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EDITORIAL

Need for Uniformity in Clinical Teachings from Global Perspective

Amit A. Saraf

"Until we get equality in education, we won’t have an equal society"  
---Sonia Sotomayor

INTRODUCTION

The journey of medicine is a long and interesting one. Dating back to the 1800s when “doctors” were actually sorcerers/witch doctors, magic and religion were inseparable and talismans, and spells actually formed an important part of the treatment. Learning from these “doctors” was on a one-to-one basis and involved a lot of trial and error. Believe it or not, the firm faith of the healers and the patients in these tricks actually made the patient feel better, the term for which was the “placebo effect.”

The roots of ancient Indian medicine can be traced back to the Vedas. Then came the “Charaka Samhita” and “Shushruta Samhita.” Around the same time, the Chinese came up with acupuncture and hydrotherapy. They also discovered multiple herbal remedies. Japanese medicine was influenced by Chinese beliefs to a great extent. Gradually with increased understanding about the human body and its physiology the foundation of modern medicine was laid. Now, all across the globe students pursue medicine, gain an understanding of the dynamics of the human body and practice it to alleviate suffering.

THE BACKGROUND

Sir William Osler said, “Medicine is learnt by the bedside and not the classroom.” In accordance with this principle, all over, in addition to reading the books, there is a clinical curriculum in place, a method to teach budding doctors how to listen to the patient and impart knowledge beyond books. The one factor that we still haven’t been able to remedy, is the standardization of this teaching. The protocols in an Indian hospital vary from the ones followed in the United States of America or the United Kingdom. To give a real-life scenario, I would urge the reader to remember the books we read for our MBBS curriculum, they were English Language Book Society books, which basically meant, books authored as per the guidelines and research-based out of the European countries. Now, remember our post-graduations days, the books we read were based on an American way of teaching and medical management, our very own, Harrisons Principles of Internal Medicine, to give an example. Therein lies the problem, the two curriculums in our own Indian scenario were from two distinct continents, which does not create a uniformity of teaching. To quote another daily life example, all of us would relate to the fact that in India we use “mg/dL” as our standard unit of measurement, whereas in the United States the acceptable standard norm of measurement is “mmol/lit.”

All these variations in medical teachings to management are in spite of the basic physiology, the cellular functions of every individual, the homeostatic mechanisms are ultimately similar irrespective of the geographical location of the individual. Being aware of these mechanisms doctors find it difficult to practice outside borders, thanks to differences in the ways of working and the protocols.

THE NEED

Why should there be uniformity in clinical teachings?

Clinical teachings are responsible for imparting the skills and knowledge to the healthcare workers which enables them to treat a patient. If healthcare professionals all around the world were to receive standard teaching, irrespective of the geographical area of practice, the quality of care provided could be maintained, and communication and collaboration across borders could be facilitated. In such a scenario, the clinical decisions taken by the professionals would be based on the best available evidence. In addition to this, there would be a better understanding regarding the cultural beliefs and linguistic background ensuring a better rapport with the patient, leading to ease in gaining the trust of the patient. All of this would eventually lead to better health outcomes, help minimize errors and ensure that no matter where the patient stays or where they receive care from, the care provided would be of the best possible quality. Standardization of medical education and care will aid the global development, maintenance, and implementation of artificial intelligence in future medical treatment modalities.

THE WAY FORWARD

How do we make this happen?

When one talks about standardizing clinical teachings across the globe, it does seem to be a daunting task but not an impossible one. Any change is met with resistance and faces hurdles but gradually as the reasoning behind the suggested change becomes known, and the associated positive outcomes start to become evident, the change is accepted wholeheartedly.

The first step in this will be creating a draft of the curriculum while taking inputs from all over, a draft that would need to be modified several times before the final version is ready to be implemented. Communication regarding the preferred teaching methods and practices amongst institutions, hosting international conferences, opening up communication portals, and hosting webinars to achieve a better understanding of the differences and ways to reduce the said differences. Sensitizing the clinicians to the cultural differences and language background so as to remove the communication barrier and create a healthy environment. Making use of virtual clinical simulators and telemedicine to bring together students from different parts of the world and imparting knowledge as per the latest guidelines.

In addition to communication and collaboration among educators, institutions, and policymakers standardization would

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How to cite this article: Saraf AA. Need for Uniformity in Clinical Teachings from Global Perspective. J Assoc Physicians India 2023;71(9):11–12.

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require a lot of effort, investment, and patience. The task might progress slowly but remember slow and steady wins the race and the victory in this aspect would translate into patients in every nook and corner of the world receiving the best, uniform, up-to-date medical care!

I think the time has come to complete India’s G20 slogan of “One Earth, One Family, One Future” and include “One Medical Care.”

**REFERENCES**


3. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5084543/

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Increased Pill Burden and Adverse Effects of Psychotropics Correlated with Poor Quality of Life and Medication Nonadherence: A Cross-sectional Drug Utilization Study at a Tertiary Care Hospital in Delhi during COVID-19 Pandemic

Ashish Kumar1, Sumita Halder2*, Shruti Srivastava3, Rachna Gupta4

Received: 15 November 2022; Revised: 31 March 2023; Accepted: 12 May 2023

ABSTRACT

Background: The coronavirus disease 2019 (COVID-19) pandemic has led to an increase in mental health problems such as depression and anxiety. This study aims to investigate the prescribing pattern of psychotropic drugs in patients with common mental disorders which might be altered during the pandemic and also whether the pandemic could alter their quality of life (QOL) and medication adherence.

Materials and methods: After prior ethical approval, a descriptive cross-sectional drug utilization study (DUS) of 200 prescriptions was undertaken to evaluate the pattern of psychotropic drug usage as per WHO (World Health Organization)/International Network of Rational Use of Drugs (INRUD) guidelines. The correlation of the average number of drugs per prescription with QOL was observed. The correlation of adverse drug reactions (ADRs) with medication adherence was also analyzed.

Results: The average number of drugs per prescription during the pre-COVID-19 and COVID-19 period was estimated to be 2.48 and 2.96. The percentage of drugs prescribed by generic name in the two different periods (pre-COVID-19 and COVID-19) was 97.40 and 95.77%. The percentage of drugs prescribed from the list of essential medicines was 89.40 and 85.12%, respectively. The percentage of prescriptions with injections was 0.45% and 0.53%, respectively for the two periods. The QOL during the COVID-19 pandemic was found to be negatively correlated to the average number of drugs per prescription (correlation coefficient = −0.61) and medication adherence was found to be poor in patients who developed ADRs with the drugs prescribed (p-value of 0.001).

Conclusion: In the tertiary care hospital described, rational drug prescribing was followed. Increase in the number of drugs per prescription was found to be associated with poor QOL and the development of ADRs led to medication nonadherence in the patients. Further studies with larger sample sizes are needed to confirm these results.

Key Points
- Evaluation of WHO (World Health Organization)/International Network of Rational Use of Drugs (INRUD) drug prescribing indicators in patients of common mental disorders in the tertiary care hospital showed compliance with rational prescribing methods.
- The prescribed daily dose (PDD)/defined daily dose (DDD) ratio of most psychotropic drugs and tricyclic antidepressants was found to be less than one.
- An increase in pill burden was found to be negatively correlated to the health-related quality of life (HRQOL) of patients.
- The present study revealed a strong association between the development of adverse drug reactions (ADRs) and medication nonadherence.

Plain Language Summary
Drug utilization studies help to assess whether the health resources are optimally utilized by the hospital. The present study was undertaken to assess whether there was any alteration in the prescription patterns of patients with common mental disorders and their influence on medication adherence and their QOL during the COVID-19 pandemic in tertiary care hospitals. We observed that the prescription patterns followed rational and safe prescribing practices before as well as during the pandemic. However, during the pandemic, the increased number of medications adversely affected the QOL of patients. The increased incidence of side effects also adversely affected medication adherence. The findings of this study should prompt healthcare policymakers and clinicians to ensure that patients are receiving appropriate pharmacological interventions to prepare for such changing situations in the future. Thus, the outcome of this study would be taken as the basis for identifying potential targets to make improvements in the prescribing patterns and drug dispensing policies of the hospital.

How to cite this article: Kumar A, Halder S, Srivastava S, et al. Increased Pill Burden and Adverse Effects of Psychotropics Correlated with Poor Quality of Life and Medication Nonadherence: A Cross-sectional Drug Utilization Study at a Tertiary Care Hospital in Delhi during COVID-19 Pandemic. J Assoc Physicians India 2023;71(9):14–18.
health outcomes. A careful assessment of this relationship is crucial to safeguard the sustainability of healthcare systems and for planning the interventions needed to improve pharmacological care.10,11

In light of the above observations, and the current COVID-19 crisis, we conducted a cross-sectional study to generate data on drug utilization in patients of common mental disorders from the psychiatry outpatient department (OPD) of a tertiary care teaching hospital with a focus on identifying the lacunae in the existing prescription and to suggest ways for improving drug prescribing patterns. The pattern of drug prescription was correlated with the QOL of the patient pertaining to disease and medication adherence.

**Materials and Methods**

**Study Design and Ethical Considerations**

A descriptive cross-sectional drug utilization study (DUS) was conducted after a prior Institutional Ethics Committee (IEC, University College of Medical Sciences, Delhi) approval. Written informed consent was taken from the patients participating in the study in their own vernacular language.

**Selection Criteria**

**Inclusion Criteria**

Patients of either sex aged between 18 and 60 years and diagnosed with common mental disorders (depression and anxiety disorders as per WHO definition) on medications for at least 4 weeks were included.

**Exclusion Criteria**

Patients with organic brain disease, having any history of substance intoxication or overdose, unable to come physically or communicate and any serious medical illness such as myocardial infarction, cerebrovascular accident, diabetic coma, or any surgical condition that requires immediate intervention were excluded. Patients with high suicide risk were excluded from the study (based on a detailed psychiatric evaluation carried out by the clinician). Additionally, the California suicide risk estimator scale12 was used wherever feasible.

**Sample Size**

A total of 200 patients were recruited (100 patients during the COVID-19 pandemic and 100 patients from pre-COVID-19 period as a comparator arm).

**Study Procedure**

The data of the patients attending the psychiatry OPD, during the period 1st January 2021 to 31st August 2022, was collected and recorded in a structured case record form. The data analysis was done as follows:

- The prescriptions were evaluated as per WHO-INRUD drug prescribing indicators.13
- The average number of drugs per prescription.
- Percentage of drugs prescribed by generic name.
- Percentage of the drugs prescribed from the list of essential medicines.
- Percentage of prescriptions with injections(s) prescribed.
- The National List of Essential Medicines 2015 of India was used to check whether the drugs prescribed were from the list.14

Prescriptions were assessed for a pattern of psychotropic drug use as per DUS metrics.15

The prescribed drugs were classified as per WHO anatomical therapeutic and chemical (ATC)—DDD classification.16

Prescribed daily dose (PDD) was calculated by taking the average of the daily dose prescribed and the PDD/DDD ratio was used to assess the overuse and underuse of prescribed drugs.

The patients were asked about the health-related QOL (HRQOL) pertaining to disease using the WHO QOL-Brief version (BREF) questionnaire17 and its correlation with an average number of drugs per prescription was done.

Adverse drug reactions (ADRs) of each patient were captured using the ADR form by the Pharmacovigilance Commission of India.18

The severity of ADR was assessed using the Hartwig’s severity assessment scale.19 For the purpose of the study, only mild (levels 1 and 2) and moderate (level 3) cases of ADR as per Hartwig severity assessment scale were included.

Medication adherence was evaluated using a self-administered medication compliance questionnaire and its correlation with an average number of side effects per prescription was done.

A complete history of the patient was recorded. Additionally, the mental status exam (MSE) was assessed. MSE was carried out under the supervision of an experienced psychiatrist.

Other variables of interest that could confound or mediate the relation were assessed including demographic profile (including age, sex, address, education, literacy, marital status, and living arrangements) and clinical parameters such as associated comorbidities.

**Statistical Analysis**

Statistical analysis was done with the IBM Statistical Package for the Social Sciences version 20.0. Continuous variables were presented as mean ± standard deviation (SD). Categorical variables were expressed as frequencies and percentages. Categorical data between the groups were compared using the Chi-squared test. The Shapiro–Wilk test has been applied to test the normality of continuous data in the case of the parametric test. The comparison of nonnormally distributed continuous variables between two groups was performed using the Mann–Whitney U test. The correlation of nonnormally distributed variables was performed using Spearman’s correlation test. For all statistical tests, a p-value < 0.05 has been taken to indicate a significant difference/association/correlation.

**Results**

**Characteristics of Study Participants**

The percentage of female and male patients was 57 and 43% during the COVID-19 period, while it was 54 and 46% during the pre-COVID-19 period, respectively. The age range was 18–60 years with the mean age being 38 ± 12.14 years during COVID-19 and 36.74 ± 11.19 years during the pre-COVID-19 period. Various other demographic characteristics of the study population are shown in (Table 1).

**Pattern of Psychiatric Disorders**

Among patients recruited during COVID-19 times, 49% were of anxiety disorders, 42% of depression, and 9% of mixed anxiety depressive disorder. Among patients recruited from the pre-COVID-19 pandemic period, 40% had anxiety disorders, 47% had depression and 13% were diagnosed having mixed anxiety depressive disorder.

**Analysis of Prescription Patterns as per WHO/INRUD Drug Use Indicators**

A total of 200 prescriptions were evaluated for drug use indicators. Around <2% of the prescriptions contained more than five drugs per prescription. Apart from psychotropic drugs, other commonly co-prescribed drugs were vitamin B complex, calcium and vitamin D3, and antacids. A comparison between various drug use indicators during COVID-19 and pre-COVID-19 pandemic is shown in (Table 2).

**Psychotropic Drugs Classification as per the WHO-ATC/DDD Index**

Defined daily dose (DDD) mentioned in the table is for the oral formulation of the drugs as mentioned in WHO-ATC/DDD index 2022.16
Table 1: Demographic characteristics of study population during pre-COVID-19 (n = 100) and during COVID-19 (n = 100)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pre-COVID-19</th>
<th>COVID-19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) ± SD</td>
<td>36.74 ± 11.19</td>
<td>38 ± 12.14</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>46</td>
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<tr>
<td>Education</td>
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<td></td>
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<tr>
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<td>35</td>
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<tr>
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</tr>
<tr>
<td>Middle school</td>
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</tr>
<tr>
<td>High school</td>
<td>19</td>
<td>11</td>
</tr>
<tr>
<td>Graduate</td>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td>Marital status</td>
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<td></td>
</tr>
<tr>
<td>Married</td>
<td>75</td>
<td>76</td>
</tr>
<tr>
<td>Divorced/widowed</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Never married</td>
<td>18</td>
<td>17</td>
</tr>
<tr>
<td>Financial status</td>
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<td></td>
</tr>
<tr>
<td>Independent</td>
<td>36</td>
<td>33</td>
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<tr>
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<tr>
<td>Working</td>
<td>45</td>
<td>34</td>
</tr>
</tbody>
</table>

A, average number of drugs per prescription; B, percentage of drugs prescribed by generic name; C, percentage of the drugs prescribed from the list of essential medicines; D, percentage of prescriptions with injections prescribed. If p < 0.05 here is a statistically significant difference between the groups. Test applied, Mann–Whitney U test.

