stroke and Borrelia antibody came positive. Whether it is an association or cooccurrence is a question.

CSF was done in all which showed lymphocytic pleocytosis. The electrophysiological tests and the MRI Brain were done according to the clinical presentation. After excluding other causes Borrelia serology was planned in all. All the patients were treated with injectable Ceftriaxone and oral Doxycycline. Some had a good response and some had a partial response to treatment. All the patients are under follow up.

ELISA is the screening test used for serological and CSF testing with a sensitivity of 21 to 98 % according to type of serology and specificity ranging from 69 to 99 %. ELISA measures overall antibody response (typically IgM and IgG) to B. burgdorferi antigens.2,6 However, because some of these antigens are cross-reactive with antigens from the host or other pathogens, specificity of the ELISA alone is not optimal. It may be false positive in other spirochetal diseases, in AIDS, Tuberculosis, Infectious mononucleosis, Bacterial endocarditis, Rocky mounted spotted fever and in Connective tissue disorders like Rheumatoid arthritis.2 Western blot is the confirmatory test but it lacks standardisation and reproducibility. To increase specificity of serology, a two-tier approach is recommended with an initial Enzyme immunoassay followed by separate immunoglobulin Western blots if the first step is positive.2,6

The limitation of this study is that the cases were not confirmed with Western blot and only the serological tests were relied upon which can be false positive. But the favourable response to the therapeutic combination adds value to the diagnosis made creating hope for otherwise hitherto untreatable cases.

Conclusion

As India is non endemic for Borreliosis, Neuroborreliosis is under reported usually. The objective of presenting this case series is to highlight the fact that, Neuroborreliosis is quite a possibility in cases with abnormal neurological presentations. A high index of suspicion is needed on the part of the treating Neurologist to consider it in the differential diagnosis in cases with diagnostic dilemma. To the best of our knowledge, this is the largest case series of Neuroborreliosis reported from India so far.

References

Circulatory Cytokine Levels as a Predictor of Disease Severity in COVID-19: A Study from Western India

Sudhir Bhandari¹, Govind Rankawat², Sandeep Mathur³, Anshul Kumar⁴, Rahul Sahlot⁴, Avinash Jain⁵

Abstract
Background: Inflammatory response in COVID-19 responsible for acute respiratory distress syndrome (ARDS) and multiorgan failure and play a major role in morbidity and mortality of patients. The present study was undertaken to assess serum level of cytokines and its association with other inflammatory markers and disease severity in COVID-19 and hence their prognostic significance.

Methods: This was a retrospective observational study of 175 admitted COVID-19 patients. The patient’s clinical data, laboratory investigations, inflammatory markers and serum level of cytokines [interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-10 (IL-10) and tumour necrosis factor α (TNFα)] were extracted from their medical records. All patients were divided into three groups viz. group A had asymptomatic patients, group B had mild to moderate ill patients and group C had severe or critical ill patients. Above parameters were analysed and comparative evaluation with severity of disease was done.

Results: In present study 55% patients were asymptomatic, 24% patients were mild to moderate illness and remaining 21% patients had severe or critical illness. Fever, cough, dyspnoea and co-morbidities including hypertension and diabetes were more common in group C. Absolute lymphocyte count (ALC), lymphocyte-monocyte ratio (LMR) showed decreasing trend whereas absolute neutrophil count (ANC), neutrophil-lymphocyte ratio (NLR), platelet-lymphocyte ratio (PLR) and eosinophil-lymphocyte (ELR) showed increasing trend with increase in disease severity. Serum IL-6 was found to be significantly higher in group C (64.98±111.18pg/mL) as compared to group B (15.51±20.66pg/mL) and group A (5.04±56.1pg/mL) (P<0.001). Receiver operating characteristic (ROC) curve for IL-6 to differentiate the patients with severe disease from asymptomatic and mild symptomatic disease showed a cut-off of 6.75pg/ml.

Conclusion: Elevated IL-6 levels lead to adverse clinical events so IL-6 level might serve as a potential prognostic marker for severity of disease in COVID-19. Inhibition of IL-6 might be helpful to prevent serious adverse events in COVID-19 infection.

Introduction
COVID-19, first pandemic in the era of modern medicine has rapidly made its way across the globe. COVID-19 is characterized by its varied clinical presentation ranging from asymptomatic to severe symptomatic disease with dyspnoea, hypoxia and multiple organ failure. Fever is the most common clinical presentation followed by fatigue, dry cough, anorexia and myalgia. Bilateral pneumonitis requiring oxygen support is the most frequent serious manifestation of SARS-CoV-2 infection. Case fatality rates vary widely from 0-3.9% in India to around 14% in Italy. The cause of this huge variability in the clinical presentation and mortality still eludes us. We are still trying to comprehend the phenotype and pathogenesis as more and more data pours in.

The body’s response to the invading pathogen is quite heterogenous, resulting in activation of innate and adaptive arm to varying extent resulting in healthy to dysfunctional immune response. Typical immune response to infection by SARS-CoV-2 comprises of release of damage-associated molecular patterns which stimulates the innate arm with release of inflammatory cytokines and chemokines and recruitment of T cells in an attempt to cordon off the infection. In case of inappropriate immune response this results in activation of inflammatory cascade and a sustained pro-inflammatory loop leading to multi-organ damage and increased morbidity and mortality. Humoral immune response in the form of IgM antibodies starts appearing at the end of the first week of infection followed by highly specific IgG antibodies which are thought to provide lasting immunity to the invading virus in an immunocompetent host. However recent data on rapid decay of anti-SARS-CoV-2 IgG has raised concerns particularly in subgroup of patients with mild disease.

Acute Respiratory Distress Syndrome (ARDS) has been found to complicate the natural history of COVID-19 and is the most frequent cause of death due to COVID-19. It has been shown to be result from increased production of pro-inflammatory cytokines including interleukin-1β (IL-1β), interleukin-6 (IL-6), tumour necrosis factor (TNFα) and chemokines like IP-10 and macrophage inflammatory protein 1α (MIP1α). The excess production of cytokines referred to as cytokine storm may present as fever and widespread lung inflammation which

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Received: 12.01.2021; Revised: 01.07.2021; Accepted: 18.01.2022
eventually culminates into ARDS, multiple organ failure and death. The immune response also consists of anti-inflammatory cytokines like Interleukin-10 (IL-10) which keeps check on pro-inflammatory response in check. Interleukin-1β (IL-1β), Interleukin-6 (IL-6), Interleukin-10 (IL-10) and tumour necrosis factor α (TNFα) were measured in all patients by chemiluminescent immunometric assay using Siemens IMMULITE 1000. The data was compiled, tabulated, interpreted and correlated to assess association of disease severity with serum level of cytokines in COVID-19 patients.

### Data Collection

COVID-19 was diagnosed based upon World Health Organization interim guidance. The patient information regarding demographic data, medical history, clinical manifestations, general physical examination, blood gas analysis, baseline laboratory findings and serum levels of cytokines were extracted from medical records for data analysis. All participants were divided into three groups on the basis of severity of disease which includes (I) Group A of asymptomatic patients, (II) Group B with mild-moderate symptoms and not fulfilling criteria for group A and C and (III) Group C comprised of patients with severe or critical disease, defined as respiratory rate ≥ 30/min at rest, oxygen saturation ≤ 93% (on blood gas analysis); arterial partial pressure of oxygen (PaO2) / fraction of inspired oxygen (FiO2) ratio ≤ 300 mmHg. Laboratory investigations included haemoglobin level, total leukocyte count (TLC), differential leukocyte count (DLC), platelet count, random blood sugar (RBS), renal function test (RFT), liver function test (LFT), electrolytes, procalcitonin (PCT), creatine phosphokinase (CPK), lactate dehydrogenase (LDH), ferritin, C-reactive protein (CRP), fibrin degradation products (FDP) and D-dimer. Latter three were measured using latex agglutination method. We also calculated the neutrophil-lymphocyte ratio (NLR), lymphocyte to monocyte ratio (LMR), eosinophil to lymphocyte ratio (ELR) and platelet to lymphocyte ratio (PLR).

### Statistical Analysis

We described the categorical variables as frequencies, rates and percentages while continuous variables were expressed as means and standard deviation. Intergroup comparison was done using one-way ANOVA while multiple comparisons were done using Tukey’s post hoc analysis. Receiver operating characteristic (ROC) curve was used to find cut-off for the patients suffering from severe disease with reference to the patients with non-severe disease. All statistical analyses were performed using IBM SPSS (Statistical Package for the Social Sciences) 22.0 trial version software (SPSS, Inc). Two-sided P values of less than 0.05 were considered statistically significant.

### Results

**Presenting manifestations and disease severity (Table 1)**

<table>
<thead>
<tr>
<th>Laboratory Parameter</th>
<th>Total (N=175)</th>
<th>Asymptomatic (Group A) (N=96)</th>
<th>Mild symptomatic (Group B) (N=43)</th>
<th>Severe Symptomatic (Group C) (N=36)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (year)</td>
<td>42.45 ± 15.37</td>
<td>38.15 ± 12.22</td>
<td>45.13 ± 14.64</td>
<td>50.70 ± 19.20</td>
<td>X²=0.5783, P=0.7488</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>135 (77.14%)</td>
<td>72 (75.00%)</td>
<td>34 (79.07%)</td>
<td>29 (80.56%)</td>
<td>0.004</td>
</tr>
<tr>
<td>Female</td>
<td>40 (22.86%)</td>
<td>24 (25.00%)</td>
<td>9 (20.93%)</td>
<td>7 (19.44%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Co-morbidities</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.019</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>46 (26.29%)</td>
<td>19 (19.79%)</td>
<td>13 (30.23%)</td>
<td>14 (38.89%)</td>
<td>0.011</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>14 (8.00%)</td>
<td>7 (7.29%)</td>
<td>6 (13.95%)</td>
<td>1 (2.78%)</td>
<td>0.133</td>
</tr>
<tr>
<td>Hypertension</td>
<td>14 (8.00%)</td>
<td>7 (7.29%)</td>
<td>6 (13.95%)</td>
<td>1 (2.78%)</td>
<td>0.019</td>
</tr>
<tr>
<td>Other disease</td>
<td>4 (4.00%)</td>
<td>4 (4.16%)</td>
<td>6 (13.95%)</td>
<td>2 (5.65%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Clinical Features</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cough</td>
<td>72 (41.14%)</td>
<td>36 (36.22%)</td>
<td>36 (100%)</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fever</td>
<td>74 (42.99%)</td>
<td>38 (38.37%)</td>
<td>36 (100%)</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Breathlessness</td>
<td>48 (27.43%)</td>
<td>14 (32.56%)</td>
<td>34 (94.44%)</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Headache</td>
<td>19 (10.86%)</td>
<td>7 (16.28%)</td>
<td>12 (33.33%)</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Running nose</td>
<td>4 (2.29%)</td>
<td>4 (9.30%)</td>
<td>0</td>
<td>2 (4.65%)</td>
<td>0.002</td>
</tr>
<tr>
<td>Sore throat</td>
<td>4 (2.29%)</td>
<td>3 (6.98%)</td>
<td>1 (2.78%)</td>
<td>2 (5.65%)</td>
<td>0.008</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>3 (1.71%)</td>
<td>2 (4.65%)</td>
<td>3 (8.33%)</td>
<td>2 (5.65%)</td>
<td>0.033</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>5 (2.86%)</td>
<td>2 (4.65%)</td>
<td>3 (8.33%)</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Dizziness</td>
<td>2 (1.14%)</td>
<td>1 (2.17%)</td>
<td>2 (5.65%)</td>
<td>0</td>
<td>0.019</td>
</tr>
</tbody>
</table>

**Table 1: Baseline characteristics of patients infected with COVID 19**

**Methods**

**Study Design**

This retrospective observational study was conducted on 175 admitted COVID-19 patients, at a tertiary care centre in Jaipur, India from 1st March 2020 to 31st May 2020. This study was approved by the institutional ethics committee and appropriate consent was taken from the recruited participants. In the present study, RT-PCR positive patients for SARS-CoV-2 with available data of cytokines level were included.

The study population included 175 COVID-19 patients, at a tertiary care centre in Jaipur, India from 1st March 2020 to 31st May 2020. This study was approved by the institutional ethics committee and appropriate consent was taken from the recruited participants. In the present study, RT-PCR positive patients for SARS-CoV-2 with available data of cytokines level were included.

**Data Collection**

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**Statistical Analysis**

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common in group C when compared to group B. However, there was no significant difference in the proportion of patients who had other clinical features like headache, running nose, sore throat, pain abdomen, diarrhea and dizziness. In our study group, 46 (26.3%) participants had one or more coexisting medical conditions. Diabetes mellitus were found to be significantly higher in group C as compared to group A (P=0.005). Diabetes mellitus and hypertension were significantly more common in group C when compared with group A and group B (P=0.005 and 0.011 respectively).

**Laboratory Parameters (Table 2 and Figures 1, 2)**

**Cytokines:** Mean IL-1β levels in group A, group B and group C were 5.71 pg/ml, 7.79 pg/ml and 7.55 pg/ml respectively with no significant difference (P-value>0.05). Titres of IL-6 were higher in group C (64.98 pg/ml) when compared to group A (5.04 pg/ml) and group B (15.51 pg/ml). On the application of Tukey’s HSD test, the difference of IL-6 was significant when group C was compared to group A (P-value <0.001) and group B (P-value <0.001) as well as Group A and group B (p value=0.93). Mean procalcitonin in group A was lowest (0.02 ng/ml ± 0.07), whereas in group B and group C procalcitonin titres were 0.44 ng/ml ± 0.72 and 0.24 ng/ml ± 0.47 respectively. Difference between groups was nonsignificant.

**Haematological parameters:** Amongst the various haematological parameters absolute lymphocyte count (ALC), lymphocyte to monocyte ratio (LMR) showed decreasing trend whereas total leukocyte count (TLC), absolute neutrophil count (ANC), neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR) and eosinophil to lymphocyte ratio (ELR) showed increasing trend with increase in disease severity. ALC, NLR, PLR, LMR and ELR could differentiate group B and C from group A (p<0.5).

### Table 2: Mean levels of various biochemical parameters with their comparative analysis in different groups

<table>
<thead>
<tr>
<th>Laboratory Parameter</th>
<th>Total</th>
<th>Asymptomatic (Group A) (N=96)</th>
<th>Mild symptomatic (Group B) (N=43)</th>
<th>Severe Symptomatic (Group C) (N=36)</th>
<th>P value</th>
<th>Group A and B</th>
<th>Group A and C</th>
<th>Group B and C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin (gm/dL)</td>
<td>12.9 ± 2.4</td>
<td>13.57 ± 1.9</td>
<td>12.22 ± 3.1</td>
<td>12.28 ± 2.5</td>
<td>0.011</td>
<td>0.024</td>
<td>0.993</td>
<td></td>
</tr>
<tr>
<td>Total Leucocyte count (x 10^9/ cumm)</td>
<td>7.18 ± 3.25</td>
<td>6.5 ± 2.27</td>
<td>7.02 ± 3.73</td>
<td>8.97 ± 3.96</td>
<td>0.313</td>
<td>&lt;0.001</td>
<td>0.277</td>
<td></td>
</tr>
<tr>
<td>Absolute neutrophil count (x 10^9/ cumm)</td>
<td>5.84 ± 3.59</td>
<td>5.22 ± 2.59</td>
<td>5.82 ± 3.86</td>
<td>7.15 ± 4.63</td>
<td>0.283</td>
<td>0.003</td>
<td>0.167</td>
<td></td>
</tr>
<tr>
<td>Absolute lymphocyte count (x 10^9/ cumm)</td>
<td>1.54 ± 1.09</td>
<td>1.88 ± 1.13</td>
<td>1.20 ± 0.97</td>
<td>1.25 ± 0.91</td>
<td>0.001</td>
<td>0.003</td>
<td>0.815</td>
<td></td>
</tr>
<tr>
<td>Neutrophil-Lymphocyte ratio (NLR)</td>
<td>6.53 ± 6.83</td>
<td>3.71 ± 2.63</td>
<td>8.75 ± 8.25</td>
<td>9.67 ± 8.40</td>
<td>0.001</td>
<td>0.001</td>
<td>0.625</td>
<td></td>
</tr>
<tr>
<td>Lymphocyte to monocyte ratio (LMR)</td>
<td>4.60 ± 3.75</td>
<td>5.48 ± 4.00</td>
<td>3.81 ± 2.70</td>
<td>3.74 ± 3.89</td>
<td>0.014</td>
<td>0.027</td>
<td>0.925</td>
<td></td>
</tr>
<tr>
<td>Eosinophil to lymphocyte ratio (ELR)</td>
<td>0.10 ± 0.08</td>
<td>0.07 ± 0.05</td>
<td>0.11 ± 0.09</td>
<td>0.13 ± 0.1</td>
<td>0.001</td>
<td>0.001</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>Platelet count (Lac/mL)</td>
<td>2.11 ± 0.85</td>
<td>1.98 ± 0.73</td>
<td>2.22 ± 0.82</td>
<td>2.32 ± 1.1</td>
<td>0.294</td>
<td>0.117</td>
<td>0.869</td>
<td></td>
</tr>
<tr>
<td>Neutrophil-Platelet ratio</td>
<td>3.19 ± 3.28</td>
<td>2.73 ± 1.14</td>
<td>3.16 ± 2.29</td>
<td>4.18 ± 6.03</td>
<td>0.141</td>
<td>0.025</td>
<td>0.308</td>
<td></td>
</tr>
<tr>
<td>Platelet-Lymphocyte ratio</td>
<td>2.32 ± 2.54</td>
<td>1.38 ± 0.90</td>
<td>2.87 ± 2.50</td>
<td>3.60 ± 3.81</td>
<td>0.001</td>
<td>0.001</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>Random Blood Sugar (mg/dL)</td>
<td>106.29 ± 53.18</td>
<td>96.57 ± 44.4</td>
<td>110.77 ± 55.6</td>
<td>122.59 ± 63.9</td>
<td>0.359</td>
<td>0.0046</td>
<td>0.603</td>
<td></td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>31.11 ± 25.15</td>
<td>24.67 ± 8.18</td>
<td>33.93 ± 31.15</td>
<td>45.45 ± 38.78</td>
<td>0.0073</td>
<td>&lt;0.001</td>
<td>0.147</td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.09 ± 1.23</td>
<td>0.91 ± 0.2</td>
<td>1.27 ± 1.76</td>
<td>1.38 ± 1.86</td>
<td>0.268</td>
<td>0.159</td>
<td>0.922</td>
<td></td>
</tr>
<tr>
<td>Procalcitonin (ng/ml)</td>
<td>0.19 ± 0.45</td>
<td>0.02 ± 0.07</td>
<td>0.44 ± 0.72</td>
<td>0.24 ± 0.47</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.157</td>
<td></td>
</tr>
<tr>
<td>Creatinine Kinase (U/L)</td>
<td>269.38 ± 850.80</td>
<td>146.42 ± 73.9</td>
<td>154.17 ± 152.3</td>
<td>412.29 ± 125.78</td>
<td>0.684</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Lactate Dehydrogenase (U/L)</td>
<td>748.76 ± 317.4</td>
<td>625.34 ± 251.2</td>
<td>784.76 ± 286</td>
<td>916.6 ± 365.2</td>
<td>0.096</td>
<td>0.001</td>
<td>0.296</td>
<td></td>
</tr>
<tr>
<td>Ferritin (ng/ml)</td>
<td>175.78 ± 235</td>
<td>135.18 ± 144.9</td>
<td>137.87 ± 197.3</td>
<td>396.54 ± 419.4</td>
<td>0.93</td>
<td>&lt;0.001</td>
<td>0.0006</td>
<td></td>
</tr>
<tr>
<td>Interleukin-1β (pg/ml)</td>
<td>6.60 ± 5.2</td>
<td>5.71 ± 3.19</td>
<td>7.59 ± 6.77</td>
<td>7.55 ± 7.16</td>
<td>0.083</td>
<td>0.174</td>
<td>0.976</td>
<td></td>
</tr>
<tr>
<td>Interleukin-6 (pg/ml)</td>
<td>19.95 ± 56.1</td>
<td>5.04 ± 4.4</td>
<td>15.51 ± 20.66</td>
<td>64.98 ± 111.18</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Interleukin-10 (pg/ml)</td>
<td>6.75 ± 8.87</td>
<td>5.36 ± 1.37</td>
<td>8.98 ± 16.44</td>
<td>7.67 ± 6.56</td>
<td>0.033</td>
<td>0.0013</td>
<td>0.6549</td>
<td></td>
</tr>
<tr>
<td>Tumour necrosis factor-α (pg/ml)</td>
<td>16.16 ± 13.13</td>
<td>12.31 ± 8.29</td>
<td>21.68 ± 17.18</td>
<td>19.84 ± 14.98</td>
<td>&lt;0.001</td>
<td>0.0004</td>
<td>0.617</td>
<td></td>
</tr>
</tbody>
</table>
not differentiate group B from C. ANC could differentiate between group A and C with higher counts in latter. Receiver operating characteristic (ROC) curve for interleukin-6 to differentiate the patients with severe disease from asymptomatic and mild symptomatic disease showed a cut-off of 6.75 pg/ml which had a sensitivity of 94.4% and specificity of 73.4%. (Figure 2).

Discussion

The major objectives of our study were to examine the correlation if any between cytokine markers, biochemical,
COVID-19 infection. In contrast, males may be more susceptible to have male predominance, indicating Huang et al., our study population also had male predominance, indicating higher virulence of the virus which led to development of severe disease in our cohort. Mean age of patients who developed severe disease was higher than patients with asymptomatic disease indicating ageing and immune senescence leads to increased morbidity. Therefore, patients aged more than 50 years need closer monitoring for the development of severe disease than their younger counterparts. This resonates with the most of the data worldwide describing more severe disease and higher case fatality rate in older age groups. Fever was the most common presenting symptom in our study population followed by cough, myalgia and dyspnoea, however out of 175 patients only 75 (41%) patients developed fever during the course of illness, emphasizing the shortcoming of thermal screening as the only test for screening for COVID-19. However, all patients with severe disease had higher frequency of fever, cough and dyspnoea, pointing towards clinical clues which should be borne in mind while trying to triage these patients.

Infection with COVID-19 is known to induce a dysregulated immune response in host. This dysregulated inflammatory response can lead to a cytokine storm and is responsible for progression to severe disease and death. Interleukin-6 is a pro-inflammatory cytokine secreted by all stromal and immune cells, and its secretion is induced by other pro-inflammatory cytokines such as IL-1β and TNF-α. A recently conducted metaanalysis by Grifoni E et al suggested that peripheral blood IL-6 levels are higher in patients with severe COVID-19 infection. In our cohort, we had similar findings indicating that interleukin-6 level was significantly increased with increasing disease severity. Gao Y et al pointed out that interleukin-6 in peripheral blood could serve as an independent marker to predict the progression of COVID-19, which is consistent with the results of this study; Although some studies in COVID-19 patients found higher IL-1β, IL-10 and TNF-α levels in severe disease but our study had contradictory findings. We found levels of TNF-alpha to be higher in symptomatic patients when compared to asymptomatic whereas there were no differences in IL-1β and IL-10 levels in three groups. Differences could be due to multiple factors including relatively younger cohort, and differences in level of disease severity. IL-10 is found to be higher expressed in patients with advanced age and hyperinflammatory response. Wan et al. described normal TNF-α levels in patients with COVID-19. Moreover, despite implications of multiple interleukins in disease pathogenesis, most studies have focussed on IL-6 and use of IL-6 inhibitors in patients with severe disease. Ferritin, another marker for inflammation and commonly implicated in macrophage activation syndromes was found to be higher in severe group, a finding consistent with most of the available literature on COVID-19. Non-specific markers of severity such as creatine kinase, lactate dehydrogenase, urea, creatinine, the incidence of relative neutrophilia and relative lymphopenia to be increased in patients suffering from severe disease. These findings may be the consequence of dysregulated immune response

![ROC Curve](image)

**Fig. 2:** Receiver operating characteristic (ROC) curve for interleukin-6 to differentiate the patients with severe disease from Non-severe disease

haematological parameters and the severity of disease and to look for a cut off value which may serve to distinguish between severe and non-severe disease. The correlation study between the cytokine markers and severity of disease led us to postulate that interleukin-6 has the potential to be used as a distinguishing marker for severe disease. We also derived a cut-off of 6.75 pg/ml from the ROC curve with a sensitivity of 94.4% and specificity of 73.4% to predict the development of severe disease. To the best of our knowledge, this is the first observational study in Indian population where COVID-19 patients were grouped according to their severity and inflammatory markers along with other laboratory parameters were compared in the different groups. Since the first diagnosed case in December 2019 from Wuhan province in China, many researchers have reported clinical and epidemiological characteristics of the patients suffering from COVID-19.

In congruence with a study by Huang et al, our study population also had male predominance, indicating males may be more susceptible to COVID-19 infection. In contrast to previous studies in which almost 33% of patients developed severe disease, in our study cohort, only 21% of patients had severe disease. This is similar to the Chinese cohort from Wuhan where they reported 19% to have severe-critical illness. This finding points towards the possibility of an unknown protective factor or decreased virulence of the virus which led to decreased incidence of severe disease in our cohort. Mean age of patients who developed severe disease was higher than patients with asymptomatic disease indicating ageing and immune senescence leads to increased morbidity.
triggered by virus infection which leads to kidney injury and cardiac dysfunction secondary to persistent inflammatory state. 19 Higher PLR, NLR, and newer emerging inflammatory indices like ELR and LMR have been shown to be associated with systemic inflammation and poor prognosis. 20, 21 LMR was found to be low in the severe group whereas other cells’ ratio PLR, NLR and ELR were found to be higher in severe group indication more severe inflammation. This is for the first time that newer indices like LMR and ELR have been assessed in COVID-19 and were found to distinguish the severe group from others.

In conclusion, we found that IL-6 might serve as a potential marker for severe disease. Our cohort showed patients who had fever, cough, dyspnoe a with higher IL-6, ferritin, LDH, total leucocyte count, absolute neutrophil count, platelet to lymphocyte ratio, neutrophil to lymphocyte ratio, eosinophil to lymphocyte ratio and lower lymphocyte to monocyte ratio has more severe disease. This study supports the hypothesis that elevated IL-6 levels lead to adverse clinical events and efforts to halt the cytokine storm with the help of anti-IL-6 drugs such as tocilizumab along with standard care may improve patient outcome.

Limitations

Our study had several limitations that might cause potential bias. As this study was a single centre, observational study with a small number of patients and the majority of patients were from a single hotspot, the results of this analysis should be confirmed in a multi-centre prospective study. Additionally, the long-term complications of COVID-19 could not be evaluated, the standardized larger cohort would be better to assess the temporal change in the immune response. Another confounding factor was possible superinfection with bacteria which might affect the immune response.

Ethical approval

This study approved by ethical and research committee of SMS medical college and Hospital, Jaipur, India.

Author contributions

S. Bhandari, S. Jain and R. Sahlot formulated the research questions, designed the study, developed the preliminary search strategy, and drafted the manuscript. A. Kumar, R. Sahlot and G. Rankawat collected the data for study and write the manuscript. S. Bhandari and A. Jain conducted the quality assessment. All authors critically reviewed the manuscript for relevant intellectual content. All authors have read and approved the final version of the manuscript.

Funding

The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not for profit sectors.

Availability of data and materials

Available from corresponding author upon reasonable request.

Declaration of competing interest

All authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential.

Acknowledgments

I would like to thanks the anonymous referees for their useful suggestion. I would like to thanks to my professionals Dr. Abhishek Agrawal, Dr. C. L. Nawal, Dr. S. Banerjee, Dr. Prakash Keswani, Dr. Sunil Mahavar, Dr. R S Chejara, Dr. Vidyadhar Singh, Dr. Kapil, Dr. Amitabh Dube, Dr. Vishal Gupta, Dr. Aradhana and team of Department of General Medicine SMS Medical college and attached group of Hospital, Jaipur for their valuable support and care of COVID-19 patients.

References


Mannose Binding Lectin Levels and its Association with Systemic Lupus Erythematosus Disease Severity: An Indian Report

Gargi Thakur1, Taruna Madan1, Prathamesh Surve2, Prasad Khadilkar2, Durga Chougule2, Anjali Rajadhyaksha3, Milind Nadkar3, Kanjaksha Ghosh2, Vandana Pradhan2*

Abstract

Background: Dysregulated serum levels of Mannan binding lectin (MBL) has a probable role in Systemic Lupus Erythematosus (SLE) pathogenesis.

Objective: To evaluate the association between serum MBL levels in SLE patients from western India with the severity of disease

Methods: SLE patients (n=70) from Western India were included. Based on MBL levels, patients were classified into four categories, viz. low (<100 ng/ml), mild (100-500 ng/ml), moderate (500-1000 ng/ml) and high (>1000 ng/ml). Correlation of serum MBL levels with disease severity was assessed using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI). MBL levels and circulating immune complex levels were detected by ELISA. C3, C4 and CRP levels were detected by nephelometer.

Results: Serum MBL levels of SLE patients (1954 ± 202.4 ng/ml) was lower than that of healthy controls (2388 ± 205.0 ng/ml). There was no significant correlation between MBL levels with severity of SLE on the basis of ACR criteria and SLEDAI scores (p>0.05). No significant difference was observed among MBL levels and SLE patients with (1847 ± 246.7) or without (1900 ± 246.8) Lupus Nephritis. SLE patients without infections (n=33) had low MBL levels (1700 ± 301.0 ng/ml) as compared with SLE patients with infection (n=37) (2189 ± 284.6 ng/ml) (p=0.30) between MBL levels with severity of SLE on the basis of ACR criteria and SLEDAI scores (p>0.05). No significant difference was observed among MBL levels and SLE patients with (1847 ± 246.7) or without (1900 ± 246.8) Lupus Nephritis. SLE patients without infections (n=33) had low MBL levels (1700 ± 301.0 ng/ml) as compared with SLE patients with infection (n=37) (2189 ± 284.6 ng/ml) (p=0.30) with disease severity, haematological manifestations and infections among SLE patients from Western India.

Conclusion: Present study indicated that low MBL levels were not associated with disease severity, haematological manifestations and infections among SLE patients from Western India.

Introduction

Autoimmunity is the breakdown of immune tolerance leading to an immune response against self-tissues and cells. Pathogenic role of autoantibodies in autoimmune diseases is well documented.1 Genesis of antibodies to self-antigens is a failure of adaptive immune surveillance. Disability to clear apoptotic cells is one of the important underlying mechanisms leading to generation of autoantibodies.2 Innate immune response is integral to clearance of apoptotic cells in vivo and in vitro.3 Mannose binding lectin (MBL), a member of collectin family playing important role to initiate clearance of apoptotic cells in vivo and in vitro.4 MBL is also known as a key molecule in host defense. It binds to polysaccharides on the surface of microorganisms by activating the complement system via MBL associated serine proteases (MASPs).5

The MBL gene has 4 exons with chromosome location on 10q11.2-q21. Polymorphisms of the MBL promoter region influence serum protein levels where five single nucleotide polymorphisms (SNPs) associated with reduced MBL serum protein levels have been reported.6 Based on the ethic differences this frequency of abnormal alleles vary.7 Association of MBL polymorphism has been reported in various autoimmune diseases like type II diabetes and rheumatoid arthritis (RA) etc. Deficiency in MBL concentration has been reported as a probable cause for Systemic Lupus Erythematosus (SLE) disease susceptibility.8-10 MBL deficiency with other associated complement components leading to abnormal antigen presentation can possibly the mechanism involved in SLE pathogenesis.11 It has been also been indicated that MBL deficiency results in defective clearance of apoptotic cells as well as predisposition to infections. Subsequently this may lead to over-expression of autoantigens leading to polyclonal activation in SLE pathogenesis.

The MBL deficiency may be as result of defective MBL gene polymorphisms and pathogenic role of anti-mannose binding lectin (anti-MBL) autoantibodies.10 In a meta-analysis, allele B of MBL and polymorphisms at the promoter region, specifically those found at positions −550 and −221, were reported as risk factors of SLE development and lower levels of MBL have increased risk for developing SLE.11 Our group has earlier reported that a SNP at MBL codon 54 (designated allele B) and polymorphisms in the MBL promoter region were risk factors of SLE disease susceptibility. Previously, elevated levels of anti-MBL autoantibodies with serum MBL deficiency had also been reported.12,13

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Received: 23.11.2021; Revised: 04.02.2022; Accepted: 08.02.2022
An association of high serum MBL levels and MBL activity have also been reported. It is hypothesized that high MBL levels result in an elevated complement activity leading to tissue and organ damage. Plasma concentrations of MBL-associated serine proteases (MASP-1, MASP-3), and MBL-associated protein (MAP44) were reported to be elevated in SLE patients. Interestingly, mild and moderate MBL deficiency was reported in Lupus Nephritis (LN) patients from Brazil. In the present study, we have evaluated an association of serum MBL levels in SLE patients from western India with the severity of disease.

**Material and Methods**

**Study Population**

This is a retrospective study conducted on 70 SLE patients from Mumbai, western India. The study was undertaken after the Institutional Ethical Committee (IEC) approval (Project no. NHM/IEC/05-2009). Informed consent was obtained from all individual participants included in the study. All patients were diagnosed according to the American College of Rheumatology (ACR) criteria and were treatment naive. As per disease severity, patients were categorized into mild, moderate and severe categories based on SLE Disease Activity Index (SLEDAI) scores (mild <8, Moderate 8 – 18, Severe >18) respectively. The patients were further categorized as Lupus Nephritis (LN) and SLE without Lupus Nephritis (Non LN). In LN patient, renal biopsies were examined by light microscopy using hematoxylin, eosin, periodic Schiff (PAS) staining and these patients were classified according to WHO criteria. Pregnant, post-menopausal women, smokers, patients with diabetes, significant hyperlipidaemia were excluded from study. After obtaining written informed consent, blood samples (5 ml) was collected. Sera were stored in aliquots at -80°C until tested. Basic investigations consisting of complete blood counts including erythrocytes, leukocytes and platelets count, ESR and peripheral smear examinations were done in all cases.

**Lab Investigations**

Serum samples were analyzed for MBL levels by using MBL oligomer ELISA kit (KIT29), BioPorto diagnostics, Denmark. MBL deficiency was defined as serum levels lower than 1000ng/ml and graded into low (<100ng/ml), mild (100-500ng/ml) and moderate (500-1000ng/ml). Serum samples with MBL levels more than 1000ng/ml were classified as ‘High’. Serum levels of hsCRP and complement like C3, C4 were detected by a Nephelometer (BN ProSpec, Dade Behring, Germany).

**Statistical analysis**

Statistical analyses were performed using GraphPad Prism version 6.0 (GraphPad Prism Software Inc., California, USA.) for Windows. Means/ percentage of participants between two groups were analyzed using two-tailed unpaired t-test. The Pearson’s correlation test was used to analyze correlations between various laboratory measures and SLEDAI scores. Fischer’s exact test was used to determine an association between laboratory investigations, clinical manifestations. The value of p ≤ 0.05 was considered statistically significant.