Table 2: WHO/INRUD core prescribing indicators during pre-COVID-19 and during COVID-19 pandemic

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Drug</th>
<th>ATC code</th>
<th>DDD (mg)</th>
<th>PDD (mg)</th>
<th>PDD (mg)</th>
<th>PDD/DDD</th>
<th>PDD/DDD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fluoxetine</td>
<td>N06AB03</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Paroxetine</td>
<td>N06AB05</td>
<td>20</td>
<td>14</td>
<td>14</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>Sertraline</td>
<td>N06AB06</td>
<td>50</td>
<td>36.5</td>
<td>32.5</td>
<td>0.73</td>
<td>0.65</td>
</tr>
<tr>
<td>4</td>
<td>Escitalopram</td>
<td>N06AB10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>Vilazodone</td>
<td>N06AX24</td>
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<td>30</td>
<td>30</td>
<td>0.75</td>
<td>0.75</td>
</tr>
<tr>
<td>6</td>
<td>Amitriptyline</td>
<td>N06AA09</td>
<td>95</td>
<td>75</td>
<td>75</td>
<td>0.5</td>
<td>0.45</td>
</tr>
<tr>
<td>7</td>
<td>Clomipramine</td>
<td>N06AA04</td>
<td>100</td>
<td>75</td>
<td>66.6</td>
<td>0.75</td>
<td>0.66</td>
</tr>
<tr>
<td>8</td>
<td>Nortriptyline</td>
<td>N06AA10</td>
<td>75</td>
<td>25</td>
<td>37.5</td>
<td>0.33</td>
<td>0.5</td>
</tr>
<tr>
<td>9</td>
<td>Mirtazapine</td>
<td>N06AX11</td>
<td>30</td>
<td>15</td>
<td>15</td>
<td>0.5</td>
<td>0.5</td>
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<tr>
<td>10</td>
<td>Olanzapine</td>
<td>N05AH03</td>
<td>10</td>
<td>6.25</td>
<td>7</td>
<td>0.625</td>
<td>0.7</td>
</tr>
<tr>
<td>11</td>
<td>Lorazepam</td>
<td>N05BA06</td>
<td>25</td>
<td>0.5</td>
<td>0.5</td>
<td>0.26</td>
<td>0.2</td>
</tr>
<tr>
<td>12</td>
<td>Alprazolam</td>
<td>N05BA12</td>
<td>1</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>13</td>
<td>Clonazepam</td>
<td>N03AE01</td>
<td>8</td>
<td>0.65</td>
<td>0.55</td>
<td>0.08</td>
<td>0.06</td>
</tr>
<tr>
<td>14</td>
<td>Clobazam</td>
<td>N05BA09</td>
<td>20</td>
<td>5</td>
<td>5</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>15</td>
<td>Etizolam</td>
<td>N05BA19</td>
<td>3</td>
<td>0.4</td>
<td>0.48</td>
<td>0.13</td>
<td>0.16</td>
</tr>
<tr>
<td>16</td>
<td>Propranolol</td>
<td>C07AA05</td>
<td>160</td>
<td>10</td>
<td>10</td>
<td>0.06</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Table 3: Commonly prescribed drugs in common mental disorder patients with their WHO-ATC classification codes, DDD, PDD, and PDD/DDD ratio

Table 4: Association of WHO-QOL-BREF Score with the Average Number of Drugs per Prescription

The mean scores of WHO-QOL physical, psychological, social, and environmental domains were 58.82 ± 10.39, 58.66 ± 9.06, 60.81 ± 8.76, and 65.45 ± 7.39, respectively. The correlation coefficient between an average number of drugs per prescription and QOL was found to be −0.61 (p-value 0.001), indicating poor QOL with an increased number of pills (Table 4).

Table 5: Association Between Medication Adherence and Number of ADRs per Prescription

Among 100 patients enrolled during the COVID-19 pandemic, 55% (n = 55) had complete adherence whereas 45% (n = 45) patients had incomplete adherence with the medications. Around 27% (n = 27) of patients had developed ADRs of mild to moderate severity with the medications. Around 22% (n = 22) of patients developed one ADR per prescription while 5% (n = 5) developed two ADR per prescription. Medication adherence was found to be poor in patients who developed ADR with the prescription (p-value of 0.001) (Table 5).

Table 6: The ADRs Observed in the Population Under Study

The common ADRs observed in the 100 patients (during the COVID-19 period) were nausea (2%), indigestion (4%), dry mouth (2%), and lethargy (4%) among others (Table 6).

DISCUSSION

In this cross-sectional DUS, we found that the common mental disorders were more prevalent among young adults (age-group 18–30 years) during the COVID-19 pandemic period. This is consistent with a global cross-sectional survey conducted by Varma et al., which revealed that younger people are more vulnerable to stress, anxiety, and depression during the COVID-19 pandemic. Similarly, it was found that common mental disorders were more prevalent among females (55.5%, n = 111) as compared to males (44.5%, n = 89). This also matches with the findings of a systematic review and meta-analysis done by Steel et al. on the global prevalence of common mental disorders.

The average number of drugs per prescription in this study was found to be 2.96 ± 1.18 and 2.48 ± 1.09 during the COVID-19 pandemic and prepandemic period, respectively. Similar drug utilization studies conducted in the South Asian region found...
the average number of psychotropic drugs per prescription was between 2.3 and 3 drugs per prescription (WHO recommended value 1.6–1.8).22,23 Since <2% of the prescriptions had more than five drugs, we can say that polypharmacy was avoided too.

In the present study, a large proportion of drugs, that is, 95.77% during the COVID-19 pandemic period and 97.40% during prepandemic period were prescribed by generic name. This percentage was slightly lower than the WHO recommendation of 100%, yet better than observations of other similar studies.24,25 Use of drugs from the national list of essential medicine was also high, that is, 85.12% during the COVID-19 pandemic period and 89.40% during prepandemic period which is higher than a similar study conducted by Sarangi et al.26 This reflects rational prescribing and proper use of resources in healthcare.

In order to improve the quality of drug use, the WHO-ATC/DDD methodology serves as a tool for drug utilization monitoring and research.27 It is important to note that the PDD can vary according to different patients and their disease factors. Moreover, PDD can vary between different countries substantially, as various studies have indicated that PDDs for most psychotropic drugs are often lower in the Asian population as compared to the Caucasian population.28–30 This is in line with the present study, where the PDD/DDD ratio of all the drugs was found to be one or less than one.

The PDD/DDD ratio of selective serotonin reuptake inhibitors such as fluoxetine, escitalopram was found to be one indicating adequate prescribing while for paroxetine, and sertraline it was less than one indicating underprescribing. The ratio for tricyclic antidepressants such as amitriptyline, nortriptyline, and clomipramine was also found to be less than one. These findings are in line with the study conducted by Furukawa et al. which concluded that treatment with low-dose tricyclics is justified as it results in fewer dropouts due to ADRs.31

The present study observed a negative correlation between HRQOL and pill burden, indicating a poor QOL with the increase in the number of drugs per prescription. Our observations were consistent with the study done by Schenker et al. which revealed that increased consumption of a number of drugs was associated with higher symptom burden and lower QOL.32 Medication nonadherence in psychiatric patients contributes to a poorer health outcome.33 A recent study done by Ejeta et al. concluded that adverse drug reaction leads to nonadherence and discontinuation of therapy.34 Another study done by Marasine et al. in patients diagnosed with depression showed that adverse drug effect with the antidepressant medication was a strong predictor of medication discontinuation.35 Results of the present study, indicate a strong association between the development of ADRs and medication nonadherence which harmonizes with the previous reports.

Being a cross-sectional drug utilization study, we could not evaluate the outcomes of therapy prescribed to the patients. As the study was conducted during the COVID-19 pandemic period hence we could not determine the HRQOL and medication adherence in patients from the prepandemic period. Another limitation of this study is that it does not cover rural areas. Some factors like drug adherence, QOL, etc. would be different in rural settings and therefore would generate different results.

Nevertheless, the catchment area of patients coming to this tertiary care hospital included the nearby rural areas as well.

**CONCLUSION**

The present study shows that in the described tertiary health care facility, rational drug prescribing was followed in accordance with various drug use indicators mentioned by WHO/INRUD. The study also establishes that an increase in the number of drugs per prescription is associated with poorer HRQOL. This study also highlights that the development of ADRs leads to poor medication adherence. Further research with a larger sample size is needed to provide more evidence on the association between drug prescription on HRQOL and medication adherence.

**REFERENCES**


---

**Table 4: Association between WHO-QOL-BREF score (HRQOL) with the average number of drugs per prescription**

<table>
<thead>
<tr>
<th>Total HRQOL score</th>
<th>Average number of drugs per prescription</th>
<th>Pearson correlation (R value)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>243.74 ± 31.76</td>
<td>2.96 ± 1.18</td>
<td>−0.611*</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*Correlation is significant at the 0.01 level (two-tailed). Note: If p < 0.05 there is a statistically significant correlation between the variables if r value is negative then there is a negative correlation between the variables and vice versa.

**Table 5: Association between medication adherence and number of ADRs per prescription**

<table>
<thead>
<tr>
<th>Number of ADRs</th>
<th>Total</th>
<th>Chi-square value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>48.9%</td>
<td>11.1%</td>
<td>100.0%</td>
</tr>
<tr>
<td>1</td>
<td>40.0%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>11.1%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Medication adherence</th>
<th>Total</th>
<th>Chi-square value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incomplete</td>
<td>22</td>
<td>18</td>
<td>5</td>
</tr>
<tr>
<td>Complete</td>
<td>51</td>
<td>4</td>
<td>0</td>
</tr>
</tbody>
</table>

| Total                | 73    | 22               | 5       | 100    |        |       |
|                      | 73.0% | 22.0%            | 5.0%    | 100.0% |        |       |

If p < 0.05 there is a statistically significant association between the variables; test applied, Chi-square test.
16. WHOCC – ATC/DDD Index, Available from: https://www.whocc.no/atc_ddd_index_and_guidelines/atc_ddd_index/
18. Pharmacovigilance, Programme of India, Available from: https://www.ipc.gov.in/PvPI/adr.html
19. Assessment of severity using the Hartwig severity scale, Available from: https://plos.figshare.com/articles/dataset/Assessment_of_severity_using_the_Hartwig_severity_scale_/5264671/1

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A Cross-sectional Survey of Public Knowledge and Perspective on Coronavirus Disease, Vaccination, and Related Research in India during the COVID-19 Pandemic

Renuka Munshi1,*, Miteshkumar Maurya2

Received: 08 March 2023; Accepted: 15 May 2023

ABSTRACT

Background and rationale: The coronavirus disease 2019 (COVID-19) pandemic has left no person unexposed to the wisdom about the need for human preparedness to tackle future pandemics irrespective of individual caste, race, religion, and education status. The extent of this change in knowledge and public perspective is difficult to measure in a populous nation like India subjected to individual freedom and cultural beliefs. Hence, we planned this study with the objective of evaluating the knowledge and perception of the Indian public towards COVID-19 disease, vaccination and research activities associated with the COVID-19 pandemic.

Materials and methods: This is an observational, single-center, cross-sectional study (n = 244) conducted after obtaining approval from the Ethics Committee for Academic Research Projects. All consenting study participants, Indian residents aged > 18 years, were administered a prevalidated and structured questionnaire and interviewed for their honest opinion. The outcome measures were to evaluate the knowledge and perception of the study participants in each of the domains of COVID-19 disease, COVID-19 vaccination, and COVID-19-related clinical research. Demographic characteristics were summarized using descriptive statistics. All analyses were done at a 5% significance level using GraphPad InStat version 3.1. Data on the proportion of participants’ responses to the questions in each of the three domains of COVID-19 disease (D), COVID-19 vaccination (V), and COVID-19-related research (R) were assessed.

Results: Study participants who knew the causative agent for coronavirus disease were 93.03% (227/244), nomenclature (77.45%, 189/244), those who could define pandemic (89.34%, 218/244), preventive measures against covid (96.31%, 235/244), lungs as the most common organ affected (96.31%, 235/244) and all answered that the origin of novel coronavirus was China. The majority of them felt that COVID-19 pandemic waves would never end (39.34%, 96/244), there was no effective drug/vaccine therapy available, and the lack of oxygen/hospital beds (39%) resulted in maximum mortality, and 47.13% (115/244) were worried about future bioterrorism. The lockdown measures were justified by 161/244 (65.98%), and 93.85% supported lockdown measures to curb the spread of infection. The improvement in air quality/environment hygiene, realizing the importance of hygiene, vaccine and disease, and spending quality time with family were the best three things to happen during the pandemic, while the loss of wages, nonavailability of medicines/vaccines/oxygen/hospital beds with mental/physical health deterioration were the worst three things experienced by people. Regarding COVID-19 vaccination, the most common reason to get vaccinated was to prevent infection/critical outcomes of COVID-19 (78.27%, 191/244); 79% already suffered COVID-19 prior to vaccination, while 68.85% suffered a COVID-19 infection after taking the vaccine which was mostly asymptomatic/mild. Almost 56.96% (139/244) participants supported compulsory vaccination for all in the larger interest of society and to prevent fatal COVID-19 outcomes. There were safety concerns mainly with accelerated approval of vaccines (4.1%, 10/244) among the public, and 32.78% (80/244) attributed limited infrastructure/vaccination centers/healthcare staff as the major challenge of a mass vaccination program with 71.72% (175/244) supporting the vehicle/home vaccination drives to meet the vaccination demand in the pandemic. Approximately 38.11% (93/244) blamed the lack of sufficient vaccine manufacturing sites in India as a major vaccine shortage. Almost 82% public knew that vaccines are incapable of providing lifelong immunity or conferring protection against multiple variants, with 34.83% desiring to get polyvalent vaccines that would provide immunity against multiple COVID-19 variants. A total of 57.37% knew about clinical research, believed that the vaccine/drug development process was slow in India (29.91%), that there was a lack of funds invested in COVID-19-related clinical research (62.29%), that there was a lack of attention was given to the alternative system of medicines, 77.86% supported accelerated drug/vaccine approval in the pandemic. Around 64.34% of the study, participants knew about the available and approved COVID-19 treatment options, such as antiviral drugs, monoclonal antibodies and vaccines. Of the total 244 study participants, 98.36% believed that clinical research is important for science to progress. When asked about their willingness to participate in COVID-19 clinical research, only 40.57% agreed, while 29% opted for non-COVID-19 related clinical research, and 29% refused to participate in any kind of clinical research. Almost 88.93% refused to take medicines without approval by drug regulatory bodies, and 54.51% agreed to participate in COVID-19 clinical research, only 40.57% agreed, while 29% opted for non-COVID-19 related clinical research, and 29% refused to participate.

Conclusion: The survey provides insight into the public awareness and perception of the pandemic that has taught all the lessons for capacity building in automation, construction of robust medical infrastructure, and the need for future preparedness.

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic has affected the lives of many people in India as well as abroad. Though this pandemic has had devastating effects globally in terms of public health and the economy, there were certain positive changes noted among the population, such as a change in attitude, knowledge, and perspective towards the maintenance of hygiene practice, a sense of solidarity in curbing the transmission of infectious disease to others by self-isolation, following quarantine norms and getting vaccinated, felt need to strengthen the healthcare infrastructure and importance of the role of health care workers, the use of the virtual online platform and use of automation in all routine activity, the need of accelerated research and new drug approval by drug regulatory authorities.1–5 The COVID-19 pandemic and vaccination left no person unexposed to the wisdom about
human preparedness towards the occurrence of such pandemics that may occur multiple times in the future, irrespective of caste, race, religion, and education status. It has been almost >1 year since the COVID-19 pandemic, and people have fully coordinated with the Indian vaccination program and learned to adopt COVID-19-appropriate behavior to ensure everyone’s safety.6 The extent of this change in knowledge and public perspective is difficult to predict or measure in a populous nation like India, subject to individual freedom and cultural beliefs.7–9 Hence we planned to conduct a questionnaire-based survey with both online as well as physical distribution of the questionnaire to evaluate the knowledge and perception of the Indian public towards the COVID-19 vaccination and COVID-19 disease and research activities associated with the COVID-19 pandemic.

**MATERIALS AND METHODS**

**Study Design**

This was a single-center, observational, cross-sectional study conducted on all consenting public participants residing in India, inclusive of those from medical and nonmedical backgrounds.

**Ethics**

The study was accorded approval from the Institutional Ethics Committee for Academic Research Projects. The study was conducted in accordance with the Indian Council of Medical Research National Ethical Guidelines for Biomedical and Health Research Involving Human Participants (2017) and Indian Good Clinical Practice (2001) guidelines.