**Results**

**Characteristics of study population**

The mean age of evaluation of SLE patients was 28.9 ± 10.8 years whereas the age of onset was 25.3 ± 9.5 years with a disease duration of 3.7 ± 3.9 years. Mean age of healthy controls was 32.0 ± 8.1 years and did not show differences with the age of SLE group at evaluation. Mean serum MBL level of SLE patients (1954 ± 202.4 ng/ml) was lower than that of healthy controls (2388 ± 205.0 ng/ml), though the difference was not statistically significant (p>0.05). Based on the serum MBL levels, the study population was classified into four categories, viz. low (<100 ng/ml), mild (100-500 ng/ml), moderate (500-1000 ng/ml) and high (>1000 ng/ml). Number of SLE patients in the category of low MBL levels (27%) was significantly higher than the healthy controls (11%) (p<0.0001) when compared with the number of SLE patients and controls with moderate levels of MBL.

**Correlation of MBL levels with disease severity**

SLE patients were divided into two groups, i.e Group I with SLE patients fulfilling <6 ACR criteria (n=55) and Group II satisfying ≥ 6 ACR criteria (n=15). Mean MBL levels of Group I (1786 ± 236 ng/ml) were not significantly different than mean MBL levels of Group II (1319 ± 484.7 ng/ml). Seven out of 15 (46.66%) of SLE patients with <6 ACR criteria were in the low MBL level group and was higher than SLE patients with ≥ 6 ACR criteria with low MBL levels (21.8%), the difference was not statistically significant (p=0.16). SLE patients were also classified into three groups on the basis of the SLEDAI score, namely mild (SLEDAI <8) (n=14), moderate (SLEDAI 8-18) (n=44) and severe (SLEDAI >18) (n=12). Mean MBL levels of ‘Severe group’ (1877 ng/ml) did not differ significantly from MBL levels of ‘Mild group’ (1140 ng/ml) or ‘Moderate group’ (2070 ng/ml). Interestingly, 7 out of 12 (58.33%) SLE patients with severe SLEDAI score showed elevated levels of MBL in comparison with 35.7% of SLE patients with mild SLEDAI score with elevated levels of MBL, though statistically insignificant (p=0.48). Based on ACR criteria, no correlation of the serum MBL levels with severity of SLE was noted (p=0.376) and SLEDAI score (p =0.157).

**Association of serum MBL levels with hematological manifestations**

Out of 70 SLE patients, 57 showed hematological manifestations. These patients were grouped into four groups depending on the low level (<10g/dl) of Hemoglobin (Anemia) (n=31), low number (<150,000) of platelets (Thrombocytopenia) (n=19), reduced number (<4500) of Leukocytes (Leukopenia) (n=5) and group that showed deficiencies for all three parameters (Auto Immune Hemolytic Anemia) (n=2). None of the groups of SLE patients with hematological manifestations were significantly different from that of SLE patients without haematological manifestations (n=29) with respect to MBL levels (p=0.625). Interestingly, the percentage of SLE patients with anemia, thrombocytopenia, leukopenia and AIHA were higher in the ‘Moderate’ group in comparison with SLE patients without hematological manifestations.

**Association of serum MBL levels with complement levels**

SLE patients with low C3 levels when compared with SLE patients with Normal C3 levels, there was no statistically significant difference for their MBL levels (p=0.09). Difference in percentage of SLE patients with low C3 levels and low MBL levels (27.02%) was not statistically significant than
Categorical data was compared using Fisher’s test. Data considered significant p<0.05.

### Table 1: Association of MBL levels with complement levels (C3,C4) and hsCRP in SLE patients

<table>
<thead>
<tr>
<th>MBL levels</th>
<th>C3 levels</th>
<th>C4 levels</th>
<th>hsCRP levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal (≥90 mg/dl)</td>
<td>Low (&lt;15 mg/dl)</td>
<td>Normal (≥15 mg/dl)</td>
</tr>
<tr>
<td>&lt;100 ng/ml</td>
<td>10 (27.0%)</td>
<td>7 (20%)</td>
<td>8 (22.9%)</td>
</tr>
<tr>
<td>100 – 1000 ng/ml</td>
<td>7 (18.9%)</td>
<td>8 (22.9%)</td>
<td>9 (25.7%)</td>
</tr>
<tr>
<td>&gt;1000 ng/ml</td>
<td>20 (54.1%)</td>
<td>20 (57.1%)</td>
<td>25 (55.6%)</td>
</tr>
<tr>
<td>p value</td>
<td>0.353</td>
<td>0.890</td>
<td>0.572</td>
</tr>
</tbody>
</table>

Categorical data was compared using Fisher’s test. Data considered significant p<0.05.

### Table 2: Association of MBL levels in Lupus Nephritis (n=45)

<table>
<thead>
<tr>
<th>MBL levels</th>
<th>LN (n=45)</th>
<th>Non-LN (n=25)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MPGN (n=28)</td>
<td>FPGN (n=2)</td>
</tr>
<tr>
<td>&lt;100 ng/ml</td>
<td>9 (32.1%)</td>
<td>1 (50%)</td>
</tr>
<tr>
<td>100 – 1000 ng/ml</td>
<td>4 (14.3%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>&gt;1000 ng/ml</td>
<td>15 (53.6%)</td>
<td>1 (50%)</td>
</tr>
<tr>
<td>p value</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

Categorical data was compared using Fisher’s test. Data considered significant p<0.05.

SLE patients with normal C3 levels and low MBL levels (15.15%) (p=0.32). Interestingly, percentage of SLE patients with low C3 levels and mild MBL levels (5.4%) was significantly lower than SLE patients with normal C3 levels and mild MBL levels (24.24%) (p<0.05). SLE patients with low C4 or low hsCRP levels when analysed based on their MBL levels were not found to be statistically different from SLE patients with Normal C4 or Normal hsCRP levels (Table 1).

**Serum MBL levels in renal histopathological findings among Lupus Nephritis patients**

Among 70 SLE participants, 45 SLE patients showed renal manifestations and were grouped as Lupus Nephritis (LN). No significant association was observed among the mean serum MBL levels and SLE patients with (1847 ± 246.7) or without (1900 ± 246.8) nephritis. Based on renal biopsies LN patients were grouped in to four major groups like Membranoproliferative glomerulonephritis (MPGN), Focal proliferative glomerulonephritis (FPGN), Diffuse proliferative glomerulonephritis (DPGN) and rapidly progressive glomerulonephritis (RPGN). No significant association of MBL levels with these subgroups of LN was observed (Table 2).

**Discussion**

The present study established a higher prevalence of SLE in individuals with low levels of serum MBL when compared with healthy controls, confirming our previous findings. Interestingly, low serum MBL levels among SLE patients show a severe form of disease with ACR criteria more than 6, whereas, high SLEDAI scores associated with elevated MBL levels though not statistically significant owing to limited sample size post stratification based on MBL levels.

Most of the studies investigating an association of serum MBL levels with severity among SLE patients had remained inconclusive owing to the fact that MBL like other complement molecules exhibiting more complex and paradoxical roles in SLE. On one hand, complement is integral to the inflammatory mechanisms in SLE resulting in tissue and organ damage while on the other, complement deficiencies result in defective clearance of apoptotic debris and / or associated recurrent infections like C1q deficiency (93%) and C4 deficiency (75%).

Our previous study had shown a prevalence of anti-MBL autoantibodies in 52 % SLE patients and 64% of these showed low MBL levels. However, significantly high plasma MBL levels were observed in SLE patients than healthy controls of Eastern-Indian origin in another study. MBL low producer genotype (LXA/LYB, LYB/LYB and LXB/LXB) was reported to be significantly higher in SLE patients as compared to healthy control in Eastern Indian Population. These reports of East Indian population and present study observations from Western Indian population possibly suggesting that these two Indian populations may have been distinct. Therefore, we evaluated the hypothesis that both MBL deficiency and high levels of MBL may associate with severity of SLE. Brazilian SLE patients had been reported to have higher frequency of mild and moderate deficiency in MBL levels as compared to controls.

Our attempts to correlate serum MBL levels with the severity of disease, indicated no association of serum MBL levels with the severity of the disease when patients were classified based on the ACR criteria and SLEDAI scoring. Recently, Trolldborg et al., had reported an association of alterations in plasma concentrations of the pattern recognition molecules of the lectin pathway in SLE patients to salient features of the disease, thus supporting that the lectin pathway contributes to pathogenesis of SLE. SLE patients positive for anti-dsDNA antibodies had significantly lower levels of MBL in plasma than patients negative for anti-dsDNA antibodies. No correlation between MBL serum levels and disease severity or cumulative damage was observed in SLE patients of Brazilian origin. An important evidence for protective role of MBL in SLE was provided by a recent study wherein, MBL supplement in mice model could ameliorate lupus nephritis. A higher morbidity and mortality had been reported among LN patients as compared with SLE without nephritis. SLE patients having MBL levels less than100 ng/ml were reported to have a two-fold increased risk of developing nephritis. An association between MBL deficiency and LN was observed in Brazilian population. Though our study did not show an association of low MBL
levels in LN, a higher percentage of LN patients with Diffused proliferative glomerulonephritis (DPGN) had low MBL levels.

A recent study in Brazilian SLE patients reported no correlation between serum MBL levels and complement (C3, C4) levels with high hsCRP levels. However, in the present study this association was not found. Hematological abnormalities such as anaemia are commonly observed in SLE patients. MBL low producer genotype was significantly associated with autoimmune hemolytic anaemia. Deficiency of MBL is reported to increase susceptibility to infectious disease in children and adults. In our study, SLE patients with lower MBL levels did not show any significant alteration in susceptibility to bacterial/ viral/ parasitic infections. Though previous studies had reported lower MBL levels with an incidence of pulmonary tuberculosis with conflicting results, present study did not observe any association of incidence of pulmonary tuberculosis in SLE patients with low MBL levels.

Limitations of the Study
Present study had limitations for not having paediatric age group patients included in this study. A larger cohort of SLE patients will highlight the effect on MBL association with disease severity and involvement into renal complications if any in these patients at the time of disease onset. Another limitation of the study was a sequential followup investigations could not be performed in these enrolled participants.

Conclusions
The present study showed that low levels of MBL significantly associate with SLE patients from Western India. MBL levels did not influence the predisposition of SLE patients to nephritis or infections. Normal C3 and lower MBL levels may contribute to increased susceptibility to SLE which needs to be evaluated in a larger cohort. Haematological manifestations that predominantly occur in SLE patients with moderate MBL levels need to be evaluated further.

Ethics Approval
This study was approved by Ethics Committee of National Institute of Immunohaematology (ICMR-NIHH), Mumbai, India. Written consent was obtained from participants.

Acknowledgements
We thank Department of Biotechnology, Government of India for funding this project. We thank all the patients for their participation in this study.

Funding
This work was supported by Department of Biotechnology, Government of India (BT/PR/2530/SPD/11-01-2009).

Conflicts of Interest/Competing interests
The authors Gargi Thakur, Taruna Madan, Prathamesh Surve, Prasad Khadilkar, Durga Chougule, Anjali Rajadhyaksha, Milind Nadkar, Kanjaksha Ghosh and Vandana Pradhan declare that they have no conflict of interest.

Abbreviations
ACR/SLICC – American College of Rheumatology; ALD-DNA – Activated lymphocyte – derived DNA; Anti-dsDNA – anti-double stranded deoxyribonucleic acid; Ciq – Complement component 1q; C3 – Complement component 3; C4 – Complement component 4; DPGN - Diffuse proliferative glomerulonephritis; ESRD – End Stage Renal Disease; FPGN - Focal proliferative glomerulonephritis; HB – Haemoglobin; hsCRP – High sensitivity C-reactive protein; LN – Lupus Nephritis; MAP44 - MBL-associated protein of 44kDa; MAPKs - Mitogen-activated protein kinases; MASP-1 - MBL-associated serine proteases -1; MASP-3 - MBL-associated serine proteases – 3; MBL – Mannose-binding lectin; MPGN - Membranoproliferative glomerulonephritis; NF-kB - nuclear factor-kB; PAS – periodic Schiff (PAS); RA - Rheumatoid Arthritis; RPNP - Rapidly progressive glomerulonephritis; SLE – Systemic Lupus Erythematosus; SNPs – single nucleotide polymorphisms; WBCs – White blood cells; Th – T helper cells.

References
Retino-Renal Dissociation in Type 2 Diabetes Mellitus: An Observational Cross Sectional Study at a Tertiary Care Center of Sub-Himalayan Region

Rajesh Kumar¹, Thakur Prashant Singh², Ajay Jaryal³, Surinder Thakur⁴

Abstract

Introduction: Diabetic retinopathy, the diabetes-specific long-term microvascular complication is an important predictor of diabetic nephropathy. Diabetes induced retinopathy mostly proceeds nephropathy in patients with Type1 diabetes; however, this sequence is not consistent in patients with Type 2 diabetes and has significant discordance.

Methods: It was a hospital-based prospective, observational study conducted at Indira Gandhi Medical College, Shimla Himachal Pradesh a tertiary care center in the sub-Himalayan region of India from July 2016 to June 2017. A total of 141 patients were recruited in this study period.

Results: 141 patients with type 2 diabetes, 83(58.9%) males,58(41.1%) females were recruited in the study. The mean duration of diabetes in this study was 5.7±6.21 years. Mean HbA1C in our study was 9.66±3.04%. 79(56.0%) patients had poor glycaemic control. Out of a total of 141 patients, DKD (albuminuria and/or reduced eGFR) was present in 67 (47.52%) patients. 33 had diabetic retinopathy.

Conclusion: The relationship between retinopathy and nephropathy in type 2 diabetic patients is not as clear as in type 1 diabetic patients. Patients with type 2 diabetes do not have diabetic nephropathy always and non-diabetic renal disease is also quite common. The absence of retinopathy, rapid progression diabetes, presence of RBC, and cast are some of the atypical findings, and patients presenting with them should be subjected to renal biopsy to rule out non-diabetic renal disease (NDRD).

Introduction

Diabetic retinopathy, the diabetes-specific long-term microvascular complication is an important predictor of diabetic nephropathy. Diabetes induced retinopathy mostly proceeds nephropathy in patients with Type1 diabetes; however, this sequence is not consistent in patients with Type 2 diabetes and has significant discordance. India, the largest and most populated country of southeast Asia and a host to the maximum number of patients with diabetes which is expected to rise from 67 million to 101 million by 2035,¹² will also have a large population of patients suffering from these vascular complications. Various studies have reported that 47-63% of patients with type 2 diabetes with retinopathy have proteinuria.³ However, the reverse is not true and 47.3% of patients of Type 2 Diabetes with renal abnormalities like proteinuria and/or renal insufficiency did not have diabetic retinopathy signifying the importance of identifying nondiabetic renal diseases (NDRD) in patients of type 2 diabetes while patients Type1 diabetes with these renal abnormalities usually have retinopathy.⁴ We studied this retino-renal dissociation and the histological pattern on renal biopsy in patients of diabetes having albuminuria (UACR>1000 mg/g) or reduced eGFR without evidence of retinopathy in a tertiary care hospital of sub-Himalayan region Himachal Pradesh.

Methods

It was a hospital-based prospective, observational study conducted at Indira Gandhi Medical College, Shimla Himachal Pradesh a tertiary care center in the sub-Himalayan region of India from July 2016 to June 2017. A total of 141 patients were recruited in this study period. The diagnosis of type 2 diabetes was made based on the American diabetes association criteria (ADA). Diabetic Nephropathy/diabetic kidney disease (DKD) was diagnosed based on measurements of urinary albumin creatinine ratio (UACR) in a spot urine sample on two occasions in the absence of acute febrile illness, UTI, diabetic ketoacidosis, uncontrolled hypertension, heart failure, short term pronounced hyperglycemia, and vigorous exercise and/or by measurement of serum creatinine and estimation of eGFR. An estimated GFR of <60 ml/min/1.73m² was defined as renal impairment. Microalbuminuria was defined as values of 30-300 mg/g of creatinine and macroalbuminuria >300 mg/g of creatinine. Retinopathy was assessed based on direct and indirect opthalmoscopy performed in every patient by an ophthalmologist after the dilation of pupils. The severity of diabetic retinopathy (DR) was further categorized into proliferative and non-proliferative diabetic retinopathy.
Table 1: Prevalence of Diabetes kidney Disease (Albuminuria and reduced eGFR)

<table>
<thead>
<tr>
<th>UACR (mg/g)</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;30mg/g</td>
<td>48(34.0%)</td>
<td>36(25.5%)</td>
<td>84(59.6%)</td>
</tr>
<tr>
<td>30-299mg/g</td>
<td>23(16.3%)</td>
<td>14(9.93%)</td>
<td>37(26.2%)</td>
</tr>
<tr>
<td>≥300mg/g</td>
<td>12(8.5%)</td>
<td>8(5.7%)</td>
<td>20(14.2%)</td>
</tr>
</tbody>
</table>

Table 2: Prevalence of Diabetes Kidney Disease (Reduced eGFR)

<table>
<thead>
<tr>
<th>eGFR (ml/min/1.73m²)</th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;60/ml/min/1.73m²</td>
<td>58(41.1%)</td>
<td>36(57.4%)</td>
<td>94(66.7%)</td>
</tr>
<tr>
<td>30-60/ml/min/1.73m²</td>
<td>17(7.8%)</td>
<td>10(7.1%)</td>
<td>27(14.9%)</td>
</tr>
<tr>
<td>15-29/ml/min/1.73m²</td>
<td>6(4.2%)</td>
<td>6(4.2%)</td>
<td>12(8.5%)</td>
</tr>
<tr>
<td>&lt;15/ml/min/1.73m²</td>
<td>2(1.4%)</td>
<td>2(1.4%)</td>
<td>4(2.8%)</td>
</tr>
</tbody>
</table>

Table 3: Prevalence of retinopathy in patients of Type 2 Diabetes

<table>
<thead>
<tr>
<th>Retinopathy</th>
<th>Male (N=83)</th>
<th>Female (N=58)</th>
<th>Total (N=141)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>67 (80.72%)</td>
<td>41 (70.69%)</td>
<td>108 (76.6%)</td>
</tr>
<tr>
<td>Mild NPDR</td>
<td>6 (7.23%)</td>
<td>8 (13.79%)</td>
<td>14 (9.93%)</td>
</tr>
<tr>
<td>Moderate NPDR</td>
<td>7 (8.43%)</td>
<td>9 (15.52%)</td>
<td>16 (11.35%)</td>
</tr>
<tr>
<td>Severe NPDR</td>
<td>1 (1.20%)</td>
<td>0 (0.00%)</td>
<td>1 (0.71%)</td>
</tr>
<tr>
<td>PDR</td>
<td>2 (2.41%)</td>
<td>0 (0.00%)</td>
<td>2 (1.42%)</td>
</tr>
</tbody>
</table>

Table 4: Dissociation of diabetic kidney disease with retinopathy (n=67)

<table>
<thead>
<tr>
<th>DKD DR (present)</th>
<th>DR (absent)</th>
<th>Total (n=141)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>13(9.2%)</td>
<td>61(43.3%)</td>
</tr>
<tr>
<td>Present</td>
<td>20(14.2%)</td>
<td>47(33.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>33(23.4%)</td>
<td>108(76.6%)</td>
</tr>
</tbody>
</table>

Fig. 1: Spectrum of Non diabetic renal disease on Renal Biopsy (n=111)

141 patients with type 2 diabetes, 83(58.9%) males, 58(41.1%) females were recruited in the study. The mean duration of diabetes in this study was 5.78±2.21 years. Mean HbA1C in our study was 9.66±0.04%. 79(56.0%) patients in our study had HbA1C more than 9.0% while 39(27.7%) had HbA1C between 7.0–9.0%. Only 23(16.3%) patients had HbA1C less than 7.0%. A total of 118 (83.7%) patients had poor glycemic control. Out of a total of 141 patients, 67(47.5%) patients had diabetic retinopathy in some form. Among patients who had DKD 67(47.5%), 20 (14.2%) had retinopathy. 13 (9.2%) patients had diabetic retinopathy without evidence of DKD in this study. Thus, a significant number of patients, 47 (33.3%) developed nephropathy without the development of retinopathy in our study, and this finding was correlated among several studies done in India, Asia, and America. R.T. Erasmus et al. showed that among patients with microalbuminuria only 18% (12/64) patients had retinopathy. In their study out of 113 patients suffering from type 2 diabetes, the prevalence rate of microalbuminuria was as high as 54% among males and 59% among females. The prevalence of retinopathy was 16%. They concluded that microalbuminuria and retinopathy may not be related. In another study only 39.3% of patients had retinopathy and dissociation was 60.7%. In a study done in Pakistan by Sajid Nisar et al, out of 86 patients who had microalbuminuria 39 (45.4%) patients also had retinopathy, however, the difference in continuous variables was analyzed by student t-test and a p-value of <0.05 was considered as statistically significant.

Results

In our study, a total of 67(47.5%) patients had diabetic kidney disease (DKD) when assessed using either micro or macroalbuminuria and eGFR (<60 ml/min/1.73m²) or both. Out of all 141 patients, 33 (23.40%) patients had retinopathy in some form. Among patients who had DKD 67(47.5%), 20 (14.2%) had retinopathy. 13 (9.2%) patients had diabetic retinopathy without evidence of DKD in this study. Thus, a significant number of patients, 47 (33.3%) developed nephropathy without the development of retinopathy in our study, and this finding was correlated among several studies done in India, Asia, and America. R.T. Erasmus et al. showed that among patients with microalbuminuria only 18% (12/64) patients had retinopathy. In their study out of 113 patients suffering from type 2 diabetes, the prevalence rate of microalbuminuria was as high as 54% among males and 59% among females. The prevalence of retinopathy was 16%. They concluded that microalbuminuria and retinopathy may not be related. In another study only 39.3% of patients had retinopathy and dissociation was 60.7%. In a study done in Pakistan by Sajid Nisar et al, out of 86 patients who had microalbuminuria 39 (45.4%) patients also had retinopathy, however,
the remaining 47 (54.6%) patients did not have retinopathy.9 We also studied renal biopsy among patients with macroalbuminuria without retinopathy. Out of 11 patients subjected to renal biopsy 6 patients had DG either stage 3 or 4 (Table 2).5 patients had findings other than DG and the pattern of biopsy among NDRD patients includes Ig A nephropathy, Hypertensive nephrosclerosis, multiple myeloma, and acute tubulointerstitial nephritis. This finding was similar among various other studies done across the globe.6

In both types of diabetes, chronic hyperglycemia is the primary cause of the disease. In type 1 diabetes hyperglycemia starts in the first decades of life and is usually the only recognized cause of nephropathy. On the contrary, in type 2 diabetes hyperglycemia starts in the third or fourth decades of life, hence kidneys have already suffered the long-term consequences of aging and other recognized promoters of chronic renal injury such as arterial hypertension, obesity, dyslipidemia, and smoking. Aging is per se a cause of progressive glomerulosclerosis and combined with the above risk factors may contribute to the nonspecific changes of the arteriolarosclerotic type which so often coexist with, and occasionally overwhelming, the typical features of diabetic glomerulopathy (DG) in type 2 diabetes.11

Due to the high prevalence of accelerated atherosclerosis involving the renal microvasculature, ischemic changes are also frequently observed in type 2 diabetes. Thus, the term diabetic nephropathy, especially in type 2 diabetes, reflects a heterogeneous mixture of different diseases that are sustained by different mechanisms and may coexist in different combinations.

Pure diabetic glomerulopathy is more frequently observed in patients with earlier onset of diabetes and evaluated at the stage of microalbuminuria (incipient nephropathy), whereas specific, vascular and tubulointerstitial changes are more prominent in older patients with macroalbuminuria, renal insufficiency (overt nephropathy), and long-lasting history of arterial hypertension. Regardless of the involved mechanisms, this heterogeneous pattern of renal diseases may explain why, unlike in type 1 diabetes, in type 2 diabetes renal involvement is not always associated with retinopathy.12 Thus all of the above studies strongly suggest the high prevalence of the nondiabetic renal disease among patients with type 2 diabetes and in our study also it was seen among 45.4% of the patients who were subjected to renal biopsy. Hence a high index of suspicion in patients with the atypical presentation is needed to rule out other causes of renal involvement among diabetics. Also, the presence or absence of retinopathy is not a good marker for the simultaneous involvement of diabetic nephropathy as suggested by data in our study. 54.5% (6 out of 11) patients on renal biopsy had diabetic nephropathy without retinopathy.

Conclusion

The relationship between retinopathy and nephropathy in type 2 diabetic patients is not as clear as in type 1 diabetic patients. Patients with type 2 diabetes do not have always diabetic retinopathy and non-diabetic renal disease is also quite common. The absence of retinopathy, rapid progression diabetes, presence of RBC, and cast are some of the atypical findings, and patients presenting with them should be subjected to renal biopsy to rule out non-diabetic renal disease (NDRD).

Limitations

This was a cross-sectional hospital-based study done on a small population, not an epidemiological study. Hence Prevalence of complications may change in a population-based epidemiological study. We have included patients with diagnosed CKD also which could lead to the falsely high prevalence of DKD.

References

Predictors of Prolonged Hospital Stay in Patients with Acute Pulmonary Thromboembolism- A Hospital Based Cohort Study

Arif Rehman Sheikh1*, Suhail Mantoo2, Sanaullah Shah3, Rayees ul Hamid Wani1, Showkat Nazir Wani4

Abstract

Aims and Objectives: Patients with pulmonary thromboembolism (PTE) are commonly admitted to hospital and generally have a prolonged hospital stay in this part of the world. We aimed to determine different clinical and laboratory parameters that are associated with prolonged hospital stay in our set-up and to analyse effectiveness of Pulmonary Embolism Severity Index (PESI) score as a predictor of prolonged hospital stay in patients with PTE.

Materials and Methods: It was a hospital based observational prospective study. Confirmed cases of PTE defined as patients with evidence of thrombus on CT pulmonary angiogram (CTPA) were included in this study. Depending on the length of hospital stay, patients were divided into two cohorts: Shorter Hospital stay (less than mean i.e., < 10 Days) and Prolonged Hospital stay (longer than mean i.e., ≥ 10 Days). Logistic regression analysis was done to identify predictors of prolonged hospital stay.

Results: 150 patients were included in the study with 67 patients (44.67%) having shorter hospital stay (<10 days) and 83 patients (55.33%) having prolonged hospital stay (≥10 days). On multivariate regression analysis, parameters that were found to be statistically significant were hypotension at presentation, decreased level of consciousness, pco2 < 30 mmHg, presence of S1Q3T3 pattern on electrocardiogram (ECG) and high risk PESI (class III-V).

Conclusion: PESI class can be effectively used to predict prolonged hospital stay in patients with pulmonary embolism. Patients with hypotension at presentation, decreased level of consciousness, pco2 less than 30 mmHg, and S1Q3T3 on ECG are more likely to have prolonged hospital stay in our healthcare setup.

Introduction

Pulmonary thromboembolism (PTE) is a relatively common and potentially fatal medical emergency. It is the third most common cardiovascular disease after acute coronary syndrome and stroke, with an incidence rate of 112 cases per 100,000 in the general population.1,2 More than 100,000 deaths are attributed to pulmonary embolism in USA annually.4

PTE is associated with a wide variety of presenting features, ranging from no symptoms to sudden death. Most Patients with PTE are admitted to hospital for their initial treatment. Pulmonary embolism related hospitalisation is associated with substantial burden of health care utilisation and associated costs. In the developed countries, pulmonary embolism related hospitalisation costs are estimated at $13,300 to $31,000 per patient annually.3

Several factors are believed to influence length of hospital stay in pulmonary embolism patients, including patient demographics, clinical characteristics, physician resistance, difficulty in risk stratification, and the type of anticoagulation received.6 However, no validated predictive models are available to estimate the length of hospital stay in pulmonary embolism patients.

There is very scarce data from the developing countries regarding pulmonary embolism associated hospitalisations and the factors affecting the length of hospital stay. The aim of this study was to identify factors that are associated with prolonged hospital stay in patients admitted due to pulmonary embolism. We also aimed to analyse the utility of Pulmonary Embolism Severity Index (PESI) score in predicting the length of hospital stay in these patients.

Materials and Methods

It was a hospital based observational prospective study, conducted at a leading tertiary care institute in north India. An approval to conduct the study was obtained from the Institutional Review Board of the hospital. An informed consent was taken from all the included patients to use their medical data for research purposes, although the study did not involve any diagnostic or therapeutic manoeuvre outside of the routine evaluation of these patients and did not entail any extra costs to them.

Patients admitted with an initial diagnosis of pulmonary embolism at the time of hospital admission between August 2015 and September 2019 were included in this study. Patients were included only if they had confirmed diagnosis of pulmonary embolism by CT pulmonary angiography. Patients with a history of pulmonary embolism prior to present hospitalisation were excluded from the study.

The in-patient medical records of all the included patients were reviewed and the length of hospital stay was calculated for each eligible patient. Hospital stay was calculated from the date of admission to the date of discharge or death. Hospital stay was
considered as a dichotomous variable based on its median and patients were divided in two groups i.e., shorter hospital stay (i.e., less than mean) and prolonged hospital stay (i.e., more than mean). Information about the patient demographics, risk factors, clinical examination, laboratory investigations, and treatment(s) given was obtained and recorded on a proforma. The discharge of the patients is decided by the treating physician in our set-up and so as to simulate the implications of this study on day-to-day clinical practice, the treating physicians were blinded to the aims of the study and the authors had no say in deciding the treatment or discharge of included patients. A Pulmonary Embolism Severity Index (PESI) score, was calculated for all patients as shown in Table 1. Based on the PESI score, patients were classified into five different classes: Class I: PESI Score of ≤65 points, Class II: 66–85 points, Class III: 86–105 points, Class IV: 106–125 point and Class V: >125 points.

Mean length of hospital stay was calculated for this cohort of patients and patients were later stratified into two cohorts: Shorter Hospital stay (less than mean i.e., < 10 Days); Prolonged Hospital stay (i.e., more than 10 Days). The demographic and clinical characteristics of patients are shown in Table 2.

The mean age of the subjects was not different between the two patient groups. In our study population, 29 patients belonged to elderly age group (defined as age greater than 65 years) and there was a statistically significant correlation of this age group with the prolonged hospital stay. Male sex and rural background of patients did not predict prolonged hospital stay.

The most common underlying risk factors in our patients with pulmonary thromboembolism were immobilization (defined as bed rest for more than 3 months), recent surgery and previous thromboembolism were immobilization (defined as bed rest for more than 3 months), chronic lung disease, malignancy, history of trauma to lower extremity and pelvis within preceding three months, recent surgery and previous history of stroke. None of these risk factors had statistically significant difference in the two cohorts.

Tachycardia (defined as heart rate of 100 per minute or more), tachypnea (defined as respiratory rate of 20 per minute or greater), hypotension (defined as blood pressure of less than 90/60 mm Hg for more than 15 minutes) and decreased level of consciousness (defined as Glasgow Coma Scale score of less than 15) at presentation were found to be significantly more prevalent in the cohort of patients with prolonged hospital stay.

Different lab parameters were compared in the two cohorts of patients and it was found that total leucocyte count (TLC) of greater than 11,000/μl, spo2 less than 90%, and pCO2 of less than 30 mm Hg were more common in patients with prolonged stay. Similarly, elevated troponin levels (measured qualitatively by rapid card tests) were significantly more common in patients with prolonged hospital stay.

Among the ECG findings, presence of sinus tachycardia, S1Q3T3, Right axis deviation (RAD) and right bundle branch block (RBBB) were significantly associated with prolonged hospital stay. Similarly, presence of dilated RA/ RV, RV hypokinesia and pulmonary hypertension (PAH) were more likely present in prolonged hospital stay.

Mean d dimer was calculated for the patients with shorter hospital stay and prolonged hospital stay. The difference between the two cohorts was not statistically significant. Use of thrombolytic agents and presence of complications were associated with prolonged hospital stay.

PESI class showed statistically significant difference between shorter hospital stay and prolonged hospital stay, with higher class being associated with more prolonged hospital stay.

**Predictors of prolonged hospital stay on multivariate analysis**

Multivariate regression analysis of statistically significant parameters was done and it was found that hypotension at presentation, altered level of consciousness, low pCO2, presence of S1Q3T3 on ECG, and a higher PESI class (class III-V) were significant predictors of prolonged stay of hospital in patients with pulmonary embolism (Table 3).

**Association of Pulmonary Embolism Severity Index (PESI) with duration of hospital stay**

The mean duration of hospital stay varied significantly with the PESI class, with patients belonging to class I having a mean stay of 6.9 days (SD=5.78 days) and patients belonging to class V having a mean of 17.1 days (SD=6.13 days) as shown in Table 4.

The calculated receiver operating characteristic (ROC) analysis for PESI score predicting prolonged hospital stay showed area under curve (AUC) of 0.763 with p value of .001. A PESI score cut-off value of 95 had a sensitivity of 81.9% and specificity of 50.0% for predicting prolonged hospital stay (Figure 1).

**Discussion**

Pulmonary embolism is one of the important causes of hospitalisation and...
Table 2: Demographic and clinical characteristics of pulmonary embolism patients with shorter versus prolonged hospital stay

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Shorter Hospital Stay (&lt;10 Days) N=67</th>
<th>Prolonged Hospital Stay (≥10 Days) N=83</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>50.9 15.3</td>
<td>53.2 15.7</td>
<td>0.342</td>
</tr>
<tr>
<td>Age &gt; 65 (years)</td>
<td>8 11.9</td>
<td>21 25.3</td>
<td>0.039*</td>
</tr>
<tr>
<td>Male sex</td>
<td>32 47.8</td>
<td>34 41.0</td>
<td>0.404</td>
</tr>
<tr>
<td>Rural population</td>
<td>34 50.7</td>
<td>48 57.8</td>
<td>0.386</td>
</tr>
<tr>
<td>Risk factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immobilisation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic lung disease</td>
<td>17 25.4</td>
<td>20 25.5</td>
<td>0.557</td>
</tr>
<tr>
<td>Previous stroke</td>
<td>3 4.5</td>
<td>8 9.6</td>
<td>0.228</td>
</tr>
<tr>
<td>Malignancy</td>
<td>11 16.4</td>
<td>12 14.5</td>
<td>0.740</td>
</tr>
<tr>
<td>Previous surgery</td>
<td>5 7.5</td>
<td>14 16.9</td>
<td>0.137</td>
</tr>
<tr>
<td>Heart failure</td>
<td>4 6.0</td>
<td>13 15.7</td>
<td>0.073</td>
</tr>
<tr>
<td>Signs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tachycardia</td>
<td>55 82.1</td>
<td>77 92.8</td>
<td>0.045*</td>
</tr>
<tr>
<td>Tachypnea</td>
<td>49 73.1</td>
<td>73 88.0</td>
<td>0.021*</td>
</tr>
<tr>
<td>Lower limb edema</td>
<td>41 61.2</td>
<td>53 63.9</td>
<td>0.738</td>
</tr>
<tr>
<td>Decreased level of consciousness</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RVales</td>
<td>27 40.3</td>
<td>39 47.0</td>
<td>0.412</td>
</tr>
<tr>
<td>Hypotension</td>
<td>1 1.5</td>
<td>48 57.8</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Fever</td>
<td>6 9.0</td>
<td>12 14.5</td>
<td>0.303</td>
</tr>
<tr>
<td>Labs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb &lt;10 g/dL</td>
<td>13 19.4</td>
<td>20 24.1</td>
<td>0.491</td>
</tr>
<tr>
<td>TLC &gt;11,000/ul</td>
<td>22 32.8</td>
<td>47 56.6</td>
<td>0.004*</td>
</tr>
<tr>
<td>Platelet count &lt; 1.5 lac/ul</td>
<td>27 40.5</td>
<td>36 43.4</td>
<td>0.704</td>
</tr>
<tr>
<td>SPO2 &lt;90%</td>
<td>32 47.8</td>
<td>63 75.9</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>PCO2 &lt;30 mm Hg</td>
<td>7 10.4</td>
<td>30 36.1</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>pH &gt; 7.4</td>
<td>50 74.6</td>
<td>54 65.1</td>
<td>0.207</td>
</tr>
<tr>
<td>+ve troponin levels</td>
<td>6 9.0</td>
<td>40 48.2</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>ECG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sinus tachycardia</td>
<td>55 82.1</td>
<td>77 92.8</td>
<td>0.045*</td>
</tr>
<tr>
<td>S1Q3T3</td>
<td>4 6.0</td>
<td>22 26.5</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>RAD</td>
<td>0 0.0</td>
<td>5 6.0</td>
<td>0.041*</td>
</tr>
<tr>
<td>RBBB</td>
<td>0 0.0</td>
<td>10 12.0</td>
<td>0.003*</td>
</tr>
<tr>
<td>Echo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dilated RA/RV</td>
<td>22 32.8</td>
<td>46 55.4</td>
<td>0.006*</td>
</tr>
<tr>
<td>RV Hypokinesia</td>
<td>5 7.5</td>
<td>18 21.7</td>
<td>0.016*</td>
</tr>
<tr>
<td>PAH</td>
<td>18 26.9</td>
<td>40 48.2</td>
<td>0.008*</td>
</tr>
<tr>
<td>Thrombolysis</td>
<td>1 1.5</td>
<td>33 39.8</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Complications</td>
<td>1 1.5</td>
<td>18 21.7</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>PESI Class</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Class I</td>
<td>6 8.9</td>
<td>2 2.4</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Class II</td>
<td>15 22.3</td>
<td>8 9.6</td>
<td></td>
</tr>
<tr>
<td>Class III</td>
<td>31 46.3</td>
<td>16 19.3</td>
<td></td>
</tr>
<tr>
<td>Class IV</td>
<td>11 16.4</td>
<td>17 20.5</td>
<td></td>
</tr>
<tr>
<td>Class V</td>
<td>4 6.0</td>
<td>40 48.2</td>
<td></td>
</tr>
</tbody>
</table>

*Statistically Significant Difference (P-value<0.05)

This study has provided the important insights into the different parameters that are associated with prolonged hospital stay in these patients.

This study shows that duration of hospital stay in patients with PTE is generally long (median 10 days) which is comparable with recent data from the RIETE registry in the European setting (mean hospital stay of 10 days). This is however significantly higher as compared to different studies from USA, which have reported a mean hospital stay of around 4-5 days. This could be partly due to different patient characteristics and partly due to different health care system facilities.

Older age has been found previously to be independently associated with longer hospital stay. Older people are at high risk of adverse clinical outcomes and treatment related complications with consequent prolonged hospital stay. In our study, mean age of patients was comparable in two cohorts, and although patients with age greater than 65 years were more frequent in prolonged hospital stay cohort, it didn’t reach statistical significance on multivariate analysis. This departure from the previously published literature could be because of the fact that the patients in our study were younger and only 19.3% of patients were above the age of 65 years.

Our study shows that hemodynamic...
instability and decreased level of consciousness at presentation is associated with prolonged hospital stay in patients with pulmonary embolism. Our findings are in contrast to previous study by Marco Paolo Donadini et al., wherein systolic blood pressure below 100 mmHg and altered mental status did not influence the length of hospital stay in patients with pulmonary embolism. A potential explanation for this finding is that these patients are kept under intense clinical surveillance for a longer time and many treating physicians are sceptical about early discharge given the worse prognosis associated with these signs.

Basic laboratory tests are available for majority of patients with pulmonary embolism and many parameters have been previously evaluated for predicting the prognosis of PE. However, there is little data about the utility of different lab parameters in predicting the duration of hospital stay. Our study shows that the proportion of patients with an elevated leucocyte counts, pCO2 less than 30, Spo2 less than 90% and elevated cardiac troponins was significantly higher in patient cohort with prolonged hospital stay. Presence of low Pco2 independently predicted a prolonged hospital stay. Though the exact reason for this observation could not be ascertained, it could be partly because of increased respiratory distress in these patients with subsequent prolonged observation period.

ECG is a readily available, inexpensive, and quickly interpretable tool and its role in prognosticating pulmonary embolism (PE) is being increasingly recognized. ECG signs that are reported as good predictors of a negative outcome included SIQ3T3, complete right bundle branch block, T-wave inversion, right axis deviation, and atrial fibrillation.12 We found in our study that SIQ3T3, Right axis deviation and right bundle branch was significantly present more in patients with a prolonged hospital stay than patients with a shorter hospital stay. On multivariate analysis, however, only the presence of SIQ3T3 was associated with a prolonged hospital outcome. As the inappropriate selection of certain high risk PE patients for early discharge results in significant post discharge adverse outcomes, physicians probably are extremely cautious while making a decision for discharge of these patients.13 Nevertheless, large scale studies are clearly indicated to define the precise correlation of these parameters and their association with prolonged hospital stay in patients admitted with pulmonary embolism.

Pulmonary Embolism Severity Index (PESI) is the most extensively validated tool devised to predict mortality in patients with PE and identify low risk patients who may be candidates for outpatient treatment. We aimed to analyse the usefulness of PESI score and PESI class in predicting the duration of hospital stay in patients with pulmonary embolism. Our study found that higher the PESI class, more is the duration of hospital stay. Also, a PESI score of >95 points predicted a prolonged hospital stay with a sensitivity of 81.9% and a specificity of 50%. This observation may help clinicians in identifying patients who may be at risk of adverse outcomes associated with prolonged hospitalisation.

Nonetheless, in addition to the above mentioned different clinical variables, some other factors may be associated with prolonged hospital stay.11 The first important factor is complexity of anticoagulant regimens. Given the fact that many patients are still receiving vitamin k antagonists in our clinical setup, the consequent need for International Normalised ratio (INR) monitoring may be an important obstacle for early discharge. In addition, early hospital discharge always relies on adequate home circumstances and easy access to healthcare facilities which is far from satisfactory in our setup.

**Limitations**

There are certain limitations in our study. First, the healthcare setup in which study was conducted is not universal and the results can’t be generalised. Secondly, attitude of treating physicians varies widely with regards to ideal time to discharge the patients with pulmonary thromboembolism and this could be one confounding factor. Finally, many patients had delay in discharge because of use of conventional oral anticoagulants, but we had no data regarding the delay caused by getting INR in the therapeutic range.

**Conclusion**

The results of this study show that certain clinical and laboratory parameters may help in identifying patients who have prolonged hospital stay. PESI score, a validated tool of prognosis and severity, can be effectively used to predict prolonged hospital stay in patients with pulmonary embolism. The findings from this study may help clinicians to take decision prospectively about duration of hospital stay for effective health care utilisation in patients with pulmonary embolism.

**References**

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- Glycomet GP 3 Forte: Each uncoated tablet contains metformin hydrochloride IP (as prolonged release form) 1500mg and glimepiride IP 3mg.
- Glycomet GP 2 Forte: Each uncoated tablet contains metformin hydrochloride IP (as prolonged release form) 1000mg and glimepiride IP 2mg.
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- ASCVD & CHF

**Across Ages**
- Young
- Elderly
- >90 Years

**Across Stages**
- Newly Diagnosed
- Early Stage
- Long Duration

**Across BMI**
- Underweight
- Normal
- Overweight
- Obese

**Across Complications**
- Nephropathy
- Neuropathy/ Diabetic Foot
- Retinopathy

**Source:**
1. JAPI 2020 68,51.-55
2. Data on File
3. Cureus 2020, 12(9): e10.7759/cureus.1070
4. Diabetes Technology & Therapeutics 2019,.2,79-84

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1) Int. J. Res. Pharm. Sci., 9(4), 1368-1373

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Assessment of Fluid Responsiveness by Changes in End Tidal Carbon Dioxide During Passive Leg Raising Test and Fluid Challenge

Rakesh Bhadade¹, Minal Harde²*, Rosemarie de Souza³, Tushar Madke⁴

Abstract

Purpose: It is rationale to predict fluid responsiveness for optimum hemodynamic management. Passive Leg Raising (PLR) causes reversible increase in cardiac output (CO) and changes in end-tidal CO₂ pressure (ETCO₂) can be considered surrogate for CO variations. We aimed to assess the variations in ETCO₂ with PLR and fluid challenge (FC) and also compared it with systolic arterial pressure (SAP), mean arterial pressure (MAP), heart rate (HR) and central venous pressure (CVP). Methodology: This Prospective study was conducted in the ICU of a tertiary care teaching public hospital. PLR was performed before FC in patients of circulatory failure on mechanical ventilation. ETCO₂ and hemodynamics were monitored and compared after PLR and FC. ROC curve of parameters, based on their Area under the Curve (AUC) was compared. MS Excel, PSPP version 1.0.1 was used for analysis. Results: Among hundred patients studied, 74 showed ETCO₂ change≥ 2 mmHg (>5%) and were fluid responders. Increase in EtcO₂ after PLR at 1 minute and FC at 30 minutes was statistically significant (p=2.73x10⁻⁷³) so is SAP(p=4.02x10⁻⁷⁵) and MAP(p=1.75x10⁻⁷⁵). AUC of predictive performance of parameters showed change in ETCO₂ (AUC ROC 0.985 [0.938 to 0.999]) had significantly outperformed CVP (AUCROC 0.822 [0.733-0.892]), SAP (AUCROC 0.793 [0.701-0.868]), MAP (AUCROC 0.810 [0.719-0.881]), HR (AUCROC 0.574 [0.471-0.673]).

Conclusion: Variations in ETCO₂ >5% induced by PLR can predict fluid responsiveness and is a reliable, non-invasive, easy, quick, and reversible method. ETCO₂ is better predictor than SAP, MAP, CVP, and HR during PLR and FC. We may recommend PLR-induced changes in ETCO₂ to predict fluid responsiveness in mechanically ventilated patients.

Introduction

Fluid therapy is one of the most challenging tasks during management of critically ill patients in the intensive care unit (ICU) or in emergency department (ED). The art of fluid management is to maintain hemodynamic stability, correct hypovolemia to prevent organ hypoperfusion without added complications of volume overload. It is rationale to predict fluid responsiveness so as to identify patients on the ascending portion of the Frank-Starling curve with preload reserve and have capacity to increase cardiac output (CO) in response to volume expansion (VE).¹

Fluid challenge is to administer a small volume of fluid (500 ml) and observe its effects on CO and can be assessed by various clinical parameters. They are heart rate (HR), systolic blood pressure (SAP), mean arterial pressure (MAP) and static and dynamic indices, central venous pressure (CVP), pulmonary artery occlusion pressure (PAOP), stroke volume variation (SVV), pulse pressure variation (PPV), ultrasonography by inferior vena cava diameter variation (IVCDV) and echocardiography.¹⁻³ However these methods are usually limited by risk of fluid overload in volume unresponsive patients, the lack of reliability of non-invasive methods and static indices, invasive indwelling devices, unavailability in ED, cost and the lack of expertise.³⁻⁵

Judicious administration of fluids requires quick and reliable identification of patients in whom volume expansion (VE) actually increases CO without added complications. Passive Leg Raising (PLR) test also known as self volume challenge is shifting patient from semi-recumbent to supine position and elevating patient’s leg by 45 degrees. This causes increase in cardiac preload due to transfer of blood from lower limbs and thus offer a dynamic assessment.⁶ Outcomes of PLR are rapid and transient hence a continuous CO measurement is required to assess the response. Changes in partial end-tidal carbon dioxide pressure (ETCO₂) obtained by capnography can be considered surrogate for cardiac output variations during constant ventilation and stable tissue CO₂ production (VCO₂). ETCO₂ monitoring is standard of care in mechanically ventilated patients which is continuous and non-invasive and it precisely reflects changes in pulmonary blood flow and CO and can be considered as a surrogate of CO monitoring.⁷⁻⁸ Thus variations in ETCO₂ induced by a PLR manoeuvre may reflect changes in the CO and hence predict fluid responsiveness.

In India most of the critical care setups may not have direct measures of CO or expertise or urgency in ED or emergency preoperative period where invasive lines and devices are unavailable or may take time
to secure. PLR can be considered a dynamic method to predict fluid responsiveness. ETCO2 monitoring module is inbuilt in most of the ICU monitors and ventilators. Hence in pursuit for practical and easy method which is dynamic measure but non invasive, we decided to conduct this study to observe variation in ETCO2 induced by PLR and FC to predict fluid responsiveness. In current prospective study we aimed to assess the variations in ETCO2 with PLR and also compared it with SAP, MAP, CVP and HR changes which were routinely used in ICU. We also correlated ETCO2 and above parameters with fluid challenge.

**Methodology**

This Prospective study was conducted in the ICU of a tertiary care teaching public hospital. Permission from the institutional ethics committee was obtained (ECARP/2017/59) to conduct the study over a period of one year. The ICU is 23 bedded, equipped with remote controlled adjustable ICU beds with multi-parameter monitor (Intellivue™ MP70 monitor, Philips Medical Systems, Best, The Netherlands) and ventilator (Dräger Savina® 300 Classic, Dräger Medical, Lubeck, Germany) and mainly caters to medical and allied speciality patients. Patients fulfilling the eligibility criteria were included in the study after taking the written informed valid consent from the patient’s legally acceptable relatives and were selected by non-probability convenience sampling method. This was an observational prospective study, PLR test was performed just before fluid challenge which was decided by the attending clinician.

We included the patients admitted in the ICU on controlled mechanical ventilation who were in circulatory failure and the attending clinician has decided to give fluid challenge to them. Circulatory failure was defined as presence of one or more of the following signs: systolic blood pressure (SBP)≤ 90 mmHg, the need of vasopressor drugs, urine output ≤ 0.5 ml/kg/hr for two hours, HR> 100 beats/minute and delayed capillary refilling. Exclusion criteria were age < 18 years, pregnancy, any contraindication to perform PLR for example deep venous thrombosis (DVT) intracranial hypertension (ICH), or lower limb fractures. Patients with shivering, fever were also excluded as metabolic CO2 production is altered in these conditions which may affect ETCO2.

The study was performed in ICU on hemodynamically unstable patients in whom a fluid challenge was planned. Hemodynamic parameters like HR, SAP, MAP, CVP and ETCO2 were monitored continuously throughout the study procedure. ETCO2 in was measured via endotracheal tube connector using a side-stream gas analyzer integrated into the patient monitor. It was performed in four sequential stages on the ICU bed using the automatic action of the bed (Figure 1). Baseline parameters were recorded with the patient in the semi-recumbent position (A). PLR manoeuvre was performed by altering the patient position to supine and raising the patient’s leg to 45° and parameters noted (B). The patient was then returned to the original semi-recumbent position. After five minutes when the hemodynamic variables reached their baseline values, fluid challenge was performed with 500 ml of crystalloid over a period of 30 minutes and parameters were measured before fluid infusion and immediately after completion of infusion. (C,D) During the course of the study procedure, patient was closely monitored and posture, ventilatory settings, vasopressor infusion and other supportive management were kept constant. The changes in ETCO2 induced by PLR and fluid challenge at various time intervals were observed and correlated. Similarly changes in SBP, MAP, CVP and HR at various time intervals were also observed and correlated after PLR and fluid challenge. Fluid responsiveness (FR) is defined as an increase of ETCO2> 5% which equals increase in CO of 12-15% or rise in systolic blood pressure ≥ 10 mmHg, after PLR or 500 ml of crystalloids which reliably reflects that variations authentically occurred.1,8

Considering an increase of ETCO2> 5% as genuine reflection of fluid responsiveness after PLR and fluid challenge, sample size was calculated using the following formula.4

\[ n = \frac{Z^2 \cdot p(1-p)}{d^2} \]

Where:
- \( n \) is the sample size
- \( z \) is the z-statistics for desired level of confidence
- \( p \) is the estimate of expected proportion with the variable of interest
- \( d \) (Precision) is the half width of desired interval

\[ n = 94.16. \text{Hence overall 100 patients were included.} \]

Qualitative data was represented in form of frequency and percentage. It included gender, fluid response status (responder/non- responder), etc. Association between qualitative variables was assessed by Chi-Square test, with Continuity Correction for all 2X2 tables and by Fisher’s Exact test for all 2X2 tables where Chi-Square test was not valid due to small counts.

Quantitative data was represented using mean ± standard deviation(SD) and Median & Interquartile range(IQR). It included age, ETCO2, HR, SAP, MAP, CVP etc at various time intervals. Comparison of quantitative data measured between fluid responders and non-responders was done using unpaired t-test. Comparison of data measured at various time intervals was done using Friedman repeated measures analysis of variance on ranks as all data failed ‘Shapiro–Wilk Normality’ test. If p-value of Friedman repeated measures analysis of variance on ranks was statistically significant, Dunn’s post hoc test for pair-wise comparison was applied. Receiver operating characteristic (ROC) curve of quantitative data, by binomial qualitative variable ‘FR’ (responders and non-responders), was generated to pair-wise compare the ROC curve of parameters, based on their Area Under the Curve (AUC).

Table 1A: Statistics of PLR/FC ETCO2 at various time intervals among the study population

<table>
<thead>
<tr>
<th>Pair compared</th>
<th>Difference of Ranks</th>
<th>q</th>
<th>p-value</th>
<th>Difference is-</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline vs. 1 min</td>
<td>314.000</td>
<td>5.313</td>
<td>≤0.05</td>
<td>Significant</td>
<td></td>
</tr>
<tr>
<td>Baseline vs. 3 min</td>
<td>177.500</td>
<td>3.003</td>
<td>&gt;0.05</td>
<td>Not significant</td>
<td></td>
</tr>
<tr>
<td>Baseline vs. 5 min</td>
<td>32.000</td>
<td>0.541</td>
<td>&gt;0.05</td>
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<td></td>
</tr>
<tr>
<td>Baseline vs. FC Baseline</td>
<td>5.500</td>
<td>0.093</td>
<td>&gt;0.05</td>
<td>Not significant</td>
<td></td>
</tr>
<tr>
<td>FC Baseline vs. FC 30 min</td>
<td>314.500</td>
<td>5.321</td>
<td>≤0.05</td>
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</tbody>
</table>


Table 1B: Statistics of PLR/FC SAP at various time intervals among the cases with and without Fluid Response

<table>
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<tr>
<th>Pair compared</th>
<th>Difference of Ranks</th>
<th>q</th>
<th>p-value</th>
<th>Difference is-</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline vs. 1 min</td>
<td>283.000</td>
<td>4.788</td>
<td>&lt;0.05</td>
<td>Significant</td>
<td></td>
</tr>
<tr>
<td>Baseline vs. 3 min</td>
<td>178.500</td>
<td>3.020</td>
<td>&gt;0.05</td>
<td>Not significant</td>
<td></td>
</tr>
<tr>
<td>Baseline vs. 5 min</td>
<td>50.000</td>
<td>0.846</td>
<td>&gt;0.05</td>
<td>Not significant</td>
<td></td>
</tr>
<tr>
<td>Baseline vs. FC Baseline</td>
<td>19.500</td>
<td>0.330</td>
<td>&gt;0.05</td>
<td>Not significant</td>
<td></td>
</tr>
<tr>
<td>FC Baseline vs. FC 30 min</td>
<td>325.500</td>
<td>5.507</td>
<td>≤0.05</td>
<td>Significant</td>
<td></td>
</tr>
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Table 2A: Statistics of PLR/FC SAP at various time intervals among the study population

<table>
<thead>
<tr>
<th>PLR SAP at-</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>IQR</th>
<th>Chi-Square</th>
<th>p-value</th>
<th>MAP variations at different intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>85.34</td>
<td>12.53</td>
<td>90.00</td>
<td>12.00</td>
<td>357.612</td>
<td>4.02E-75</td>
<td>MAP variations at different intervals</td>
</tr>
<tr>
<td>At 1 min</td>
<td>89.68</td>
<td>13.24</td>
<td>94.00</td>
<td>14.00</td>
<td>85.34</td>
<td>12.93</td>
<td>90.00 12.00 Statistical significance</td>
</tr>
<tr>
<td>At 3 min</td>
<td>92.28</td>
<td>12.82</td>
<td>94.00</td>
<td>14.00</td>
<td>85.34</td>
<td>12.93</td>
<td>90.00 12.00 Statistical significance</td>
</tr>
<tr>
<td>At 5 min</td>
<td>86.19</td>
<td>12.88</td>
<td>90.00</td>
<td>12.00</td>
<td>85.34</td>
<td>12.93</td>
<td>90.00 12.00 Statistical significance</td>
</tr>
<tr>
<td>FC Baseline</td>
<td>85.31</td>
<td>12.93</td>
<td>90.00</td>
<td>12.00</td>
<td>85.34</td>
<td>12.93</td>
<td>90.00 12.00 Statistical significance</td>
</tr>
<tr>
<td>FC after 30 minutes</td>
<td>91.28</td>
<td>13.78</td>
<td>94.00</td>
<td>16.00</td>
<td>85.31</td>
<td>12.93</td>
<td>90.00 12.00 Statistical significance</td>
</tr>
</tbody>
</table>

Table 2B: Pair wise comparison between PLR/FC SAP at various time intervals

<table>
<thead>
<tr>
<th>Pair compared</th>
<th>Difference of Ranks</th>
<th>q</th>
<th>p-value</th>
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<td>Baseline vs. FC Baseline</td>
<td>19.500</td>
<td>0.330</td>
<td>&gt;0.05</td>
<td>Not significant</td>
<td></td>
</tr>
<tr>
<td>FC Baseline vs. FC 30 min</td>
<td>325.500</td>
<td>5.507</td>
<td>≤0.05</td>
<td>Significant</td>
<td></td>
</tr>
</tbody>
</table>


Appropriate statistical software, including but not restricted to MS Excel, PSP version 1.0.1 was used for analysis. An alpha value (p) of <0.05 was used as the cut-off for statistical significance.

Results

During the study period of one year hundred patients studied, of them 54(54%) were males and 46(46%) were females with a mean age of 51.24 years. Seventy four patients (74%) showed increase in ETCO2 of > 2 mmHg (5%) after PLR maneuver at 1 minute. At five minutes after PLR maneuver, all the parameters returned to baseline in all patients and then FC was started for over 30 minutes. Same 74 patients, who responded to PLR showed increase in ETCO2 of > 2 mmHg (5%) after FC at 30 minutes. These 74 patients were considered fluid responders and the remaining 26 patients were non-responders. Distribution of demographic data showed no significant difference between fluid responders and non-responders with respect to age (p= 0.822), gender (p= 0.370), signs of circulatory failure, and ventilatory settings. Indications for fluid challenge in the study population were tachycardia in 96%, hypotension 94%, vasopressor administration in 78% patients, oliguria in 62%, and delayed capillary refilling with skin motting in 53% of study population.

Increase in ETCO2 after PLR and FC at different intervals was compared and found to be statistically significant (p= 2.73×10⁻⁷³). (Table 1A) Post Hoc pair-wise analysis showed that difference in ETCO2 was statistically significant between PLR at baseline versus PLR at 1 minute and, FC at baseline versus FC after 30 minutes. This indicates that the ETCO2 significantly increases at 1 minute after PLR and 30 minutes after FC compared to baseline and the change is comparable (Table 1B).

Systolic arterial pressure (SAP) changes at various time intervals after PLR and FC (Table 2A) showed statistically significant difference (p= 4.02×10⁻⁷³). Pair-wise comparison of SAP showed a statistically significant difference after PLR from baseline to 1 minute (p< 0.05) and after FC from baseline to 30 minute (p< 0.05) (Table 2B).

MAP variations at different intervals after PLR and FC was compared and showed statistically significant difference (p= 1.75×10⁻⁰⁵) (Table 3A). Pair-wise comparison of MAP at time intervals showed statistically significant difference between PLR at baseline versus 1 minute and, FC at baseline versus 30 minutes (Table 3B).

CVP and HR changes at different intervals after PLR and FC showed statistical significance (p= 1.14×10⁻⁰⁴) and (p= 3.02×10⁻³⁶) respectively (Tables 4 A and B). However there was no statistically significant difference following pair-wise comparison of CVP and HR after PLR and FC at any intervals. This suggests that though the absolute values of CVP and HR are significant, but change in CVP or HR after PLR or FC was not significant at any interval.

In the ROC curve, AUC of predictive performance of different hemodynamic parameters was compared after PLR at 1 minute because change after PLR was most significant at 1 minute (Figure 2). The change in ETCO2(AUC ROC 0.985 [0.938 to 0.999]) had significantly outperformed change in CVP(AUC ROC 0.822 [0.733-0.892]), change in SAP(AUC ROC 0.793 [0.701-0.868]), change in MAP(AUC ROC 0.810 [0.719-0.881]), and change in HR (AUC ROC 0.574 [0.471-0.673]).

Discussion

Maintaining haemodynamic homeostasis in critically ill patients is very crucial as fluid therapy is a double edged sword. Intravascular volume therapy should be customized according to the patient’s requirement by goal-directed therapy. There is strong recommendation for use of volume responsiveness appraisal in hemodynamic monitoring as it...
Table 3A: Statistics of PLR/FC MAP at various time intervals among the cases with and without Fluid Response

<table>
<thead>
<tr>
<th>PLR MAP at-</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>IQR</th>
<th>Chi-Square</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>68.32</td>
<td>8.60</td>
<td>69.50</td>
<td>10.00</td>
<td>350.002</td>
<td>1.75E-73</td>
</tr>
<tr>
<td>At 1 min</td>
<td>71.25</td>
<td>8.68</td>
<td>73.00</td>
<td>10.00</td>
<td>350.002</td>
<td>1.75E-73</td>
</tr>
<tr>
<td>At 3 min</td>
<td>69.56</td>
<td>8.56</td>
<td>71.00</td>
<td>10.75</td>
<td>350.002</td>
<td>1.75E-73</td>
</tr>
<tr>
<td>At 5 min</td>
<td>68.41</td>
<td>7.98</td>
<td>70.00</td>
<td>10.00</td>
<td>350.002</td>
<td>1.75E-73</td>
</tr>
<tr>
<td>FC Baseline</td>
<td>68.16</td>
<td>8.63</td>
<td>70.00</td>
<td>11.75</td>
<td>350.002</td>
<td>1.75E-73</td>
</tr>
<tr>
<td>FC after 30 minutes</td>
<td>72.77</td>
<td>9.42</td>
<td>75.00</td>
<td>11.00</td>
<td>350.002</td>
<td>1.75E-73</td>
</tr>
</tbody>
</table>


Table 3B: Pair wise comparison between PLR/FC MAP at various time intervals

<table>
<thead>
<tr>
<th>Pair compared-</th>
<th>Mean Difference</th>
<th>Std. Error</th>
<th>p-value</th>
<th>Difference is-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline vs. 1 min</td>
<td>266.500</td>
<td>14.245</td>
<td>≤ 0.05</td>
<td>Significant</td>
</tr>
<tr>
<td>Baseline vs. 3 min</td>
<td>120.000</td>
<td>6.414</td>
<td>≤ 0.05</td>
<td>Significant</td>
</tr>
<tr>
<td>Baseline vs. 5 min</td>
<td>14.500</td>
<td>0.775</td>
<td>No</td>
<td>Not significant</td>
</tr>
<tr>
<td>Baseline vs. FC Baseline</td>
<td>8.000</td>
<td>0.428</td>
<td>&gt; 0.05</td>
<td>Not significant</td>
</tr>
<tr>
<td>FC Baseline vs. FC Baseline</td>
<td>327.000</td>
<td>17.479</td>
<td>≤ 0.05</td>
<td>Significant</td>
</tr>
</tbody>
</table>

Table 3C: Statistics of PLR/FC CVP at various time intervals among the cases with and without Fluid Response

<table>
<thead>
<tr>
<th>PLR CVP at-</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>IQR</th>
<th>Chi-Square</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>7.13</td>
<td>2.35</td>
<td>7.00</td>
<td>3.75</td>
<td>262.51</td>
<td>1.14E-54</td>
</tr>
<tr>
<td>At 1 min</td>
<td>7.98</td>
<td>1.78</td>
<td>8.00</td>
<td>3.00</td>
<td>262.51</td>
<td>1.14E-54</td>
</tr>
<tr>
<td>At 3 min</td>
<td>7.49</td>
<td>2.13</td>
<td>8.00</td>
<td>3.75</td>
<td>262.51</td>
<td>1.14E-54</td>
</tr>
<tr>
<td>At 5 min</td>
<td>7.16</td>
<td>2.33</td>
<td>7.00</td>
<td>3.75</td>
<td>262.51</td>
<td>1.14E-54</td>
</tr>
<tr>
<td>FC Baseline</td>
<td>7.29</td>
<td>2.22</td>
<td>7.00</td>
<td>4.00</td>
<td>262.51</td>
<td>1.14E-54</td>
</tr>
<tr>
<td>FC after 30 minutes</td>
<td>8.59</td>
<td>1.39</td>
<td>8.50</td>
<td>2.00</td>
<td>262.51</td>
<td>1.14E-54</td>
</tr>
</tbody>
</table>

Table 3D: Statistics of PLR/FC HR at various time intervals among the cases with and without Fluid Response

<table>
<thead>
<tr>
<th>PLR HR at-</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>IQR</th>
<th>Chi-Square</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>117.21</td>
<td>15.51</td>
<td>118.00</td>
<td>24.50</td>
<td>176.482</td>
<td>3.02E-36</td>
</tr>
<tr>
<td>At 1 min</td>
<td>114.36</td>
<td>15.47</td>
<td>113.50</td>
<td>22.50</td>
<td>176.482</td>
<td>3.02E-36</td>
</tr>
<tr>
<td>At 3 min</td>
<td>114.82</td>
<td>15.48</td>
<td>116.00</td>
<td>23.75</td>
<td>176.482</td>
<td>3.02E-36</td>
</tr>
<tr>
<td>At 5 min</td>
<td>115.44</td>
<td>15.70</td>
<td>116.50</td>
<td>24.00</td>
<td>176.482</td>
<td>3.02E-36</td>
</tr>
<tr>
<td>FC Baseline</td>
<td>118.10</td>
<td>15.58</td>
<td>118.00</td>
<td>23.75</td>
<td>176.482</td>
<td>3.02E-36</td>
</tr>
<tr>
<td>FC after 30 minutes</td>
<td>114.61</td>
<td>15.91</td>
<td>115.00</td>
<td>23.75</td>
<td>176.482</td>
<td>3.02E-36</td>
</tr>
</tbody>
</table>


Improves critical care outcome, reduces complications and decreases length of hospital stay.4 Hence we prospectively studied 100 patients with signs of circulatory failure, for prediction of fluid responsiveness by observing variation in ETCO2 induced by PLR and compared it with FC.

Among 100 patients, 74 showed rise in ETCO2 ≥ 5% after PLR and were fluid responders while 26 patients were non-responders and the same effects were reproduced after FC. Demographics, baseline hemodynamic variables, signs of circulatory failure, and ventilatory settings were comparable between fluid responders and non-responders. PLR mimics a FC by transferring a blood from the lower extremities toward the heart and resultant CO changes in volume responders were reflected in ETCO2 variations. Thus PLR induced rise in ETCO2 directly reflected increase of CO suggesting volume responsiveness is a non-invasive, easy, quick reliable method and it was also supported with increase in SAP and MAP. The results observed by PLR at 1 minute were mirrored in fluid challenge test at 30 minutes and hence PLR is recommended which is self fluid challenge.

A systematic review, have shown that the change in CO after PLR is a reliable and accurate method to predict fluid responsiveness.4 PLR must be performed correctly and sequentially (Figure 2) by adjusting the automatic bed and not manually which increases the test’s sensitivity and reliability.9-11 Toupin et al confirmed that ΔETCO2 ≥ 2 mmHg is predictive of fluid responsiveness.9 Monnet X et al have confirmed a direct and linear relationship between CO and ETCO2 variations and could be regarded as a surrogate for assessing the effects of PLR.9-12

In the present study, ETCO2 changes after PLR and FC and pair wise comparison of ETCO2 at different time intervals were statistically significant. Increase in ETCO2 from baseline to 1 minute after PLR and from baseline to 30 minute after FC was highly significant. This suggests that a change in ETCO2 that occurs after 30 minutes of FC can be obtained by using PLR at 1 minute and that too without fluid. This confirms that PLR acts as a self volume challenge by transfer or blood from lower limbs and same effects observed at 30 minutes after 500 ml fluid administration. We observed that the effects of PLR are transient and reversible as ETCO2 values peaked at 1 minute and after assuming semirecumbent position decreased rapidly and at 5 minute they were almost equal to baseline. Thus PLR manoeuvre is a quick, reversible, easy-to-perform test and gives same results as FC without any complications. Monnet X et al confirmed that PLR gives self volume challenge and predicts responsiveness and the effects are rapidly reversible, avoiding the risks of fluid overload.10 Studies observed that PLR is a dynamic technique with adequate sensitivity and specificity to predict fluid responsiveness by increase in ETCO2 ≥ 5% having rapid and reversible effects.13 Many studies the echoed same findings.14-16 Recent meta-analysis by Huang H et al mentions that the ETCO2 findings.14-16 Recent meta-analysis by Huang H et al mentions that the ETCO2 variations were reasonable in predicting fluid responsiveness during the PLR in patients on mechanical ventilation where direct CO measurements were unachievable or inconvenient under many clinical conditions.17

SAP and MAP changes and pair-wise comparison of SAP and MAP at different time intervals after PLR and FC showed statistical significance so as change from baseline to 1 minute after PLR and from baseline to 30 minute after FC. The PLR can increase preload and when heart is volume responsive, increase in CO which will reflect as increase in SAP and MAP. Toupin mentioned that a combination
of increase in ETCO₂ ≥ 2 mmHg and SBP ≥ 10 mmHg induced by PLR was predictive of fluid responsiveness. Boulain T et al found that radial artery pulse pressure significantly increased after PLR. However Pickett JD et al found that non-invasive BP monitoring were not sensitive or specific predictors of fluid responsiveness after PLR. Qi H et al mentions there was no significant correlation between the ΔMAP% and ΔCI% in sepsis.

CVP and HR changes after PLR and FC showed statistically significant difference, but their pair-wise comparison showed no statistical significance at any intervals. This suggests that although the absolute values of CVP and HR are statistically significant, change in CVP or HR after PLR or FC is not significant at any interval. Many studies have mentioned that the use of CVP may not be sensitive to guide fluid therapy. In a meta-analysis Huang H et al confirmed that static variables poorly predict fluid responsiveness as compared to dynamic indicators.

Comparison of AUC of different variables used to assess fluid responsiveness was done after PLR at 1 minute because change after PLR was significant at 1 minute. The change in ETCO₂ (AUC ROC 0.865 [0.938 to 0.999]) had significantly outperformed change in CVP, SAP, MAP and HR. (Figure 2) Thus ETCO₂ is better and reliable predictor of fluid responsiveness than SAP, MAP, CVP, and HR during PLR and can be considered as a substitute of direct CO measurement. Lakhal K et al tested minimally invasive surrogates for CO and found that increases in the ETCO₂ did significantly better than other parameters. Monnet X and Huang H et al confirmed that the ETCO₂ variations were reasonable in predicting fluid responsiveness during the PLR in patients on mechanical ventilation.