**Sample Size Calculation**

For our cross-sectional study, the sample size was calculated to be n = 354 based on Tandon et al. study with a response rate of 36% for knowledge assessment as the primary objective and a 5% margin of error (d) using a sample size formula \( n = \frac{Z^2 \cdot p(1-p)}{d^2} \). This was calculated to get their responses. The nationality as Indian was ensured through the contact numbers for online participants and identity proof for offline enrolment.

**RESULTS**

**Demographics**

The validated structured three-domain questionnaire was circulated among 354 study participants with a response rate of almost (68.93%). The sample size was \( n = 244 \) from those who consented and gave the response used for the final analysis. The demographic characteristics of the study participants are tabulated in Table 1.

**COVID-19 Disease Pandemic-based Question**

There were 13 (D1–D13) COVID-19 disease pandemic-related questions in the pan-India survey circulated all over India. Almost 93% (227/244) of the study participants could correctly answer the causative agent for the COVID-19 pandemic as a novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus, but still, 4 and 3% of the study population attributed the cause to bacterial infection and influenza adenovirus infection respectively that indicates the lack of awareness about the cause of COVID-19 pandemic among some individuals. When asked about the meaning of the number “19” used in COVID-19 and the number “2” in the SARS-CoV-2, 77.45% (189/244) responded correctly, reflecting good knowledge regarding the pandemic. When questioned about the non-ending waves of the COVID-19 pandemic, 39.34% (96/244) were optimistic that the COVID-19 pandemic waves would end someday though 23.77% (58/244) believed that the COVID-19 pandemic waves would never end and that people would have to learn to stay with masks, physical distancing, booster vaccination and other precautions indefinitely while 36.88% (90/244) found it difficult to predict anything conclusive. Only 89.34% (218/244) of the study participants could correctly define the term “pandemic” as a disease outbreak across the country and continents, but few responded that it meant spread within the country (5.73%), local area outbreak (1.63%) and one that causes increase mortality (3.27%). Almost 96.31% (235/244) of the respondents were aware of vaccination, wearing masks, physical distancing, and use of hand sanitizers as the precautionary measures to prevent COVID-19 infection. That the lungs are the first organs to be affected in SARS-CoV-2 infection was correctly answered by 96.31% (235/244). The majority (38%) believed that an increase in mortality during the COVID-19 pandemic was mainly due to the lack of effective drugs/therapy and oxygen/hospital beds. Few attributed the reasons for deaths to no effective vaccine available/not taken vaccine (8.19%), less attention given to non-covid serious diseases (6.96%), lack of health care staff (6.96%), and poverty (2%). All the study participants were very well aware of the country source origin of the novel coronavirus as China. Almost 47.13% (115/244) of the study participants believe the possibility of any pandemic due to virus/infectious agents (bioterrorism) could reoccur in the future. 65.98% (161/244) of the study participants were of the opinion that the lockdown restrictions during the COVID-19 pandemic were not a breach of individual freedom to movement, but still, 45/244 (18.44) criticized this measure to curb the pandemic, while few (15.57%) were neutral. A total of 229/244 (93.85%) of the study population were in favor of movement restrictions of lockdown to prevent/break the chain of virus transmission, but 6.14% (15/244) supported the freedom of movement to earn their livelihood rather than dying of starvation due to loss of wages. Among the best three things that happened during the COVID-19 pandemic were improvement in air quality/environmental hygiene (61.88%), people realizing the importance of hygiene in practice, vaccination, virus pathogenesis (59.42%), and all could spend quality time with family (50.41%). The worst three things that occurred during the pandemic were people losing their jobs/wages (74.59%), nonavailability of medicines, vaccines, oxygen/intensive care unit (ICU) beds (67.21%), and mental/physical health deterioration (58.61%). The other responses are given in detail in Table 2.

**COVID-19 Vaccine-based Questions**

There were 13 COVID-19 vaccination-based questions (V1–V13) circulated through our questionnaire survey. About 89.34% (218/244) of study participants did not suffer any major/serious medical condition/disease before receiving the COVID-19 vaccine. Most of the people (78.27%) took vaccination to prevent COVID-19 infection, with 22.13% (54/244) being infected once, twice (2.86%), and more than two times (2.86%). For those infected with the novel SARS-CoV-2 variant postvaccination, symptoms were asymptomatic (1.63%), mild...
Public Knowledge and Perspective on Coronavirus Disease

Table 1: Demographic characteristics of the study participants

<table>
<thead>
<tr>
<th>Demographic characteristics</th>
<th>The proportion of study participants (N = 244) n/N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>118 (48.36)</td>
</tr>
<tr>
<td>Female</td>
<td>126 (51.63)</td>
</tr>
<tr>
<td>Age-wise distribution of respondents are:</td>
<td></td>
</tr>
<tr>
<td>18–20 years</td>
<td>62 (25.41)</td>
</tr>
<tr>
<td>21–30 years</td>
<td>103 (42.21)</td>
</tr>
<tr>
<td>31–40 years</td>
<td>40 (16.39)</td>
</tr>
<tr>
<td>41–50 years</td>
<td>23 (9.42)</td>
</tr>
<tr>
<td>51–60 years</td>
<td>16 (6.55)</td>
</tr>
<tr>
<td>State-wise distribution of study participants (N = 244)</td>
<td></td>
</tr>
<tr>
<td>Madhya Pradesh</td>
<td>6</td>
</tr>
<tr>
<td>Karnataka</td>
<td>3</td>
</tr>
<tr>
<td>Tamil Nadu</td>
<td>2</td>
</tr>
<tr>
<td>Maharashtra</td>
<td>201</td>
</tr>
<tr>
<td>Andhra Pradesh</td>
<td>1</td>
</tr>
<tr>
<td>12th grade passed</td>
<td></td>
</tr>
<tr>
<td>10th grade passed</td>
<td></td>
</tr>
<tr>
<td>Below 10th grade</td>
<td></td>
</tr>
<tr>
<td>Did you take the COVID-19 vaccine?</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>240 (98.36)</td>
</tr>
<tr>
<td>No</td>
<td>4 (1.63)</td>
</tr>
<tr>
<td>Number of patients taking a vaccine for COVID-19</td>
<td></td>
</tr>
<tr>
<td>Single dose</td>
<td>8 (3.27)</td>
</tr>
<tr>
<td>Two doses</td>
<td>190 (77.86)</td>
</tr>
<tr>
<td>Booster dose</td>
<td>46 (18.85)</td>
</tr>
<tr>
<td>Type of COVID-19 vaccine taken</td>
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</tr>
<tr>
<td>Covishield</td>
<td>189 (77.45)</td>
</tr>
<tr>
<td>Covax</td>
<td>45 (18.44)</td>
</tr>
<tr>
<td>Sputnik</td>
<td>4 (1.63)</td>
</tr>
<tr>
<td>Moderna messenger ribonucleic acid (RNA) vaccine</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Covovax</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Don't know</td>
<td>3 (1.22)</td>
</tr>
<tr>
<td>Not applicable (not taken vaccine)</td>
<td>3 (1.22)</td>
</tr>
<tr>
<td>Adverse events after taking any of the doses of COVID-19</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>130 (53.27)</td>
</tr>
<tr>
<td>No</td>
<td>114 (46.72)</td>
</tr>
<tr>
<td>Adverse events</td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td>89 (70.1)</td>
</tr>
<tr>
<td>Body ache</td>
<td>77 (60.6)</td>
</tr>
<tr>
<td>Headache</td>
<td>39 (30.7)</td>
</tr>
<tr>
<td>Injection site pain, redness, swelling or bruise</td>
<td>56 (44.1)</td>
</tr>
<tr>
<td>Other adverse events</td>
<td></td>
</tr>
<tr>
<td>COVID infection</td>
<td></td>
</tr>
<tr>
<td>Lichen planus</td>
<td></td>
</tr>
<tr>
<td>Menstrual cycle disturbance</td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td></td>
</tr>
<tr>
<td>Dullness of mood</td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td></td>
</tr>
<tr>
<td>Education status</td>
<td></td>
</tr>
<tr>
<td>Below 10th grade</td>
<td>4 (1.63)</td>
</tr>
<tr>
<td>10th grade passed</td>
<td>55 (22.54)</td>
</tr>
<tr>
<td>12th grade passed</td>
<td>51 (20.90)</td>
</tr>
<tr>
<td>Undergraduate passed</td>
<td>28 (11.47)</td>
</tr>
<tr>
<td>Graduate passed</td>
<td>12 (4.91)</td>
</tr>
<tr>
<td>Postgraduate passed and above</td>
<td>94 (38.52)</td>
</tr>
</tbody>
</table>

Table 2: COVID-19-related Research-based Questions

<table>
<thead>
<tr>
<th>Questions</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Is it clear that the vaccine would protect from multiple Covid-19 variants?</td>
<td>34.83%</td>
</tr>
<tr>
<td>Would it protect against the multiple variants of COVID-19?</td>
<td>81.96%</td>
</tr>
<tr>
<td>Lack of awareness related clinical research to evaluate public knowledge on the research practice</td>
<td>57.37%</td>
</tr>
<tr>
<td>Lack of infrastructure in mass vaccination programs reaching the public</td>
<td>32.78%</td>
</tr>
</tbody>
</table>

(22.54%), moderate (6.96%), and severe (0.81%). However, when asked about if there should be compulsory vaccination for all for the larger benefit of society, 56.96% (139/244) participants were in favor of reasons to benefit society by breaking the virus transmission chain and protecting from severe/fatal COVID-19 outcomes. However, 17.21% (42/244) of the study participants were not in favor of compulsory vaccination of individuals for the larger interest of society and believed that every individual had the right to decide whether or not to be vaccinated. Reasons for not taking the COVID-19 vaccine included safety concerns about the vaccines due to the accelerated regulatory approval process (4.1%), hearing about safety issues (adverse events) among those vaccinated (3.68%), 2.45% were of the belief that COVID-19 disease would not have an adverse impact on them and 2.45% were confused about which vaccine to take. The important hurdles in mass vaccination programs reaching the public were limited infrastructure/vaccination centers and healthcare staff (32.78%, 80/244) and lack of awareness programs by the government and covid knowledge deficiencies (25.41%, 62/244) in view of public opinion followed by insufficient vaccine production (29.51%), multiple doses of vaccine (7.37%, 18/244), and cost factor (4.91%, 12/244). About 71.72% (175/244) were in support of both home and vehicle vaccination drives for the larger benefit interest of the society to tackle the pandemic, while 14.75% declined this option due to associated safety concerns. Lack of manufacturing sites (38.11%, 93/244) was considered to be the main cause of the shortage of vaccine production in India, followed by vaccines being exported outside the country (30.73%, 75/244) as the second major cause. Approximately 82.37% believed that the vaccine would not confer life-long immunity against COVID-19, nor would it protect against the multiple variants of COVID-19 (81.96%, 200/244). The quality of vaccines that everyone would appreciate the most were those that protect against multiple COVID-19 variants (34.83%, 85/244), offered lifelong immunity/immunity for maximum duration (29.1%, 71/244), and safety was preferred over efficacy by 19.26% (47/244) public. A detailed evaluation of the COVID-19 vaccination-related response is provided in Table 3.

COVID-19-related Research-based Questions

There were 13 questions for COVID-19-related clinical research to evaluate public knowledge about the research practice in India. Almost more than half (57.37%) of the study population could define and understand the meaning of clinical research. Vaccine and drug development processes were slow in India during covid pandemic, as suggested by 29.91% of the respondents, but the majority (41.39%) did not support this view, while 26.68% failed to comment. A total of 62.29% (152/244) agree that India has invested sufficient funds to promote clinical research during the pandemic. No attention was provided to alternative systems of medicine as 47.95% of the public understand the meaning of clinical research. There were 13 questions for COVID-19-related Research-based Questions. There were 13 questions for COVID-19-related Research-based Questions. There were 13 questions for COVID-19-related Research-based Questions. There were 13 questions for COVID-19-related Research-based Questions.
### Table 2: COVID-19 disease pandemic-based questions

<table>
<thead>
<tr>
<th>S. No.</th>
<th>COVID-19-related clinical research-based questions</th>
<th>Options</th>
<th>Number of participants with correct responses (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>What is the causative agent for COVID-19 disease/infection according to your knowledge</td>
<td>(a) Novel SARS CoV-2</td>
<td>227 (93.03)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) Influenza and adenovirus</td>
<td>7 (2.86)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c) Fungal infection</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(d) Bacterial infection</td>
<td>10 (4.1)</td>
</tr>
<tr>
<td>D2</td>
<td>What is 19 in COVID-19 and 2 in SARS-COV-2 indicates according to you</td>
<td>(a) 19 for receptor and 2 is variant</td>
<td>18 (7.37)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) 19 is the year of the COVID outbreak, and 2 is for ACE-2 receptor</td>
<td>189 (77.45)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c) 19 for variants and 2 for subtypes</td>
<td>9 (3.68)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(d) Don't know</td>
<td>24 (9.83)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(e) Never heard of this</td>
<td>4 (1.63)</td>
</tr>
<tr>
<td>D3</td>
<td>Do you expect that the COVID-19 pandemic waves will never end, and we have to live with masks, physical distancing, and booster vaccinations with all safety measures?</td>
<td>(a) Yes</td>
<td>58 (23.77)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) No</td>
<td>96 (39.34)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c) Difficult to predict</td>
<td>90 (36.88)</td>
</tr>
<tr>
<td>D4</td>
<td>The pandemic refers to a disease outbreak that spreads</td>
<td>(a) Within the country</td>
<td>14 (5.73)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) In the local area of stay</td>
<td>4 (1.63)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c) Cause a lot of deaths</td>
<td>8 (3.27)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(d) Across countries and continents</td>
<td>218 (89.34)</td>
</tr>
<tr>
<td>D5</td>
<td>What are the best measures to follow to prevent COVID-19 Infection?</td>
<td>(a) Vaccination</td>
<td>7 (2.86)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) Wear masks</td>
<td>1 (0.41)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c) Physical distancing</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(d) Use hand sanitizers</td>
<td>1 (0.41)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(e) Taking antiviral drugs</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(f) All the four above</td>
<td>235 (96.31)</td>
</tr>
<tr>
<td>D6</td>
<td>Which organ does the novel coronavirus usually infect first?</td>
<td>(a) Kidney</td>
<td>1 (0.41)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) Heart</td>
<td>8 (3.27)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c) Lungs</td>
<td>235 (96.31)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(d) Brain</td>
<td>0 (0)</td>
</tr>
<tr>
<td>D7</td>
<td>What do you think the mortality in the COVID-19 pandemic is more due to?</td>
<td>(a) Lack of oxygen and hospital beds</td>
<td>94 (38.52)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) Lack of healthcare staff</td>
<td>13 (5.32)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c) Due to non-covid diseases</td>
<td>17 (6.96)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(d) Due to poverty</td>
<td>5 (2.04)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(e) Due to no vaccine available/not taking the vaccine</td>
<td>20 (8.19)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(f) No effective drugs/therapy available</td>
<td>95 (38.93)</td>
</tr>
<tr>
<td>D8</td>
<td>From which country is the suspected source of the novel coronavirus first came in the year 2019?</td>
<td>(a) India</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) United States of America</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c) United Kingdom</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(d) China</td>
<td>244 (100)</td>
</tr>
<tr>
<td>D9</td>
<td>Do you expect a pandemic due to any viral or infectious agent anytime in the future?</td>
<td>(a) Yes</td>
<td>115 (47.13)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) No</td>
<td>25 (10.24)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c) Cannot predict</td>
<td>104 (42.62)</td>
</tr>
<tr>
<td>D10</td>
<td>The lockdown decision during the COVID-19 pandemic is a breach of individual freedom to movement. Do you agree with this statement?</td>
<td>(a) Yes</td>
<td>45 (18.44)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) No</td>
<td>161 (65.98)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c) Cannot say</td>
<td>38 (15.57)</td>
</tr>
<tr>
<td>D11</td>
<td>Given an option, A. would you like to follow lockdown/restricted movement norms to prevent infection spread or B. Let others lose life/get infected due to infectious diseases just to sustain your livelihood/family during any pandemic.</td>
<td>(a) A</td>
<td>229 (93.85)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) B</td>
<td>15 (6.14)</td>
</tr>
</tbody>
</table>
Public Knowledge and Perspective on Coronavirus Disease