Study had limitations due to unavailability of CO monitoring for comparison and our observations are specific to the population we studied that is patients with circulatory failure on controlled mechanical ventilation however strength being the large sample size.

Thus from the present study we conclude that variations in ETCO₂ 2 mmHg (>5%) induced by PLR can predict fluid responsiveness. Changes induced by PLR at 1 minute were mirrored with FC at 30 minutes. The effects of PLR induced self fluid challenge returned to baseline at 5 minutes. ETCO₂ is reliable and better predictor of fluid responsiveness than SAP, MAP, CVP, and HR during PLR and FC. Thus PLR induced rise in ETCO₂ is a reliable, non invasive, easy, quick, reversible and reproducible method to predict fluid responsiveness. Thus we may recommend PLR-induced changes in ETCO₂ to predict fluid responsiveness in mechanically ventilated patients.

### References

10. Monnet X, Bataille A, Magalhaes E, Barrois I, Le Corre M,

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**Fig. 2: Comparison of ROC curves of different variables at 1 minute after PLR**

<table>
<thead>
<tr>
<th>Variable</th>
<th>AUC</th>
<th>SE</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLR_ETCO₂_CHANGE_Baseline_to_1_min</td>
<td>0.985</td>
<td>0.00768</td>
<td>0.938 to 0.999</td>
</tr>
<tr>
<td>PLR_CVP_CHANGE_Baseline_to_1_min</td>
<td>0.822</td>
<td>0.0324</td>
<td>0.733 to 0.892</td>
</tr>
<tr>
<td>PLR_SAP_CHANGE_Baseline_to_1_min</td>
<td>0.793</td>
<td>0.0408</td>
<td>0.701 to 0.868</td>
</tr>
<tr>
<td>PLR_MAP_CHANGE_Baseline_to_1_min</td>
<td>0.810</td>
<td>0.0409</td>
<td>0.719 to 0.881</td>
</tr>
<tr>
<td>PLR_HR_CHANGE_Baseline_to_1_min</td>
<td>0.574</td>
<td>0.058</td>
<td>0.471 to 0.673</td>
</tr>
</tbody>
</table>
A Case Control Study of Risk Assessment of Diabetes and Nephropathy with eNOS (T786C and 27bp VNTR) Gene Polymorphisms

Anju1*, Harpreet Singh2, OP Kalra3, Daya Shankar4, Anil Kumar4

Abstract

Objectives: To determine the association of eNOS (T786C and 27bp VNTR) gene polymorphism with the risk of type II diabetes mellitus and diabetic nephropathy in North India.

Methods: The prospective case control study was conducted over a period of 18 months. A total of 100 patients of Type 2 Diabetes Mellitus (A1: 50 cases without Diabetic nephropathy-DN and 50 cases with DN) aged 18-75 years and 50 healthy adults as control (Group B) were included. The endothelial nitric oxide gene variant (T786C and 27bp VNTR) genotypes and alleles were studied. Odds ratio with 95% CI was calculated for genotype and alleles for the occurrence of diabetes and DN. p value of less than 0.05 was considered as significant.

Results: With Bb as reference (27bp VNTR), the odds ratio for Ab in the three groups (A1,A2,B) was 2.243, 1.545 and 0.746 respectively; and for Aa was 3.043, 3.058 and 1.878 respectively; with TT as reference (T786C), it was 1.573, 1.55 and 1.055 respectively for TC; and for CC it was 2.121, 2.063 and 2.348 respectively. The OR was comparable among the study groups and control for all genotypes and alleles (p>0.05).

Conclusion: In conclusion, there was a trend towards higher predilection of DN with aa genotype and a allele in 27 VNTR, CC genotype and C allele of -786T>C polymorphism however it was not found to be statistically significant. Future large sample studies are required to account for the ethnic variation for a clearer association of the genes and their associated risk with Diabetes and its complications.

Introduction

Diabetes mellitus (DM) has become a common public health problem with changing lifestyles worldwide. Insulin resistance forms the backbone of the disease with increasing oxidative stress and inflammation forming the key pillars in the occurrence of complications (microvascular and macrovascular).1

Diabetic nephropathy (DN) accounts for a serious complication leading to kidney failure in the end stages. It is

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Received: 23.05.2021; Revised: 21.11.2021; Accepted: 18.01.2022
Table 1: Comparison of baseline demographic and clinical investigation findings

<table>
<thead>
<tr>
<th>Variables</th>
<th>A-1(n=50)</th>
<th>A-2(n=50)</th>
<th>A (n=100)</th>
<th>B (n=50)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age(in years)</td>
<td>Mean ± SD 56.2 ± 10.59</td>
<td>53.8 ± 9.02</td>
<td>55 ± 9.86</td>
<td>38.4 ± 9.04</td>
<td>A1 vs A2:0.098</td>
</tr>
<tr>
<td>Median (25th-75th percentile)</td>
<td>56 (50.5-63)</td>
<td>55 (50-58)</td>
<td>56(50-62)</td>
<td>38(30-46)</td>
<td>A vs B:&lt;.0001</td>
</tr>
<tr>
<td>Gender</td>
<td>Female 14 (28%)</td>
<td>19 (38%)</td>
<td>33 (33%)</td>
<td>30 (60%)</td>
<td>A1 vs A2:0.288</td>
</tr>
<tr>
<td></td>
<td>Male 36 (72%)</td>
<td>31 (62%)</td>
<td>67 (67%)</td>
<td>20 (40%)</td>
<td>A vs B:.002</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>Mean ± SD 25.38 ± 2.75</td>
<td>27 (25-28)</td>
<td>25 (23-26.75)</td>
<td>A vs B: &lt;.0001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Median (25th-75th percentile) 26 (24.25-27)</td>
<td>27 (26-30)</td>
<td>26 (25-28)</td>
<td>A vs B: &lt;.0001</td>
<td></td>
</tr>
<tr>
<td>Fasting blood sugar (mg/dL)</td>
<td>Mean ± SD 195.72 ± 53.74</td>
<td>198.24 ± 86.12</td>
<td>196.98 ± 71.43</td>
<td>91.6 ± 5.82</td>
<td>A1 vs A2:0.22</td>
</tr>
<tr>
<td></td>
<td>Median (25th-75th percentile) 187.5 (150-223.5)</td>
<td>156 (140-210)</td>
<td>172 (140.75-222.5)</td>
<td>A vs B:&lt;.0001</td>
<td></td>
</tr>
<tr>
<td>Postprandial glucose levels (mg/dL)</td>
<td>Mean ± SD 31.892)</td>
<td>64 (20.798-92)</td>
<td>106 (98-112)</td>
<td>A vs B: &lt;.0001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Median (25th-75th percentile) 25.5 (24-28)</td>
<td>900 (700-1100)</td>
<td>75 (25.75-900)</td>
<td>A vs B:&lt;.0001</td>
<td></td>
</tr>
<tr>
<td>HbA-1c (%)</td>
<td>Mean ± SD 8.45 ± 1.6</td>
<td>9.01 ± 2.62</td>
<td>8.73 ± 2.18</td>
<td>5.16 ± 0.27</td>
<td>A1 vs A2:0.658</td>
</tr>
<tr>
<td></td>
<td>Median (25th-75th percentile) 8.1 (7.225-9)</td>
<td>8.15 (7.1-10.525)</td>
<td>8.15 (7.175-9.425)</td>
<td>5.2 (5-5.4)</td>
<td>A vs B:&lt;.0001</td>
</tr>
<tr>
<td>eGFR (ml/min)</td>
<td>Mean ± SD 91.48 ± 11.31</td>
<td>25.28 ± 18.72</td>
<td>58.38 ± 36.65</td>
<td>105.8 ± 7.96</td>
<td>A1 vs A2:&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>Median (25th-75th percentile) 92 (90-95.75)</td>
<td>20.4 (12.958-31.892)</td>
<td>64 (20.798-92)</td>
<td>106 (98-112)</td>
<td>A vs B:&lt;.0001</td>
</tr>
<tr>
<td>Spot urine for albumin creatinine ratio (mg/g creatinine)</td>
<td>Mean ± SD 25.38 ± 2.75</td>
<td>1055.08 ± 866.16</td>
<td>540.23 ± 799.42</td>
<td>27.76 ± 31.41</td>
<td>A vs A2:&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>Median (25th-75th percentile) 25.5 (24-28</td>
<td>900 (700-1100)</td>
<td>75 (25.75-900)</td>
<td>23 (23-24)</td>
<td>A vs B:&lt;.0001</td>
</tr>
<tr>
<td>24 hour urine albuminuria (mg/day)</td>
<td>Mean ± SD 31.02 ± 35.45</td>
<td>1068.48 ± 875.29</td>
<td>549.75 ± 807.23</td>
<td>24.54 ± 23.9</td>
<td>A vs A2:&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>Median (25th-75th percentile) 27 (25.25-28)</td>
<td>900 (710-1100)</td>
<td>127 (27-900)</td>
<td>25 (23-26)</td>
<td>A vs B:&lt;.0001</td>
</tr>
<tr>
<td>Serum creatinine(mg/dL)</td>
<td>Mean ± SD 0.9 ± 0.14</td>
<td>3.6 ± 2.26</td>
<td>2.25 ± 2.09</td>
<td>0.81 ± 0.18</td>
<td>A1 vs A2:&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>Median (25th-75th percentile) 0.9(0.800 - 1</td>
<td>3.1(2.100 - 5)</td>
<td>1.2(0.900 - 3.100)</td>
<td>0.8(0.700 - 0.900)</td>
<td>A vs B:&lt;.0001</td>
</tr>
</tbody>
</table>

characterised by deranged filtration mechanism, structural alterations and changes in the metabolism. Due to the variations in the diabetics who develop DN despite glycaemic control, it has been suggested that genetic factors play an important role apart from environmental factors.

In the event of ongoing advancements in genetic methods, several group of genes which increase the risk of DN has been identified which has been categorised into: Lipid metabolism [acyl-coenzyme A carboxylase beta (ACACB) and adiponectin (ADIPOQ) genes]; glucose metabolism [glucokinase regulatory protein (GCKR) and transcription factor 7-like 2 (TCF7L2)]; angiogenesis (erythropoietin (EPO) promoter gene and vascular endothelial growth factor A (VEGFA)]; renal structure and function [4.1 protein ezrin, radixin, moesin (FERM) domain-containing 3 (FRMD3), and shroom3 (SHROOM3)]; Renin-angiotensin pathway [angiotensin-converting enzyme (ACE) and angiotensin II receptor type 1 (AGTR1)]; inflammation and oxidative stress related genes [engulfment and cell motility protein 1 (ELMO1), TGF-β, and nitric oxide synthase 3 (NOS3, eNOS)] and others [solute carrier family 12 member 3 (SLC12A3), and RAB38/CTSC, and potassium voltage-gated channel subfamily J member 11 (KCNJ11)].

Interestingly, among the set of various susceptibility genes, eNOS gene has been given due attention. This is mainly because eNOS plays a significant role in controlling the blood pressure and the vascular tone, thereby influencing the endothelial function. The presence of this gene in preglomerular vessels in diabetics has led to it being an important pathophysiologic denominator for DN. Moreover, this fact has been progressively studied for targeted therapy with “nitric oxide synthases (NOS) inhibitors” to treat DN and other diseases like hypertension and atherosclerosis.

The eNOS gene is located on “chromosome7q35–36”, has 26 exons which covers 21 kb and is chiefly expressed in the endothelium. This gene is required in the synthesis of nitric oxide (NO) from L-arginine under the action of the enzyme endothelial NO synthase (NOS). In normal individuals, the role of NO is to maintain the vascular homeostasis by acting as a vascular dilator.

Studies have evidenced that that variant genes of eNOS [eNOS 4a/b (27bp- variable number tandem repeat:VNTR), T-786C, G894T(glu 298Asp) and G986T] may affect the synthesis of NO whereby decreased NO levels may increase the chances of glomerular diseases and onset of DN; however there remains discrepancy in this association of eNOS variants and DN based on the co-morbidities, glycaemic controls, ethnicity and race of the patients.

Of the various studies eNOS polymorphisms, the present study aimed to see the association of eNOS (T786C and 27bp VNTR) gene polymorphism with the risk of type II diabetes mellitus and diabetic nephropathy in North India.

**Methods**

The prospective case control study was conducted in the Department of Medicine, Nephrology and biochemistry in Rohtak over a period of 18 months. A total of 100 patients of Type 2 Diabetes Mellitus aged 18-75 years (Group A) and 50 healthy adults as control (Group B) were included. Group A patients were divided into two subgroups: A-1: 50 Type 2 Diabetes mellitus patients with no evidence of Diabetic nephropathy; A-2: 50 Type 2 Diabetes mellitus patients with evidence of Diabetic nephropathy.

The cases with diagnosis of type 2 diabetes were consecutively selected among the patients who visited the OPD in department of medicine after employing the diagnostic criteria as laid down by the ADA guidelines.
Table 2: 27 VNTR genotype and alleles, Odds ratio and 95% CI in the 27 VNTR genotype and alleles distribution between A-1 subgroup, A-2 subgroup and control Group after adjusting for age and gender

<table>
<thead>
<tr>
<th>27 VNTR genotype and alleles</th>
<th>A-1</th>
<th>A-2</th>
<th>B</th>
<th>p value, Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>27 VNTR genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bb</td>
<td>25 (50%)</td>
<td>26 (52%)</td>
<td>30 (60%)</td>
<td>1.0 (Reference)</td>
</tr>
<tr>
<td>Ab</td>
<td>19 (38%)</td>
<td>15 (30%)</td>
<td>17 (34%)</td>
<td>(0.686 to 7.329)</td>
</tr>
<tr>
<td>Aa</td>
<td>6 (12%)</td>
<td>9 (18%)</td>
<td>3 (6%)</td>
<td>(0.429 to 21.585)</td>
</tr>
</tbody>
</table>

27 VNTR alleles

<table>
<thead>
<tr>
<th>B</th>
<th>69 (69%)</th>
<th>67 (67%)</th>
<th>77 (77%)</th>
<th>1.0 (Reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>31 (31%)</td>
<td>33 (33%)</td>
<td>23 (23%)</td>
<td>0.108, 2.06</td>
</tr>
</tbody>
</table>

DNA extraction and genotyping of eNOS gene polymorphisms

For DNA extraction and genotyping, 2ml of venous blood was collected in an EDTA vial from patients of study group and control. Genomic DNA was extracted from blood lymphocytes using the proteinase K and phenol chloroform extraction procedure. Two eNOS SNPs, namely, (-786) T>C and 27 VNTR (variable number tandem repeat) were genotyped using the polymerase chain reaction or polymerase chain reaction–restriction fragment length polymorphism (PCR / PCR–RFLP) methods. Ten micro liter of PCR products of (-786) T>C were digested by MspI restriction enzyme for four hours. However, 27 VNTR of eNOS gene was characterized by presence of either four 27-bp repeats (a allele) or five 27-bp repeats (b allele) and were genotyped using PCR assay with primers as previously used by Wang et al. The digested PCR products were resolved on 2–3% agarose gels stained with ethidium bromide. Ten micro liter of above PCR reaction were digested by MspI for four hours at 37 degree Celsius. Then digested product was resolved on 3% agarose gel. PCR product of 162 bp and 61 bp correspond to T allele. However, PCR product of 116 bp, 61 bp and 46bp were observed in C allele.

Statistical analysis

The data entry was done in the Microsoft EXCEL spreadsheet and the final analysis was done with the use of Statistical Package for Social Sciences (SPSS) software ver 21.0. The presentation of the Categorical variables was done in the form of number and percentage (%). On the other hand, the presentation of the continuous variables was done as mean ± SD and median values. The data normality was checked by using Kolmogorov-Smirnov test. The cases in which the data was not normal, we used non parametric tests. The comparison of the variables which were quantitative in nature were analysed using Mann-Whitney Test (for two groups). The comparison of the variables which were qualitative in nature were analysed using Chi-Square test. Independent t-test was used to compare the duration of diabetes among the cases. Adjusted odds ratio with 95% CI was calculated for genotype and alleles after adjusting for age and gender. For statistical significance, p value of less than 0.05 was considered as significant.

Results

Table 1 shows the comparison of baseline demographic, clinical and laboratory variables. The mean of the age of the cases were 55 ± 9.86 years (56.2 ± 10.59 in A1 and 53.8 ± 9.02 in A2, p=0.098) and of controls was 38±9.04 years. Median (IQR) of age (years) in Group A was 56 (50-62) years which was statistically significantly higher as compared to control Group 38 (30-46) years (p value<0.001). The male:female ratio was 2:1 in study Group while it was 3:2 in control Group (p=0.002).

The mean body mass index (kg/m²) was 26.62±2.92 kg/m² in study Group (25.38 ± 1.81 in A1 and 27.86 ± 3.3 in A2, p<.0001) while in control Group it was 24.96±2.84 kg/m² (p value 0.0005). The glycemic values (fasting blood sugar mg/dl), postprandial blood sugar (mg/dl); and glycated haemoglobin were statistically significantly higher in study Group (p value<0.001) were comparable among A1 and A2. The mean duration (SD) of diabetes among the cases (A1 and A2) was comparable (5.7 ± 1.3 vs 6.2 ± 2.1 years, p=0.155).

The mean eGFR in Group A2 (25.28 ± 18.72 ml/min) was significantly lower as compared to Group A1 (91.48 ± 11.31 ml/min), p=0.0001; and overall the controls had significantly more eGFR (105.8 ± 7.96 ml/min). The mean 24-hour urine albuminuria was also significantly more in Group A2 (1068.48 ± 875.29 mg/day) as compared to Group A1 (31.02 ± 35.45 mg/day), p<0.0001; both of which were significantly more than controls (24.54 ± 2.39 mg/day). Even the spot ACR was significantly

Volunteers from the hospital staff or the patient relatives who accompanied the cases. Since, the diabetic population was mainly old aged whereas the controls population enrolled were the healthy patient relatives (who accompanied the patients) and hospital staff (predominantly nurses) who were relatively young aged; the age and gender matching was not done in cases and controls (as it was time consuming).

Diabetic nephropathy was diagnosed by measuring 24 hour urine albumin. A 24 hour urine albumin more than 300 mg/day and spot albumin creatinine ratio (ACR>300 mg/g creatinine) was used for diagnosis of diabetic nephropathy.13 Any patient with Hepatic diseases (AST/ALT > 2times upper limit), systemic disease that might affect kidney function, and pregnancy were excluded.

After ethical clearance and obtaining written informed consent and explaining them the purpose and protocol of the study, all the study subjects were subjected to detailed clinical history, complete general physical as well as systemic examination. Demographic features and clinical parameters of subjects and control were recorded especially age (years), Body Mass index (kg per metre square), BP (mm hg), time since onset of disease (years), Biochemical characteristics include- Blood glucose (fasting and post prandial), lipid profile, liver function, renal function, HBA1C, urine complete examination, 24 hour urine and spot urine for albuminuria,. Any other relevant investigation like serum electrolytes, chest x-ray, and electrocardiography was done as and when required. The endothelial nitric oxide gene variant were studied in both study Group and control Group.

The data entry was done in the Microsoft EXCEL spreadsheet and the final analysis was done with the use of Statistical Package for Social Sciences (SPSS) software ver 21.0. The presentation of the Categorical variables was done in the form of number and percentage (%). On the other hand, the presentation of the continuous variables was done as mean ± SD and median values. The data normality was checked by using Kolmogorov-Smirnov test. The cases in which the data was not normal, we used non parametric tests. The comparison of the variables which were quantitative in nature were analysed using Mann-Whitney Test (for two groups). The comparison of the variables which were qualitative in nature were analysed using Chi-Square test. Independent t-test was used to compare the duration of diabetes among the cases. Adjusted odds ratio with 95% CI was calculated for genotype and alleles after adjusting for age and gender. For statistical significance, p value of less than 0.05 was considered as significant.
Table 3: 786 T>C genotype and alleles, Odds ratio and 95% CI in the 786 T>C genotype and alleles distribution between the A-1 subgroup, A-2 subgroup and control group after adjusting for age and gender

<table>
<thead>
<tr>
<th>786 T&gt;C genotype and alleles</th>
<th>A-1 (50%)</th>
<th>A-2 (46%)</th>
<th>B (60%)</th>
<th>p value, Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>786 T&gt;C genotype</td>
<td>A-1 vs B</td>
<td>A-2 vs B</td>
<td>A-1 vs A-2</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>25 (50%)</td>
<td>23 (46%)</td>
<td>30 (60%)</td>
<td>1.0 (Reference)</td>
</tr>
<tr>
<td>TC</td>
<td>23 (46%)</td>
<td>23 (46%)</td>
<td>18 (36%)</td>
<td>0.472 (1.573)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.458 to 5.398)</td>
</tr>
<tr>
<td>CC</td>
<td>2 (4%)</td>
<td>4 (8%)</td>
<td>2 (4%)</td>
<td>0.575 (2.121)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.153 to 20.927)</td>
</tr>
<tr>
<td>786 T&gt;C allele</td>
<td></td>
<td></td>
<td></td>
<td>1.0 (Reference)</td>
</tr>
<tr>
<td>T</td>
<td>73 (73%)</td>
<td>69 (69%)</td>
<td>78 (78%)</td>
<td>0.371 (1.529)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.603 to 3.877)</td>
</tr>
<tr>
<td>C</td>
<td>27 (27%)</td>
<td>31 (31%)</td>
<td>22 (22%)</td>
<td>1.055 (2.414)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(0.182 to 23.433)</td>
</tr>
</tbody>
</table>

more in Group A2 (1055.08 ± 866.16 mg/g) as compared to Group A1 (25.38 ± 2.75 mg/g), p<0.0001; both of which were significantly more than controls (27.76 ± 31.41 mg/g). The mean serum creatinine values were also significantly higher in cases as compared to controls (2.25 ± 2.09 vs 0.81 ± 0.18 mg/dL) with group A2 having significantly more mean value as compared to group A1 (3.6 ± 2.26 vs 0.9 ± 0.14, p<0.0001)

27VNTR

Genotype: In all the Groups (both study and control) the majority was bb genotype of 27 VNTR (50% in A1, 52% in A2 and 60% in control Group) followed by ab in 38% in A1, 30% in A2 and 34% in control Group (p>0.05). Alleles: 27 VNTR alleles were b in majority of subjects (69% in A1, 67% in A2 and 77% in control Group) without any statistically significantly difference (p value>0.05).

After adjusting for age and gender (to remove confounders of age and gender), with Bb as reference, the adjusted odds ratio (95% CI) for Ab in the three groups (A1, A2, B) was 1.573(0.458 to 5.398), 1.55(0.488 to 4.921) and 1.055(0.462 to 2.414) respectively and for CC was 2.121(0.153 to 29.427), 2.063(0.182 to 23.433) and 2.348(0.35 to 15.736) respectively; with none of the adjusted OR reached statistical significance. On comparison of allele between the Groups (with T as reference), no correlation was seen between the T and C allele with adjusted odds ratio of 1.529(0.603 to 3.877), 1.482(0.614 to 3.582) and 1.147(0.616 to 2.136) respectively (Table 3).

Discussion

The present study is one of the few Indian studies to demonstrate the role and association of NOS3–786T>C and 27bp VNTR polymorphism of the eNOS gene in predicting the risk ratio of DN in patients with diabetes. The study results showed that eNOS 786T>C base pair VNTR and CC genotype of eNOS 786T>C which confer to a lower level of NO synthesis, were seen in very few individuals in comparison to the other studies as the genetics is mainly attributed to the population types and ethnic background.4 (4) DN is influenced by various modifiable environmental factors apart from the production of NO by eNOS maintains the overall vascular homeostasis to some extent,1 which may allow the insignificant association of decreased production of NO in some cases as seen in the present study.

In the present study, we found that variable genotype of eNOS T786C and 27bp VNTR showed no significant risk association with type 2 DM or DN. The findings were inline with the previous study on Egyptian population1 and Japanese population2 who found no association of eNOS T786C with DM or DN.

Our findings were in direct contrast to the previous study done in North Indian population by Ahluwalia et al.3,4 which showed that -786) >C and 27VNTR variants of the eNOS gene carried a high risk of nephropathy in Type 2 diabetics. The study also observed an increased risk in DN as compared to controls (p value 0.009). Such significant association has also been seen in other ethnicities such as Mexican American patients,5 Iranian patients,6 and Chinese patients with diabetes.7

So overall the results remain conflicting and subject to the study population type. Interestingly after reviewing the previous studies, we propose certain points of differences that may have led to the insignificant results in the study. (1) the control population in the study were not age and gender matched with the controls, although we adjusted the confounders in the final analysis (2) Aa genotype of 27 VNTR and CC genotype of eNOS 786T>C which confer to a lower level of NO synthesis, were seen in very few individuals in comparison to the studies that have shown a significant association. For example, in the study by Moguib O et al, CC genotype was not recorded in any patient. This fact have been reinstated in one of the study,22 which showed that the relative distribution and frequency of T786C variant of eNOS gene is higher in Caucasians(42.0%) as compared to Africans Americans (18%) and Asians (14%). (3) Another reason for negative results in the study can be due to different ethnicity in comparison to other studies as the genetics is mainly attributed to the population types and ethnic background.4 (4) DN is influenced by various modifiable environmental factors apart from the production of NO by eNOS maintains the overall vascular homeostasis to some extent,1 which may allow the insignificant association of decreased production of NO in some cases as seen in the present study.

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genetics like duration, smoking, BMI and treatment and (5) the genetic polymorphisms other than the ones analysed in the study may also have had a significant impact on the study population as all of them were not matched and controlled. Studies have shown that interaction of various variants within the haplotypes may influence the diseases rather than just the single polymorphisms.26

Even the mechanisms that are responsible for the association between eNOS polymorphisms and diabetes (DN) is not yet fully understood. Though the variants of eNOS gene cause defective NO synthesis; thereby increasing the susceptibility to renal disease;27 still, such changes in eNOS expression has not been always found to correlate with NO synthesis. It may be because NO synthesis by eNOS depends not only on the gene expression but also on the availability of the substrate and cofactors.28 In addition, NO synthesis may also be decreased due to the increasing inhibitors of eNOS gene such as ADMA.29

Beside the non-significant association of eNOS with DN; we also did not find any association of eNOS genotypes and alleles with type 2 diabetes. This may be due to a recessive mode of inheritance of the studied genes which failed to express if not homozygous.

The strengths of the study were that ours was a case control study where we determined two associations simultaneously: diabetes and non-diabetes and DN vs non-DN, and adjusted for age and gender in the final analysis. Secondly, we studies two genetic polymorphisms for the same gene. Though the results were on the negative side, we were able to elucidate various facts about the studied genes.

The study results must be interpreted in view of certain limitations. Firstly, though eNOS affects NO synthesis and thereby the vascular tone, the present study did not report presence or absence of vascular disease (retinopathy/ peripheral vascular disease/ ischemic heart disease). Secondly, there was no follow-up of the patients. Third, other genetic polymorphisms affecting the diabetics were not taken into account. Lastly, ours being a referral centre, actual area of the population subserved was not standardised to a particular geographic area. Since the association of eNOS gene variants were analysed in only a limited number of patients, the authentication of the results need more extensive analysis. Overall, the present study results contribute, at least partially, to better understanding of the genetic background of DN.

Conclusion
In conclusion, there was a trend towards higher predilection of DN with certain genotype and a allele in 27 VNTR, CC genotype and C allele of -786T>C polymorphism however it was not found to be statistically significant. Diabetes being a polygenic trait requires identification and assessment of multiple genes together to adjust for the confounding caused by the various gene polymorphisms and epigenetic mechanisms (e.g., DNA methylation, chromatin histone modifications, and functional noncoding RNAs). Future large sample studies are required to be conducted on a varied database to take into account for the ethnic variation which may give a much clearer association of the genes and their associated risk with Diabetes and its complications.

References
Indian Reality of Clinical Practice and Patient Profile in Diabetes Care: Lessons from the IMPACT survey

Shashank R Joshi1*, Subhash K Wangnoo2, Subhankar Chowdhury3, Hemraj B Chandalia4, Bipin Sethi5, Ambika G Unnikrishnan6, Abdul Hamid Zargar7, Ashok Kumar Das8, Ajay Kumar9, Sanjay Kalra10, Viswanathan Mohan11

Abstract
India shoulders a heavy burden of diabetes mellitus (DM), the management of which is suboptimal globally.

Objectives: Insulin Management: Practical Aspects in Choice of Therapy (IMPACT) survey was designed to gain insight into the ground (in-clinic) reality of DM management by physicians in India.

Methods: A survey consisting of 12 multiple-choice questions was conducted by SurveyMonkey®, focusing on practice profile, patient profile, and other aspects of DM management.

Results: The survey included 2424 physicians. Majority of them were general physicians (58.5%) followed by diabetologists (31.1%). Most (49.2%) of the respondents specified that the ideal time for a DM consultation is 15 min. However, 73.4% of them provided consultation of <10 min because of heavy patient load. Nearly half of the respondents reported that their patients consumed a diet with carbohydrate content of 60% to 80%, and 79.4% of them admitted that <50% of their patients adhered to dietary advice. About 73.5% of the respondents believed controlling fasting plasma glucose (FPG) level alone would not adequately control postprandial plasma glucose (PPG) level, and 93.0% of them preferred an insulin therapy at the initiation that controls both FPG and PPG levels.

Conclusion: Limited consultation time, high-carbohydrate diet, and a need for choosing insulin regimens that provide control for both PPG and FPG levels are some ground realities of DM management in India. These realities need to be factored in while choosing treatment options to achieve the desired glycemic control and improve the status of diabetes care.

Introduction
India has 74.2 million adult people with diabetes mellitus (DM), which is the second-largest population across the world. Unfortunately, the burden of DM is steadily increasing across the globe, and by 2045, India is projected to have 124.9 million people with DM.1 Despite the availability of the latest tools and medicines and the best efforts of physicians, DM control is less satisfactory in India. DiabCare India 2011 survey reported that even after patients received treatment for type 2 diabetes mellitus (T2DM) for around 6 years, their mean glycated hemoglobin (HbA1c), fasting plasma glucose (FPG), postprandial plasma glucose (PPG), and random blood glucose (RBG) were far above the recommended targets.2 Uncontrolled glycemia can put the patients at risk of developing various micro- and macro-vascular complications.

Due to urbanization and socioeconomic transition, Indians are switching from traditional foods to energy-intense, nutrient-poor, and high-carbohydrate diets. Also, sedentary occupations and low levels of recreational physical activity are increasingly being adopted. Moreover, a lack of knowledge and awareness about DM in India, particularly in rural areas, adds up to the burden. For instance, in India, over 50% of the public is unaware of DM, and among those aware of DM, only 51.5% know that DM affects other organs as well.3 India has a doctor-to-population ratio of around 0.69:1000, which is lower than the World Health Organization (WHO) norm of 1:1000.4 This leads to overburdened doctors working long hours to keep up with the patient inflow, and as a result, in-clinic time available for consultation per patient would become less.

Delay in insulin initiation can also contribute to poor diabetes control.5 Fear of hypoglycemia and weight gain; social stigma; and inability to inject insulin, monitor blood glucose (BG), and titrate insulin doses are some of the barriers that delay insulin initiation and intensification.6

With the current knowledge of the disease and the treatment options available, DM management can be significantly improved by evolving and adopting an approach to DM care that suits the Indian context. This can be done through collective efforts by government agencies, healthcare professional bodies, practicing doctors, and pharmaceutical companies. Providing care in tune with the Indian reality can bridge barriers to therapeutic inertia for timely insulin initiation. Leveraging the current possibilities, the

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Received: 20.03.2022; Accepted: 30.03.2022
Novo Nordisk Education Foundation (NNEF), in collaboration with like-minded partners, had launched the “IMPACT India: a 1000-Day Challenge” program to improve the status of DM care in India with an Indian patient-centric approach for its management. Under the guidance of the steering committee members of the IMPACT India initiative, a survey was conducted amongst physicians in India, focusing on their practice profile, patient profile, and the other aspects of DM management with an aim to gain insight into the ground (in-clinic) reality of DM management being done by them.

Materials and Methods

Questionnaire Design

An expert group developed a 12-question survey. The questionnaire was created on the website of a web-hosting company, SurveyMonkey®, the survey was anonymized, and the responses were confidential throughout the process. The questionnaire consisted of three parts (Table 1) and was presented in the form of multiple-choice questions. The survey was made available to participants between June 1, 2019, and January 27, 2020, at https://www.surveymonkey.com/r/HQ29DPH. The questionnaire is provided in the supplement (Annexure A).