Table 3: COVID-19 vaccination-based questions

<table>
<thead>
<tr>
<th>S. No.</th>
<th>COVID-19-related Clinical research-based questions</th>
<th>Options</th>
<th>Number of participants with their responses (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V1</td>
<td>Why did you take the vaccine for COVID-19? Select the most important reason from the options below.</td>
<td>(a) To prevent covid infection/critical outcome</td>
<td>191 (78.27)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) To prevent transmission to others</td>
<td>35 (14.34)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c) Government will restrict movement and deprive us of benefits</td>
<td>13 (5.32)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(d) Peer pressure</td>
<td>2 (0.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(e) Not applicable/not taken vaccine yet</td>
<td>3 (1.22)</td>
</tr>
<tr>
<td>V2</td>
<td>Did you suffer from COVID-19 infection before the COVID vaccines were available or before taking the COVID vaccine?</td>
<td>(a) Yes</td>
<td>48 (19.67)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) No</td>
<td>193 (79.09)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c) Not applicable (not taken vaccine)</td>
<td>3 (1.22)</td>
</tr>
<tr>
<td>V3</td>
<td>Did you get infected even after receiving the COVID-19 vaccine?</td>
<td>(a) Yes</td>
<td>73 (29.91)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) No</td>
<td>168 (68.85)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c) Not applicable (not taken vaccine)</td>
<td>3 (1.22)</td>
</tr>
<tr>
<td></td>
<td>If yes, how many times were you detected positive for COVID-19 disease after COVID-19 vaccination</td>
<td>(a) 0</td>
<td>70 (28.68)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) 1</td>
<td>54 (22.13)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c) 2</td>
<td>7 (2.86)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(d) 8</td>
<td>7 (2.86)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(e) Not applicable</td>
<td>13 (5.32)</td>
</tr>
<tr>
<td>V4</td>
<td>If you were infected with COVID-19 post-COVID vaccination (COVID test positive), was the disease mild/moderate/severe based on the severity of the infection? (based on your assessment)</td>
<td>(a) Asymptomatic</td>
<td>4 (1.63)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) Mild</td>
<td>55 (22.54)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c) Moderate</td>
<td>17 (6.96)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(d) Severe</td>
<td>2 (0.81)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(e) No infection postvaccination</td>
<td>149 (61.06)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(f) Not applicable (not taken vaccine yet)</td>
<td>17 (6.96)</td>
</tr>
<tr>
<td>V5</td>
<td>What do you think, should there be compulsory vaccination for all?</td>
<td>(a) It’s our right to take the decision to take vaccination</td>
<td>42 (17.21)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) Benefit society by reducing the transmission</td>
<td>46 (18.85)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c) Protect from severe/fatal covid-19 outcomes</td>
<td>17 (6.96)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(d) Both b and c</td>
<td>139 (56.96)</td>
</tr>
</tbody>
</table>

Contd…
<table>
<thead>
<tr>
<th>S. No.</th>
<th>COVID-19-related Clinical research-based questions</th>
<th>Options</th>
<th>Number of participants with their responses (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>V6</td>
<td>If you have not taken the vaccine, please tell us your reasons for not taking the vaccine [can tick more than one answer]; if you have taken the COVID-19 vaccine, kindly tick as vaccine taken. [you can tick multiple options] if any reasons other than above, please specify as text below</td>
<td>(a) I don’t think coronavirus will have an adverse impact on me</td>
<td>6 (2.45)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) Against the rule of my religion</td>
<td>3 (1.22)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c) Safety concerns about the vaccine as it got approval too quickly</td>
<td>10 (4.09)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(d) Safety concerns due to some serious and non-serious adverse events in those vaccinated</td>
<td>9 (3.68)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(e) Lack of sufficient information about the vaccine</td>
<td>5 (2.04)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(f) No slots available for vaccines, and those available are paid</td>
<td>2 (0.81)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(g) Vaccine is chimpanzee adenovirus vectored and has the source of animal origin for manufacturing</td>
<td>1 (0.41)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(h) Vaccine may interfere with my genetics (deoxyribonucleic acid/RNA)</td>
<td>2 (0.81)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(i) No time to go and take the vaccine</td>
<td>1 (0.41)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(j) Confused about which vaccines to take as multiple vaccines are available</td>
<td>3 (1.22)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(k) I have seen others vaccinated and still getting COVID-19 infection</td>
<td>6 (2.45)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(l) Do not understand the clinical trial data of covid vaccines</td>
<td>2 (0.81)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(m) Not applicable</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(n) Any others specify</td>
<td></td>
</tr>
<tr>
<td>V7</td>
<td>What is the most difficult part of any mass vaccination program to reach the public, in your opinion?</td>
<td>(a) Cost</td>
<td>12 (4.91)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) Availability (insufficient production)</td>
<td>72 (29.51)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c) Multiple doses of vaccine</td>
<td>18 (7.37)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(d) Limited infrastructure/vaccination centers and healthcare staff</td>
<td>80 (32.78)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(e) No awareness program by the government/lack of public knowledge</td>
<td>62 (25.41)</td>
</tr>
<tr>
<td>V8</td>
<td>What do you think about the home and on-vehicle vaccination drive by the Government of India, where healthcare workers vaccinate critically ill patients?</td>
<td>(a) Not to promote as too difficult to manage adverse events</td>
<td>36 (14.75)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) Both home and vehicle vaccine drives are justified to meet the need of a large population</td>
<td>175 (71.72)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c) Home vaccination drive is better than a vehicle drive</td>
<td>26 (10.65)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(d) A vehicle vaccination drive is better than the home drive</td>
<td>7 (2.86)</td>
</tr>
<tr>
<td>V9</td>
<td>What do you think is the main cause of the shortage of vaccine production in India?</td>
<td>(a) Lack of sufficient manufacturing sites</td>
<td>93 (38.11)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) Lack of raw materials required for vaccine production</td>
<td>51 (20.90)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c) Most vaccines were exported outside of India</td>
<td>75 (30.73)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(d) Vaccines expired as the vaccination program was conducted in phases</td>
<td>25 (10.24)</td>
</tr>
<tr>
<td>V10</td>
<td>Did you suffer or have any medical condition/disease in the past before taking COVID-19 Vaccine? If yes, what disease/comorbidities you had before taking the vaccination (e.g., asthma or any kind of allergy, heart disease, epilepsy, kidney disease, surgeries, etc.)</td>
<td>(a) Yes</td>
<td>26 (10.65)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) No</td>
<td>218 (89.34)</td>
</tr>
<tr>
<td>V11</td>
<td>Do you think your vaccination provides you with lifelong immunity against COVID-19 disease</td>
<td>(a) Yes</td>
<td>43 (17.62)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) No</td>
<td>201 (82.37)</td>
</tr>
<tr>
<td>V12</td>
<td>Do you think once you have taken the vaccination, it will offer you immunity against all variants of the COVID-19 virus?</td>
<td>(a) Yes</td>
<td>44 (18.03)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) No</td>
<td>200 (81.96)</td>
</tr>
<tr>
<td>V13</td>
<td>What quality of vaccine would you like the most?</td>
<td>(a) Protection against multiple variants</td>
<td>85 (34.83)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(b) Maximum efficacy, safety later</td>
<td>7 (2.86)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c) Safety first, then efficacy</td>
<td>47 (19.26)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(d) Immunity for maximum duration/lifelong immunity</td>
<td>71 (29.09)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(e) Free of cost to the public</td>
<td>12 (4.91)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(f) Single-dose vaccination to reduce follow-ups/missed dose issues</td>
<td>22 (9.01)</td>
</tr>
</tbody>
</table>

Mask/social distancing/steam inhalation to prevent any infections.
during pandemic times based on interim data results. When asked about the medicines approved by the Indian regulatory authority, 64.34% knew that vaccines, antiviral drugs and monoclonal antibodies were approved during the pandemic times and 98.36% confirmed that clinical research is important for science and humanity to progress with respect to any disease with pandemic potential. 48.36% of the public were willing to participate in any COVID-19-related clinical research, while 40.57% preferred to participate in COVID-19 research over non-COVID-19 research. About 88.93% of the study participants refused to take any drug to treat COVID-19 without approval from the regulatory authority. The biggest hindrance in the progress of the clinical research has been attributed to lack or misuse of finances (35.24%, 86/244) followed by lack of infrastructure/qualified research staff for science and humanity to progress with respect to any disease with pandemic potential.

Table 4: COVID-19-related clinical research-based questions

<table>
<thead>
<tr>
<th>S. No.</th>
<th>COVID-19-related clinical research-based questions</th>
<th>Number of participants with correct responses (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>What is clinical research or clinical trials? (a) Research done in humans to use drugs in animals (b) Research done in humans to approve drug use in humans (c) Research done to understand the pathogenesis of virus infection in vitro (d) Research to develop new molecule for COVID treatment</td>
<td>24 (9.83) 140 (57.37) 53 (21.72) 27 (11.06)</td>
</tr>
<tr>
<td>R2</td>
<td>According to you, was the vaccine and drug development process slow in India during the outbreak of the COVID-19 pandemic? (a) Yes (b) No (c) Cannot comment</td>
<td>73 (29.91) 101 (41.39) 70 (28.68)</td>
</tr>
<tr>
<td>R3</td>
<td>During the COVID-19 pandemic, do you think India had invested sufficient funds to promote clinical research on COVID in India? (a) Yes (b) No</td>
<td>152 (62.29) 91 (37.29)</td>
</tr>
<tr>
<td>R4</td>
<td>Do you think that there was no attention provided to alternative systems of medicine (Ayurveda, Homoeopathy, Unani, Siddha) during COVID-19 research during the pandemic? (a) Yes (b) No</td>
<td>117 (47.95) 127 (52.04)</td>
</tr>
<tr>
<td>R5</td>
<td>Do you think that the early approval of vaccines and new drugs by the medicine approval authority of India based on interim data from clinical trials during the COVID-19 pandemic is justified during a pandemic? (a) Yes (b) No</td>
<td>190 (77.86) 54 (22.13)</td>
</tr>
<tr>
<td>R6</td>
<td>What preventive or curative therapy has been approved by the medicine approval authority of India for COVID-19 disease based on clinical research? (a) Vaccines (b) Monoclonal antibodies (c) Antiviral drugs (d) All the above</td>
<td>76 (31.14) 4 (1.63) 7 (2.86) 157 (64.34)</td>
</tr>
<tr>
<td>R7</td>
<td>Do you think that clinical research is important for science and humanity to progress with respect to any kind of pandemic? (a) Yes (b) No</td>
<td>240 (98.36) 4 (1.63)</td>
</tr>
<tr>
<td>R8</td>
<td>Would you participate in COVID-19-related clinical research for the development of evidence-based therapy for COVID-19? (a) Yes (b) No (c) Cannot say</td>
<td>118 (48.36) 53 (21.72) 73 (29.91)</td>
</tr>
<tr>
<td>R9</td>
<td>If you are given an option to participate in clinical research, what kind of research you would like to participate in priority? (a) COVID-19 related (b) Non-COVID-19 related (c) Would not participate in any</td>
<td>99 (40.57) 72 (29.51) 73 (29.91)</td>
</tr>
<tr>
<td>R10</td>
<td>Are you ready to take drugs for the treatment of COVID-19 without any medicine approval Authority of India approval for the same? (a) Yes (b) No</td>
<td>27 (11.06) 217 (88.93)</td>
</tr>
<tr>
<td>R11</td>
<td>In your opinion, what is the biggest hindrance to the progress of COVID-19-related clinical research activity in India? (a) Lack or misuse of funding support (b) Lack of infrastructure/qualified research staff (c) Nondisclosure of data and results publicly (d) Lack of advertisement for public participation (e) Low literacy rate</td>
<td>86 (35.24) 64 (26.22) 39 (15.98) 24 (9.83) 31 (12.70)</td>
</tr>
<tr>
<td>R12</td>
<td>If you are given an opportunity to participate in clinical research to actively take COVID-19 virus infection under controlled hospital settings and help in research to look for the efficacy of treatment or therapy, would you like to participate/venture into this kind of research? (a) Yes (b) No</td>
<td>133 (54.51) 111 (45.49)</td>
</tr>
<tr>
<td>R13</td>
<td>Do you think we lag behind other countries in research and development as per your experience during the current COVID-19 pandemic? (a) Yes (b) No</td>
<td>157 (64.34) 87 (35.65)</td>
</tr>
</tbody>
</table>
staff (26.22%, 64/244) and nondisclosure of study data/results publicly (15.98%, 39/244). Interestingly, 54.51% (133/244) of the study participants were willing to get infected with the COVID-19 virus under controlled research settings (human challenge research study) to investigate novel treatment/s for COVID-19 infection. A total of 64.34% of the study participants believe that India lags behind other countries in terms of research and development, as experienced during the COVID-19 pandemic. A detailed evaluation of the COVID-19-related clinical research response is provided in Table 4.

Discussion
The COVID-19 pandemic has taught people how to live by taking the necessary precautionary measures. Individuals have realized the importance of personal/community hygiene practices, the importance of vaccination and research as well. There are many studies that have evaluated the public perspective and attitude regarding the COVID-19 pandemic. Singh et al.’s knowledge, attitude, and practice (KAP) study (n = 694) on the Indian population revealed that 63.5% had good knowledge, 74% showed positive attitudes, and 93.2% practiced COVID-19-appropriate safe behavior.11 Poddar et al.’s study on n = 521 study participants revealed overall good knowledge, attitude and practice towards COVID-19 disease.12 A questionnaire-based survey among the Indian population conducted by Singh et al. during April–May 2020 showed a moderate level of COVID-19-related knowledge, attitudes, and practices in the Indian population.13 A cross-sectional study from Saudi Arabia by Al-Hanawi et al., conducted with 3,388 participants, revealed that men have less knowledge, less optimistic attitudes, and less good practice toward COVID-19 than women, with older people having better knowledge and practices than younger people.14 Kusum et al. described the determinants for COVID-19 vaccination acceptance and hesitancy among Indians, with 64.9% showing willingness to take COVID-19 vaccine, while 11.8% called coronavirus disease a hoax, 7.4% didn’t feel the need to get vaccinated and 5.6% didn’t want the vaccine to interfere with the natural immune system of the human body.15 Kumari et al. conducted eight focussed group discussions (n = 43), which revealed that young, working professionals and senior citizens referred to less reliable/incomplete sources of information regarding the COVID-19 vaccine, like advertisements and social media with no positive association found between the level of knowledge and positive attitude (acceptance) towards COVID-19 vaccination16 Bhartiya et al.’s KAP study on COVID-19 vaccination acceptance in western Indian population (n = 1342) also revealed that 79% were willing to take COVID-19 vaccine while 2% were still reluctant to take the vaccine with vaccine hesitancy being mainly due to the risk of serious adverse events following immunization.17 Anderson et al.’s study highlights the knowledge gap among the global population about clinical research, with 90% believing that clinical research is safe and 49% accepting that clinical trials disrupt their daily routine.18 Vittal et al.’s study on 257 medical students revealed that the students appreciated the importance of clinical research and would like it taught in the early part of the medical curriculum.19 Balamurugan et al. showed that more than 50% of the participants believed that there is no risk in clinical research, and 60% were not aware of what clinical research is.20 Figer et al.’s study in n = 453 Mumbai participants revealed that socioeconomic strata and age were the two important determinants behind clinical trial awareness.21

Study Limitations
Though the study was synthesized to target populations from all over India, the maximum enrolled study participants were from the state of Maharashtra. Moreover, our study enrolled a maximum population literate enough to read the informed consent documents and the questionnaire. The responses collected from across the states were possible through an online Google form link as it was difficult to physically reach out to every Indian state.

Conclusion
The survey provides insight into the public awareness and perception of the pandemic that has taught us all the lessons to learn, capacity building, constructing robust infrastructure, and the need for future preparedness.

Ethics Committee Approval
The study was approved by the Institutional Ethics Committee for Academic Research Projects [ECARP/2021/182].

Authors Contribution Statement
• Dr Renuka Munshi: Conceptualization, literature review, editing, reviewing and finalizing the manuscript.
• Dr Miteshkumar Maurya: Conceptualization, literature review, data collection, preparing and editing the manuscript.

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References

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Editor-in-Chief, JAPI
Study of Association of Chromosomal Region 1Q21–23 with Rheumatoid Arthritis and Their Correlation with Severity of Disease

Liyakat Ali Gauri1, Manoj Kumar Meena2, Ummmed Singh3*, Nikita Manoj4, Nadeem Liyakat5, Ramratan Yadav6, Ambreen Liyakat7, Nisha8

Received: 02 January 2023; Revised: 15 May 2023; Accepted: 18 May 2023

ABSTRACT

Background: The understanding of the pathophysiology of rheumatoid arthritis (RA) has taken a major step forward with the research of new illness-related genes and further deciphering the involved molecular. Gene variants like human leukocyte antigen (HLA)—DRB1 and PTPN22 1858T act as individual risk factors for RA. It also serves as a risk factor for the rate of progression of joint destruction and clinical manifestations in autoimmune diseases like RA. The focus of this study is to find out the association of chromosomal region 1q21–23 with RA and its connection with disease severity using the disease activity score (DAS) and distribution frequency of the prevalent alleles of such genes in an already recruited group of patients/controls of India, specifically Northwest Rajasthan.

Materials and methods: This was a case-control study wherein every patient of RA aged 16 years and above diagnosed with RA as per the 2010 American College of Rheumatology (ACR) and the European League against Rheumatism (EULAR) revised criteria for RA in Outpatient Department (OPD) and Inpatient Department (IPD) patients were included. Blood samples of the study population were drawn at Sardar Patel Medical College (SPMC), Bikaner (rheumatology OPD), along with the cooperation of Birla Institute of Technology and Science (BITS), Hyderabad (Department of Biological Sciences) from July 2009 to January 2012. A total of 100 controls (without any previous history of disease) and 135 cases were selected considering inclusion and exclusion criteria. Clinical data along with laboratory parameters like complete blood count, serum electrolytes (sodium, potassium, calcium, and chloride ions), blood sugar, blood urea with serum creatinine, lactate dehydrogenase (LDH) isoenzymes assay, serum glutamic-oxaloacetic transaminase (SGOT)/serum glutamic pyruvic transaminase (SGPT) ratio, serum γ-glutamyl transferase (GGT) level, serum amylase, arterial blood gas (ABG), total serum proteins were evaluated and recorded from the patients.

Results: Our study showed control group has a mean age of 45.11 ± 4.12 years. The case and control groups did not have significant differences in any of the clinical variables. 99% of cases show joint deformity. Allelic frequencies of the D1S318 polymorphism in cases were found significant in sizes 198, 204, 208, and 210, while it was found insignificant in sizes 192, 196, 200, 202, and 206. No correlation was found in allelic frequencies of the D1S318 polymorphism in cases and controls.

Conclusion: Bigger cohort studies will allow better genomic elucidation of clinically defined intermediate phenotypes evaluated in RA patients by virtue of the autoimmune origin of the disease and its diverse symptoms in patients. Genetic-molecular studies can be a milestone for adopting effective personalized treatment for such progressively debilitating diseases.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory illness that causes symmetric polyarthritis. It is the commonest type of inflammatory form of arthritis, which frequently causes joint destruction and resultant physical impairments. Since RA is a systemic condition, it can cause a wide range of extraarticular symptoms, including tiredness, vasculitis, subcutaneous nodules, pericarditis, pulmonary system involvement, peripheral nerve involvement, and hematological dysregulations.

The understanding of general and clinical studies over the last 20 years has completely changed how RA is diagnosed and treated. In recent years, S antibodies against cyclic citrullinated peptides (CCPs) have gained recognition as important biomarkers with diagnostic and prognostic value. The capacity to identify joint inflammation and degeneration in RA has increased with developments in magnetic resonance imaging and ultrasound technology. With the discovery of novel disease-associated genes and a newer understanding of the molecular pathways involved, our understanding of the pathophysiology of RA has advanced significantly. The clear benefit of the specifically targeted newest group of biologics has drawn attention to how important it is to comprehend these various pathways. Despite these advances, a major obstacle to the treatment and prevention of RA is the lack of a thorough understanding of its primary pathogenic pathways.

Three instances per 10,000 people are diagnosed with RA each year. Under the age of 15, onset is uncommon, and up until the age of 80, incidence increases with advancing years. Women are impacted three to five times more often than males by this condition, which has a 1% prevalence rate. Around 2–3% of first-degree relatives have the condition, while 15–20% of monozygotic twins have disease genetic concordance.

Family history is a significant risk factor since it is significantly correlated with the hereditary tissue type major histocompatibility complex (MHC) antigen human leukocyte antigen (HLA)—DR4 (notably DR0401 and DR0404).

Clinical signs of RA, as well as the rate at which joint destruction progresses in this inflammatory illness, are all influenced by the HLA-DRB1 and PTPN22 1858T gene variations. The immune-pathogenetic models of numerous different genes, including PAD4, CTLA4, MIF, and SLC22A4, are also up for discussion. The clinical implications of certain RA-related gene polymorphisms with the application of pharmacogenetic principles to a range of
therapeutic approaches include traditional disease-modifying anti-rheumatic medications and novel biological agents. Pharmacogenetics is an expeditiously developing field of study that promises to soon produce medicines that are personalized to each patient’s genetic profile. Most likely, a number of disease-related genes work together to cause RA. Whole genome linkage studies eventually focused on one particular locus, cytoband 1q21–23, which is related to various autoimmune illnesses in search of those that might contribute to RA. Finding these genes throughout the entire genome took a lot of work.

After performing the usual array of genetic association analyses, the scientists discovered one mutation in the FCRL3 gene, a signal nucleotide polymorphism (SNP) 169C T, that was highly related to RA. Cell investigations revealed that the SNP-169C allele greatly boosted the expression of the FCRL3 gene, which has an impact on B-cells, one of the two important immune system cells. RA is the result of this faulty immunological response. A higher vulnerability of this mutation for other autoimmune diseases, including lupus, has also been discovered, according to the research team. The objective of the current study is to evaluate the relationship between RA and its chromosomal region 1q21–23 and other autoimmune diseases, including lupus, etc.

Materials and Methods

All Outpatient Department (OPD) and Inpatient Department (IPD) patients who were available (just for blood samples) were enrolled in this case-control study. After obtaining the patients’ or their guardians’ informed consent, blood samples from the study population were taken at the rheumatology OPD and the department of medicine at Sardar Patel Medical College (SPMC), Bikaner, along with aids from the biological sciences department at Birla Institute of Technology and Science (BITS) Pilani, Hyderabad, between July of the year 2009 to January of the year 2012. In controls, samples from subjects who were of a similar age and gender were gathered using the same criteria for blood collection.

Inclusion Criteria

All patients aged 16 years and above and suffering from RA as per the definition of the 2010 American College of Rheumatology (ACR) and the revised European League Against Rheumatism (EULAR) criteria were included in the study.

Exclusion Criteria

- Rheumatoid arthritis (RA) patients with other critical illnesses like malignancy, renal failure, and liver failure were excluded from the study.
- Patients encountered overlap syndrome, that is, RA patients with concurrent connective tissue disorders like systemic lupus erythematosus, scleroderma, etc.

Diagnostic Criteria

The EULAR and the ACR 2010 have revised the 1987 ACR classification criteria for RA, which is mentioned below:

<table>
<thead>
<tr>
<th>Classification criteria for RA</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Joint involvement</td>
<td></td>
</tr>
<tr>
<td>One large joint (shoulder, elbow, hip, knee, ankle)</td>
<td>0</td>
</tr>
<tr>
<td>2–10 large joints</td>
<td>1</td>
</tr>
<tr>
<td>1–3 small joints (metacarpophalangeal joint, proximal interphalangeal joint, thumb interphalangeal, metatarsophalangeal joint, wrists)</td>
<td>2</td>
</tr>
<tr>
<td>4–10 small joints</td>
<td>3</td>
</tr>
<tr>
<td>&gt;10 joints (at least 1 small joint)</td>
<td>5</td>
</tr>
<tr>
<td>Serology</td>
<td></td>
</tr>
<tr>
<td>Negative rheumatoid factor (RF) and negative anti-citrullinated peptide (CCP) antibody</td>
<td>0</td>
</tr>
<tr>
<td>Low-positive RF or low-positive anti-CCP antibodies (≤3 times upper limit normal (ULN))</td>
<td>2</td>
</tr>
<tr>
<td>High-positive RF or high-positive anti-CCP antibodies (≤3 times ULN)</td>
<td>3</td>
</tr>
<tr>
<td>Acute-phase reactants</td>
<td></td>
</tr>
<tr>
<td>Normal C-reactive protein (CRP) and normal erythrocyte sedimentation rate (ESR)</td>
<td>0</td>
</tr>
<tr>
<td>Abnormal CRP or abnormal ESR</td>
<td>1</td>
</tr>
<tr>
<td>Duration of symptoms</td>
<td></td>
</tr>
<tr>
<td>&lt;6 weeks</td>
<td>0</td>
</tr>
<tr>
<td>≥6 weeks</td>
<td>1</td>
</tr>
</tbody>
</table>

Methods

A total of 100 controls with no previous history of RA and 135 diagnosed cases of RA were included in the study after considering predefined inclusion and exclusion criteria. Detailed clinical history of the patients along with proper family history was gathered as per preformed proforma.

Blood Sampling Procedure

Before the patient and volunteer were discharged from the project, competent medical professionals drew venous blood samples using sterile disposable syringes. The samples were then immediately converted into already labeled blood collection vials with 0.5 M of ethylenediaminetetraacetic acid (EDTA) (anticoagulant) and delivered via cold chain to the lab. Then collected blood samples were centrifuged at 2000 RPM for about 8 minutes to separate the serum from the sample, which was then placed in a simple vial and kept at −20°C until it was passed over to the specific genetic lab.

Analytical Method

Clinical Chemistry

Clinical details and laboratory parameters, including complete blood count, blood sugar, serum electrolytes including serum calcium, blood urea with creatinine, lactate dehydrogenase (LDH) isoenzymes assay, serum glutamic-oxaloacetic transaminase (SGOT)/serum glutamic pyruvic transaminase (SGPT) ratio, serum γ-glutamyl transferase (GGT), serum amylase, arterial blood gas (ABG), total proteins were evaluated as per kit manufacturer’s instructions.

Method for Isolation and Quantification of Deoxyribonucleic acid (DNA)

The usual approach was used to isolate DNA from both fresh and preserved blood samples, with a few minor adjustments made for the lab environment. Using a ultraviolet spectrophotometer with optical densities of 260 and 280 nm, DNA was measured and further validated after being seen on a 0.8% agarose gel for quality assurance.

To achieve the aim and objectives of this study, we proceed to the following steps:

- DNA isolation.
- DNA quantification.
- Polymerase chain reaction (PCR).
- Statistical analysis of data.

DNA Isolation

The isolation of DNA is the basic step of every molecular genetics research. It involves lysis of red blood cells, subsequently followed by lysis of white blood cells, extraction of protein, and finally precipitation of DNA using chilled ethanol.
**Stock Solutions**

- To 70 mL of distilled water, 18.61 gm of EDTA was added. By adding NaOH pellets and directing the magnetic stirrer, the pH was brought down to 8. A 100 mL final volume was achieved by including distilled water.
- To get the final volume equal to 100 mL, add 100 mL of distilled water to the 80 mL of dissolved 29.22 gm of sodium chloride (NaCl). The remedy was autoclaved and kept at room temperature.
- A total of 300 cc of distilled water was used to dissolve 60.57 gm of tris base. With concentrated hydrochloric acid (HCl), the pH was brought to 8.0, and using distilled water final volume was set to 500 mL.

**Practical Options**

- For making a final volume of 500 mL, combine 4.0 gm of NaCl, 1 gm of potassium chloride, 5.75 gm of sodium dihydrogen phosphate, and 1 gm of potassium dihydrogen phosphate.
- Mix 10 mL of tris HCl, 8 mL of NaCl, 0.4 mL of 0.5 M EDTA, and 100 mL of distilled water to make the final volume IX solution is created before use by dilution of 10× solution by 10 times with distilled water.
- A total of 1 gm of sodium dodecyl sulfate was mixed in 8 mL distilled water forming a resultant volume of 10 mL in order to prepare 10% weight by volume.
- To create an mL of 70% ethanol solution, 3 mL of distilled water was taken to dilute 7 mL of absolute ethanol. DNA was allowed to air dry at room temperature after ethanol was decanted.
- For 4 hours at 56°C, 500 µL of millipore water was used to dissolve DNA. DNA that had been dissolved was kept at 4°C until use.

**Spectrophotometer**

Quantification of DNA is done by measuring the optical density (OD) of DNA at 260 nm.

DNA concentration of a sample is calculated using the below-mentioned formula

\[ \text{OD} \text{ of DNA at 260 nm} \times (50 \mu g/mL) \times (\text{dilution factor}) \]

Measuring the OD of the sample at 280 nm helps to quantify the purity of DNA. The ratio of approximately 1.8 at an OD of A260/A280 nm shows adequate DNA preparation, while a ratio of >1.2 signifies the need for reextraction of DNA.

**Genomic DNA and PCR Amplification**

In 5 mL of EDTA, blood lymphocytes’ DNA was extracted. The PCR is a quick and easy in vitro approach for enzymatic synthesis of specific DNA sequences, and software named Primer 3 (freely available on the internet) was used to design the necessary primer sequence.

**Standardization of the PCR**

A typical PCR reaction mixture of IX taq buffer + 0.25 mM deoxynucleoside triphosphates + 0.5 pmol/µL per primer + 0.5 units taq would be created for 50–100 ng/pL of target DNA. The primers will be subjected to PCR at 94°C for 4 minutes then by 30 cycles of 94°C for 1 minute and annealing (with 48–68°C temperature range) for 1 minute, followed by extension of DNA at 72°C for 1 minute, with the final step of extension taking place for 10 minutes at 72°C. Electrophoresis at 60V in 2% agarose gel at 100V as per the anticipated length of the restriction fragments under study.