Statistical methods

Data were retrieved from SurveyMonkey®, and descriptive statistics were used for the analysis of

<table>
<thead>
<tr>
<th>Table 1: Questionnaire design</th>
<th>Questionnaire design</th>
</tr>
</thead>
<tbody>
<tr>
<td>Part Subject</td>
<td>Subset</td>
</tr>
<tr>
<td>A</td>
<td>Indian reality of diabetes practice</td>
</tr>
<tr>
<td></td>
<td>Included four questions to know:</td>
</tr>
<tr>
<td></td>
<td>The specialty of physicians</td>
</tr>
<tr>
<td></td>
<td>The average number of people with diabetes seen per day</td>
</tr>
<tr>
<td></td>
<td>The average time of consultation given, and</td>
</tr>
<tr>
<td></td>
<td>The ideal consultation time required by the physicians.</td>
</tr>
<tr>
<td>B</td>
<td>Indian reality of people with diabetes</td>
</tr>
<tr>
<td></td>
<td>Included two questions to know:</td>
</tr>
<tr>
<td></td>
<td>The percentage of carbohydrate consumption by Indian patients</td>
</tr>
<tr>
<td></td>
<td>Adherence towards dietary advice.</td>
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<tr>
<td>C</td>
<td>Indian reality of managing diabetes</td>
</tr>
<tr>
<td></td>
<td>Included six questions to understand:</td>
</tr>
<tr>
<td></td>
<td>Factors considered while prescribing insulin therapy</td>
</tr>
<tr>
<td></td>
<td>Status of diabetes at insulin initiation</td>
</tr>
<tr>
<td></td>
<td>Possibility of switching a doctor when insulin is advised</td>
</tr>
</tbody>
</table>

Annexure A: Survey questionnaire with responses

<table>
<thead>
<tr>
<th>Questions</th>
<th>Answer choices</th>
<th>Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1: You are currently practicing as:</td>
<td>a. Endocrinologist</td>
<td>4·1%</td>
</tr>
<tr>
<td></td>
<td>b. Diabetologist</td>
<td>31·1%</td>
</tr>
<tr>
<td></td>
<td>c. Physician</td>
<td>58·5%</td>
</tr>
<tr>
<td></td>
<td>d. Others (please specify)</td>
<td>6·3%</td>
</tr>
<tr>
<td>Q2: Number of patients with diabetes you see in a day:</td>
<td>Up to 10</td>
<td>30·6%</td>
</tr>
<tr>
<td></td>
<td>10–30</td>
<td>50·0%</td>
</tr>
<tr>
<td></td>
<td>30–50</td>
<td>16·0%</td>
</tr>
<tr>
<td></td>
<td>50–100</td>
<td>3·1%</td>
</tr>
<tr>
<td></td>
<td>&gt;100</td>
<td>0·2%</td>
</tr>
<tr>
<td>Q3: What do you think should be the ideal consultation time per patient with diabetes?</td>
<td>5 min</td>
<td>3·4%</td>
</tr>
<tr>
<td></td>
<td>10 min</td>
<td>32·7%</td>
</tr>
<tr>
<td></td>
<td>15 min</td>
<td>49·2%</td>
</tr>
<tr>
<td></td>
<td>30 min</td>
<td>14·8%</td>
</tr>
<tr>
<td>Q4: In reality, due to very heavy patient load, what is the average consulting time you are able to spend with a diabetic patient?</td>
<td>&lt;5 min</td>
<td>11·9%</td>
</tr>
<tr>
<td></td>
<td>5–10 min</td>
<td>61·5%</td>
</tr>
<tr>
<td></td>
<td>10–30 min</td>
<td>25·9%</td>
</tr>
<tr>
<td></td>
<td>&gt;30 min</td>
<td>1·0%</td>
</tr>
<tr>
<td>Q5: What is the percentage of carbohydrates consumed in a meal by your diabetes patients?</td>
<td>40–50%</td>
<td>15·8%</td>
</tr>
<tr>
<td></td>
<td>50–60%</td>
<td>34·6%</td>
</tr>
<tr>
<td></td>
<td>60–70%</td>
<td>39·5%</td>
</tr>
<tr>
<td></td>
<td>70–80%</td>
<td>10·1%</td>
</tr>
<tr>
<td>Q6: What percentage of your patients adhere to the dietary advice?</td>
<td>&lt;25%</td>
<td>30·6%</td>
</tr>
<tr>
<td></td>
<td>25–50%</td>
<td>48·8%</td>
</tr>
<tr>
<td></td>
<td>50–75%</td>
<td>17·8%</td>
</tr>
<tr>
<td></td>
<td>&gt;75%</td>
<td>2·8%</td>
</tr>
<tr>
<td>Q7: At insulin initiation, the Western guidelines suggest starting with basal insulin focusing on FPG alone. What do you practice with your patients in the context of Indian realities?</td>
<td>Focus efforts to address FPG alone</td>
<td>7·0%</td>
</tr>
<tr>
<td></td>
<td>Focus efforts to address both FPG and PPG levels</td>
<td>93·0%</td>
</tr>
<tr>
<td>Q8: When do you start insulin in your patients based on duration of type 2 diabetes?</td>
<td>&lt;3 years from diagnosis</td>
<td>17·9%</td>
</tr>
<tr>
<td></td>
<td>3–5 years from diagnosis</td>
<td>26·2%</td>
</tr>
<tr>
<td></td>
<td>5–7 years from diagnosis</td>
<td>28·7%</td>
</tr>
<tr>
<td></td>
<td>7–10 years from diagnosis</td>
<td>18·4%</td>
</tr>
<tr>
<td></td>
<td>&gt;10 years from diagnosis</td>
<td>8·8%</td>
</tr>
<tr>
<td>Q9: When do you start insulin in your patients based on glycemic control?</td>
<td>At a HbA1c of &gt;7% when multiple OADs do not help achieve targets</td>
<td>15·4%</td>
</tr>
<tr>
<td></td>
<td>At a HbA1c of &gt;8% when multiple OADs do not help achieve targets</td>
<td>36·3%</td>
</tr>
<tr>
<td></td>
<td>At a HbA1c of &gt;9% when multiple OADs do not help achieve targets</td>
<td>40·9%</td>
</tr>
<tr>
<td></td>
<td>At a HbA1c of &gt;10% when multiple OADs do not help achieve targets</td>
<td>7·5%</td>
</tr>
<tr>
<td>Q10: In starting and continuing insulin therapy, what is the most important parameter you consider?</td>
<td>An insulin regimen with the best glycemic control</td>
<td>10·3%</td>
</tr>
<tr>
<td></td>
<td>A simple insulin regimen</td>
<td>7·1%</td>
</tr>
<tr>
<td></td>
<td>An insulin regimen which can balance efficacy, safety, and convenience</td>
<td>82·6%</td>
</tr>
<tr>
<td>Q11: In your estimate, what percentage of your patients with diabetes have switched doctors when advised insulin.</td>
<td>Yes</td>
<td>26·3%</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>73·7%</td>
</tr>
</tbody>
</table>
| Q12: Unlike Western world (protein-based non-vegetarian meals), India has high carbohydrate-based meals (rice/roti). Under such circumstances, do you believe that controlling FPG will adequately control PPG? | Abbreviations: FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; OADs, oral antidiabetic drugs; PPG, postprandial plasma glucose.
the survey findings.

**Results**

A total of 2424 physicians from all over India responded to the survey, which included 58.5% physicians, 31.1% diabetologists, 4.1% endocrinologists, and 6.3% others as declared by the respondents. Half (50.0%) of the respondents reported that they were consulted by 10 to 30 patients per day in their diabetes practice. The majority (49.2%) of the respondents specified that the ideal time required for consultation of one patient was 15 min, whereas others (14.8%) felt that it should be 30 min. However, a higher proportion (73.4%) of the respondents spent <10 min per patient (Figure 1).

Nearly half of the respondents reported that their patients consumed a diet with the carbohydrate content of 60.0% to 80.0% (Figure 2). Also, a majority (79.4%) of them conveyed that <50% of their patients adhered to diabetes-friendly dietary advice.

More than half (54.9%) of the respondents reported that the initiation of insulin therapy generally happened between 3 and 7 years after the diagnosis of T2DM in their patients. Moreover, 77.2% of the respondents mentioned that they initiate insulin therapy at HbA1c level between 8% and 10%, when multiple oral antidiabetic drugs (OADs) would not help achieve HbA1c goal. Unfortunately, up to 30% of the people with diabetes switched doctors when advised insulin therapy, as reported by 67.3% of the respondents.

A higher proportion (73.5%) of the respondents believed that controlling FPG alone would not adequately control the PPG level because Indians with T2DM mainly consume high-carbohydrate-based meals, unlike the Western population. A greater percentage (93.0%) of the respondents did not agree with the Western guidelines, which suggest initiating only with basal insulin, focusing on FPG alone; they rather preferred an insulin therapy that would control both FPG and PPG levels (Figure 3). Additionally, most respondents (82.6%) preferred to consider an insulin regimen that could balance efficacy, safety, and convenience while initiating and continuing insulin therapy (Figure 4).

**Discussion**

The IMPACT survey included a mix of endocrinologists, diabetologists, and physicians. Most of the respondents were consulted by 10 to 30 patients with diabetes per day. In addition, these respondents were consulted by other patients as well.

The epidemiology of diabetes in Indians follows the rule of two-thirds rather than the rule of halves. The rule of two-thirds is also evident from the IMPACT survey. More than two-thirds (73.4%) of the IMPACT survey respondents reported giving a maximum of 10 min for consultation of a person with DM, and approximately two-thirds (64.0%) of the IMPACT survey respondents believed that the
According to the Second Diabetes Attitudes, Wishes and Needs (DAWN2) study, Indians with DM ranked their physicians the best in providing patient-centered care, whereas the physicians ranked themselves as second.11,12 Better interaction between the treating physician and the patient can help a greater implementation of lifestyle modifications.

A real-world study conducted by Raj et al. in India reported that the primary barriers to insulin therapy are hypoglycemia (25.9%), stress (17.1%), and fear of injection (10.3%).13 A study by Mokta et al., conducted in Himachal Pradesh, reported that only 7.9% of patients agree to take insulin at the first suggestion.14 The present survey revealed that as per two-thirds (67.3%) of the respondents, up to 30% of their patients would switch doctors when advised insulin. This underscores the need for more emphasis on awareness and social marketing of insulins in the community at large.

The joint clinical practice recommendations from Research Society for the Study of Diabetes in India and Endocrine Society of India (RSSDI-ESI 2020) suggest initiating insulin when patients fail to achieve HbA1c target with three OADs or if a patient is intolerant to any individual agent or combination of agents.15 In the DiabCare India 2011 study of 6168 patients, 35.2% of them were on insulin therapy, either alone or in combination with OADs.2 This is similar to a cross-sectional study of 4947 adult participants with diagnosed DM (all types) in the National Health and Nutrition Examination Survey (NHANES) that showed 31.8% white Americans were on insulin therapy between 2005 and 2012.16 Khunti et al. in an observational study conducted in ten countries found that out of the 17,374 patients who were on multiple OADs, 41% had HbA1c ≥9% and 22% had HbA1c ≥10%. The study also reported that average HbA1c at the time of initiation of insulin therapy was 8.9%, with mean duration of OAD therapy being 8.5±6.6 years.17 In the IMPACT survey, more than half (54.9%) of the respondents conveyed that by the time insulin therapy was initiated in their patients, it was already 3 to 7 years after the diagnosis of DM. This wider range could be because insulin initiation is mainly done based on individual requirements. Insulin therapy was initiated when HbA1c was >8% as per 36.3% of the respondents and when HbA1c was >9% as per 40.9% of the respondents. The Tresiba® Real-world Use Study (TRUST)18 and the Study of MAnagement of diabetes with Ryzodeg® Treatment (SMART)19 reported that approximately 50% of the people with DM have poor glycemic control (HbA1c ≥9%) despite being on multiple OADs. An earlier study by Moses et al. reported that despite poor DM control with OADs, insulin therapy was initiated after mean diabetes duration of 7 years,20 whereas an audit of insulin usage in an Indian cohort of patients enrolled over 10 years (2006–16) showed that insulin was initiated after mean diabetes duration of 8.8±6.4 years.21 Timely insulin initiation can help in attaining good glycemic control and can change the natural history of diabetes.15,22 A study by Mokta et al. found that within 2 months of the initiation of insulin therapy in newly diagnosed T2DM treatment-naive patients, the mean FPG, PPG, and HbA1c significantly (P < 0.001) reduced from 267±276 mg/dL, 408±101 mg/dL, and 11.5±1.4% to 107±10 mg/dL, 145±24 mg/dL, and 7.3±0.8%, respectively.22

Most of the Western guidelines, such as the American Diabetes Association/European Association for the Study of Diabetes (ADA/EASD), ADA, and American Association of Clinical Endocrinologists/American College of Endocrinology (AACE/ACE), suggest considering basal insulin while initiating insulin therapy.24-26 This may suit the Western setting of a low-carbohydrate diet, wherein the PPG contribution to overall HbA1c is low at higher quartiles.27 In contrast, the Indian diet mainly consists of high-carbohydrate content, which can lead to poor glycemic control, especially poor PPG control, thereby leading to macro- and micro-vascular complications.28 Wang et al. reported in a study that in Asians with T2DM, PPG is as important as FPG, even in those patients in whom HbA1c levels are high.29 Similarly, the majority (73.5%) of IMPACT survey respondents believed that because of the high-carbohydrate content in the Indian diet, controlling the PPG level alone will not control the PPG level adequately.
controlled trials by Chan et al., revealed that basal insulin effectively lowered the FPG level in people with T2DM, which was uncontrolled with OADs. Although a similar reduction in the FPG level was seen in both groups, fewer Asian patients achieved the target of HbA1c <7% compared with non-Asians, suggesting the need for addressing both PPG and FPG levels in an Asian population. Hence, addressing the FPG alone by initiating basal insulin may not help many people with diabetes in an Indian setting to achieve the desired treatment goals. The current IMPACT survey also supports this fact as 93% of the respondents said that they focus their efforts on controlling both FPG and PPG levels. A previous survey done among Indian physicians had also reported premix insulin as the preferred choice for patients with high-carbohydrate diet. Physicians in India prefer premix insulin due to several reasons, such as better glycemic control than basal insulin, low risk of hypoglycemia, and convenience and ease of administration. As a result, local guidelines, such as the RSDSI-ESI 2020 clinical practice recommendations for the management of T2DM, do note the Indian reality of high-carbohydrate diet and recommend insulin co-formulation and premix insulin as a choice for initiating insulin therapy.

Conclusion

In summary, the IMPACT survey highlights several Indian realities of T2DM management. Limited consultation time, high-carbohydrate diet, and the need for an insulin regimen that offers control of both PPG and FPG levels are some of the ground realities of DM management in India. Being mindful of these realities, while planning therapeutic strategies, can help achieve desired outcomes.

References

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A Clinical Profile of Patients with Hyperuricemia and the Relationship between Hyperuricemia and Metabolic Syndrome: A Cross-sectional Study at a Tertiary Hospital in the Indian Population

Anjali Rajadhyaksha¹, Nitin Sarate², Nilesh Raghorte³, Sushrut Ingawale²*

Abstract

Introduction: Metabolic syndrome is a constellation of interrelated risk factors that increase the risk of cardiovascular diseases (CVD) and diabetes mellitus. The increase in prevalence of hyperuricemia was considered to be directly related to increasing incidence of obesity and Metabolic Syndrome in developing and developed countries. Hyperuricemia is defined as serum uric acid of 6.0mg/dl and 7.0mg/dl for females and males respectively.

Aims and Objectives: To study correlation of hyperuricemia with metabolic syndrome or its components.

Materials and Methods: An observational, cross sectional single centre study with 316 patients fulfilling inclusion and exclusion criteria was carried out.

Results: Out of 316 patients, 202 (63.9%) were males and 114 (36.1%) were females. 138 (43.7%) were from rural areas and 178 (56.3%) were from urban areas. 126 (39.9%) patients had an active lifestyle and 190 (60.1%) had a sedentary lifestyle. Mean waist circumference among 114 females was 82.10 cm and among men was 87.07 cm. 113 patients fulfilled the criteria for central obesity with the mean uric acid level of 8.14 mg/dl (p=0.001); Mean uric acid level of patients without central obesity was 7.36 mg/dl. 113 patients fulfilled the criteria for hypertriglyceridemia with mean s.uric acid level 8.24mg/dl (p=0.0440). 124 had elevated blood pressure with mean s.uric acid 8.28 mg/dl (p=0.004). Patients with normal blood pressure had a mean value of s. uric acid 7.86 mg/dl. 33.44% fulfilled the criteria for metabolic syndrome (41.23% of total females and 32.10% of total males). Odds ratio was 1.28 and 0.864 for females and males respectively.

Conclusion: Prevalence of metabolic syndrome in patients with hyperuricemia was 35.4%. More common in females than males. Hyperuricemia is more prevalent in patients with a sedentary lifestyle. Hyperuricemia positively correlates with central obesity, blood pressure, hypertriglyceridemia and hyperglycemia. Hence, it is of utmost importance to screen patients of hyperuricemia for metabolic syndrome or its components to prevent mortality and morbidity associated with CVDs.

Introduction

Metabolic syndrome (MetS) is a complex of interrelated risk factors including obesity, hyperglycemia, hypertension, decreased high density lipoprotein and hypertriglyceridemia. Current literature suggests a strong association with development of diabetes, coronary artery disease, stroke and chronic kidney disease. Risk factors for hyperuricemia (HU) are male gender, high meat consumption, alcohol consumption, increasing age, chronic kidney disease, drugs like cyclosporine, diuretics etc. In India the prevalence of both MetS and HU are increasing with demographic shift from rural to urban, but the relation between Serum Uric Acid (SUA) levels and MetS has not been fully studied yet.

A major effort has been made to describe association between Serum uric acid level (SUA), Hypertension and MetS but there are still areas of uncertainty. SUA as a risk factor of hypertension has been extensively studied. However, there is a need to gain knowledge about differences between subgroups, including different age groups and various categories of obesity. As focus on individually targeted strategies is growing in modern medicine and currently also used in anti-hypertensive treatment, options may expand when impact of SUA is further explored in relation with MetS. Hence we planned this study with the aim to study the clinical profile of patients with HU and understand its relation with MetS.

Materials and Methods

It was a single-center, cross-sectional, observational study over a period of 18 months at the medical wards of Seth G.S. Medical College and K.E.M. Hospital, Parel, Mumbai which is a public tertiary care teaching hospital. After Institutional Ethics Committee approval [Ref no: EC/201/2015], those patients satisfying the inclusion and exclusion criteria were recruited. The sample size for this study was calculated according to the prevalence of HU with 95% confidence interval which was 316 patients. Inclusion criteria were: Patients with age>12 years found with SUA level >7mg/dl

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Received: 22.02.2022; Accepted: 25.03.2022

Original Article
(men) and >6mg/dl (women). Exclusion criteria were: Patients with cancer, tumor lysis syndrome, solid organ transplant, chronic renal failure and those on medication which cause HU. A total of 316 patients were recruited after written informed consent.

The data was recorded in a case record form which included data on following domains: (1) patient demographic details like age, gender, etc.; (2) relevant history and clinical examination findings; (3) anthropometric profile like height, weight, body mass index (BMI), waist and hip circumference; (4) investigations like hemoglobin, complete blood counts, serum creatinine, lipid profile, SUA and fasting and post-meal blood sugar levels, etc.

Definitions

1. Hyperuricemia (HU): Serum UA (≥102 cm in men, ≥ 88 cm in women) (5)
2. Metabolic Syndrome (MetS): The AHA/NHLBI modified NCEP/ATP III criteria was used to determine presence of MetS at study visit. Patients were diagnosed to have MetS if three or more of the following criteria were present. (i) Arterial hypertension (Systolic BP ≥130mm of Hg or diastolic BP ≥85 mm of

Table 2: Anthropometric Profile and Clinical Examination

<table>
<thead>
<tr>
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<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A) Height, cm, meansSD</td>
<td></td>
</tr>
<tr>
<td>Male (M)</td>
<td>165.95±5.66</td>
</tr>
<tr>
<td>Female (F)</td>
<td>156.40±5.63</td>
</tr>
<tr>
<td>B) Weight, kg, meansSD</td>
<td></td>
</tr>
<tr>
<td>Male (M)</td>
<td>63.86±9.89</td>
</tr>
<tr>
<td>Female (F)</td>
<td>57.75±11.63</td>
</tr>
<tr>
<td>C) Body Mass Index (BMI), kg/m², meansSD</td>
<td></td>
</tr>
<tr>
<td>Male (M)</td>
<td>23.17±3.08</td>
</tr>
<tr>
<td>Female (F)</td>
<td>23.48±4.45</td>
</tr>
<tr>
<td>D) Waist Circumference (WC), cm, meansSD</td>
<td></td>
</tr>
<tr>
<td>Male (M)</td>
<td>87.07±7.80</td>
</tr>
<tr>
<td>Female (F)</td>
<td>82.10±9.29</td>
</tr>
<tr>
<td>E) Hip Circumference (HC), cm, meansSD</td>
<td></td>
</tr>
<tr>
<td>Male (M)</td>
<td>96.00±7.76</td>
</tr>
<tr>
<td>Female (F)</td>
<td>95.61±10.69</td>
</tr>
<tr>
<td>F) Heart rate, beats/min, meansSD</td>
<td></td>
</tr>
<tr>
<td>Male (M)</td>
<td>87.06±9.54</td>
</tr>
<tr>
<td>Female (F)</td>
<td>86.95±9.90</td>
</tr>
<tr>
<td>G) Respiratory Rate, cycles/min, meansSD</td>
<td></td>
</tr>
<tr>
<td>Male (M)</td>
<td>16.88±3.72</td>
</tr>
<tr>
<td>Female (F)</td>
<td>16.54±3.41</td>
</tr>
<tr>
<td>H) Systolic Blood Pressure (SBP), mm of Hg, meansSD</td>
<td></td>
</tr>
<tr>
<td>Male (M)</td>
<td>120.74±17.23</td>
</tr>
<tr>
<td>Female (F)</td>
<td>120.09±18.14</td>
</tr>
<tr>
<td>J) Diastolic Blood Pressure (DBP), mm of Hg, meansSD</td>
<td></td>
</tr>
<tr>
<td>Male (M)</td>
<td>77.67±11.59</td>
</tr>
<tr>
<td>Female (F)</td>
<td>77.40±12.27</td>
</tr>
</tbody>
</table>

Hg or drug treatment for hypertension), (ii) Increased fasting glucose (≥100mg/dl or drug treatment for diabetes), (iii) Reduced high-density lipoprotein (HDL) Cholesterol (<40 mg/dl in men, <50mg/dl in women or drug treatment of Low HDL cholesterol), (iv) Increased triglycerides (≥150mg/dl or drug treatment of hypertriglyceridemia), and (v) Increased waist circumference (≥102 cm in men, ≥ 88 cm in women) (5)

Statistical Analysis

The data was compiled, tabulated using Microsoft Excel 2010. Statistical analysis was conducted with SPSS 23.0 (SPSS Inc., Chicago, IL, USA). Results for Quantitative Variables like age, SUA levels are presented as mean (±SD) or median (IQR) as per normality of distribution by the Shapiro-Wilk test. Results for Qualitative Variables like gender, diet, examination abnormality, etc are presented as frequencies and percentages. The association between dependent variables and independent factors was analyzed with the help of Chi Square test, t-tests, ANOVA and Kendall tau-b test as per need. Level of significance is considered at 95% confidence interval (p<0.05).

Results

Patients demography and clinical characteristics (Table 1)

Over a period of 18 months, 316 patients who fulfilled the inclusion and exclusion criteria were recruited. Most of the patients were between 12-30 years (55.72%). The mean age was 53.17 years (SD, ±15.08), ranging from 13 to 86 years. Age-group distribution was: <40 years 67(21.2%), 41-50 years 55 (17.4%), 51-60 years 88 (27.8%), 61-70 years 71(22.5%) and >70 years 35 (11.1%). 202 (63.9%) were males, the rest were females. There was male predominance with sex ratio being 1.77:1. The mean age of female patients was 54.46 years (SD, ±14.70) and mean age for male was 52.45 years (SD, ±15.27); p=0.25. 138(43.7%) patients were residing in rural areas and 178(56.3%) were from urban areas with a ratio of 1:1.29 (Rural:Urban). 177 (56%) patients had mixed diet and 139(44%) patients had vegetarian diet. Of 316 patients 86 (27.2%) were smokers, 65 (20.6%) were alcoholic and 84 (26.6%) were tobacco chewers. When asked about lifestyle, 126(39.9%) patients had an active lifestyle, while 190 (60.1%) had a sedentary lifestyle (ratio, active: sedentary, 1:1.5).

Anthropometric Profile and Clinical Examination (Table 2)

Mean height was female 156.40 cm (SD,±5.63) and male 165.95 cm (SD,±5.66). Mean weight was female 57.75 kg (SD,±11.63) and male 63.86 kg (SD,±5.66). Mean BMI (kg/m²) for females was 23.48 (SD,±4.45) and for male it was 23.17 (SD,±3.08). Mean waist circumference of females was 82.10 cm (SD,±9.29) and of males was 87.07 cm (SD,±7.60). Mean hip circumference of females was 77.67 cm (SD,±11.59) and of males was 87.07 cm (SD,±7.80).
for neurological examination and 12(3.79%) for abdominal examination. Majority of the patients had a normal systemic examination.

**Laboratory Profile (Table 3)**

The mean (±SD) of various investigations are depicted in Table 3 to describe the study population. Statistically significant differences among gender were found in values of hemoglobin, SUA, HDL cholesterol and post-meal blood sugars.

**Relationship of Hyperuricemic with MetS and its components (Tables 4 and 5)**

1. **Central obesity by waist circumference**: Out of 316 patients with HU 113 fulfilled criteria for central obesity with mean SUA level was 7.96(±1.34)mg/dl and patients without central obesity mean SUA level was 8.21(±1.24)mg/dl [p=0.026].

2. **Hypertriglyceridemia**: Out of 316 patients with HU 99 fulfilled criteria for hypertriglyceridemia with mean SUA level in patients with hypertriglyceridemia was 8.24(±1.29)mg/dl and patients without hypertriglyceridemia mean SUA level was 7.93(±1.27)mg/dl [p=0.044].

3. **HDL-Cholesterol**: Out of 316 patients with HU 89 had decreased HDL-C ratio with mean SUA level in patients with normal HDL-C mean SUA level was 8.29(±1.14)mg/dl and patients with normal HDL-C mean SUA level was 7.93(±1.26)mg/dl [p=0.022].

4. **Hypertension**: Out of 316 patients with HU 124 had hypertension with mean SUA level in patients with hypertension was 8.28(±1.24)mg/dl and patients with normal blood pressure mean SUA level was 7.87(±1.29)mg/dl [p=0.004].

5. **Hyperglycemia**: Out of 316 patients with HU 138 had hyperglycemia with mean SUA level in patients with hyperglycemia was 8.21(±1.24)mg/dl and patients with normal blood sugar mean SUA level was 7.87(±1.29)mg/dl [p=0.026].
mean SUA level was 7.89(±1.30)mg/dl [p=0.026].

6. MetS: Of 316 patients of HU 112(35.4%) fulfilled the criteria for MetS. Odds ratio for MetS was 1.479. Among these 47(41.2%) were females, the rest males. Gender specific odds ratio for females was 1.278 and for male 0.864. Mean SUA level for MetS (n=112) was 8.34(±1.23) mg/dL and those without MetS (n=204) was 7.86(±1.28) mg/dL. Table 5 shows correlation of SUA and number of components satisfied for MetS criteria (p=0.020 by ANOVA). With a non-parametric test of significance Kendall tau-b, there was a very weak positive correlation between uric acid level and metabolic syndrome (τb=0.148) which was statistically significant (p<0.001).

In our study, the prevalence of MetS in HU is 35.4% with females having higher prevalence (41.2%) than males (32.2%). It is lower than the prevalence found in the study conducted by Ramachandra et al, which found prevalence to be 41%, but more than Misra A et al noted 29.9%. Community-based study from Eastern India has measured a prevalence of MetS of 31.4%, with females having a much higher prevalence (48.2%) than males (16.3%). A study conducted in a semi-urban area in South India showed that the prevalence of MetS is 29.7% (26.5% in men and 31.2% in women). Our study showed higher prevalence of MetS in females as compared to males (41.2% versus 32.2%) supported by various studies done in different parts of the country.

HU is an increasingly common medical problem not only in advanced countries, but also in developing countries. It has been described that HU is associated with MetS components such as obesity, dyslipidemia, hyperglycemia and hypertension. The purpose of our study was to investigate the HU and the association between SUA levels and the various MetS components. In our study, overall HU is much more common in males (63.9%) than females (36.1%). This result is comparable with Conen et al research which had prevalence of 35.1% and 8.7% in males and females respectively. The pathogenic mechanism may be due to estrogen promoting SUA excretion Sumino At al. So it is more important for men to prevent HU. Prevalence of HU in rural areas was 43.7% and in urban areas was 56.3% with a ratio of 1:1.29 with prevalence is significantly higher in patients from urban areas. HU had more prevalence in patients with mixed diet (56%) with high purine and meat than in patients with vegetarian diet (44%). But the most important risk factor which is modifiable was lifestyle; a significant number of patients with HU had a sedentary lifestyle (60.1%) than patients who had an active lifestyle (39.9%). These findings in our study are comparable with different studies done such as Fung At al, Choi HK At al regarding purine rich foods, dairy and protein intake, Gaflo At al on serum urate and its relation with alcoholic beverage intake in men and women. In our study each individual component of MetS also has significant association with HU. Central obesity and hypertension had more significant association in females (p=0.001, 0.001). These findings are consistent with the study conducted by Misra At al which shows that Abdominal obesity is quite prevalent in South Asians, with females outnumbering males. Several studies conducted previously indicated that body fat, abdominal adiposity, and cardiovascular risk are higher in South Asians compared to Caucasians at similar BMI and lower average WC levels. In a WHO consultation on obesity in Asia and Pacific regions in 2000, it lower cut-offs for diagnosis of overweight and obesity were suggested, i.e. 23 kg/m2 and 25 kg/m2 or greater, respectively. A more recent WHO consultation in 2004 suggested that the proportion of Asian people with a high risk of type 2 diabetes mellitus (T2DM) and CVD is considerable at BMI values lower than the existing WHO cut-off point for overweight (25kg/m2). Hypertension is generally accepted to be the weakest association with insulin resistance compared to other factors that make up the MetS, although studies have shown that a substantial percentage of hypertensive individuals have insulin resistance.
In our study, the data indicates that serum triglyceride is markedly associated with HU (p<0.05). This was more significant in male (p<0.032). Conen et al and Schachter et al showed the same results (p<0.05). (13, 14) HU and hypertriglyceridemia are suggested to be associated with insulin resistance.27 The association between insulin resistance, HU, and hypertriglyceridemia are complicated. This may be expected as the uric acid production is linked to glycolysis and that glycolysis is controlled by insulin. It was shown in our study that SUA is negatively correlated with serum HDL-C (p<0.05), again this had more significant association in male (p<0.001). This finding is consistent with Rho et al.28 The mechanisms of this may be due to the relationship between decreased HDL-C levels and insulin resistance syndrome (Schmidt et al). Dyslipidemia is common in South Asians who have lower HDL and higher levels of small, dense low-density lipoprotein (LDL) compared to Caucasians across all strata of the society.29 Elevated serum TG is more common in urban Indians and migrant Indians compared to the rural population.30 The NCEP ATP III criterion is most likely to identify insulin resistance is the TG/HDL ratio, but large-scale clinical trials to determine its relative risk for development of CVD or its relationship with the MetS in different ethnicities are lacking. In our study, the data indicate that hyperglycemia is markedly associated with HU (p=0.0260). This was also more significant in male (p=0.006).

Studies had shown that the prevalence of type 2 diabetes is particularly high in Indians, with the prevalence of insulin resistance in healthy, young lean Indian men being 3-4 times higher than lean men in other ethnic groups.31 In a study which was conducted in South African Indian individuals with MetS, elevated fasting blood glucose was found to be the most frequently occurring criterion (IDF, 87%; NCEP ATP III, 83%); while a study in South-East Asian country noted that mortality risk for T2DM in Asian Indians was higher when compared with Chinese and Malays, although the latter had a higher prevalence of impaired glucose tolerance and IFG, suggesting that IFG carries a greater risk for CVD mortality in Asian Indians due to faster progression to type 2 diabetes.32

As diabetes is considered as the major factor underlying the progression of vascular disease, the IDF definition utilizes a fasting glucose cut-off of 100 mg/dl, a level consistent with the American Diabetes Association’s revised 2003 definition of IFG which was also accepted by NCEP in 2004.

In our study it was found that mean SUA level in patients with MetS was 8.343 mg/dl in comparison to patients who did not fulfill the criteria of MetS mean SUA level was 7.857 mg/dl. With a non-parametric test of significance Kendall tau-b, there was a very weak positive correlation between SUA level and MetS (ρ=0.148) which was statistically significant (p<0.001). When SUA level is plotted on the Y-axis with the number of components of MetS on the X-axis there is significant correlation present which shows that SUA level increases with the number of components of MetS increases.

Conclusion

In our study, the prevalence of metabolic syndrome in patients of hyperuricemia was 35.4%, higher in females than males. Prevalence of hyperuricemia was more in males than females. Hyperuricemia was more prevalent in urban as compared to rural area and also in patients with sedentary lifestyle compared to active lifestyle. Hyperuricemia was positively correlated with BMI, Central Obesity, Blood pressure, triglyceride levels and hyperglycemia and negatively correlated with HDL-C Ratio. Central obesity and hypertension has more significant association in female patients with hyperuricemia. Hypertriglyceridemia, decreased HDL-C ratio and hyperglycemia has more significant association in male patients with hyperuricemia. This study showed a weak positive correlation between serum uric acid level and number of components fulfilled for the criteria for diagnosing Metabolic syndrome. Given the high prevalence of MetS among the Indian population, more research is required to examine the role of SUA in the pathogenesis of MetS.

Limitations

Our study was a cross-sectional study. A longer prospective follow-up study would give a better understanding of association between HU and MetS. The SUA levels in the population <40 years were higher than expected, but the number of components fulfilled for metabolic syndrome criteria was higher for the population >40 years. This probably leads to weaker correlation between SUA and Metabolic syndrome. Therefore further studies can be planned into investigating HU in population <40 years.

Ethical statement

This study was approved by the Institutional Ethics Committee with reference number EC/201/2015.

References

Spectrum of Neurological Illnesses in Pregnancy – An Observational Study from a Tertiary Care Centre of Eastern India

Subhadeep Gupta¹, Uddalak Chakraborty², Atanu Chandra³, Arpan Dutta⁴, Jyotirmoy Pal⁴, Biman Kanti Ray⁵, Goutam Gangopadhyay⁶

Abstract

Introduction: Neurological disorders in pregnancy may be observed in patients with a pre-existing neurological disorder; patients developing a primary neurological disorder during the course of pregnancy or puerperium; and patients with primary medical disorders presenting with neurological manifestations.

Objectives: The objectives of the study were to find out the magnitude of neurological disorders in pregnancy in a tertiary care hospital along with assessment of proportion of women with particular disorders among total number of neurological disorders during the course of pregnancy or puerperium (6 weeks after child birth) and also to elicit the effect of neurological disorders on pregnancy outcome, if any.

Methods: A prospective observational longitudinal study was carried out in a tertiary care centre of Eastern India from July 2018 to June 2020 including all pregnant women attending the department of Obstetrics and Gynaecology. We screened 886 pregnant women, out of which 91 cases were identified and investigated. For the purpose of comparison of fetal and maternal outcome, 91 control subjects were chosen from the screened patients in a randomized fashion, so that the baseline characteristics of the two groups were comparable.

Results: In our study, 10.3% population had neurological disorders, among which 30.8% had primary headache, 3.2% had secondary headache, 8.5% had neurological low back pain, 19.1% had epilepsy, 6.4% had cerebrovascular disorders, 27.6% had peripheral neuropathy, 4.2% had other disorders such as neuropsychiatric Wilson’s disease, myasthenia gravis and compressive myelopathy. Moreover, 10.2% of the total study population was hypertensive and 2.9% were diabetic.

Conclusion: 10.3% mothers did have some neurological disorder, the commonest of which was migraine (primary headache) followed by carpal tunnel syndrome (peripheral neuropathy) and neurological low back pain. Overall fetomaternal outcomes were favorable barring cerebro-vascular disorder and Posterior reversible encephalopathy syndrome (PRES). We recommend screening for neurological disorder from early pregnancy for early detection and appropriate management of that condition.
Introduction

The physiology of pregnancy leads to changes in neurological disease presentation that may not be typical in non-pregnant state. In addition, neurological diseases follow specific pattern during pregnancy that, if known, can provide guidance and reassurance.1 Neurological disorders in pregnancy may be seen in patients who had a pre-existing neurological disorder; or in patients who developed a primary neurological disorder during the course of pregnancy or puerperium or in patients with primary medical disorders presenting with neurological manifestations. Primary headaches especially migraine are much common compared to secondary headaches.2 Apart from headaches, neurological disorders in pregnancy may be encountered in the form of cranial and peripheral neuropathy, neurological low back pain and epilepsy.1-3 There is existing paucity of literature regarding the spectrum of neurological illness in pregnancy from Eastern India.

Materials and Methods

This study was conducted to find out the magnitude of neurological disorders in pregnancy in a tertiary care hospital followed by assessment of proportion of women with particular neurological disorders among the total number of neurological disorders during the course of pregnancy or puerperium (6 weeks after child birth) and also to elicit the effect of neurological disorders on pregnancy outcome, if any. We conducted a hospital based prospective and longitudinal observational study in the Departments of Obstetrics and Gynaecology along with Department of Neuromedicine of RG Kar Medical College and Hospital, Kolkata from July, 2018- June, 2020. We included all cases with pre-existing neurological illness, neurological ailments which appeared de novo during pregnancy as well as neurological complications attributable to therapeutic management of pregnancy. After obtaining ethical clearance of Institutional Ethical Committee, we screened 886 pregnant women attending department of Obstetrics and Gynaecology, out of which 91 cases were identified and investigated. For the purpose of comparison of fetal and maternal outcome, 91 control subjects were
chosen from the screened patients in a randomized fashion, so that the baseline characteristics of the two groups were comparable. The study population included pregnant women screened at the antenatal clinic as well as those admitted through emergency.

The data were obtained by history taking, adequate clinical examination and relevant investigations pertinent to the neurological illness. The acquired data were statistically analysed by SPSS 20.0.1 and Graph Pad Prism 5. Chi-square test, Fischer’s test were used for assessment of proportions while Z test was done to signify differences between proportions and correlation was established using Pearson’s correlation test. A p value ≤ 0.05 was considered to be statistically significant.

**Results**

As per our study population was concerned, among 886 cases, 91(10.3%) mothers had neurological disorder. The proportion of mothers having neurological disorder was significantly low.

Among the neurological disorders, 29(30.8%) patients had primary headache, 3 (3.2%) patients had secondary headache, 8 (8.5%) patients had neurological low back pain (LBP), 18 (19.1%) patients had epilepsy, 6 (6.4%) patients had cerebrovascular disorder, 26 (27.6%) patients had peripheral neuropathy and 4 (4.2%) patients had others (1 Wilson’s disease, 1 myasthenia gravis, 2 compressive myelopathy) (Figure 1).

Among the patients with primary headaches, 20(69.0%) patients had migraine. Proportion of migraine patients was significantly higher than other primary headaches. All patients in secondary headache group presented with eclampsia related posterior reversible encephalopathy syndrome (PRES) and all of them had abnormality in brain imaging. Among the patients with neurological LBP, 1(12.5%) patient had L1-L2 radiculopathy, 2 (25.0%) patients had L3-L4 radiculopathy, 2(25.0%) patients had L5-S1 radiculopathy and 3(37.5%) patients had S1-S2 radiculopathy. The distribution of spectrum of epilepsy revealed that 13(72.2%) patients had idiopathic GTCS, 2(11.1%) patients had idiopathic partial seizure, 1(5.6%) patients had neurocysticercosis and 2(11.1%) patients had old calcified tuberculosis in epilepsy group. Among the group of patients with peripheral neuropathy, 23(88.5%) patients had carpal tunnel syndrome, 1(3.8%) patient had sciatic nerve injury during episiotomy repair and 2(7.6%) patients had cranial nerve palsy.

All 5(100.0%) patients with cerebrovascular disorders had ischemic stroke. 2(40.0%) patients had stroke in middle cerebral artery territory and 3(60.0%) patients had stroke in posterior circulation.

Among the other neurological illness in this study, we had a patient of Wilson’s disease on copper chelating therapy (Figure 2 A, B), who had a spontaneous vaginal delivery with optimal feto-maternal outcome. Another patient had Myasthenia gravis, who had an assisted vaginal delivery with no adverse effects on mother or the fetus. Two patients presented with compressive myelopathy, of which...
According to different studies, the prevalence of hypertension in pregnancy was found to be 6.9-7.8% (p<0.0001). Prevalence of hypertension as 90 (10.2%) patients had hypertension compared to 60 (7.4%) controls. The difference was significant (p<0.0001). It was found not to affect feto-maternal outcome; expressed in terms of mode of delivery, gestational age at delivery, neonatal birth weight, APGAR score at birth (1 min and 5 mins), need for nursery admission and maternal condition at discharge.12,13

Adeney KL et al in their case control study showed that the patients with history of migraine was associated with 1.8 fold increased risk of preeclampsia.12 In our study 15% of the migraineurs had associated hypertension. The incidence of secondary headache in our study was 3.2% among the diseased population and 0.3% in the study population. The incidence of neurological low back pain in our study was 8.5% in the diseased population and 0.9% in the study population. 91 (91.9%) patients had mechanical LBP (p<0.0001). It was found not to affect adversely the feto-maternal outcome. LSCS rates in both the groups were comparable and the cause of LSCS was purely due to obstetric indications and not due to LBP. Neonatal outcome was also favorable and all the mothers were discharged in stable condition. The incidence of epilepsy was 19.1% in the diseased group and 2% in the study group. Idiopathic GTCS was the most common cause of epilepsy during pregnancy accounting for 72.2% of the cases, followed by idiopathic partial seizures (11.1%), old calcified tuberculoma (only 2 cases) and neurocysticercosis (only 1 case). The outcome of pregnancy was favorable in all cases of epilepsy as there was no statistically significant difference between the two groups regarding mode of delivery, mean age at delivery, birth weight, need of nursery admission and APGAR score at 1 and 5 mins.

The incidence of cerebrovascular disorders was 6.4% in the diseased population and 0.7% in the entire study population. Stroke accounted for 5 cases while cerebral venous sinus thrombosis was observed in 1 case. All 5 patients had eclampsia resulting in ischemic stroke. Proportion of ischemic stroke patients was significantly higher than haemorrhagic 66.7% of the cases underwent LSCS compared to 33.3% in the control group. Summarizing the incidence from multiple studies, the incidence of stroke is estimated to be

### Table 3: Comparison of literature showing spectrum of neurological illness in pregnancy

<table>
<thead>
<tr>
<th>Disease</th>
<th>Condition</th>
<th>To et al.14 (n=161) (%)</th>
<th>Janaki et al.15 (n=161) (%)</th>
<th>Agarwal et al.16 (n=161) (%)</th>
<th>Srinivasan et al.17 (n=161) (%)</th>
<th>Gupt et al.18 (n=161) (%)</th>
<th>Present study (n=94) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache</td>
<td>Not mentioned</td>
<td>Not mentioned</td>
<td>Not mentioned</td>
<td>Not mentioned</td>
<td>Not mentioned</td>
<td>Not mentioned</td>
<td>94 (32.4)</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>102 (63.5)</td>
<td>30 (30.9)</td>
<td>5 (5.7)</td>
<td>17 (23.5)</td>
<td>22 (28.9)</td>
<td>18 (19.1)</td>
<td>6 (6.4)</td>
</tr>
<tr>
<td>Cerebrovascular disorders</td>
<td>7 (4.3)</td>
<td>48 (49.5)</td>
<td>72 (26.7)</td>
<td>41 (60.3)</td>
<td>9 (11.9)</td>
<td>6 (6.4)</td>
<td>2 (2.2)</td>
</tr>
<tr>
<td>CNS infections</td>
<td>-</td>
<td>4 (4.1)</td>
<td>5 (5.7)</td>
<td>3 (4.4)</td>
<td>12 (15.8)</td>
<td>1 (1.06)</td>
<td>1 (1.06)</td>
</tr>
<tr>
<td>CNS tumors</td>
<td>12 (7.5)</td>
<td>7 (7.2)</td>
<td>7 (7.2)</td>
<td>16 (23.5)</td>
<td>1 (1.06)</td>
<td>1 (1.06)</td>
<td>8 (8.5)</td>
</tr>
<tr>
<td>Eclampsia</td>
<td>19 (11.8)</td>
<td>Not included</td>
<td>Not included</td>
<td>Not included</td>
<td>Not included</td>
<td>Not included</td>
<td>Not included</td>
</tr>
<tr>
<td>Demyelinating diseases</td>
<td>4 (2.5)</td>
<td>1 (1.03)</td>
<td>1 (1.1)</td>
<td>3 (4.4)</td>
<td>1 (1.3)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Peripheral neuropathies</td>
<td>12 (7.5)</td>
<td>1 (1.03)</td>
<td>2 (2.3)</td>
<td>1 (1.5)</td>
<td>1 (1.3)</td>
<td>26 (27.6)</td>
<td>-</td>
</tr>
<tr>
<td>Metabolic encephalopathies</td>
<td>Not included</td>
<td>Not included</td>
<td>Not included</td>
<td>Not included</td>
<td>28 (36.8)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cord affections</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5 (7.4)</td>
<td>1 (1.3)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Movement disorders</td>
<td>Not mentioned</td>
<td>Not mentioned</td>
<td>Not mentioned</td>
<td>Not mentioned</td>
<td>Not mentioned</td>
<td>Not mentioned</td>
<td>Not mentioned</td>
</tr>
<tr>
<td>Neuromuscular junction disorders</td>
<td>Not mentioned</td>
<td>Not mentioned</td>
<td>Not mentioned</td>
<td>Not mentioned</td>
<td>Not mentioned</td>
<td>Not mentioned</td>
<td>Not mentioned</td>
</tr>
<tr>
<td>Neurological LBP</td>
<td>Not mentioned</td>
<td>Not mentioned</td>
<td>Not mentioned</td>
<td>Not mentioned</td>
<td>Not mentioned</td>
<td>Not mentioned</td>
<td>1 (1.06)</td>
</tr>
<tr>
<td>Incidence of neurological disorders</td>
<td>326 per 10000 deliveries</td>
<td>Not studied</td>
<td>Not studied</td>
<td>Not studied</td>
<td>335 per 10000 deliveries</td>
<td>10,600 per 10000 deliveries</td>
<td>-</td>
</tr>
</tbody>
</table>

One was due to vertebral hemangioma (Figure 2C, D) and another one was attributed to Pott’s spine; in both the cases Cesarean section was performed under spinal anesthesia with optimal feto-maternal outcome.

A comparison between case and control group with respect to parity, comorbidity and feto-maternal outcome has been formulated in Table 1.

A subgroup analysis of mothers with neurological disorders in comparison to maternal outcome (Figure 3) and fetal outcome (Figure 4) has been elucidated in Table 2.

### Discussion

Proportion of primiparity was significantly high (p<0.0001). As high as 90 (10.2%) patients had hypertension (p<0.0001). Prevalence of hypertension in pregnancy was found to be 6.9-7.8% in different studies.4 According to CDC prevalence of hypertension in female of age group of 20-34 yrs is 6.8%. In our study magnitude of hypertension was even higher in mothers having migraine, 3 (15.0%) out of 18 mothers with migraine had hypertension (p=0.04444). The prevalence of hypertension among our cases exceeded the prevalence of headache.

Proportion of Diabetes mellitus 26 (2.9%) patients was significant. Systemic analysis of worldwide data suggests GDM prevalence ranged from 0.40 to 24.3% and pre-existing DM (type I and 2) ranged from 0 to 0.7%.9,11

We found that 91 (10.3%) patients had neurological disorder (Z=33.448; p<0.0001). Primary headache (30.8%), epilepsy (19.1%) and peripheral neuropathy (27.6%) formed the bulk were the others were secondary headache (3.2%), low back pain (8.5%), cerebrovascular disorders (6.4%), myasthenia gravis (1.05%), Wilson’s disease (1.05%), spinal intradural tumor (1.05%) and CNS tuberculosis (1.05%). Table 3 shows the comparative analysis of different studies done worldwide.

In our study the incidence of primary headache was 30.8% among the diseased population and 3.3% in the study population. Among the causes of primary headache, migraine was found to be most common (69%) followed by tension type headache (27.6%) and cluster headache (3.4%). According to the study primary headache was not found to adversely affect feto-maternal outcome; expressed in terms of mode of delivery, gestational age at delivery, neonatal birth weight, APGAR score at birth (1 min and 5 mins), need for nursery admission and maternal condition at discharge.12,13
The incidence of peripheral neuropathy was 27.6% in the diseased group and 2.9% in the entire study population. Carpal tunnel syndrome was found to be the commonest cause. Carpal tunnel syndrome is one of the commonest neurovascular disorders feto-maternal outcome in pregnancy. The study population shows a considerable number of epilepsy patients, though contrary to popular believe outcome is good and at par with normal pregnancies. Only in two subgroups, namely PRES and cerebrovascular disorders feto-maternal outcome is not that bright in our study. However intensive care management both for mother and neonate can still reverse the situation. Few diseases like meningitis and metabolic encephalopathy which were not encountered in our study also require prompt diagnosis and aggressive management. Now a days it can be appropriately said that when a women become pregnant the question is no longer whether to continue or discontinue pregnancy with such disorder, rather the issues are early detection and appropriate management of the neurological disorder to have a favorable outcome with respect to both mother and child.

**References**

19. Wilterdink JL, Easton JD. Cerebral ischemia in pregnancy. Adv Neurol 2001; 88:25-34 per 1, 00,000 deliveries, the hospital based study our sample size is smaller and based on prior studies which did not look into all the neurological problems. Our screening questionnaire is not internationally accepted. Its validity and reliability was judged based on a small pilot study. The causal relationship of neurological complications and pregnancy in our cases could not be established in our cases in absence of a multivariate analysis.
Autoimmune Hemolytic Anemia in Chronic Liver Disease following COVID-19 Episode

Aravind Rajeev¹, NN Padmakumar²

Abstract
This report describes a case of Autoimmune Hemolytic Anemia that was possibly induced by COVID-19 in a patient with history of Chronic Liver Disease and Diabetes Mellitus. Autoimmune responses like hemolytic anemia are known to be triggered by viral infections. COVID-19 is reported to be inducing Autoimmune Hemolytic Anemia in susceptible individuals. This is a case report of a 65 year old male with history of chronic alcoholism, who tested positive for COVID-19 infection which was treated as per protocols and was uncomplicated at the time of discharge. After about three months he presented with complaints of breathlessness which on laboratory evaluation revealed Direct Coombs test positive hemolytic anemia. Anemia improved with blood transfusion and steroid administration but patient eventually developed hepatorenal syndrome and expired.

Introduction
Autoimmune blood diseases have been reported following viral infections. Infections with recent SARS-CoV-2 virus have also been reported to induce such autoimmune responses. Such incidences are fatal in patients with comorbidities and unfavorable general condition at the time of presentation. Autoimmune hemolytic anemia (AIHA) is a general term for anemia caused by hemolysis due to an autoantibody attack on red blood cells. It is caused by immunoglobulins binding to red blood cells mediating destruction. It is associated with lymphoproliferative diseases, autoimmune diseases, infections (HIV, Mycoplasma pneumoniae, hepatitis C, Epstein-Barr virus, and cytomegalovirus) and medications.¹ Alcoholic Liver Disease and viral hepatitis are also known associations. Infection with SARS-CoV-2 leads to the syndrome of Coronavirus Disease 2019 (COVID-19). Several case reports and case series have implicated an association between COVID-19 infection and AIHA.¹

Case Report
A Male patient aged 65 years with unremarkable medical history except a history of drinking approximately 800 mL (90g of pure alcohol) per day for approximately 35 years was admitted in the hospital. He had complaints of abdominal discomfort for the last 2 year. For these complaints, he tried home remedies and alternative medicines at a local medical facility on an outpatient basis. He then developed flu-like symptoms and was diagnosed to have COVID-19 for which he was admitted and managed conservatively as per treatment protocols. Clinical presentation and laboratory workup during the period of admission was otherwise uneventful. He was asymptomatic at discharge. No history of any other infections or vaccinations in the following period. After about 3 months, he presented at the hospital with complaints of shortness of breath and fatigue and abdominal discomfort. He was having tachycardia and tachypnoea. Physical examination revealed normocephalic head, icteric sclera, clear conjunctiva with pallor and normal tongue. Skin was pale. On neurological examination, he was alert and oriented. Cranial nerves were grossly intact. Musculoskeletal system demonstrated full, active range of motion in all extremities on gross evaluation. There was no clinically evident lymphadenopathy. Ascites and swelling in the legs were observed. On systemic examination, Cardiovascular system: Normal heart sounds, Respiratory system: Normal vesicular breath sounds, Central Nervous System: Higher mental functions normal and Per abdomen: soft, nontender, mildly distended, bowel sounds heard, hepatomegaly present.

 Routine Blood investigations confirmed anemia. Peripheral smear showed microcytic anemia with spherocytes, thrombocytopenia with normal platelet morphology, no atypical cells or parasites. Direct Antiglobulin Test (DAT/Coomb’s) was positive and he was diagnosed with AIHA. ANA screen was negative. HbA1c was 7.20. The

Table 1: Lab parameters of the patient

<table>
<thead>
<tr>
<th>Parameter</th>
<th>On Admission</th>
<th>Third Week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin</td>
<td>6.5g/dL</td>
<td>8.5g/dL</td>
</tr>
<tr>
<td>RBC</td>
<td>1.02million/mm³</td>
<td>3.28million/mm³</td>
</tr>
<tr>
<td>PCV</td>
<td>17.7%</td>
<td>28.4%</td>
</tr>
<tr>
<td>Platelets</td>
<td>100,000/mm³</td>
<td>80,000/mm³</td>
</tr>
<tr>
<td>Serum Creatinine</td>
<td>1.5mg/dl</td>
<td>7.3mg/dl</td>
</tr>
<tr>
<td>Serum Urea</td>
<td>117mg/dL</td>
<td>347mg/dL</td>
</tr>
<tr>
<td>AST</td>
<td>62 U/L</td>
<td>68U/L</td>
</tr>
<tr>
<td>ALT</td>
<td>115 U/L</td>
<td>64U/L</td>
</tr>
<tr>
<td>Albumin</td>
<td>2.27 g/dL</td>
<td>2.62g/dL</td>
</tr>
<tr>
<td>Total Bilirubin</td>
<td>2.41mg/dL</td>
<td>7.44mg/dL</td>
</tr>
<tr>
<td>Direct Bilirubin</td>
<td>0.89mg/dL</td>
<td>4.11mg/dL</td>
</tr>
<tr>
<td>Indirect Bilirubin</td>
<td>1.52</td>
<td>3.33mg/dL</td>
</tr>
<tr>
<td>Serum LDH</td>
<td>853.8 U/L</td>
<td>380 U/L</td>
</tr>
</tbody>
</table>

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Received: 12.11.2021; Accepted: 24.01.2022
After hospitalization, intravenous administration of diuretics and albumin alleviated ascites. Blood sugar levels were controlled with insulin therapy. He was treated with transfusion of Fresh Frozen Plasma, Erythropoietin injection and prophylactic antibiotics. Repeated transfusions were needed to maintain his blood cells and hemoglobin count. The patient was administered prednisolone (60 mg/day). Signs and symptoms of anemia were alleviated with transfusions and steroids. However, the liver failure progressed. Supplementary and supportive medicines for liver and renal failure were added. On the third week of admission patient became disoriented and showed signs of hepatic encephalopathy, which duly progressed from stage 1 to 4. Renal function also deteriorated. Patient was hypotensive and bradycardic and at his condition nephrologist invalidated hemodialysis. In the third week of hospitalization, he died of hepatorenal syndrome.

**Discussion**

At this juncture India is past two waves of COVID-19 pandemic and vaccination drive is going on. With a huge population in post infectious stage our next hurdles will be tackling the post infectious complications of the disease. Viral diseases are known to trigger AIHA. Cases of COVID-19 induced AIHA are also reported. Two criteria must be met to diagnose AIHA: serologic evidence for an autoantibody and clinical or laboratory evidence for hemolysis. Serologic evidence for autoantibody is provided by positive DAT. A CBC with peripheral smear, bilirubin, LDH are used to evaluate hemolysis. The presence of spherocytes in blood films is a stronger indicator of AIHA. Reports of cases of AIHA with autoimmune hepatitis, viral hepatitis and alcoholic liver disease have been documented. AIHA in association with autoimmune hepatitis is suggested to be due to cross reactivity with viruses such as HAV and EBV in susceptible individuals. The pathogenesis of AIHA secondary to viral infection may involve activation of B cells in response to the viral infection, production of autoantibodies in response to an exogenous antigen that is similar to autoantigens, activation of macrophages by cytokines expressed after viral infection, and acceleration of phagocytosis of erythrocyte-bound autoantibodies.

The clinical spectrum of symptomatic COVID-19 cases ranges from mild to critically ill. Autoimmune disorders including immune thrombocytopenia, Guillain-Barré and antiphospholipid syndrome have been recognized in the context of COVID-19. A study reported that among COVID-19 patients, 14% of anemic ICU admissions and 9% of other anemic admissions tested positive for DAT and spherocytosis.

Hepatorenal syndrome (HRS) is a common complication of advanced cirrhosis, characterized by renal failure and major disturbances in circulatory function. Renal failure is caused by intense vasoconstriction of the renal circulation. The diagnosis of HRS is currently based on the exclusion of other causes of renal failure. The prognosis is very poor, particularly when there is rapidly progressive renal failure (type 1).

The patient in our report had history of chronic alcohol intake. Low serum albumin was suggestive of chronic liver disease which was confirmed by sonographic findings. On evaluation he had AIHA, thrombocytopenia and diabetes mellitus. Splenic sequestration and decreased growth factors due to liver disease is to be considered as a cause of thrombocytopenia. Evan’s syndrome precipitating ITP along with AIHA is another possibility. Multiple transfusions with blood products were needed to maintain blood cell levels. Corticosteroids are the cornerstone of treatment in warm AIHA and the patient responded to it. But he eventually developed hepatic encephalopathy and hepatorenal syndrome. The underlying liver disease made interpretation of lab results difficult and prognosis of the disease worse in this case. Episodes of post infectious autoimmune diseases have been reported within weeks after the triggering infection. Considering that, initial presentation of our patient might have been at a later and worsened stage. A routine evaluation in post infectious stage in susceptible comorbid individuals and prompt suspicion of the disease will hopefully give a better outcome for patients.

**Conclusion**

This report describes a case of Autoimmune Hemolytic Anemia that was possibly induced by COVID-19 in a patient with history of Chronic Liver Disease and Diabetes Mellitus. COVID-19 is reported to be inducing AIHA in susceptible individuals. AIHA responds to steroids and blood transfusion. The outcome of the disease depends on underlying comorbidities of the patient and general condition at the time of presentation.

**Acknowledgements**

This report did not receive specific grants from any funding agencies in the public, commercial, or non-profit sectors.

**References**

Recent years are witnessing emergence of newer and newer International and national Health/Diseases awareness days in a calendar year. These days are usually set by a major organization like WHO, UN or government. Dates are health related, and help raise awareness of health conditions, medical illnesses, research, key health or ethical issues. It also mobilizes support for action from local community to the international stage.

There are many days in this category, but WHO Member States have mandated focus on the 9 days as “official” Global public health day like: World TB Day (24th March), World Health Day (7th April) along with others. India is actively promoting World Yoga day (21st June).

It is difficult to define rare diseases in the world. In US, a rare disease is defined as a condition that affects fewer than 200,000 people. This would translate to 800,000 people in India as per population. Definition was created by US Congress in the Orphan Drug Act of 1983. Rare diseases became known as orphan diseases because drug companies were not interested in adopting them to develop treatments. The Orphan Drug Act created financial incentives to encourage companies to develop new drugs for rare diseases.

There are 7,000 Genetic and Rare Diseases (GARD) in the world. GARD organization has maintained a list and detailed database of rare diseases and other terms to help people find reliable information.

Rare Disease Day is observed on the last day of February to raise awareness for rare diseases and improve access to treatment for such individuals and their families. The first Rare Disease Day was coordinated and held in European nations and Canada by the European Organization for Rare Diseases on February 29, 2008. Date was chosen because February 29 is a “rare day” and 2008 was the 25th anniversary of the Orphan Drug Act.

Due to vast number of rare diseases there is limited public, patient and physician knowledge and the signs/symptoms to look for. Physicians may only have come across some of these rare conditions once (if at all) during their career. It is said that rare diseases can often hide behind the symptoms of common illnesses.

Diagnosis is therefore challenging and misdiagnosis as unusual form of common diseases, forces patients to see multiple doctors and undergo multiple tests.

Despite this, old adage: “Uncommon manifestations of common disease are more common than rare disease” has proved right, time and again for many.

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Hepatocellular Carcinoma Evaluation and Management for Physicians: Joint Gastroenterology Research Society and Association of Physicians of India Guidelines

Abstract
Hepatocellular carcinoma (HCC) is the most common cause of, and accounts for almost 90% of all liver cancers. Data from India is limited especially due to cancer not being a reportable disease and in view of wide variation in diagnostic modalities. This document is a result of a consensus meeting comprising Hepatologists, Interventional Radiologists, Hepatobiliary surgeons, medical and surgical Oncologists nominated by the Association of Physicians of India and Gastroenterology Research Society of Mumbai. The following Clinical Practice Guidelines for practicing physicians is intended to act as an up to date protocol for clinical management of patients with hepatocellular carcinoma. The document comprises seven sections with statements and sub-statements with strength of evidence and recommendation.

Introduction
Hepatocellular carcinoma (HCC) is the most common cause of, and accounts for almost 90% of all liver cancers. Advancing age increases the risk of acquiring HCC with the peak age being around 70 years across populations barring China and sub-Saharan Africa where there is a trend towards a younger age group. The mortality burden attributable to HCC is immense, ranking 4th in the list of cancer deaths globally with almost 0.8 million death per annum. Data from India is limited especially due to cancer not being a reportable disease and wide variation in diagnostic modalities – imaging, cytology or histology and blood markers like alpha-fetoprotein. The source of information hence is hospital and population based cancer registries. This gives a projected cumulative risk of HCC in both sexes for HCC to be 1 in 277 persons, over 0-74 years of life. The age adjusted incidence rates (AAIR) for HCC are variable within India. In the previous ICMR- population based registry from 2008, Sikkim (7.5/100,000) and Mizoram (6.4/100,000) reported the highest AAIR. The data from tertiary care centers in India in the past 2 decades is indicative of the fact that 70-97% of the cases of HCC had underlying cirrhosis, most common cause being hepatitis B.

With this background it becomes essential that community practice is undertaken with more vigilance in terms of identifying the HCC early and prompt effective therapy to achieve good outcomes. In order to achieve this, the physician should have knowledge of the setting, clinical clues, available data from National Cancer Registry Program in India as of June 2020 is based on 28 population based cancer registries and 58 hospital based cancer registries. This gives a projected cumulative risk of HCC in both sexes combined for 2020 to be 1 in 277 persons, over 0-74 years of life. The age adjusted incidence rates (AAIR) for HCC are variable within India. In the previous ICMR- population based registry from 2008, Sikkim (7.5/100,000) and Mizoram (6.4/100,000) reported the highest AAIR. The data from tertiary care centers in India in the past 2 decades is indicative of the fact that 70-97% of the cases of HCC had underlying cirrhosis, most common cause being hepatitis B.

With this background it becomes essential that community practice is undertaken with more vigilance in terms of identifying the HCC early and prompt effective therapy to achieve good outcomes. In order to achieve this, the physician should have knowledge of the setting, clinical clues,....
surveillance and diagnostic methods, staging of HCC and various therapeutic measures including indications for liver transplantation.

Consensus Process

This document represents the outcome of consensus meeting held virtually on 24th December 2020, with invited experts in the field of hepatology, interventional radiology, medical and surgical oncology and hepatobiliary surgery from across the country, nominated by the Association of Physicians of India and Gastroenterology Research Society of Mumbai. Participants at the meeting presented literature and recommendations on specific areas of the disease, each of which was deliberated by a panel of experts. At the end of each section, the members voted on the recommendations using the nominal voting technique as per the standard guidelines. The quality of evidence has been adapted from the Grades of Recommendation, Assessment, Development and Evaluation (GRADE) system (Table 1). The final draft of recommendation was circulated among the experts and was approved. This article aims to provide a comprehensive guideline for physicians in daily clinical practice addressing the challenges faced in hepatocellular carcinoma diagnosis and treatment in resource limited settings.

Clinical clues: When to suspect HCC?

Cirrhotic patients form the major proportion of “at risk group” for HCC especially in adults. In the pediatric population, HCC in non-cirrhotic liver is seen in 26-62%. It is postulated that overall one-third of cirrhotics will develop HCC in their lifetime. Literature from the Indian subcontinent reports the incidence of HCC to be 1.6% per year in cirrhotics. Most frequent causes are hepatitis B and C, alcohol, intake and aflatoxin exposure as per western literature.1 With growing insight into NAFLD, studies have suggested an increased incidence of HCC in this group of patients especially with associated metabolic syndrome, diabetes and obesity. Interestingly, there is an additive effect of these factors when superimposed upon chronic viral hepatitis.

Hepatocellular carcinoma is often asymptomatic in early stages, and the advent of symptoms may be ominous. The triad of right upper quadrant pain, palpable mass and weight loss may be seen in symptomatic patients.2 Abdominal distention and abdominal pain are the more common symptoms according to different series and new onset of such symptoms in a chronic liver disease should raise suspicion.3,4 Bruit over the liver is seen in a small proportion but when present signifies advanced stage and is deemed as a reliable diagnostic sign.5 Decompensation of cirrhosis with jaundice, hepatic encephalopathy or ascites may occur due to development of HCC.6 Rapid development or worsening of portal hypertension may suggest invasion of the tumor into the portal structures. Non cirrhotics on the other hand have an insidious presentation and present more often with malaise, anorexia, wasting, right upper quadrant pain, and abdominal distention. Sudden severe pain, hypotension, shock and abdominal distention can occur in the event of a catastrophe like rupture of a peripherally located tumor into the peritoneum or bleeding into a tumour.7 Bone, lung and abdominal viscera are the common sites of distant metastasis. Bone pain may at times be the initial manifestation of HCC.8 Paraneoplastic syndromes like watery diarrhea, due to gastrin and vasoactive intestinal peptide secretion, hypoglycemia, hypocalcemia and feminization are described as rare features.9 Those in whom HCC is identified prior to presentation as acute decompensation are known to be better responsive to aggressive chemotherapy and have a better outcome.10

Statements

1.1. Early detection of hepatocellular carcinoma is associated with better outcomes. Attempts should be made to diagnose HCC in the asymptomatic phase during surveillance. (evidence moderate, recommendation strong)

1.2. HCC should be suspected in patients with acute decompensation of cirrhosis, sudden deterioration of liver biochemistry, new onset right hypochondriac discomfort in a cirrhotic, new onset ascites or worsening of ascites, acute variceal bleed and appearance or increasing size of any new lesion on imaging in a cirrhotic (evidence moderate; recommendation strong)

Surveillance of HCC

Surveillance refers to the periodic application of a diagnostic test to individuals at specific risk of developing a given disease. Hepatocellular carcinoma (HCC) is the fourth leading cause of cancer related deaths worldwide10 and is the fourth leading cause of cancer related mortality in men in India.11 Although there is lack of data on HCC from a national registry, data from registries in various cities of India, have shown a significant increase in the incidence of HCC.12 HCC is associated with very poor prognosis except for the subgroup of the patients who are diagnosed at an early stage and hence, are amenable to curative therapy in the form of resection, ablation or liver transplantation. However, majority of the patients of HCC are diagnosed at a later stage in India precluding use of curative therapy. Hence, there is a need for active surveillance of patients at risk to diagnose HCC at an early stage which will translate to reduction in disease-related mortality.

Outcome of surveillance

Surveillance of HCC helps in detection of the tumor at an early stage and helps in timely intervention to reduce to mortality. To study the benefit of surveillance in hepatitis B (HBV) carriers, the first randomized control trial (RCT) was done in China, in which 17,820 patients with chronic HBV infection were randomized to surveillance with six monthly ultrasound and serum alpha fetoprotein (AFP) measurement versus no surveillance. The surveillance group had a greater number of patients who were diagnosed at a subclinical stage (76.3% vs. 0, P < 0.01), greater number of patients who underwent resection for HCC (70.6% vs. 0, P < 0.05) and a higher 1-year and 2-year survival rate among those diagnosed with HCC (88.1% and 77.5%, respectively), compared to 0% at 1 year for HCC patients in the no-surveillance group (P < 0.01).13 Subsequently, the same group conducted another RCT among 18,816 HBV carriers and reported a higher early tumor detection, higher rate of curative treatment along with lower mortality among patients randomized to surveillance for HCC.14

However, there is lack of a similar RCT in patients with cirrhosis of liver and the data from surveillance of HBV
carriers cannot be extrapolated to this population, hence, a meta-analysis consisting of 47 studies (including 15,158 patients with cirrhosis) was published, which demonstrated higher tumor detection rate (odds ratio [OR], 2.08; 95% CI, 1.80–2.37) and overall survival (OR, 1.90; 95% CI, 1.67–2.17) in patients undergoing periodic surveillance.25 In a retrospective study from India, patients undergoing surveillance were more likely to be diagnosed at an earlier stage and had higher survival (Stage 0/A: 15.6 ± 14.2 months vs. Stage B/C: 9.43 ± 19.7 months vs. Stage D: 5.59 ± 11.9 months; p = 0.0006). While treatment for HCC improved overall survival, only 28% of eligible patients received treatment, explaining the lack of survival benefit noted in the surveillance group.26 Taking all these data into consideration, surveillance is recommended in the population at risk for developing HCC.

**Target populations**

**Cirrhotic patients**

The annual incidence of HCC is 2.0 – 6.6% in patients with cirrhosis & in patients of HCC, underlying cirrhosis is found in 80 – 90% of cases.27,28 Cost-effectiveness studies in western patients have shown that surveillance for HCC in cirrhotics would be beneficial if the incidence is 1.5%/year or greater, irrespective of etiology while the threshold in non-cirrhotics for the same is 0.2%.29,30 Hence, surveillance is currently recommended in all patients of cirrhosis due to any etiology. But, presence of advanced liver failure (Child–Pugh class C) prevents effective HCC therapies from being employed. A study from Italy compared the effect of surveillance in Child-Pugh class B and class C patients. In Child-Pugh class B, the median survival was 17.1 (95% CI 13.5–20.6) in the surveillance group versus 12.0 (95% CI 9.4–14.6) months in the no surveillance group (p = 0.022). But, in Child-Pugh class C, the median survival was 7.1 (95% CI 2.1–12.1) months in the surveillance group versus 6.0 (95% CI 4.1–7.9) months in the no surveillance group (P = 0.740).31 Hence, individuals with Child–Pugh class C should be assessed for liver transplantation. If patient is not a candidate for liver transplantation surveillance has no additional benefit.

**Non-cirrhotic patients**

Patients with chronic hepatitis B virus (HBV) infection are at risk of HCC development even in the absence of cirrhosis. The risk varies with geographical distribution (higher in Asia and Africa than Western countries), higher levels of HBV replication, age and gender (males higher than females).32-35 These patients have lower risk compared to patients with cirrhosis but have higher risk than general population which increases with age. In a cohort study of males belonging to multiple race and age groups, risk of HCC was highest among Asian Pacific Islanders, followed by whites and African Americans. Also, regardless of race, annual incidence of HCC was more than 0.2% for all patients older than 40 years with high levels of alanine aminotransferase.36 A similar HCC incidence rate of 0.2 per 100 person-years has been observed in inactive carriers with chronic HBV infection from East Asian countries.37 Asian females > 50 years of age and patients with family history of HCC are also at increased risk of HCC. Hence, surveillance should be offered in the above subset of patients as these patients can be offered curative resection for HCC.

Patients with chronic hepatitis C (HCV) and bridging fibrosis in the absence of cirrhosis are also at risk of being under-staged and thus at significant risk of HCC.37 Transient elastography is a promising tool in such patients which can help in stratifying HCC risks in patients with active viral replication,38,39 enabling surveillance for patients with high risk. However, routine surveillance in non-cirrhotic HCV patients in not recommended.

HCC can develop in patients of non-alcoholic steatohepatitis (NASH) without cirrhosis and in a meta-analysis, non-cirrhotic NASH subjects were at greater odds of developing HCC than non-cirrhotic subjects of other etiologies (OR 2.61, 95% CI 1.27–5.35, P = 0.009).40 However, the incidence of HCC in this group is not expected to be high enough to be candidates for universal surveillance, given the quantum of prevalence of NAFLD in the general population. As the number of NASH patients is on rise, there is a clear need to prospectively acquire information on cohorts of patients with NASH, in order to define high-risk patients who should undergo surveillance.