**Results**

Our study showed that the control group had a mean age of 45.11 ± 4.12 years. To subtract the risk of flaws in the severance of groups considering the delayed onset of disease, controls were deliberately selected from higher age groups. Table 1 depicts the data on clinical variables of both case and control groups which did not differ significantly in any of the clinical variables. While comparing systolic blood pressure (SBP) (135.64 ± 4.70 vs 130.93 ± 10.10; t = −0.669, p = 0.5044) and diastolic BP (DBP) (80.42 ± 6.54 vs 83.47 ± 10.77; t = 0.865, p = 0.388) of cases and controls, no odds were observed. The level of mean cholesterol was observed to be greater among cases, while there were no significant odds in the mean cholesterol level of cases and controls (165.21 ± 50.91 vs 160.84 ± 12.97; t = −0.739 p = 0.4612). The respiratory rate (RR) and the pulse rate (PR) were also

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**Table 1: Comparison of clinical profile among cases vs the control group**

<table>
<thead>
<tr>
<th></th>
<th>Cases N Mean ± standard deviation (SD)</th>
<th>Unrelated Controls N Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>135 43.01 ± 13.23</td>
<td>100 45.12 ± 4.13</td>
</tr>
<tr>
<td>SBP</td>
<td>135 135.65 ± 4.71</td>
<td>100 130.94 ± 10.10</td>
</tr>
<tr>
<td>DBP</td>
<td>135 80.43 ± 6.55</td>
<td>100 83.47 ± 10.78</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>135 165.22 ± 50.92</td>
<td>100 160.85 ± 12.98</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>78 157.01 ± 96.72</td>
<td>–</td>
</tr>
<tr>
<td>Urea</td>
<td>135 40.46 ± 25.03</td>
<td>2 44.50 ± 20.61</td>
</tr>
<tr>
<td>Total protein</td>
<td>135 6.50 ± 1.77</td>
<td>2 6.40 ± 3.11</td>
</tr>
<tr>
<td>Albumin</td>
<td>81 2.72 ± 0.79</td>
<td>–</td>
</tr>
<tr>
<td>Globulin</td>
<td>89 3.54 ± 2.00</td>
<td>–</td>
</tr>
<tr>
<td>Albumin/globulin ratio</td>
<td>82 0.98 ± 1.26</td>
<td>–</td>
</tr>
<tr>
<td>Creatinine</td>
<td>62 0.68 ± 0.75</td>
<td>–</td>
</tr>
<tr>
<td>PR</td>
<td>135 79.11 ± 13.00</td>
<td>8 82.50 ± 27.18</td>
</tr>
<tr>
<td>RR</td>
<td>135 18.01 ± 8.48</td>
<td>8 16.87 ± 3.09</td>
</tr>
</tbody>
</table>
similar in both groups and on comparison, no significance was noted. Socioeconomically, most of our cases, being females, were homemakers (66%), while the remaining 24% were either an agriculturalist or freelancers. The dietary pattern of recruited groups showed that most of them were vegetarian (68%), with only 32% being nonvegetarian. On analysis for the occurrence of rheumatoid nodules, only little number of cases (3%) were found to have rheumatoid nodules. While evaluating for joint deformity, nearly 59% of cases had a joint deformity. Almost 86% of the cohort had elevated (>6 mg/L) CRP levels.

### Discussion

Every human cell typically contains one pair of identical chromosomes. However, in the case of deletion syndrome of 1q21.1, as a portion of the sequence of chromosomes is missing, one of the pair is incomplete. The length of one chromosome is normal, whereas that of the other is insufficient.

In the notation, 1q21.1, “1” stands for chromosome 1, while “q” for the long arm of the chromosome, and “21.1” is for the portion of the long arm where the deletion is located. This syndrome's root cause is a deletion in the distal region of the 1q21.1 gene, which is a type of copy number variation (CNV). The size of 1q21,1, which ranges from 141.5 Megabase (Mb) to 147.9 Mb, is roughly 6 Mb. The 1q21.1 deletion syndrome typically arises due to the involvement of the distal area; however, overlap with the TAR area is still possible. The phenotype of CNV is quite varied. 1q21.1 has two sites where CNVs can be situated: the proximal area or TAR area (144.1–144.5) and the distal area (144.7–145.9). Although there are many multiple repeats of the same structure in 1q21.1, 25% of the structure is unique. The sequence contains certain gaps, and as of this writing, nothing further is known about those DNA sequences.

**Short Tandem Repeats (STR) Markers**

Simple sequence repeats, also called STRs or microsatellites, are DNA sequences that repeat and have between two and six base pairs. It is a particular variety of variable number of tandem repeat (VNTR), which is employed as a molecular marker in genetics, kinship, population, and other investigations. VNTRs can also be used to investigate gene duplication or deletion. Additionally, microsatellites have been linked to human diseases, particularly cancer and neurological disorders. When compared to other neutral regions of DNA, microsatellites have a larger rate of mutation that can mostly be explained by slippage during DNA replication on a single DNA strand. Although genomic microsatellite distributions are linked to recombination locations, most likely because repeated sequences are involved in recombination rather than as a result of it, mutation may also occur during recombination during meiosis. Proofreading processes in the nucleus can correct some slippage errors, but some mutations can evade correction. Factors include the size of the repeat unit, the number of repetitions, the existence of variant repeats, the frequency of transcription in the region of the DNA repeat, and others. Reduced polymorphism can result from the interruption of microsatellites, which may be caused by mutation. Tables 2 and 3 show the distribution of alleles for the dinucleotide repeat, D1S1489, and D1S318 polymorphisms in patients and controls.

**Table 3: Allelic frequencies of the D1S318 polymorphism in cases and controls**

<table>
<thead>
<tr>
<th>Size (bp)</th>
<th>Controls (90)</th>
<th>Cases (111)</th>
<th>Cases vs controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>154</td>
<td>20 (0.22)</td>
<td>24 (0.21)</td>
<td>Not significant (NS)</td>
</tr>
<tr>
<td>162</td>
<td>1 (0.011)</td>
<td>2 (0.018)</td>
<td>NS</td>
</tr>
<tr>
<td>166</td>
<td>3 (0.033)</td>
<td>4 (0.036)</td>
<td>NS</td>
</tr>
<tr>
<td>168</td>
<td>26 (0.288)</td>
<td>30 (0.27)</td>
<td>NS</td>
</tr>
<tr>
<td>170</td>
<td>7 (0.077)</td>
<td>8 (0.072)</td>
<td>NS</td>
</tr>
<tr>
<td>172</td>
<td>16 (0.177)</td>
<td>20 (0.180)</td>
<td>NS</td>
</tr>
<tr>
<td>174</td>
<td>10 (0.11)</td>
<td>13 (0.102)</td>
<td>NS</td>
</tr>
<tr>
<td>176</td>
<td>7 (0.07)</td>
<td>9 (0.08)</td>
<td>NS</td>
</tr>
<tr>
<td>178</td>
<td>1 (0.011)</td>
<td>1 (0.009)</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Table 2: Comparison of Allelic frequencies of the D1S5498 polymorphism among cases and controls**

<table>
<thead>
<tr>
<th>Size (bp)</th>
<th>Controls (90)</th>
<th>Cases (107)</th>
<th>Cases vs controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>192</td>
<td>1 (0.006)</td>
<td>1 (0.009)</td>
<td>NS</td>
</tr>
<tr>
<td>196</td>
<td>1 (0.006)</td>
<td>1 (0.009)</td>
<td>NS</td>
</tr>
</tbody>
</table>
| 198      | 39 (0.217)    | 11 (0.010)  | 95% confidence interval (CI) = 1.52–22.23; $\chi^2 = 4.290; p = 0.0383$
| 200      | 27 (0.150)    | 7 (0.07)    | NS               |
| 202      | 74 (0.411)    | 48 (0.45)   | NS               |
| 204      | 23 (0.128)    | 24 (0.023)  | 95% CI = 3.21–19.10; $\chi^2 = 6.659; p = 0.0099$
| 206      | 11 (0.061)    | 11 (0.010)  | NS               |
| 208      | 2 (0.011)     | 1 (0.01)    | 95% CI = 3.5–18.2; $\chi^2 = 7.492; p = 0.0062$
| 210      | 2 (0.011)     | 2 (0.02)    | 95% CI = 2.1–7.3; $\chi^2 = 5.421; p = 0.0199$
10 mg/L are generally considered indicative of an infectious or inflammatory process. The expression of CRP increases rapidly after an inflammatory stimulus with resultant serum levels <500 times baseline. This makes CRP an ideal marker for the diagnosis and monitoring of inflammatory processes. The CRP gene contains SNPs that have been discovered to be connected to differences in basal CRP levels. Due to significant linkage disequilibrium of the CRP gene, correlations of CRP levels with haploid genotype are more precise than those of single SNPs. Lower basal CRP levels are correlated with rs1205 variants. Some bigger studies, like the third National Health and Nutritional Examination Survey and the Framingham Heart Study (FHS), have also reported the correlation between plasma CRP levels and CRP genotypes. The FHS data were analyzed using 13 SNPs, and it was discovered that only two haplotypes, identified by the SNPs rs3091244 and rs1205, were related to CRP levels. Earlier research conducted in our group did not find a link between the C allele at rs1205 and lower (20%) serum CRP levels, but it did find a link between the CG genotype and a reduced prevalence of RA in the Asian Indian community. The correlation between rs1205 and higher CRP levels was also larger in individuals with higher body mass index at the same time.

The second most common high density lipid apolipoprotein is apoprotein (apo) A-II, but it is still unclear what it does. Since there are no known effects of deficiency of apoA-II in humans, it has been considered as a minor apolipoprotein long before. In a small number of cases, excessive expression of apoA-II in transgenic mice resulted in coupled dyslipidemia and insulin resistance. Because of this, interest in this protein has greatly increased, and also the fact that the apoA-II gene is located on chromosome 1q23, which is already a hotspot for searching genes associated with familial combination dyslipidemia, insulin resistance, and type 2 diabetes mellitus (T2DM). It has now become a focus of interest for research on these complexes after signs of a link between the 1q21–q24 region of the chromosome and insulin resistance or T2D. However, no such polymorphism that raises apoA-II levels in people has been discovered so far. The expression of plasma apoA-II concentration may be controlled by other nonstructural loci. Saturated fat consumption by an individual also raises plasma apoA-II levels. Our knowledge of the connection between apoA-II mutations and amyloidosis has been expanded by a number of investigations, including both humans and animals. The importance of comprehending apoA-II’s activity and metabolism is highlighted by an elevated plasma concentration of the protein, which may lead to the development of familial combination hyperlipidemia or T2DM. To reach this understanding, genetic investigations in adequately described instances and genomic and proteomic methods in cell and mouse models may be helpful.

**Conclusion**

We concluded that allelic frequencies of the D15498 polymorphism in cases and were found significant in sizes 198, 204, 208, 210, while it was found insignificant in sizes 192, 196, 200, 202, and 206. No correlation was found in Allelic frequencies of the D15318 polymorphism in cases and controls. Bigger cohort studies will allow better genomic elucidation of clinically defined intermediate phenotypes evaluated in RA patients with the virtue of the autoimmune origin of the disease and its diverse symptoms in patients. Genetic studies can be a milestone for adopting effective personalized treatment for such progressively debilitating diseases.

**References**

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Sputum Neutrophil Gelatinase-Associated Lipocalin as a Biomarker in Asthma-COPD Overlap

Ajay Babu1, Huliraj Narayanswamy2, Archana Baburao3

Received: 18 January 2023; Accepted: 27 March 2023

ABSTRACT

Background: Asthma COPD overlap (ACO) is a consensus-based phenotype having characteristics of both COPD and asthma. Distinguishing ACO from other diseases is even more important as it is related to low health-related quality of life, augmented exacerbation rate and hospital admission, a rapid deterioration in lung function, and increased morbidity and mortality. But it cannot be diagnosed explicitly based on spirometry tests, patient demographics, radiology, or by-sputum cytology. There is an unmet need to develop biomarkers.

Objectives: To assess the role of sputum neutrophil gelatinase-associated lipocalin (NGAL) as a biomarker of ACO.

To find the correlation between sputum NGAL levels with forced expiratory volume 1 (FEV1) and exacerbation rate in ACO.

To find the correlation between sputum NGAL level with sputum neutrophils and eosinophils in ACO.

Materials and methods: In this comparative correlational study, 180 subjects were enrolled into four groups with 45 patients each with asthma, COPD, ACO, and healthy nonsmokers respectively. Involving taking detailed history and demographics, sputum was analyzed for the differential count and NGAL.

Results: Asthma COPD overlap (ACO) cases had high sputum NGAL levels; the second was the COPD group, and the last in the case asthma group. Nonsmokers had notably lower readings than the diseased. Out of three, receiver operating characteristic (ROC) figures, the validity of NGAL was best in selecting patients of ACO than COPD and asthma. The area under curve (AUC) was highest for ACO and less than the acceptable limit for the remaining two. NGAL cut-off value of 2473 pg/mL had 80% sensitivity and 50% specificity for ACO.

Conclusion: The present study investigated the sputum NGAL levels as a biomarker in ACO identified by the syndromic approach. Sputum NGAL, a biomarker associated with airway inflammation in airway diseases, was supportive of clinically differentiating ACO from asthma to COPD.

MAIN POINTS

- Asthma COPD overlap is associated with augmented exacerbation rate, hospital admission, and a rapid deterioration in lung function leading to high morbidity and mortality.
- Diagnosis of ACO is based on a syndromic approach. Hence there is an unmet need for a new biomarker for the clinical diagnosis of ACO.
- Asthma COPD overlap had notably increased sputum NGAL levels when compared to COPD and asthma.

INTRODUCTION

Asthma COPD (chronic obstructive pulmonary disease) overlap (ACO) is defined as a condition with persistent airflow limitation and several clinical features that coincide with asthma and COPD.1 An exact definition is not yet developed due to a lack of evidence about its clinical phenotypes and underlying mechanisms. A high number of patients who have chronic respiratory symptoms have a diagnosis and characteristics of both asthma and COPD.2

Distinguishing between asthma and COPD is essential due to the distinct treatments available and the resulting clinical outcomes concerning illness and mortality rates.3 While asthma and COPD differ in terms of their inflammation patterns, immunological mechanisms, and the degree of reversibility in airflow limitation, there is a substantial population of patients who experience symptoms and signs of both disorders.4

Initially, “asthma-COPD overlap syndrome” was defined as the overlap of the symptoms of COPD and asthma in some patients. It was later revised to ACO from ACOs by Global Initiative for Asthma (GINA) since it covers a group of patients with variable intersection levels. This is most seen in asthmatic smokers. In COPD patients, it is very uncommon to find good postbronchodilator reversibility (BDR) in spirometry.5 Most of the inflammatory pathways of asthma and COPD are markedly different; both are seen in ACO. Sputum analysis showed both neutrophils and eosinophils in ACO. Asthma, COPD, and ACO can differ in their biomarker profile. Diagnosis of ACO cannot be made only based on lung function tests, patient demographics, sputum cell counts, or imaging of the lungs.6–8 There is a prerequisite to developing novel diagnostic biomarkers for the clinical assessment of ACO.

In ACO, there is increased neutrophilic airway inflammation and airway epithelial injury. So sputum neutrophil gelatinase-associated lipocalin (NGAL), as a biomarker in ACO, together with the patient’s clinical features, spirometry, and radiology, will be greatly helpful in diagnosing ACO patients.

MATERIAL AND METHODS

This comparative correlational study was done in the Department of Respiratory Medicine, Kempegowda Institute of Medical Sciences and Research Center, Bengaluru, Karnataka, India, over 2 years. Both male and female patients in the age-group of 40–75 years with stable asthma, COPD, and ACO on regular medications were included in the study. Diagnosis of asthma was based on GINA and defined as reversible airflow obstruction with a postbronchodilator forced expiratory volume 1 (FEV1)/FVC < 0.7, with an increase in FEV1 of ≥ 12% or 200 mL. COPD diagnosis was based on Global initiative for obstructive lung disease (GOLD) guidelines and defined by incompletely reversible airflow obstruction with a postbronchodilator FEV1/FVC < 0.7. ACO was identified by the features

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How to cite this article: Babu A, Narayanswamy H, Baburao A, Sputum Neutrophil Gelatinase-Associated Lipocalin as a Biomarker in Asthma-COPD Overlap. J Assoc Physicians India 2023;71(9):34–38.
that it shares with asthma and COPD according to GINA guidelines and GOLD strategy—with more significant variability in airflow and airflow obstruction that is not fully reversible.

Based on the medical history, self-reported questionnaire data, standard guidelines, and inclusion criteria, 180 study subjects diagnosed with asthma, COPD, and ACO according to GINA, GOLD, and syndromic approach, respectively, were enrolled in the study and compared to healthy asymptomatic nonsmokers. The sample size was calculated using the formula:

\[
N = \frac{Z^2 \times V(AUC)}{d^2}
\]

\(N\) = minimum sample size required.
\(Z\) = critical ratio of the confidence interval at 5% error Z = 1.96.
\(V\) = Variance of the area under the curve.
\(d\) = Allowable margin of error in the estimation of sensitivity—taken as 10% of the area under curve (AUC) = 0.07.