**Modalities of surveillance**

**Radiographic surveillance tests**

Ultrasoundography (USG) of liver is regarded as a standard surveillance test. It is the most commonly used method being non-invasive, relatively inexpensive, and without any associated risk of radiation. In an RCT of USG with or without AFP for surveillance in HBV patients, the sensitivity of ultrasound was 84% for any stage HCC and 63% for early-stage HCC.41 However, in patients with cirrhosis, USG may have a suboptimal performance due to the presence of fibrous septa and regenerative nodules, which appear as a coarse pattern on ultrasound and may mask the presence of a small tumor. In a meta-analysis, the sensitivity and specificity of USG for detection of HCC at any stage were 84% (95% CI, 76%–92%) and 91% (95% CI, 86%–94%), respectively, but, the pooled sensitivity of ultrasound was only 47% (95% CI, 33%–61%) for detection of early-stage HCC.42 Hence, it is recommended that USG of liver for HCC surveillance should be done by an expert radiologist.

Cross sectional imaging modalities, such as computerized tomography (CT) or magnetic resonance imaging (MRI) are expected to have a higher accuracy for diagnosis of HCC. However, sensitivity of cross-sectional imaging is similar to USG. In an RCT comparing 6-monthly USG and yearly triphasic CT for HCC surveillance, biannual US was found to be more sensitive (71.4%) when compared to CT (66.7%) with lower overall cost.43 In a study from India on economic evaluation of HCC surveillance showed that 6-monthly USG with AFP and yearly CT is not cost effective both from the hospital and the patient perspective.44 MRI-based surveillance with liver specific contrast has been shown to have higher sensitivity for detection of early HCC compared with ultrasound (83.7% vs 25.6%; P < 0.001) with fewer false-positive findings (3.0% vs 5.6%; P = 0.004).45 However, use of MRI is limited by high cost, higher time consumption & low accessibility.

**Sero logical surveillance tests**

AFP is the most common and best-studied biomarker for HCC with a level of 20 ng/ml being the most commonly used cut-off for further evaluation. However, biomarkers alone have low sensitivity for surveillance. In a
study evaluating the biomarkers AFP had the best area under the receiver operating characteristic curve (0.80, 95% confidence interval [CI]: 0.77–0.84), followed by des-gamma carboxyprothrombin (DCP) (0.72, 95% CI: 0.68–0.77) and lectin-bound AFP (AFP-L3%) (0.66, 95% CI: 0.62–0.70) for early-stage HCC and the sensitivity of AFP was 66%.46

Combination of radiological and serological tests

Although current serological biomarkers are not sensitive when used alone, their addition to USG for surveillance has been proposed as a mean of increasing the sensitivity of surveillance. In meta-analysis on surveillance imaging and AFP for detection of HCC in cirrhosis, USG with AFP demonstrated a higher sensitivity of 63% (95% CI, 48%–75%) for early-stage HCC compared to sensitivity of 45% (95% CI, 30%–62%) with USG alone (P = 0.002).42

Interval of surveillance

HCC surveillance is recommended to be performed every 6 months. To determine the optimal interval of surveillance for HCC, a retrospective analysis of prospectively maintained multi-center Italian database was performed, which showed a better overall median survival of 40.3 months in the 6-monthly surveillance group, compared to 30 months in the 12-monthly surveillance group (P = 0.03).47 A subsequent RCT was conducted to evaluate if reducing the surveillance interval to 3 months led to improved outcomes. Although the 3-months surveillance group had higher incidence of non-malignant lesions, similar number of patients in both 3-months and 6-months group were detected with HCC at an early stage (79% vs. 71%; P = 0.40) and similar proportions received curative therapies (62% vs 58%; P = 0.88).48 Overall, these data provide support for the 6-month surveillance interval.

Statements

2.1. Following group of patients are at high risk for HCC and should undergo HCC surveillance (evidence moderate; recommendation strong):

- Cirrhotic patients, Child-Pugh stage A and B
- Cirrhotic patients, Child-Pugh stage C awaiting liver transplantation
- Non-cirrhotic HBV patients (male > 40 years, female > 50 years, or family history of HCC)

2.2. Surveillance should include USG and serum AFP (evidence moderate; recommendation strong).

2.3. Surveillance should be done at an interval of 6 months (evidence moderate; recommendation strong).

Diagnosis of HCC

Investigations for diagnosis of HCC

Regenerative nodules developing in cirrhotic liver derive their blood supply from portal vein predominantly like that of liver parenchyma.49 As the nodule changes to dysplastic and HCC its predominant blood supply comes from hepatic artery.49 This forms the basis of diagnosis of HCC by non-invasive methods using multiphase CT or MRI scans.49 Although radiology plays a pivotal role in non-invasive diagnosis of HCC sometimes biopsy is necessary.

Radiological diagnosis of HCC

Imaging modalities available for diagnosis of HCC include multiphase computed tomography scan (CT), multiphase magnetic resonance imaging (MRI) with extracellular contrast, contrast enhanced ultrasound(CEUS) and multiphase magnetic resonance imaging(MRI) using hepatobiliary contrast agents. Technical requirements for CT, MRI and CEUS are as mentioned below.50

1. Multiphase CT scan : CT scanner should have > 8 detector rows. Following phases should be captured
   a. Non contrast phase b) Arterial phase (average 30 seconds with bolus tracking) c) Venous phase at 65 seconds d) Delayed phase at 240 seconds with injection rate minimum 4 ml/s. Slice thickness should be of 3 mm with no overlap. Multiplanar reformations should be done whenever necessary.50

2. Multiphase MRI scan with extracellular contrast: It should be at least 1.5 Tesla. There should be torso phased array coil. Following images should be captured Unenhanced T1 weighted, b)T2 weighted imaging, c) Contrast in T1 weighted imaging with arterial phase, portal venous phase and delayed phase like multiphase CT scan. Slice thickness should be <5 mm. Additional imaging techniques often used include diffusion weighted imaging, subtraction imaging, fat suppression and multiplanar acquisition.50

3. Multiphase MRI scan with hepatobiliary contrast: Gadoxetate disodium and Gadobenate dimeglumine are used in hepatobiliary phase imaging. These contrast agents are taken up by hepatocytes and excreted from the kidney and from the liver through the bile duct. As a result, liver parenchyma is intensely enhanced showing definite hyperintensity in the hepatobiliary phase in addition to the diagnosis based on blood supply. There is transitional phase 2-5 minutes after contrast injection. Hepatobiliary phase occurs 20 minutes after injection for Gadoxetate disodium and needs 1-3 hours delay for Gadobenate dimeglumine. Nodules without liver parenchymal cells, such as liver cancer, are visualized as hypointense.50

4. Multiphase contrast enhanced ultrasound(CEUS): It is performed with intravenous injection of a microbubble contrast agent. Real-time imaging is performed continuously for the 1st minute to capture the arterial phase. This is followed by intermittent scanning every 30–60 seconds for up to about 5 minutes to evaluate washout. It requires expertise. Significant disadvantage of CEUS is that it can’t scan entire liver at a time like CT or MRI.50

Imaging features of HCC

Typical features of hepatocellular carcinoma include non-rim arterial phase hyperenhancement (APHE) and washout in portal venous phase. Enhancing capsule appearance visible on portal venous or delayed phase. Threshold growth is defined as an increase in size of mass by > 50 % in
< 6 months, measured in same phase sequence and plane (if possible). To measure the size of lesion largest outer edge to outer edge dimensions should be taken. Non-invasive diagnosis of hepatocellular carcinoma is applied for liver lesions of > 1 cm in cirrhotic patients due to high pre-test probability. Several ancillary features are described which increase likelihood of a lesion being HCC and include hyperintensity on T2-weighted MRI, hyperintensity on diffusion-weighted MRI, intra-lesional fat, lesional iron sparing, corona enhancement, presence of capsule, mosaic architecture, nodule-in-nodule architecture, intra-lesional haemorrhage.

Comparison of different imaging modalities is as enlisted in Table 2 and approach to a nodule in cirrhotic liver is depicted in Figure 1.

CEUS has low sensitivity for detection of lesion as compared to CT and MRI but has higher specificity as compared to CT and MRI especially for small nodules (< 20 mm) 92.9% vs. 76.8% vs 83.2%. CEUS as second imaging modality has highest specificity 76.8 (after MRI) and 70.7 (after CT). MRI with hepatobiliary contrast agents compared to MDCT and MRI with IV contrast had higher specificity for diagnosis of lesions < 1 cm (78% vs 71% vs 71%) and 1-2 cm (94% vs 91% vs 89 %). For the lesions less than < 1 cm repeat imaging with same modality which diagnosed liver space occupying lesion after 4-6 months to see change in character or size. Special imaging techniques CEUS or MRI with hepatobiliary contrasts should be used if available in case of high suspicion for HCC in cirrhotic patients. In lesions of 10–20 mm in size, the combination of CT and MRI had a specificity of 100%, but a sensitivity of 55.1%. CE-EUS can be considered in patients with a low estimated GFR< 30 mL/min/1.73m².

Liver imaging reporting and data system (Li-RADS) provides standardization for hepatocellular carcinoma (HCC) imaging. Li-RADS defines eight unique diagnostic categories (LR 1 to 5, LR-M for malignant but not specific for HCC, LR-TIV for tumour in vein, LR-TR for treated lesion) based on imaging appearance that reflect the probability of HCC or malignancy with or without tumour in vein. LR-3 is indeterminate for HCC. LR-4 is probable and LR-5 is
Table 3: Staging systems as per American Hepato-Pancreato-Biliary Association (AHPBA) HCC consensus conference 2010

<table>
<thead>
<tr>
<th>Clinical</th>
<th>Pathological</th>
<th>Transplant</th>
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<tbody>
<tr>
<td>1. Okuda staging</td>
<td>1. American Joint Committee on Cancer (AJCC) / International Union Against Cancer (UICC)</td>
<td>1. UNOS modified TNM staging system</td>
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<tr>
<td>2. IPBPA (International Hepato-Pancreato-Biliary Association)</td>
<td>2. Liver Cancer Study Group of Japan (LCSG) staging system</td>
<td>2. UCSF extended criteria</td>
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<tr>
<td>3. CLIP (Cancer of the Liver Italian Programme Score)</td>
<td>3. Japanese Integrated Staging (JIS) score (includes the LCSGJ)</td>
<td>3. Pittsburgh scoring system</td>
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<td>4. BCLC (Barcelona Clinic Liver Cancer)</td>
<td>4. Modified JIS</td>
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<td>5. Revised BCLC</td>
<td>5. New Liver Cancer Study Group of Japan</td>
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<td>6. CUPI (Chinese University Prognostic Index)</td>
<td>6. TNM (Tumour Node Metastasis) staging</td>
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<td>7. American Liver Tumor Study Group (ALTSG) modified Tumor-Node-Metastasis classification</td>
<td>7. Early HCC prognostic score</td>
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<td>8. Groupe d’Etude et de Traitement du Carcinome Hepatozellulaire (GRETCH)</td>
<td>8. Tokyo score</td>
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Table 4: Comparison of different staging systems for Hepatocellular carcinoma

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<th>Variables</th>
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<tr>
<td></td>
<td>Okuda</td>
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<td>Child Pugh score</td>
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<td>Total Bilirubin</td>
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<td>Alkaline phosphatase</td>
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<td>Alphafetoprotein</td>
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<td>Tumour size</td>
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<td>Number of nodules</td>
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<td>TNM stage</td>
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<td>Portal vein thrombosis</td>
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<td>Metastasis</td>
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<td>Portal Hypertension</td>
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<tr>
<td>General status</td>
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</table>

Abbreviations: CLIP: Cancer of the Liver Italian Program score, BCLC: Barcelona Clinic Liver Cancer, GRETCH: Groupe d’Etude et de Traitement du Carcinome Hepatozellulaire, CUPI: Chinese University Prognostic Index, MESIAH: Model to Estimate Survival in Ambulatory HCC patients, HKLC: Hong Kong Liver Classification, JIS: Japanese International Staging system

definite for HCC. LiRADS are applied to cirrhotic patients with lesion > 1 cm only. LiRADS 5 category has 96-100% specificity for diagnosis for HCC.

Role of 18F-FDG-PET scan

Although useful investigation in other malignancies it has very limited role in primary diagnosis of HCC. This is because HCC is not FDG avid tumour and well differentiated HCC has no FDG uptake. However it is useful in diagnosis of recurrent disease and facilitate the selection of patients for surgical resection or liver transplantation by detecting metastatic disease.

Tumour markers

Alfa-fetoprotein (AFP) is the only tumour marker which has undergone extensive evaluation. At cut-off of 500 ng/ml it has 50% sensitivity and >90% specificity in detecting presence of HCC in patients with background liver disease. However only 10-20% of early tumours has elevation of AFP. AFP can be falsely elevated in patients with exacerbation of underlying liver disease as in Hepatitis B flares. It is a better marker in monitoring response to therapy and detecting recurrence.

Newer biomarker like PIVKA II in combination with alfa-fetoprotein has sensitivity and specificity of 94% and 98% in diagnosis for HCC. Long non coding RNAs have sensitivity of 0.83 and specificity of 0.80 for diagnosis of HCC. These biomarkers still need extensive evaluation.

Role of biopsy in diagnosis

Routine use of biopsy is not recommended for diagnosis of HCC in patients with cirrhosis. In cirrhotics, LiRADS 5 category has 96-100% specificity for diagnosis for HCC. Biopsy is associated with risk of bleeding in 3-4% cases and severe bleeding requiring transfusion in 0.5% cases. Risk of needle track seeding of tumour cells is about 2.7%. Sampling errors can occur for small lesions < 2 cm. Biopsy is required to confirm the diagnosis of hepatocellular carcinoma.

1. Inconclusive imaging findings in patients with cirrhosis.
2. Lesions in non-cirrhotic liver.
3. Lesions with vascular liver diseases.
4. Elevation of CA 19-9, CEA or mixed HCC-cholangiocarcinoma.
5. Liver lesion without evidence of HCC risk factors.

In suspected nodules with negative biopsy close observation with follow up imaging at 3-4 month interval should be done.

Statements

3.1. The cornerstone of diagnosis of HCC is radiology. Multiphase CT and multiphase MRI are modalities of choice for evaluation of liver lesions in cirrhotic patients. (evidence high; recommendation strong)

3.2. Classical features on radiology include non-rim arterial phase hyperenhancement (APHE) AND washout in portal venous or delayed phase, enhancing capsule appearance on multiphase CT or MRI. (evidence high; recommendation strong)

3.3. Diagnosis of HCC by LiRADS is applicable to patients with cirrhosis only. (evidence high; recommendation strong)

3.4. PET scan should not be used for primary diagnosis of HCC. (evidence moderate; recommendation strong)

3.5. Normal AFP doesn’t rule out presence of HCC. (evidence moderate; recommendation strong)

3.6. Biopsy or FNAC is not necessary for diagnosis of HCC routinely in cirrhotic patients. Decision to biopsy should be taken based on multidisciplinary team meeting discussion. (evidence low; recommendation strong)

Staging of Hepatocellular Carcinoma

Hepatocellular carcinoma is a heterogeneous neoplasm with broad spectrum of biological behavior. Optimal staging strategies will result in uniformity in treatment selection and prognostication along with better comparison between study and treatment outcomes. Staging depends on various prognostic variables related to the patient factors, tumor related factors and the anatomical extent of the disease, treatment efficacy and interaction between them (Table 5). Liver function is the most important
The Okuda staging system was the first recognized system developed in 1985. In 2010, American Hepato-Pancreato-Biliary Association (AHPBA) consensus conference mentions use of 18 different clinical staging systems which were categorized into clinical, pathological and transplant grading systems (Table 3). There has been considerable evolution in the staging system depending on the prognostic variable predicting survival and guiding treatment decisions. Figure 2 and Tables 3 and 4 demonstrate evolution of important staging systems and outline their comparison. The Okuda, CLIP (Cancer of the Liver Italian Program score), MESIAH (Model to Estimate Survival in Ambulatory HCC patients), and ITA.LI.CA (Italian Liver Cancer) predict survival and the BCLC (Barcelona Clinic Liver Cancer), HKLC (Hong Kong Liver classification), and Alberta staging systems guide treatment decisions. Group-modified Tumor-Node-Metastasis classification, and the CUPI Chinese University Prognostic Index) are the other clinical staging systems that have been developed. Other systems are neither well validated nor widely adopted.

BCLC algorithm is widely accepted and endorsed by American Association for the Study of Liver Disease (AALSD) and the European Association for the Study of the Liver (EASL) for staging and management guidelines. HKLC has better discriminatory and prognostic power in the Eastern population. In a hypothetical Kaplan–Meier survival analysis, the median overall survival of those treated by HKLC was 16.56 months compared with 8.88 months when treated by BCLC. In BCLC-B patients classified as HKLC-II (BCLC-B/HKLC-II), for whom BCLC staging recommends TACE, the survival benefit of radical curative therapies over TACE was substantial (5-year survival probability, 52.1% vs 18.7%; P < .0001 by log-rank test). In BCLC-C patients classified as HKLC-II (BCLC-C/HKLC-II), for whom BCLC staging recommends systemic therapy, the survival benefit of radical curative therapies over systemic therapy was even more pronounced (5-year survival probability, 48.6% vs 0%; P < .0001 by log-rank test). However, in BCLC-C patients classified as HKLC-III (BCLC-C/HKLC-III), the survival benefit of TACE over systemic therapy was

### Table 5: Prognostic Variables in Hepatocellular carcinoma

<table>
<thead>
<tr>
<th>Patient Factors</th>
<th>Tumor factors</th>
<th>Liver factors</th>
<th>Etiology as factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. General medical conditions</td>
<td>1. Number and size of tumour</td>
<td>1. Child Turcotte Pugh score</td>
<td>1. Alcohol</td>
</tr>
<tr>
<td></td>
<td>4. Vascular invasion</td>
<td>4. Active inflammation</td>
<td></td>
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<tr>
<td></td>
<td>5. Serum AFP</td>
<td>5. Functional hepatic reserve</td>
<td></td>
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<tr>
<td></td>
<td>6. DNA aneuploidy (DNA index)</td>
<td>6. PIVKA-II serum levels</td>
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</tr>
<tr>
<td></td>
<td>7. Genotype</td>
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<tr>
<td></td>
<td>8. VEGF levels†</td>
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*: Protein induced by vitamin K absence/antagonism II; †: Measured by maximal removal rate of glycolated human serum albumin (GSA-Rmax); &: Model for End Stage Liver Disease; €: Vascular endothelial growth factor

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**Fig. 2**: Evolution of staging system in HCC Timeline for development of various clinical HCC staging systems

**Fig. 3**: Barcelona Clinic Liver Cancer (BCLC) staging system for HCC

determinant in survival in HCC patients with advanced liver disease whereas quality and type of treatment are important predictors of outcome in those without or limited liver disease.55-67
CTP score: Add score of each parameter to obtain total score
- Class A: 5–6
- Class B: 7–9
- Class C: 10–15

minimal but significant (3-year survival probability, 9.7% vs. 1.7%; P < .001 by log-rank test). With regard to early stage disease, there was no difference in median survival when comparing those treated by guidelines by BCLC or HKLC. However, more treatment episodes were triaged to this group by HKLC than BCLC and adherence was higher in HKLC than in BCLC. Patkar et al. showed that intermediate stage of BCLC staging system in 90% of surgically treated patients with HCC, despite BCLC guidelines recommending chemoembolization as a preferred treatment strategy for that stage. Therefore, it can be stated that BCLC is a widely accepted staging system but HKLC is more pragmatic in deciding treatment especially in early and intermediate staged tumor. HKLC provides separate classification of locally advanced tumor (stage 3b) and tumor with extrahepatic venous invasion or metastasis (stage 4). HKLC also has a unique stage Va for transplantable early HCC associated with Child C cirrhosis and ECOG >1. Multifocal tumors or intrahepatic vascular invasion are not considered contraindication for surgical resection under this system.

**Statements**

4.1. Staging of HCC is important for optimal treatment selection and prognostication. (evidence moderate; recommendation strong)

4.2. Staging system performance is highly variable as it depends upon many factors including patient demographics, type and extent of liver disease and treatment. (evidence moderate; recommendation strong)

4.3. BCLC is a widely accepted staging system but HKLC is more practical in deciding treatment especially in early and intermediate staged tumor. (evidence moderate; recommendation strong)

**Impact of Liver function on decision making in management of HCC**

HCC is a complex disorder with the background liver function also affecting outcomes. Most HCC patients have underlying primary liver disease. Decompensation of chronic liver disease precludes optimal management of HCC and portends a poor prognosis. Liver dysfunction may arise as a complication of liver directed therapy in HCC. As there are multiple treatment options available, understanding the HCC and underlying primary liver disease, liver function status and multidisciplinary treatment decision is important in these patients.

**Methods of assessment of Liver function**

1. Clinical features
2. Child-Turcotte-Pugh Score
3. Biochemical tests - ALBI score
4. Model for End-Stage Liver Disease Score (MELD)
5. Portal Hypertension assessment – HVPG, Radiological evaluation
6. FLR (Future Liver remnant)
7. Non-invasive measures of fibrosis and function - ICG 1

**Clinical signs and symptoms which suggest deranged liver function in HCC**

- Presentation as acute abdominal pain – Portal vein thrombosis or bleeding into tumor
- Presence of ascites
- Jaundice

**Child-Turcotte-Pugh (CTP) Score**

CTP score was developed in 1964 by Child and Turcotte (Table 6) to predict postoperative outcome in patients undergoing Porto-caval shunt. Later in 1973 it was modified by Pugh. So, it was not developed to assess liver function. Nevertheless, it is commonly used in decision making for management of HCC. Ideal candidate for surgical resection is patient with CTP A status. For same size lesion, stage of HCC changes with change in CTP status. As an example, a patient with CTP C cirrhosis irrespective of tumor size and extrahepatic metastasis status are classified as stage D according to BCLC staging system (Figure 3) and stage 5 according to HKLC staging system (Figure 4).

**Drawback of CTP score**

a. Subjective parameters (ascites, Hepatic encephalopathy)

b. “Floor and Ceiling effect” - The CTP scoring system is not able to discriminate the risk of liver failure in patients with low scores of 5–6 points in class A, that is, the “floor effect”.

Patients with CTP C exhibit a wide
range of cirrhosis severity, that is, the “ceiling effect”.79

Bioc hemal Test – ALBI (Albumin bilirubin) score

Johnson et al, proposed a new evidence-based objective liver function grading system based only on the serum albumin and bilirubin levels, the so-called ALBI grading system.79 ALBI score is calculated using formula

ALBI Score = (log10 bilirubin × 0.66) + (albumin × − 0.085)

Patients are classified in 3 grades as, ALBI grade I if score ≤ − 2.60, grade II if score is between − 2.59 to − 1.39 and grade III if score > − 1.39. ALBI score is useful in all stages of HCC. In early stages of HCC, patient with CTP A status undergoing surgical resection ALBI grade I has better outcome and lesser post-operative liver failure than ALBI grade II. ALBI grade II is also useful to better predict survival prior to TACE than CTP score in intermediate stage and superior to CTP in identifying patients at risk of early mortality & overall survival prior to Sorafentin.

MELD (Model for end stage liver disease) score

MELD score was developed by Patrick Kamath et al in 2002 at Mayo clinic to predict survival post TIPSS in refractory ascites or variceal bleeding.80 MELD score is calculated as: MELD = 3.78×ln[serum bilirubin (mg/dL)] + 11.2×ln[INR] + 9.57×ln[serum creatinine (mg/dL)] + 6.43

If the patient has been dialyzed twice within the last 7 days, then the value for serum creatinine used should be 4.0 mg/dL. Any value less than one is given a value of 1 (i.e. if absolute value of bilirubin is 0.8, a value of 1.0 is used). Presently, MELD score is used for organ allocation system in HCC. There is no superiority of MELD over CTP for patients who are not amenable to liver transplantation. Risk of decompensation increases post resection for patients with MELD score > 9.

Portal Hypertension Assessment

Clinically significant portal hypertension is defined as a hepatic vein pressure gradient greater than 10 mm Hg or the presence of esophageal varices or splenomegaly associated with a platelet count lower than 100 × 109/L.75 Assessment of portal hypertension can be done by measuring HVPG (drawback – invasive method) or indirect method like presence of varices on endoscopy or presence of collaterals in abdomen without any obvious vascular thrombosis. Presence or absence of portal hypertension has major impact on decision making and outcome in patient with HCC as shown in Figure 5.81

FLR (Future Liver Remnant)

FLR predicts the liver remnant after hepatectomy. Adequate FLR is essential to prevent post-hepatic liver failure (PHLF). FLR is calculated using CT volumetric analysis. The critical minimum FLR - 20% in normal livers and 25% in cirrhotic livers (CTP A without portal hypertension).82 The values are variable as per CTP status and portal hypertension. Though FLR is taken as marker for liver function however it is surrogate marker and liver volume varies in individual with age and weight.

ICG 15 (Indocyanine green 15)

ICG is dye with an inert, water-soluble dye. It has a protein binding close to 95%. It measures hepatic function and liver perfusion. After intravenous injection (the dosage of 0.5 mg/kg body weight), ICG is almost completely bound to proteins, is distributed in the blood within 2 to 3 min: Volume of distribution is very close to plasma volume and half-life is very short (3 to 5 min, longer in case of hepatic dysfunction), ICG is excreted unchanged and almost completely (97%) into the bile in a non-conjugated form. The results are expressed either as retention at 15 min (ICG R15) or plasma disappearance rate (ICG PDR). ICG R15 value is increased with hepatic dysfunction due to defective excretion. ICG R15 value should be 10-20% for major hepatectomy and 40% for minor hepatectomy.83 Test is not done in patients with serum bilirubin > 3 mg/dl due to false low values as both are excreted by same carrier in liver.

Application of Liver function test to decide best treatment modality for patient with HCC

A. Indication for surgical resection
Patient with
• CTP status A or early B (<8)
• MELD score ≤ 9
• No portal hypertension ( not an absolute C/I. Limited resection can be done)

B. Indication of Liver Transplantation
• Any patient with cirrhosis – Treatment of choice
• Patients who are not candidates for LR or RFA due to the severity of the liver disease
• Patients who present transplantable tumor recurrence or bad prognostic factors on pathological examination after curative treatment

C. Radio frequency ablation (RFA)

Indication: Best option for BCLC Stage 0 or stage I patient who may or may not candidate for surgical resection with lesion preferably <2cm

Contraindication
• Lesion is near to the main biliary tree, abdominal organs, or vasculature
• Ascites
• Platelet < 50000, INR > 1.5
• Bilirubin > 3 mg/dl
• Subcutaneous location

D. TACE

Indication
• Stage B BCLC
• Preserved liver function CTP A/B
• Stage 0/A: nor feasible for LR/LT
7 (no ascites), PS 0
• Single or Multinodular lesion
• No vascular invasion
• No extrahepatic spread
• Stage 0/A: nor feasible for LR/LT

Contraindications for TACE

Liver related factors
• Decompensated cirrhosis with CTP score > 8
• Portal venous thrombosis
• Tumor related factors
• Extensive tumor involving the both lobe of liver
• Extrahepatic metastasis
• Other factors
• Tumor > 10 cm
• Biliary dilatation
• Poor performance status

E. TARE

Indication: BCLC B patients who are
• Poor candidates for TACE
• Larger tumors (>2 segments) with portal vein invasion
• Progressive disease post-TACE
• CTP score < 7
Liver directed therapies for HCC and complication after hepatectomy and is associated with increased risk of underlying liver disease. MELD ≥9 therapy and require optimization of poor candidates for any tumor directed (evidence high; recommendation strong)

Assessment of liver function in patients with HCC is done using Clinical status of the patient and liver function. Contraindications: • Lesion located near stomach or small intestine • Lesion located near stomach or small intestine • Ascites • CTP A or CTP early B • Multiple tumors or large lesion • Severe Renal, Lung or CVS comorbidity

F. SBRT (Stereotactic body radiation therapy)

Indications:
• Lesion located on liver surface or large lesion or accompanying PVT

Contraindications:
• Adequate other organ function
• A good PS (ECOG <2)
• Child-Pugh A liver disease
• Adequate other organ function

5.3. Liver resection is treatment of choice for patient with non-cirrhotic HCC. (evidence high; recommendation strong)

Therapy in HCC

Management of HCC is complex, requiring optimization of the underlying liver disease and cancer directed therapy. The underlying liver disease in HCC may preclude optimal cancer directed therapy, eventually affecting outcomes. Also therapy for cancer may lead to decompensation of underlying liver disease. The presence of clinically significant portal hypertension may also impact treatment allocation. Hence, evaluation and management of underlying liver disease is of paramount importance in patients with HCC.

Hepatitis B (HBV) and Hepatitis C (HCV) are the most common causes of HCC in India. Antiviral therapy for HBV is associated with reduction in rates of decompensation in patients with HBV related chronic liver disease. Antiviral therapy with drugs with high barrier to resistance like Entecavir have been shown to improve CTP score in up to 50% decompensated HBV related chronic liver disease. Tenofovir in a previous comparative study has shown to improvement in CTP and MELD score in decompensated chronic liver disease. Antivirals reduce the risk of viral reactivation after Trans-arterial chemo-embolisation (TACE), Stereotactic body radiotherapy (SBRT) and surgery. Antivirals also reduce risk of HCC recurrence by virological control after surgery or liver transplantation. Continuation of antivirals lifelong for these patients will be useful.

Therapy for Hepatitis C is more challenging in the background of HCC. Directly acting antivirals (DAAs) have made therapy for hepatitis C safe and extremely efficacious at a low cost and finite duration of 12 to 24 weeks. DAAs are known to reduce risk of decompensation in HCV related chronic liver disease by almost 62% in a previous study of almost 26000 patients from the USA. DAAs also reduce risk of HCC in patients with chronic hepatitis C by almost 72%. They are also associated with improvement in CTP and MELD scores in patients with HCV related cirrhosis. Despite conflicting data on recurrence of HCC after cure in patients who received DAAs, Singhal et al. and Romano et al. have showed that the risk of HCC recurrence may be lower in patients who achieve Sustained Virological Response (SVR).

Issues remain the timing of HCV therapy with respect to tumor directed therapy. Also the likely benefits of HCV therapy may not accrue to patients with limited life expectancy and hence should be avoided in patients with advanced cancer with limited life span.

The rate of SVR is lower in patients with HCC (89.6%) than in those without HCC (93.5%) due to various factors. With respect to the timing of therapy, DAAs in early HCC (BCLC 0,A) can be considered after complete eradication of tumor. DAAs can be started 3 to 6 months after complete cancer control. For candidates undergoing Liver transplant, HCV treatment prior to LT can be considered in a) MELD 23-27 b) Those eligible for down-staging therapies prior to LT c) If wait-list for LT is long. In intermediate stage and advanced stage HCC (BCLC B & C), individualizing HCV therapy is needed and must be prioritized for patients with patients with higher life expectancy. Referral to a hepatologist for management of HCV is imperative.

Patients with other etiologies of liver disease like autoimmune hepatitis and Budd Chiari syndrome with HCC should be referred to an expert center for management by a hepatologist and multidisciplinary team. Management of complications of advanced liver
disease like ascites, gastrointestinal bleeding and hepatic encephalopathy take credeence over tumor directed therapy in HCC.106

Treatment allocation and multidisciplinary management of HCC

The BCLC and HKLC systems of staging account for patient performance status, underlying liver disease and tumor characteristics. The treatment allocation is based on these staging systems (Figures 6 and 7).100 Curative therapies include surgical resection of tumor and liver transplantation.101 Surgical resection may involve anatomical resection along the lobar or segmental boundaries, or non-anatomical resection of the tumor. Surgical resectability is decided based on size and number of underlying tumors (which impacts recurrence) and future liver remnant and status of underlying liver disease (which impact risk of liver failure after hepatectomy). Ablative therapies include radiofrequency ablation (RFA), microwave ablation (MWA), irreversible electroporation (IRE) and cryoablation. These are curative modalities used for patients with small tumors (<3 cm). RFA is the most frequently used ablative modality.102 RFA has been shown to provide results similar to surgery for tumors <2 cm in size. Hence in patients with small tumors (BCLC 0), careful patient selection between ablation and surgical resection is a must.

Trans-arterial therapies include transarterial chemoemobolization using doxorubicin or trans-arterial radioembolisation using radioactive Yttrium-90 microspheres (20-35 micrometre size). These are potentially curative modalities used as bridging therapies for downstaging prior to transplant or surgical resection. They are often used as palliative modalities in BCLC B or C patients to prolong survival. While TACE involves use of larger beads leading to ischemia in the tumor, TARE uses microspheres to deliver radiation doses of up to 120 Gy within the tumor, while the rest of the liver does not exceed 70 Gy.103 Stereotactic body radiotherapy (SBRT) is an emerging palliative therapy for HCC, where in focused doses of external beam radiation are given to the tumor with lesser impact on neighbouring normal parenchyma. Although primarily used for palliation, emerging data suggests its role as a downstaging modality.104 Systemic therapies have grown manifold since the SHARP trial with Sorafenib in 2006.105 Sorafenib showed an Overall survival (OS) benefit of approximately 3 months over placebo in patients with advanced HCC. Sorafenib has remained the systemic treatment of first choice till recently. Regorafenib became available as a second line agent for patients who progress on Sorafenib based on data from the RESORCE trial. The last 4 years have seen approvals for multiple agents for use in HCC. Lenvatinib, another multitkiase inhibitor is approved as a first line agent in HCC. Cabozantinib and Ramcirimub have been approved by the FDA as second line agents. Immunotherapy with Nivolumab is approved for patients who failed Sorafenib.106 Recently, Atezolizumb and Bevacizumab was found to be superior to Sorafenib as a first line agent, increasing Progression free survival (PFS) and OS (OS) in patients with HCC in a phase III randomised controlled trial.107 All these agents are primarily approved for use in patients with preserved liver function (Child A) without any decompensation. Hence, caution should be exercised for their use in patients with advanced liver disease.108

Combination of multiple treatment modalities in HCC may improve outcomes in patients by targeting multiple pathways and lowered chances of resistance. Also combination may allow for the best treatment effects with least side effects. Multiple modalities may be used in patients prior to resection and in unresectable tumors.108 Treatment planning and allocation require the coordinated efforts of hepatologists, surgical oncologists, medical oncologists, radiation oncologists, interventional radiologists and palliative care physicians. In a previous study by Sinn et al, there was a survival benefit in patients who were managed by an MDT versus those who were not (71.2% vs 49.4% 5-year survival, p<0.001) with adjusted Hazard Ratio of 0.47.109 In another previous study, the proportion of patients receiving curative therapy changed from 6-19% to 31-45% when MDT was involved in treatment planning.110 In a previous study from the Veterans Administration database, treatment planned through a MDT was associated with better survival in patients with HCC (Adjusted Hazard Ratio 0.83).111 In another study, the odds of receiving any treatment were higher for patients who had their therapy planned via a MDT independent of MELD, AFP and tumor stage.112 MDTs reduce changes of arbitrary decisions and evidence based decisions are made as agreement of members becomes important.113 The survival benefit of MDT is particularly marked in patients with poor liver function (ALBI 2 or 3), higher AFP (>200 ng/ml) and intermediate or advanced HCC (BCLC B or C). Hence, patients should be referred to a centre where MDT based treatment allocation is done. MDTs should be actively involved in follow up of these patients.