As more subjects reported and were eligible, 180 were enrolled in the study. Study subjects were enrolled into four groups—group I, asthma, \(n\) = 45; group II, COPD, \(n\) = 45; group III, ACO, \(n\) = 45; and group IV, control, \(n\) = 45. Patients with an exacerbation and use of oral corticosteroids in the past 4 weeks, active pulmonary tuberculosis, chronic lung diseases other than asthma, COPD and ACO, diabetes mellitus, malignancy, and renal diseases were excluded from the study. Informed written consent was taken from all subjects and ethical clearance was obtained from the Institutional Ethics Committee (KIMS/IEC/D-24/2020).

Baseline data of all study subjects were collected, including demographics, duration of symptoms, smoking history, history, treatment history, occupational history, biomass exposure, number of exacerbations in the last year, medication history, chest X-ray findings, and spirometry. Baseline blood investigations were done, including complete blood count, renal function test, and absolute eosinophil count. To estimate sputum differential counts, sputum cytospin preparations were prepared by cytospin and centrifuged at 450 rpm for 6 minutes. Slides were stained with hematoxylin and eosin stains for cell differentiation. We assessed detailed cellular profiles in sputum consisting mainly of eosinophils and neutrophils.

Induced sputum was collected for the differential count and NGAL estimation. Collected sputum was treated with dithioerythritol and then gently vortexed at room temperature for homogenization. The supernatant was stored at −80°C until the NGAL assay. NGAL was estimated using a human NGAL ELISA kit (product code BEK-1166 BIOSPES CO.LTD. Catalog no: BEK1166, Size 96T, Range 156 pg/mL-10,000 pg/mL).

**Statistical Analysis**

Data analysis was done using Statistical Package for the Social Sciences (SPSS) for Windows version 20.0 (IBM SPSS Statistics). The data collected during the study were analyzed using descriptive statistics such as percentage, range, mean, standard deviation, correlation analysis, and inferential statistics like analysis of variants with post hoc tests like the Bonferroni test and Kruskal–Wallis test. Correlations of the sputum markers with demographics, lung function, and sputum cell profiles were calculated by Spearman’s rank correlations. Multiple Linear regression analysis was done to evaluate the impact of variables like age, body mass index (BMI), FEV1/FEV ratio, and pack-years of cigarette smoking on sputum NGAL value. Receiver operating characteristic (ROC) curve analysis was performed to assess the sensitivity, specificity, and diagnostic accuracy of the biomarker NGAL. Statistical significance was set at \(p < 0.05\).

**RESULTS**

Around 180 subjects were enrolled in the study in four groups after satisfying the inclusion and exclusion criteria. Table 1 demonstrates the demographic and laboratory data of the study subjects. Sputum NGAL levels were significantly different among the four groups. Mean sputum NGAL levels in asthma patients were 2196.55 ± 1308.46 pg/mL, while in COPD patients, it was 2792.76 ± 1461.43 pg/mL; in ACO, it was 3746.68 ± 1376.17 pg/mL, and in the control group, it was 1625.79 ± 1484.96 pg/mL. There was a statistically significant difference between sputum NGAL levels in ACO patients compared to other groups (\(p \leq 0.001\)) (Fig. 1). Even though there was an increasing trend of sputum NGAL values in ACO, it did not differ significantly between COPD and asthma. There was 1 weak negative correlation between sputum NGAL and FEV1 (\(r = -0.336\)) (Fig. 2), FEV1/FVC (\(r = -0.150\)) (Fig. 3), and FEV1/FVC% (\(p = -0.247\)) (Fig. 4), whereas it had a positive correlation with sputum eosinophils (\(r = 0.443, p = 0.00\)) (Fig. 5) and sputum eosinophils (\(r = 0.183, p = 0.014\)). Sputum NGAL values were significantly higher in individuals having exacerbations of symptoms compared to those who did not (3225.23 vs. 2219.33, \(p < 0.001\)).

**Sputum Neutrophil gelatinase-associated lipocalin (NGAL)** as a biomarker had maximum validity in identifying cases of ACO than COPD and asthma. The AUC was highest for ACO (0.731) (Fig. 6). It was less than the acceptable limit for the remaining two (AUC 0.65).
Discussion

Our study assessed the levels of sputum NGAL as a tool for discerning ACO from asthma to non-ACO COPD. A set of patients with both asthma and COPD features were represented by the global term ACO. More than a few criteria have been proposed for diagnosing ACO since 2008.9 –16 Most of these criteria were simple and required spirometry and a previous diagnosis of COPD. This led to overreckoning of the ACO cases. Various diagnostic criteria were published over the years. In a study by Jo et al., he compared the prevalence of ACO found with multiple diagnostic criteria in the very same people.17 The prevalence was wide-ranging from 31% of modified Spanish criteria10 to 48% with PLATINO criteria.18 A consensus document was released by GOLD and GINA in 2016 for the diagnosis of ACO, recommending a syndromic method to stereotype ACO.2, 3 Because of the universal acceptance of GINA and GOLD, we implemented this tool in our study to estimate the size of ACO. Recent guidelines describe salient characteristics of ACO instead of providing a definite definition.

Table 1: Baseline characteristics of study subjects

<table>
<thead>
<tr>
<th>Variables</th>
<th>Asthma (n: 45)</th>
<th>COPD (n: 45)</th>
<th>ACO (n: 45)</th>
<th>Nonsmoker (n: 45)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>49.59 ± 9.00</td>
<td>64.20 ± 10.63</td>
<td>55.96 ± 8.73</td>
<td>55.47 ± 10.55</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gender (female/male)</td>
<td>21/24</td>
<td>4/41</td>
<td>16/29</td>
<td>20/25</td>
<td>0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.62 ± 2.84</td>
<td>21.58 ± 2.75</td>
<td>21.94 ± 2.51</td>
<td>21.96 ±2.57</td>
<td>0.18</td>
</tr>
<tr>
<td>Smoking (pack year)</td>
<td>1.14 ± 4.42</td>
<td>28.78 ± 13.31</td>
<td>8.40 ± 12.86</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Biomass fuel exposure, n (%)</td>
<td>3 (6.6)</td>
<td>4 (8.88)</td>
<td>4 (8.88)</td>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>ICS uses n (%)</td>
<td>30 (66.6)</td>
<td>25 (55.55)</td>
<td>27 (60)</td>
<td>0</td>
<td>0.58</td>
</tr>
<tr>
<td>LABA use n (%)</td>
<td>30 (66.6)</td>
<td>31 (68.8)</td>
<td>27 (60)</td>
<td>0</td>
<td>0.59</td>
</tr>
<tr>
<td>LAMA use n (%)</td>
<td>0</td>
<td>11 (24.44)</td>
<td>12 (26.66)</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Family history of asthma, n (%)</td>
<td>8 (17.77)</td>
<td>0</td>
<td>6 (13.33)</td>
<td>0</td>
<td>0.006</td>
</tr>
<tr>
<td>Allergic rhinitis n (%)</td>
<td>11 (24.44)</td>
<td>0</td>
<td>11 (24.44)</td>
<td>1 (2.22)</td>
<td>&lt;0.012</td>
</tr>
<tr>
<td>Post bronchodilator FEV1/FVC (%)</td>
<td>64.27 ± 4.58</td>
<td>64.33 ± 3.92</td>
<td>64.22 ± 4.06</td>
<td>87.04 ± 6.48</td>
<td>0.99</td>
</tr>
<tr>
<td>FEV1 (%)</td>
<td>68.91 ± 8.58</td>
<td>67.71 ± 10.10</td>
<td>65.08 ± 10.06</td>
<td>93.80 ± 9.00</td>
<td>0.82</td>
</tr>
<tr>
<td>FVC (%)</td>
<td>91.82 ± 6.65</td>
<td>84.55 ± 5.66</td>
<td>85.13 ± 12.33</td>
<td>93.46 ± 8.43</td>
<td>0.21</td>
</tr>
<tr>
<td>BDR (%)</td>
<td>12.89 ± 1.44</td>
<td>6.34 ± 3.30</td>
<td>13.71 ± 2.01</td>
<td>5.40 ± 2.59</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>6MWD (m)</td>
<td>461.06 ± 36.08</td>
<td>416.88 ± 35.28</td>
<td>359.75 ± 42.43</td>
<td>483.53 ± 48.28</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Distance saturation product m%</td>
<td>432.96 ± 35.46</td>
<td>383.40 ± 37.76</td>
<td>334.56 ± 42.68</td>
<td>459.66 ± 46.75</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hyperinflation in chest X-ray (n)</td>
<td>4</td>
<td>45</td>
<td>20</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Exacerbations in the past 1 year (n)</td>
<td>8</td>
<td>29</td>
<td>32</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sputum neutrophils%</td>
<td>46.84 ± 5.01</td>
<td>76.47 ± 8.59</td>
<td>69.42 ± 6.95</td>
<td>39.04 ± 8.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sputum eosinophils%</td>
<td>10.36 ± 1.86</td>
<td>3.67 ± 1.04</td>
<td>8.84 ± 2.18</td>
<td>3.08 ± 1.14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AEC</td>
<td>713.16 ± 223.36</td>
<td>210.49 ± 150.18</td>
<td>352.40 ± 239.56</td>
<td>88.17 ± 117.86</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sputum NGAL (pg/mL)</td>
<td>2194.25 ± 1293.60</td>
<td>2808.30 ± 1474.09</td>
<td>3746.68 ± 1376.17</td>
<td>1625.79 ± 1484.96</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Results are presented as mean ± standard deviation. AEC, absolute eosinophil count; BDR, bronchodilator reversibility; ICS, inhaled corticosteroids; LABA, long-acting β-adrenoceptor agonist; LAMA, long-acting muscarinic antagonist; 6MWD, 6-minute walk distance.

Table 2: ROC curves, sensitivity and specificity of sputum, NGAL among study subjects

<table>
<thead>
<tr>
<th>Parameters</th>
<th>AUC</th>
<th>Cut off</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACO</td>
<td>0.731</td>
<td>2473 (pg/mL)</td>
<td>80</td>
<td>50</td>
</tr>
<tr>
<td>COPD</td>
<td>0.471</td>
<td>2644 (pg/mL)</td>
<td>51</td>
<td>47</td>
</tr>
<tr>
<td>Asthma</td>
<td>0.298</td>
<td>2593 (pg/mL)</td>
<td>40</td>
<td>40</td>
</tr>
</tbody>
</table>

Fig. 5: Scatter plot showing a positive correlation between sputum neutrophils and sputum NGAL as indicated by the rising trend line

Fig. 6: ROC curve for ACO

BMI, FEV1/FVC ratio, BDR, and pack-years of cigarette smoking on sputum NGAL value. Age, FEV1/FVC ratio, and smoking severity were significant risk factors for derangement in sputum NGAL value. As BMI and BDR were not significantly associated with the NGAL values, they were excluded to see the final predictor capacity of the remaining variables. Adjusted R² for these variables of 0.556 indicates a 55.6% change in NGAL values could be explained by these variables. The regression equation for the model is, NGAL = 82.42 + 0.328 × age (in years) + 0.404 × smoking in pack years—0.665 × FEV1/FVC%.
With this syndromic approach, 16 asthma and 29 COPD patients in our study were diagnosed to have suffered from ACO. In our study, the prevalence of ACO in the asthmatic group was 35% lower than COPD group (64%).

For ACO, four different pathways have been suggested. Early-life airflow limitation can last into adolescence and adulthood, and if other risk factors, like smoking, are present, ACO is more likely than severe asthma. Patients with COPD with significant histories of smoking or other exposures and late-onset asthmatic symptoms exhibit a second pathway. Adults with asymptomatic airway hyperresponsiveness who develop chronic airflow limitation consistent with a COPD diagnosis represent a third asthma-dominant pathway. A fourth pathway is also known, which recognizes the connection between early life risk factors and small lungs and the likelihood of developing asthma and fixed airflow limitation. As a result, from the molecular genetics perspective, ACO can represent a very diverse group of patients.

Diagnosing ACO is essential for several reasons. Almost all the studies showed that ACO had a bad prognosis compared with asthma alone or COPD alone. They had more severe symptoms with impaired lung function, poor disease control, increased rate of aggravation of symptoms, and hospital admission, resulting in a more significant financial burden and worst outcomes.19–21 So, it is vital to differentiate this subgroup of patients early and treat them suitably. Recent guidelines do not advise inhaled corticosteroids (ICS) as a first-line treatment for COPD; ICS is recommended for patients with asthma or those who need triple therapy for COPD; ICS is recommended for patients with asthma alone or COPD alone. They had ACO had a bad prognosis compared with asthma alone or COPD alone. They had a more severe symptoms with impaired lung function, poor disease control, increased rate of aggravation of symptoms, and hospital admission, resulting in a more significant financial burden and worst outcomes.19–21

Diagnosing ACO is essential for several reasons. Almost all the studies showed that ACO had a bad prognosis compared with asthma alone or COPD alone. They had more severe symptoms with impaired lung function, poor disease control, increased rate of aggravation of symptoms, and hospital admission, resulting in a more significant financial burden and worst outcomes.19–21 So, it is vital to differentiate this subgroup of patients early and treat them suitably. Recent guidelines do not advise inhaled corticosteroids (ICS) as a first-line treatment for COPD; ICS is recommended for patients with asthma or those who need triple therapy for COPD; ICS is recommended for patients with asthma alone or COPD alone. They had ACO had a bad prognosis compared with asthma alone or COPD alone. They had a more severe symptoms with impaired lung function, poor disease control, increased rate of aggravation of symptoms, and hospital admission, resulting in a more significant financial burden and worst outcomes.19–21 So, it is vital to differentiate this subgroup of patients early and treat them suitably. Recent guidelines do not advise inhaled corticosteroids (ICS) as a first-line treatment for COPD; ICS is recommended for patients with asthma or those who need triple therapy for COPD; ICS is recommended for patients with asthma alone or COPD alone. They had ACO had a bad prognosis compared with asthma alone or COPD alone. They had a more severe symptoms with impaired lung function, poor disease control, increased rate of aggravation of symptoms, and hospital admission, resulting in a more significant financial burden and worst outcomes.19–21 So, it is vital to differentiate this subgroup of patients early and treat them suitably. Recent guidelines do not advise inhaled corticosteroids (ICS) as a first-line treatment for COPD; ICS is recommended for patients with asthma or those who need triple therapy for COPD; ICS is recommended for patients with asthma alone or COPD alone. They had ACO had a bad prognosis compared with asthma alone or COPD alone. They had a more severe symptoms with impaired lung function, poor disease control, increased rate of aggravation of symptoms, and hospital admission, resulting in a more significant financial burden and worst outcomes.19–21 So, it is vital to differentiate this subgroup of patients early and treat them suitably. Recent guidelines do not advise inhaled corticosteroids (ICS) as a first-line treatment for COPD; ICS is recommended for patients with asthma or those who need triple therapy for COPD; ICS is recommended for patients with asthma alone or COPD alone. They had ACO had a bad prognosis compared with asthma alone or COPD alone. They had a more severe symptoms with impaired lung function, poor disease control, increased rate of aggravation of symptoms, and hospital admission, resulting in a more significant financial burden and worst outcomes.19–21

Therefore, by attaching to the siderophores that scavenge for iron, NGAL aids in lowering the amount of accessible iron necessary for bacterial development. Activated neutrophils release matrix metalloprotease 9 (MMP-9), and by binding to this MMP-9, NGAL prevents its deactivation. It leads to protracted effects on collagen degradation.24 Due to their propensity for acute and chronic respiratory infections and the idea that a protease antiprotease imbalance plays a significant role in the pathogenesis of emphysema and airway wall remodeling, these actions of NGAL in ACO patients, which include decreased bacterial growth and increased matrix degradation, are of particular interest. So based on the properties of NGAL to reflect neutrophil activation, antibacterial, and matrix-degrading properties, it was hypothesized that NGAL could be a systemic marker of ACO.25,24

In our study, sputum NGAL values were significantly higher in individuals with exacerbations of symptoms compared to those who did not, like other studies,23–25 and positively correlated with sputum neutrophils. It supports the relationship between increased sputum neutrophils and NGAL in inflammation, especially in patients with ACO. Infections are one of the main reasons for the exacerbations in these patients. Therefore, the sputum NGAL levels can be associated with airway inflammation and low-grade microbial colonization, making patients with ACO susceptible to acute viral and bacterial infections and exacerbating diseases. Studies have shown significantly elevated NGAL levels in female subjects with ACO compared to those with non-ACO COPD, and emphysema index was positively associated with NGAL levels in female subjects.17 However, 64.4% of the diagnosed ACO cases were male in this study. Comparatively, the number of male patients suffering from ACO, asthma, and COPD was significantly more than females. Thus, this is statistically significant, inherently, the number of patients from each gender was different, to begin with. The difference observed could also be because of that. The mean age of study subjects suffering from ACO, asthma, and COPD differed significantly. The mean age of patients in each group is ACO = 55.96, asthma = 49.59, and COPD = 64.20. COPD and ACO were manifestations of a higher age than asthma.