Palliative care in patients with HCC

Palliation for patients with advanced disease is of utmost importance, to maintain an adequate quality of life and ensure comfortable end-of-life care. Palliative care pathways should be established with palliative care experts, with hepatologists and physicians playing a pivotal role. Palliative care requires management of symptoms of HCC or advanced liver disease, psychosocial support, planning advanced care facilities and delivery of services, both inpatient and domiciliary.114 Pain remains a predominant symptom in advanced HCC. Palliation of pain can be done by Paracetamol up to 3 grams per day. Oral or intravenous paracetamol can be administered.115 Non-steroidal anti-inflammatory drugs (NSAIDs) are avoided in patients in advanced liver disease due to risk of nephrotoxicity, gastrointestinal bleeding and increased risk of decompensation.116,117 Opioids like tramadol can be used as a step up for analgesia. However, opioids can lead to drowsiness and constipation, which can worsen symptoms of advanced liver disease.118 Hence caution should be exercised in the use of opioids in these patients. Occasionally use of osmotic laxatives with opioids may be needed to tackle constipation. Bone metastases are common in patients with HCC, seen in 25-39% of those with extrahepatic metastases. These patients present primarily with pain. Palliative radiation therapy can help alleviate pain in these patients. Median doses of up to 40 Gy in various fractions can help improve pain in 81.4% patients.119,120

Nutritional optimization with...
6.5. Treatment should be individualized based upon severity of liver disease, tumor characteristics and stage, comorbidities and the performance status. (evidence moderate, recommendation strong)

6.6. In patients with advanced malignancy (BCLC D/ HKLC Vb), only supportive care is considered. Establishment of appropriate palliative care pathways for patients in different stages of HCC is a must. Chemotherapy should not be given to these patients. (evidence moderate, recommendation strong)

Role of Liver Transplantation in HCC

Hepatocellular carcinoma (HCC) represents the most prevalent primary liver tumor in the world and is consequently a relevant health issue considering its morbidity and mortality. Liver transplantation (LT) is the best and curative treatment for early-stage HCC, since it simultaneously treats the tumor and the underlying liver disease which is the main risk factor for the development of new tumor. Number of patients transplanted for HCC is increasing, with LT for HCC representing 15-50% of all LT performed in most centres. 129

In India First Dead Donor Liver Transplantation (DDLT) was done in 1995 and First Living Donor Liver Transplantation (DDLT) was done in 1998. 130 As per BCLC (Barcelona Clinic Liver Cancer) staging, patients with Early stage (A) disease and as per Hong Kong Liver Clinic (HKLC), patients with early tumor (Stage I & IIa), Liver transplantation is to be considered.

The landmark study of Mazzaferrro in 1996 established DDLT as a viable option for the treatment of HCC. 131 Study showed that when transplantation was restricted to patients with early HCC (defined as single lesion ≤ 5 cm, up to three separate lesions, none larger than 3 cm, no evidence of gross vascular invasion, and no regional nodal or distant metastases), a four-year survival rate of 75% could be achieved with recurrence rate < 10-15%. This criteria have become known as the Milan criteria and have been widely applied around the world in the selection of patients with HCC for LT. Study held at the University of California in San Francisco (UCSF), HCC patients transplanted with extended criteria of Single nodule ≤ 6.5 cm; or ≤ 3 nodules with the largest ≤ 4.5 cm and total sum of diameters ≤ 8 cm (known as UCSF criteria), outcome were similar to those transplanted within Milan criteria. 132

When waiting time for LT is ≥ 6 months, neoadjuvant therapies given to reduce the dropout risk due to tumour progression is known as bridging therapy. It depends on the tumor location, size, number, hepatic function. 1 Different Modalities include liver resection, percutaneous ethanol injection (PEI), radiofrequency ablation (RFA), microwave ablation, transarterial chemoembolization (TACE), radio-embolization, and stereotactic radiotherapy. However, there is no recommendation for one particular form of neoadjuvant therapy over the other. 133 In 48 patients within the Milan criteria, none of the patients treated with TACE had tumor progression or were withdrawn from the waiting list. 131

Therapy used to bring patients within acceptable criteria when their tumor burden exceeds the standard criteria for LT is known as Down staging therapy. Successful down staging of HCC to within Milan criteria reduces tumor recurrence and patients beyond the Milan criteria (≥ T3) should be considered for grafting after an effective downstage of the disease. 134

HCC tumor recurrence following LT is estimated to be approximately 8%-20%. 131,134,135 As per one of prognostic scoring systems [Risk Estimation of Tumor Recurrence After Transplant (RETREAT)] probability of recurrence, with a risk < 3% corresponding to a score = 0 and ≥ 75% with a score ≥ 5.

Statements

6.1. Management of liver disease and its complications impacts outcomes in patients with HCC. (evidence high; recommendation strong)

6.2. In patients with Hepatitis B related CLD and HCC, therapy for hepatitis B should be initiated with antivirals with high barrier to resistance (Entecavir or Tenofovir) and continued life-long. (evidence moderate; recommendation strong)

6.3. Therapy for Hepatitis C should be individualized based on stage of HCC and underlying liver function. (evidence moderate; recommendation strong)

6.4. Several therapies including curative therapies like liver transplantation, surgical resection and ablative therapies, and other therapies like transarterial chemoembolisation (TACE) or radioembolisation (TARE), Stereotactic body radiotherapy and systemic chemotherapy are available for management of HCC. Multidisciplinary management of HCC is needed with a team of hepatologists, surgical oncologists, medical oncologists, radiation oncologists and interventional radiologists. Referral to a center with MDT for management of HCC is recommended (evidence high, recommendation strong)

6.5. Liver transplantation should be considered for patients with advanced liver disease and tumor characteristics and stage, comorbidities and the performance status. (evidence moderate, recommendation strong)

6.6. In patients with advanced malignancy (BCLC D/ HKLC Vb), only supportive care is considered. Establishment of appropriate palliative care pathways for patients in different stages of HCC is a must. Chemotherapy should not be given to these patients. (evidence moderate, recommendation strong)

References

4. Acharya SK. Epidemiology of Hepatocellular Carcinoma in...
COVID-19 and Mucormycosis in India: A Study on Implicated Risk Factors

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Sir,

Mucormycosis represents a group of infections caused by species of the Mucoraceae family, belonging to the genus Rhizopus. The spores of this fungi are ubiquitously distributed, acquired by an individual at any point in life. But the disease is caused when there are underlying or acquired defects in immunity.

The most common type of disease was found to be Rhino-orbito-cerebral mucormycosis. Diagnosis can be made on clinical grounds, although imaging via CT/MRI of PNS and Orbits can provide a conclusive evidence.

COVID-19 in India, is being complicated by this opportunistic fungal pathogen, especially in the second wave of the pandemic. Early diagnosis and treatment is key to managing this invasive fungal infection. Hence, a survey was conducted for a better understanding of the commonly implicated risk factors in causing this mini-epidemic of Mucormycosis in India. This survey was conducted on 171 patients in Gandhi Hospital, Hyderabad, on inpatients diagnosed with Rhino-orbito-cerebral mucormycosis between 21/5/2021 to 30/5/2021.

The mean age of the patients was 48.88 ± 10.42.

113 (66.08%) were Males and 58 (33.92%) were females.

All patients (100%) reported a history of Covid-19 infection, either prior infection (71.34%) or active infection (28.65%).

The mean interval between onset of Covid and onset of initial symptoms of mucormycosis is 20.47 ± 10.13 days.

76.02% of patients had comorbidities, 32.16% were diabetic and hypertensive, 33.33% had only diabetes and 10.53% had only hypertension. 23.98% of patients had no comorbidities.

16.8% and 10.2% patients gave a history of consumption of alcohol and tobacco respectively.

Majority of patients were on insulin therapy. 62.57% patients had uncontrolled sugars.

18.71% patients reported the initial symptom of Mucormycosis to be Unilateral facial pain, whereas 18.13% had Eyelid swelling and 15.79% patients had headache. The other common initial symptoms were blackish nasal discharge (9.36%), retroorbital pain (9.36%), toothache (9.36%), unilateral facial swelling (7.6%), eye discharge (5.85%), blurring of vision (4.09%), and unilateral facial numbness (1.75%).

26.43% were treated at home and 73.57% were treated in hospital settings for COVID-19 infection.

78.36% patients recorded a history of usage of Steroids (iv/oral) as a part of treatment for COVID-19, whereas 21.64% had no steroid usage.

90 (52.63%) patients recorded a history of usage of antivirals. 62 patients were treated with Remdesivir, and 28 patients with Favipiravir.

101 (61.2%) patients were treated with Oxygen due to Covid pneumonia, 5 (2.51%) patients required non-invasive ventilation. 37.29% patients did not report usage of both.

138 (79.47%) patients were not vaccinated, 21 (13.06%) patients took a single dose of Covishield, 3 (1.76%) took both doses of Covishield and 9 (5.71%) took a single dose of Covaxin.

In the second wave of the COVID-19 pandemic in India, the unprecedented rise in Mucor cases is alarming. The most important predisposing factor seems to be COVID-19 infection by itself, and usage of steroids and prior history of Diabetes precipitating its occurrence.

79.47% patients were not vaccinated with either of the vaccines presently available in India (Covishield/ Covaxin). Vaccination, as such, is expected to decrease the severity of COVID-19 infection, thereby it should, theoretically, decrease the incidence of Mucormycosis.

An increased number of patients with moderate to severe COVID infection have developed mucormycosis, although 26.43%, who were treated at home have also fallen prey to it.

The use of steroids in mild cases without hypoxemia and utilization of higher doses of steroids must be discouraged. Furthermore, in the absence of a clear benefit, the use of Tocilizumab and other drugs altering immune system must be discouraged.

Judicious use of steroids, along with a tight control of blood sugars is critical in preventing the occurrence of this deadly fungus.

References


Association between Glycemic Control and Drug Therapy Concordance in Individuals with Type 2 Diabetes Mellitus: A Cross-sectional Study

Sujeet Jha¹, Manoj Kumar Verma², Samreen Siddiqui³, Om Prakash Sahani⁴, Devarati Majumdar⁵, Sanjita Das⁶, Avijit Mazumder⁷, Swati Waghdhare⁸

¹Sr. Director & HOD, 'Deputy Manager-Clinical Pharmacy, ²Research Manager, Max Healthcare, New Delhi; ³Clinical Pharmacist, Medanta Hospital, Gurgaon, Haryana; ⁴Director & Chief-Pharmacy, Max Healthcare, New Delhi; ⁵Prof. & HOD-Pharmacology, ⁶Director & Prof. Pharmacy, Noida Institute of Engineering & Technology (Pharmacy Institute), Greater Noida, Uttar Pradesh; ⁷Consultant Endocrinology, Max Healthcare, New Delhi;

Sir,

Diabetes Mellitus continues to be an increasing health problem worldwide affecting individuals of all ages. Despite being recognized as a major chronic illness, the concordance with antidiabetic medication is poor. The aim of this study was to determine the association of glycemic control with...
adherence to drug therapy in patients with Type 2 Diabetes Mellitus.

This prospective cross-sectional questionnaire-based study was conducted from December 2014–October 2015 at the outpatient department of Endocrinology, Diabetes and Metabolism at Max Super Specialty Hospital, Saket, New Delhi. A total of 1200 patients were screened during the 6 month-period based on the inclusion and exclusion criteria. Four different questionnaires were used in this study; Diabetes Knowledge Test (DKT), Diabetes Self-Management Questionnaire (DSMQ), Morisky Medication Adherence Scale, and Culig Adherence Scale based Questionnaire.

A total of 1200 patients were screened during the study period. Among these, 510 were diagnosed with thyroid disorder, 230 had gestational diabetes, 170 were diagnosed with other endocrine disorders, and 59 were unwilling to participate. Out of these, 231 patients were included in the study with 143 males (61.9%) and 88 females (38.1%). We observed that 38.5% of the patients were highly adhered to their medication schedule for controlling diabetes, 47.6% showed medium adherence, and 13.9% had a low concordance with their anti-diabetic medication. Additionally, individuals with concordance to anti-diabetic medication were more likely to achieve fasting blood glucose control compared to others. Many reasons have been attributed to non-adherence of the drug regimen. These include age, duration of illness, multidrug therapy, level of education, lack of knowledge, Forgetfulness has been one of the significant causes of irregularity in follow-up of patients. In our study, the most common reason for non-compliance of drug therapy observed was forgetfulness (44.6%).

Table 1 shows the correlation of Morisky Medication Adherence Scale with Hba\textsubscript{1c} and Fasting blood sugar. A statistically significant correlation between Fasting blood sugar and Morisky Medication Adherence Scale was seen among the study population (p-value < 0.001). Results from the study revealed that patients who were compliant with their drug therapy and for the management of diabetes were achieving normal blood glucose levels. The awareness of diabetes among the study population was assessed using a Diabetes Knowledge Test Score. This score categorized people into any of the three groups based on the individual’s knowledge. Group 1 (Score: 0–9) comprised of individuals with less awareness regarding diabetes. Group 2 (Score: 10–19) included subjects with medium awareness, whereas Group 3 (Score: 20–25) comprised of individuals having a high awareness.

Diabetes Self-Management Questionnaires were distributed among the study participants to assess the level of self-management and determine its correlation with glycemic control (Hba\textsubscript{1c} fasting blood sugar, and post-prandial blood sugar). A statistically significant correlation was observed between fasting blood sugar and diabetes knowledge test score (p-value = 0.010).

We concluded that technology could be utilized to send reminders to patients for taking medication as prescribed by the clinician. Medical professionals including caregivers and pharmacists should create awareness amongst patients with diabetes regarding adherence to medication. This would help to achieve better clinical outcomes linked with diabetes.

**References**


Clinico-demographic Comparison between First and Second Wave of Hospitalised Covid 19 Infections. A Retrospective Observational Study from Tertiary Care Institute of Eastern India

Deependra Kumar Rai¹, Sanjay Kumar Pandey²

¹Additional Professor & Head, Department of Pulmonary Medicine, ²Additional Professor & Head, Department of PMR, AIIMS, Patna, Bihar

Sir,

This study was performed with primary objective to compare clinico-demographic characteristics of hospitalised COVID-19 patients between first and second wave of pandemic in India. We also assess the clinical presentation and severity of COVID-19 infection among patient who received covid vaccine. This study included all the patient admitted in May 2021 (second wave) and compared to patients of July-August 2020 (first wave). Patient received at least one dose of vaccine categorised in to vaccination group, otherwise Non vaccinated group. Statistical analysis: Demographic, clinical and treatment outcome data were analysed using SPSS version 21.0 (SPSS Inc., Chicago, IL, USA).

Total 140 patients admitted in month of May 2021 were compared with 813 patients of 1st wave admitted during months of July-Aug 2020 for demographic, clinical and treatment variable. Table 1 summarised clinical profile of COVID-19 patient of two waves. The mean age of the study patients was slightly higher in the second wave as compared to the first wave which was not found statistically significant. This is in contrary to an Indian study¹ published recently which showed lower mean age in second wave compared to first wave. Regarding age distribution, there was increase in the proportion of patients in age groups 41–60 year (46.42% vs 45.87%, P <0.05), >60 years (40% vs 28.29%, P <0.05) during second wave compared to first wave. There was more proportion of female affected during second wave (30.71% vs 21.40%, P <0.05). Present study also showed significantly higher
Table 1: Demographic, clinical and treatment comparison between second to first wave of covid 19 infection

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>2nd wave (n=140)</th>
<th>1st wave (n=813)</th>
<th>Z value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>53.34 ± 16.37</td>
<td>50.96 ± 15.3</td>
<td>0.1701</td>
<td></td>
</tr>
<tr>
<td>&lt;20</td>
<td>05 (3.57)</td>
<td>02 (0.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-40</td>
<td>14 (10)</td>
<td>204 (25.09)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>41-60</td>
<td>65 (46.42)</td>
<td>373 (45.87)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;60</td>
<td>56 (40)</td>
<td>234 (28.29)</td>
<td>2.6644</td>
<td>0.00782</td>
</tr>
<tr>
<td>Male</td>
<td>97 (69.28)</td>
<td>639 (78.59)</td>
<td>2.4268</td>
<td>0.0151</td>
</tr>
<tr>
<td>Female</td>
<td>43 (30.71)</td>
<td>174 (21.40)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Covid vaccination (At least one dose)</td>
<td>32 (9 covishield, 23 Covaxin)</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical symptom</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td>108 (77.41)</td>
<td>613 (75.39)</td>
<td>0.4439</td>
<td>0.6594</td>
</tr>
<tr>
<td>Cough</td>
<td>99 (70.71)</td>
<td>547 (67.28)</td>
<td>0.8028</td>
<td>0.4237</td>
</tr>
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<td>Dyspnea</td>
<td>103 (73.57)</td>
<td>396 (48.70)</td>
<td>5.4404</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Covid Severity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild-Moderate</td>
<td>61 (43.57)</td>
<td>630 (77.49)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>79 (56.42)</td>
<td>183 (22.50)</td>
<td>8.3026</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Comorbidity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At least one comorbidity</td>
<td>70 (50)</td>
<td>414 (50.92)</td>
<td>-0.2017</td>
<td>0.8418</td>
</tr>
<tr>
<td>Diabetes</td>
<td>45 (32.14)</td>
<td>268 (32.96)</td>
<td>-0.912</td>
<td>0.3643</td>
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<td>Hypertension</td>
<td>47 (33.57)</td>
<td>245 (30.13)</td>
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<td>0.7028</td>
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<td>CKD</td>
<td>07 (5)</td>
<td>36 (4.42)</td>
<td>-1.374</td>
<td>0.1706</td>
</tr>
<tr>
<td>CAD</td>
<td>05 (3.57)</td>
<td>50 (6.15)</td>
<td>1.846</td>
<td>0.0643</td>
</tr>
<tr>
<td>COAD</td>
<td>09 (6.42)</td>
<td>26 (3.19)</td>
<td>1.877</td>
<td>0.0601</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>08 (5.71)</td>
<td>80 (9.84)</td>
<td>-1.5571</td>
<td>0.1187</td>
</tr>
<tr>
<td>Malignancy</td>
<td>02 (1.42)</td>
<td>12 (1.47)</td>
<td>-0.0431</td>
<td>0.9681</td>
</tr>
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<td>Treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxygen inhalation</td>
<td>107 (76.42)</td>
<td>421 (51.78)</td>
<td>5.4184</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ICU</td>
<td>55 (39.28)</td>
<td>234 (28.78)</td>
<td>2.4972</td>
<td>0.0124</td>
</tr>
<tr>
<td>Mortality</td>
<td>29 (20.71)</td>
<td>186 (22.87)</td>
<td>-0.5658</td>
<td>0.5688</td>
</tr>
</tbody>
</table>

 CKD, Chronic kidney disease; CAD, Coronary artery disease; COAD Chronic obstructive airway disease; ICU, Intensive Care Unit

proportion of patient with severe disease (56.42% vs 22.50%, P <0.05). Second wave witnessed significantly higher proportion of patient require oxygen therapy (76.42% vs 51.78%, P <0.05) and Intensive care unit (ICU) care (39.28% vs 28.78%, P <0.05). There was no significant difference for mortality between two waves (20.71% vs 22.87%, P >0.05)). In second wave patient vaccinated with at least one dose of either covaxin or covishield (n-32) in comparison to non-vaccinated (n-108) were significantly lower requirement of Intensive care unit (18.18% vs 45.79%, P <0.05). Although proportion of patient with dyspnoea, severe disease, or oxygen therapy was lower among vaccinated group but statistically not significant. There was no significant difference for mortality between two group.

The major limitation of the study was small sample size which was not enough powered to show some difference specially mortality and age distribution, therefore, our results should be taken with caution. This is a retrospective, single centre study may not reflect true picture, need large study to characterised the patients of second wave. But this is first study to characterised covid 19 patients of first and second wave and also to look for effect of covid vaccination in infected individual.

Conclusion

Second wave of covid 19 were significantly more affected female, higher proportion of dyspnoea and severe disease among hospitalized patients. There was no difference for mortality between these two waves. Vaccinated individual had significantly lesser ICU requirement but no significantly difference found for mortality.

References


Looking beyond A1c in Newly Onset Diebetic Patients during Covid 19 Era: A Cross Sectional Study

Shailendra Kumar Singh1, Rina Singh2, Santosh Kumar Singh3

1Endocrinologist, Pediatrician, Endocrine Clinic, Varanasi, Uttar Pradesh; 2Endocrine Center, Patna, Bihar

Sir,

Prevalence of diabetes mellitus (DM) is increasing and so is the complication. Complication in DM is related to hyperglycemia, hypertension, dyslipidemia, obesity, hyperuricemia and others. Various trials show that if we control hyperglycemia in early phase of disease than we can prevent the complication. Despite availability of various drugs many patients with long duration of diabetes are not on the target. IDMPS 7 data, in which I was also one of the investigators, from India shows that only 25.2% of Type 2 diabetic patients are on target. Recent data also shows that many newly onset DM patients are also not on target despite good insulin reserve. Reasons are many such as higher baseline A1c (>8%), poor adherence to medication, lower level of education and others. It has been seen that those patients with high baseline A1c (>8%) are the group who predominantly did not attained the target of A1c <7%. This prompts us to think that these patients might be phenotypically different than those with lower baseline A1c. We conducted a cross sectional study with aim to see whether patients with higher (≥9%) baseline A1c are phenotypically different than those with lower (<9%) baseline A1c. Furthermore our aim was to know the prevalence of various level of hyperglycemia in newly onset DM. No studies from Eastern part of India till now have been performed in this area.

We enrolled 319 (M:225; F: 94) consecutive newly diagnosed diabetic patients attending our clinic from February 2020 and May 2021. Baseline and demographic profile (Means±SD) of study subjects were presented in Table 1. Patients were stratified into Group A(A1c <9%) and Group B(A1c ≥9%) based on A1c level. Mean value of age, BMI (body mass index), A1C, WC(waist circumference), SBP(systolic blood pressure), DBP (diastolic blood
pressure), TC (total cholesterol), LDL (low density lipoprotein), TG (triglyceride), HDL (high density lipoprotein), uric acid, vitamin D and e GFR (glomerular filtration rate) in group A and group B were 46.87 and 46.19 years, 27.20 and 25.92 kg/M², 7.5 and 11.59 %, 97.81 and 96.94 cm, 132.67 and 128.69 mmHg, 85.21 and 83.86 mmHg, 182.35 and 186.17 mg/dl, 109.8 and 110.97 mg/dl, 41.78 and 41.09 mg/dl, 5.52 and 4.92 mg/dl, 17.77 and 19.64 mg/ml and 108.28 and 110.17 ml/min respectively.

It this study BMI and uric acid were significantly more in Gr A as compared to Gr B. Prevalence of hypertension (≥140/90 mmHg), VDD (vitamin D deficiency, <20 ng/ml), central obesity (WC in male≥90 cm and female ≥80 cm) and hyperuricemia (uric acid in Male ≥7, Female ≥6 mg/dl) were significantly more in Gr A as compared to Gr B. Second aim of study was to know the prevalence of various level of hyperglycemia at time of diagnosis. For this patients were stratified into three groups based on A1c level. Prevalence of patients with A1c <9%, between 9-12% and >12% were 38.56%, 38.56% and 22.88% respectively. In this study mean A1c at time of diagnosis was 10.01% while in Raghvan et al (precovid era study) it was (9.1±2.3%).4 Higher A1c in our study could be due to late presentation as a result of Covid 19 lockdown. Mean age at diagnosis was 46.46±10.7 years in present study while it was 55.6±13 years in Pantalone et al(USA).5 This shows that Indian develops DM a decade earlier.

To conclude patients with A1c ≥9% at time of diagnosis are phenotypically different. They are characterized by lower BMI, lower uric acid level and less prevalence of hypertension, central obesity and hyperuricemic. These all features are indicators of insulin deficiency. So Gr B patients should be treated differently than Gr A with insulin secretagogue along with insulin sensitizer. Since 61.44% of patients at time of diagnosing during Covid 19 era have high burden of hyperglycemia (A1c ≥9%), so early diagnosis of diabetes is urgently required. Routine screening of high-risk population and educational campaign about symptom and sign of diabetes is one way to achieve it, as many patients are asymptomatic.

### Table 1: Patient Data

<table>
<thead>
<tr>
<th>Parameter</th>
<th>All</th>
<th>Gr A</th>
<th>Gr B</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>319</td>
<td>123</td>
<td>196</td>
<td>-</td>
</tr>
<tr>
<td>M/F</td>
<td>225/94</td>
<td>77/46</td>
<td>148/48</td>
<td>-</td>
</tr>
<tr>
<td>AGE</td>
<td>46.46±10.7</td>
<td>46.87±10.07</td>
<td>46.19±11.09</td>
<td>&lt;0.575</td>
</tr>
<tr>
<td>BMI</td>
<td>26.42±4.18</td>
<td>27.20±3.88</td>
<td>25.92±4.29</td>
<td>&lt;0.006</td>
</tr>
<tr>
<td>A1c</td>
<td>10.01±2.52</td>
<td>7.5±0.85</td>
<td>11.59±1.86</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>141(44.20%)</td>
<td>63(31.22%)</td>
<td>78(39.79%)</td>
<td>&lt;0.045</td>
</tr>
<tr>
<td>Hyperuricemia</td>
<td>41(12.85%)</td>
<td>24(19.51%)</td>
<td>17(6.67%)</td>
<td>&lt;0.004</td>
</tr>
<tr>
<td>Central obesity</td>
<td>274(85.89%)</td>
<td>113(91.87%)</td>
<td>161(82.14%)</td>
<td>&lt;0.015</td>
</tr>
<tr>
<td>VDD</td>
<td>207(64.89%)</td>
<td>92(74.79%)</td>
<td>115(58.67%)</td>
<td>&lt;0.003</td>
</tr>
<tr>
<td>WC</td>
<td>97.28±9.34</td>
<td>97.81±8.52</td>
<td>96.94±7.99</td>
<td>&lt;0.045</td>
</tr>
<tr>
<td>SBP</td>
<td>130±18.35</td>
<td>132±18.31</td>
<td>128±18.29</td>
<td>&lt;0.110</td>
</tr>
<tr>
<td>DBP</td>
<td>84.3±10.9</td>
<td>85.2±10.7</td>
<td>83.8±11.0</td>
<td>&lt;0.27</td>
</tr>
<tr>
<td>TC</td>
<td>184.7±42.2</td>
<td>182.3±39.9</td>
<td>186.1±43.9</td>
<td>&lt;0.424</td>
</tr>
<tr>
<td>LDL</td>
<td>110.5±37.44</td>
<td>109.8±35.31</td>
<td>110.97±38.79</td>
<td>&lt;0.783</td>
</tr>
<tr>
<td>TG</td>
<td>195.9±13.4</td>
<td>190.2±19.51</td>
<td>199.4±10.8</td>
<td>&lt;0.491</td>
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<tr>
<td>HLD</td>
<td>41.36±6.2</td>
<td>41.78±6.91</td>
<td>41.09±6.89</td>
<td>&lt;0.526</td>
</tr>
<tr>
<td>Uric Acid</td>
<td>5.15±1.31</td>
<td>5.52±1.28</td>
<td>4.92±1.28</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Vitamin D</td>
<td>18.92±10.1</td>
<td>17.77±10.32</td>
<td>19.64±9.77</td>
<td>&lt;0.109</td>
</tr>
<tr>
<td>eGFR</td>
<td>109.45±14.43</td>
<td>108.28±12.47</td>
<td>110.17±15.52</td>
<td>&lt;0.232</td>
</tr>
</tbody>
</table>

### References


### Covid-19 Induced Myopathy and Encephalopathy in the Same Patient

**Rudrajit Paul**, **Rathindranath Sarkar**

1. Consultant Physician, Ruby General Hospital, Kolkata, West Bengal; 2Ex-HOD Medicine, Medical College, Kolkata, West Bengal

Sir,

The Covid-19 pandemic, caused by SARS-CoV2 virus, has given rise to a myriad of clinical features. While respiratory symptoms dominate the clinical picture and are mainly responsible for the mortality, the other body systems are also involved to a variable degree. We here describe an extremely rare case of neurological manifestation of Covid with minimal respiratory involvement.

A 69 year old female patient was admitted with high grade fever for five days, along with gradual drowsiness for three days. She had had no episodes of seizure or myoclonus. The fever was intermittent, rising up to 103°F on occasions. There was no complaint of dyspnoea or cough. At presentation, the patient was restless (RASS score +2), responding partially to commands and had urinary retention. SpO₂ was 96% in room air. Plantar response was bilaterally flexor. In view of the ongoing epidemic, Covid-19 RT-PCR was sent. A CT scan of the brain was apparently normal. CSF study could not be done due to restlessness.

The RT-PCR was positive with...
CT value of 21. Meanwhile, HRCT of thorax was done (Figure 1), which showed only mild lung involvement. As initial blood culture was negative and laboratory reports, including Procalcitonin, TyphiDot IgM, Malaria Antigen and Scrub Typhus IgM were all negative, the present illness was presumed to be entirely due to Covid infection. The patient was started on i.v. Dexamethasone and i.v. Remdesivir (till then, it was approved for use in Covid). Associated supportive care like low molecular weight Heparin was also started; but no antibiotic was given. By second day of treatment, the patient became fully conscious and communicative. Her catheter could also be removed. Thus, it was a case of Covid encephalopathy, responding to treatment.

But the course of disease was not over yet. As the patient became fully conscious on day 2 of admission, she started complaining of severe body ache. Her shoulders and thighs were painful to touch. She also could not get up from bed due to pain and weakness. The fever had subsided by then. Dengue and Chikungunya serologies were negative. Her CPK level was 1055 IU/L (N<195). She was not on statins and had not been given any i.m. injection during the hospital stay. So, following the temporal profile of the illness, this was a case of myositis due to Covid-19 infection. Electromyography showed presence of fibrillations. Muscle biopsy was refused by the patient and also avoided in view of the anticoagulation treatment.

The Remdesivir was stopped after 5 days. I.v. Dexamethasone (8 mg OD) was continued for a total of 10 days. She had gradual decrease in muscle pain. Finally, at discharge, she was fully conscious with no residual neurodeficit. She could also walk out of the hospital, although some proximal weakness remained. At follow up, after one month, she had no residual muscle weakness. CPK also came down to the normal range.

SARS-CoV2 is rarely associated with encephalopathy. In a case series from Switzerland, the incidence of encephalopathy in admitted Covid patients was around 4%. In that series, 90% of the encephalopathy cases were associated with severe respiratory involvement. But in our patient, the respiratory involvement was minimal. One feature noted in this Swiss study was raised CSF albumin. But in our patient, CSF study was abandoned due to agitation. CSF RT-PCR is usually negative in encephalopathy. In another meta-analysis, it was reported that Covid encephalopathy is commoner above 50 years of age. This encephalopathy is hypothesized to be caused by metabolic and/or hypoxic insult rather than direct cytopathic effect of the virus.

Myopathy in Covid has rarely been reported. Often, it takes the form of silent elevation of CPK. Generalized muscle weakness, including respiratory muscles, is more common than myalgia. This myopathy may be responsible for difficult weaning of ventilated Covid patients. However, in our case, there was both myalgia and weakness, although respiratory muscles were not involved. In a study from Germany, autopsy of Covid patients revealed inflammatory myopathy in more than half the cases. Like encephalopathy, this myopathy is also postulated to be immune mediated.

We present this case to sensitize clinicians to these rare manifestations of Covid. Especially in cases like ours where initial respiratory symptoms are minimal, a high index of suspicion is needed.

References

KEEP THE BRAIN-GUT BOND STRONG

In IBS with Anxiety

Librax
Chlordiazepoxide 5 mg • Clidinium Bromide 2.5 mg Tablets

DOSAGE:
1 TABLET T.I.D’ 1-1-1 FOR 3 MONTHS

Established Safety & Efficacy
Textbook recommended
Over 3 decades of trust
Globally available

For the use of Registered Medical Practitioner or Hospital or Laboratory only


COMMUNICATION: hasten treatment and prevent the risk of serious reactions, including death. Patients should be instructed to take the recommended dose of Librax as prescribed by a healthcare professional. Monitor patients closely for signs and symptoms of toxicity, including sedation, dizziness, drowsiness, and lightheadedness. If any of these symptoms occur, the patient should discontinue use of the medication and seek medical attention immediately.

Indication: Librax is indicated for the treatment of anxiety disorders, including anxiety disorders associated with depression. Librax is also indicated for the treatment of agitation associated with dementia of the Alzheimer type.

ADVERSE REACTIONS: The most common adverse reactions associated with Librax therapy include sedation, dizziness, drowsiness, and lightheadedness. Other possible adverse reactions include constipation, dry mouth, blurred vision, and drowsiness. In rare cases, severe allergic reactions (anaphylaxis) have been reported. Librax may also cause difficulty in focusing, adjusting to light, and other visual disturbances. If any of these symptoms occur, the patient should discontinue use of the medication and seek medical attention immediately.

WARNING: Librax is not recommended for use in patients with a history of hypersensitivity to chlordiazepoxide or clidinium bromide. Librax is contraindicated in patients with known sensitivity to any of the components of the product.

Use: Librax is indicated for the treatment of anxiety disorders, including anxiety disorders associated with depression. Librax is also indicated for the treatment of agitation associated with dementia of the Alzheimer type.

For additional information, please contact: Medical Affairs Division, Abbott Laboratories, 10th Floor, Godrej ECC, Flora No. C-68, BCC, Near MCA Club, Bandra (E), Mumbai - 400051, www.abbottnia.co.in

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Cardiol Thor (2017) 6 : 13-32

Abridged Prescribing Information:
Composition: Each Olmesar 10/20/40 tablet contains Olmesartan 10/20/40mg. Indication: Hypertension. Dosage: Adult: 20 mg once daily when used individually, increase to 40 mg after 2 weeks of therapy if required. Children (age of 6 to 16 years): 10 mg once daily for patients who weigh 20 to < 35 kg or 20 mg once daily for patients who weigh ≥ 35 kg. Increase to a maximum of 20 mg for patients who weigh < 35 kg or 40 mg once daily for patients who weigh ≥ 35 kg after 2 weeks of therapy if required. Contraindications: Hypersensitivity to Olmesartan, co-administration with aliskiren in diabetic patients, 2nd and 3rd trimester of pregnancy, biliary obstruction. Special Precautions: Assess renal function, BP & volume status during initiation of therapy & dose escalation periodically thereafter. Use with caution in elderly. Children < 1 year of age must not receive Olmesartan for hypertension. In patients whose renal function may depend on activity of the renin-angiotensin-aldosterone system (eg, patients with severe CHF), treatment may be associated with oliguria &/or progressive anemia, rarely resulting in acute renal failure &/or death. Symptomatic hypotension may be anticipated after initiation of treatment in patients with an activated renin-angiotensin system, such as volume &/or salt-depleted patients. In patients with unilateral or bilateral renal artery stenosis, increases in serum creatinine or BUN may occur. Adverse Drug Reaction: Most commonly observed adverse reaction is Hyperuricaemia, Dizziness, Headache other ADRs may be Rhinitis, Pharyngitis, Cough, Pain, Angioedema, Pruritus, Rash, Urticaria, Hyperkalaemia, Hypotenison & Muscle spasm.

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