There are only very few studies to date on biomarkers that differentiate ACO from other chronic airway diseases. NGAL is a novel biomarker in determining between ACO and non-ACO COPD; also, like in our study, using sputum as the sample might enhance the accuracy in diagnosing airway conditions, making it more effective in identifying specific respiratory diseases. This study showed that sputum NGAL is a promising biomarker in diagnosing ACO. Our study has a few limitations, like the small sample size and from a single center. Hence the results are required to be validated with more extensive cohort studies. Future studies are needed to divulge the biochemical differences in chronic airway diseases.

**Conclusion**

Our study shows that sputum NGAL can be used as a diagnostic tool in ACO and shows its role as a valuable biomarker in differentiating ACO from other chronic obstructive airway diseases. The sputum biomarker levels may reflect changes in cellular composition and lung function during disease progression.

**References**

3. From the Global Strategy for the Diagnosis, Management, and Prevention of COPD, Global Initiative for Chronic Obstructive Lung Disease Journal of the Association of Physicians of India, Volume 71 Issue 9 (September 2023)
Sputum Neutrophil Gelatinase-Associated Lipocalin as a Biomarker in Asthma-COPD Overlap


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Dr. Agam C Vora
Hon. General Secretary
A Prospective Observational Study of Autoimmune Encephalitis in Northwestern India

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Accepted: 25 April 2023

INTRODUCTION

Autoimmune encephalitis (AIE) is a group of “potentially reversible” and noninfectious causes of unexplained encephalitis.1 In 2007, California Encephalitis Project concluded that anti-N-methyl-D-aspartate receptor (anti-NMDAR) encephalitis is more than four times as frequent as viral encephalitis.2 The discovery of various neuronal and onconeural antibodies has shifted the paradigm of diagnosis and treatment of unexplained encephalopathies. The last two decades have witnessed a significant advancement in the diagnosis and management of AIE. The central nervous system (CNS) is an “immune privileged” site owing to the presence of the blood–brain barrier (BBB). It prevents the invasion of foreign substances and also secret cytokines like interleukin 1,3,6,8,10, endothelin-1, granulocyte-macrophage colony-stimulating factor, monocyte chemoattractant protein-1, etc.3 The circumventricular organs lack the BBB and hence act as a linkage between the peripheral and the central circulation. The microglia in the CNS also contribute to the active defense mechanism. The antibody-mediated CNS disorders can broadly be classified into—paraneoplastic syndrome disorder (PND) and AIE. The PNDs are relatively rare, affect the older population, are associated with systemic malignancies, has antibody against an intracellular neuronal antigen, has a monophasic course, and have a poor prognosis. On the contrary, AIE commonly affects young adults and children, is sometimes associated with malignancy, has antibodies against neuronal cell surface antigen, relapse in 20% of cases, and has a good response to immunotherapy.4,5

A third group of disorders has antibodies against intracellular synaptic proteins and may be paraneoplastic or nonparaneoplastic (stiff person syndrome, antiglutamic acid decarboxylase antibody-associated syndromes).

The AIE has diverse clinical presentations like psychiatric (depression, anxiety, psychosis, hallucination, attention deficit hyperactivity disorder, obsessive–compulsive disorder, anorexia, and bulimia), neurological (altered sensorium, memory impairment, language disturbances, and movement disorders like orofacial dyskinesia, dystonia, tics, parkinsonism, catatonia, oculogyric crisis, temporal lobe epilepsy, myoclonus, ataxia, and sleep disturbances), metabolic disturbances (hyponatraemia) and autonomic dysfunction (tachycardia, fluctuating blood pressure, and hypo or hyperhidrosis). Sometimes there may be a prodrome of fever and flu-like symptoms. The clinical feature is further supplemented by magnetic resonance imaging (MRI) brain, electroencephalogram (EEG), reactive cerebrospinal fluid (CSF), hypometabolism in functional imaging and confirmed by detection of specific autoantibody in serum or CSF, or both. Antibody-negative AIE is also common when the diagnosis is made on the basis of clinical features supported by MRI brain, EEG, CSF findings, and response to immunotherapy. However, the term “antibody negative AIE” is a misnomer as the vast array of antibodies related to AIE is not possible to diagnose with the limited number of available tests. The improvement after immunotherapy is an additional clue for diagnosis.3,5,6

ORIGINAL ARTICLE

A Prospective Observational Study of Autoimmune Encephalitis in Northwestern India

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Received: 19 January 2023; Accepted: 25 April 2023

ABSTRACT

Objectives: Autoimmune encephalitis (AIE) is a group of rare, increasingly recognized, potentially reversible, noninfectious causes of unexplained encephalitis. It affects any age-group and has a plethora of clinical presentations, the most common being the neuropsychiatric manifestation. The diagnosis of this entity at the right time and proper treatment with immunotherapy can save many lives. In this study, we describe the demographic profile, clinical spectrum, diagnosis, and treatment of 42 patients with features of AIE.

Materials and methods: This is a prospective study where 42 cases were selected from a tertiary care center in Northwestern India. Patients with suspected AIE underwent detailed clinical assessment, routine blood tests, magnetic resonance imaging (MRI) brain, electroencephalography (EEG), cerebrospinal fluid (CSF) study, and autoimmune profile in blood and CSF. Screening for malignancy was done in all patients with computer tomography (CT) thorax and abdomen and tumor markers.

Results: Among 42 patients, males, and females were almost equally affected. The mean age of onset was 31 years. Anti-N-methyl-D-aspartate receptor (anti-NMDAR) Encephalitis was the commonest of all AIE (57%) followed by anti-leucine-rich glioma inactivated-1 (anti-LGI-1) related AIE (11.9%), anti-contactin-associated protein 2 (anti-CASPR2) related AIE (4.7%), and steroid-responsive encephalopathy associated with autoimmune thyroiditis (SREAT) related to antithyroid peroxidase (anti-TPO) antibody (2.3%). Neuropsychiatric manifestation is the commonest. The seizure was noted in around 72% of patients, the commonest in the anti-NMDAR group. Faciobrachial dystonic seizure (FBDS) was noted in all five anti-LG-1-1 encephalitis patients. CSF abnormalities were seen in 33.3% of patients in the form of pleocytosis or raised protein, or both. MRI abnormality was seen in 52% of patients. EEG was abnormal in 10% of patients, and delta brush was noted in three anti-NMDAR patients. All patients received immunotherapy in the form of intravenous immunoglobulin (IVig) or pulse IV methylprednisolone (IVMPS), or both. Two patients nonresponsive to IVig and IVMPS received rituximab. Almost all patients responded to immunotherapy.

Conclusion: Autoimmune encephalitis (AIE), a potentially treatable immune-responsive entity, is a common neurological problem and may be an answer to a large number of cases having unexplained encephalitis. Good clinical acumen and knowledge are required for early diagnosis and treatment of this potentially reversible disorder.

How to cite this article: Sharma B, Paul M, Bagaria AK. A Prospective Observational Study of Autoimmune Encephalitis in Northwestern India. J Assoc Physicians India 2023;71(9):39–44.
The different forms of AIE described to date are anti-NMDAR encephalitis, anti-voltage-gated potassium channels (VGKC) related AIE which includes antileucine-rich glioma inactivated-1 (anti-LGI-1) related and contactin-associated protein 2 (CASPR-2) related AIE, steroid-responsive encephalopathy associated with autoimmune thyroiditis (SREAT), Gamma aminobutyric acid (A and B related AIE, anti-basal ganglia (D2 receptor) related AIE, Alpha amino 3 hydroxy-5 methyl- isoxazole propionic acid receptor related AIE, glycine receptor (GlyR) 1 related encephalitis, progressive encephalomyelitis with rigidity and myoclonus mediated by inhibitory GlyR receptor antibody, Ophelia syndrome, dipeptidyl-peptidase–like protein 6 antibody-associated encephalitis and encephalopathy with Ig-like cell adhesion molecule 5. NMDAR encephalitis is the most common and most elaborately described AIE.

Results

Demography

There was a total of 42 patients; 32 patients had positive autoimmune antibodies with encephalitis, confirming the diagnosis of AIE 10 patients (23.8%) in this study were “antibody-negative AIE” diagnosed on the basis of clinical features, MRI brain, EEG, CSF, and response to immunotherapy. The incidence was equal in males and females (M:F 1:1); the mean age at onset was 31.22 years, and the duration of illness was 4.78 months (ranging from 15 days to 3 years). A total of 24 patients were positive for anti-NMDAR antibody (57%), five were positive for anti-LGI-1 (11.9%), and two were positive for anti-CASPR2 antibody (4.7%), and one was positive for anti-TPO antibody (2.3%). The remaining 10 patients (23.8%) were antibody-negative AIE (Fig. 1). Clinical features neuropsychiatric symptoms were the commonest symptom seen in 96% of patients. Hallucinations were present in 52%, mutism in 62.5%, short-term memory impairment in 60%, movement disorders in 59.5%, and language abnormalities in the form of decreased fluency, impaired comprehension, or both were present in 16.6% of patients. Seizures were present in 30 (72%) patients, out of which two (4%), had myoclonic jerks and nine (21.4%) had focal seizures (Fig. 2).

Associated infections four patients (9.5%) patients were diagnosed with herpes simplex virus (HSV) encephalitis just prior to the presentation. They had an initial response to intravenous acyclovir. But after a few days had a recurrence of symptoms. A repeat MRI was the same, but seeing the recurrence, CSF anti-NMDAR antibody was sent, which came to be a positive investigation.

Routine hematological and biochemical investigations were within normal limits in all, except hyponatremia was present in three patients with anti-LGI1 antibodies. EEG was abnormal in 23.8% of patients out of which diffuse slowing of the background activity was seen in five (12%), epileptiform discharges in two (4.7%), and extreme delta brush in three (7.1%) patients. CSF was abnormal in 14 (33.3%) patients (Fig. 3) in the form of lymphocytic pleocytosis, raised protein in CSF, or both. MRI brain was abnormal in a total of 22 (52.3%) patients. Out of which, 15 patients (35.7%) had typical medial temporal lobe hyperintensities, either unilateral or bilateral (Fig. 4). A typical MRI finding in the form of subcortical hyperintensities was present in seven (17.1%) patients. A total of 20 patients had normal MRIs. Abnormalities were seen in 80% of LGI1 patients and 75% of patients with anti-NMDA antibody positivity. CT-thorax and abdomen were done to exclude malignancy.
Interestingly none of the patients in our study showed ovarian teratoma. There were 24 patients with anti-NMDA encephalitis, and four of them were children aged between 3.9 and 9 years (three girls and one boy) with female preponderance. The mean age at onset was 19.89 years, and the mean duration of presentation was 4.46 months (between 15 days and 1.5 years). The most common clinical features were behavioral abnormalities in the form of irritability, aggressiveness, restlessness, and failure to recognize relatives (95%), cognitive impairment (30.9%), and seizures (87.5%). The other features were visual hallucinations (50%), movement disorders (70.8%), sleep disturbances in the form of insomnia (43%), and myoclonic jerks (2.3%). Movement disorders in the form of orofacial dyskinesias (25%), chorea (8.33%), limb dystonia (25%), and catatonia (20.8%) were also seen. EEG findings were abnormal in 29.1%. One (4.1%) patient had epileptiform discharges. Whereas three (42.8%) patients had diffuse slowing of background activity and three (42.8%) patients had evidence of delta brush (Fig. 5). CT-abdomen did not show evidence of ovarian teratoma in any patient. MRI brain showing unilateral T2 FLAIR hyperintensity in the medial temporal lobe was present in five patients (20.8%), whereas three patients (12.5%) had bilateral hyperintensities. Apart from these, a typical MRI finding in the form of subcortical hyperintensities was present in four patients (16.6%). CSF was abnormal in 14 (71.4%) patients. Five (11%) patients with AIE had lymphocytic pleocytosis with normal protein and sugar. Seven patients had lymphocytic pleocytosis with raised proteins and normal sugar. In 9.5% of patients, CSF was positive for HSV polymerase chain reaction (PCR) before the presentation. All patients were treated with intravenous immunoglobulin (IVIg) followed by oral steroids or azathioprine. Nine patients received pulse injections of intravenous methylprednisolone (IVMPS) along with IG followed by oral steroids. The decision to combine IVIg and IVMPS was dependent on the severity of the disease at presentation and also on the history of HSV encephalitis. Two patients received rituximab due to nonresponsiveness to initial therapy with IVIg and IVMPS. One patient recovered fully in follow-up at 3 months, and one patient was lost to follow-up. So, 22 out of 24 patients had significant improvement, especially in terms of cognition, behavioral disturbances, seizures, and movement disorders with first-line immunotherapy (IVIg and IVMPS).

Anti-voltage-gated potassium channels (VGKC) related AIE anti-LGI-1 related AIE anti-LGI-1 AIE was seen in five patients in this series. The mean age at onset of illness was 61 years with the mean duration of illness at 2.9 months. All patients had behavioral and cognitive disturbances in the form of irrelevant talk, confusion, irritability, memory impairment, speech abnormality, and faciobrachial dystonic seizures (FBDS). EEG was abnormal in only one patient with a slow background. Hyponatremia was present in three patients (60%). MRI brain was abnormal in four patients with involvement of bilateral medial temporal region, hippocampus, and insula. All patients received IVIg. Pulse injection of IVMPS was given in two patients along with IVIg. Significant improvement was noted in behavior and FBDS during follow-up visits in all. The patient except for one patient who expired after showing improvement due to aspiration pneumonia.

Anti-contactin-associated protein 2 (Anti-CASPR2) related AIE anti-CASPR2 positive AIE was seen in two male patients in this study. The mean age at onset of illness was 17 years. Both patients had behavioral dysfunction and cognitive decline with myoclonic jerks. EEG was abnormal in only one patient with a slowing background. Both the patients received IVIg and pulse injections of IVMPS. Both the patients responded to immunotherapy, but later one was lost to follow-up SREAT.

One female patient in this study had SREAT with anti-TPO antibody (AB) positive in high titer with presentation having a seizure, myoclonus, and abnormal behavior for 3 weeks. EEG showed background slowing. MRI brain was normal. CSF protein was raised (86 mg/dL) with 10 cells. Serum T3, T4, and TSH were normal. The patient responded well to IV Methylprednisolone, followed by oral steroids.

**DISCUSSION**

The first description of AIE was given in the year 1968 with the occurrence of limbic encephalitis (LE) in the case of remote small-cell lung carcinoma. Considered to have a poor prognosis until the year 2001 when immune therapy responsive anti-VGKC related LE was described. AIE are autoimmune neurological disorders with symptoms of limbic and extra-limbic dysfunction in association with antibodies against cell surfaces or synaptic antigens.
The discovery of an array of autoantibodies in the last 2 decades has brought into light a number of AIE with a variety of clinical features. As it is well responsive to immunotherapy, early diagnosis, and treatment are of utmost importance.

There are many case series on AIE worldwide. However, Indian literature is still expanding on this subject. The most common AIE in the series is anti-NMDAR encephalitis (57%) which is the most prevalent AIE worldwide. The male–female ratio in this series is almost equal, with slight male preponderance, contrary to the previous studies where it is more common in children and young females. In this series, only 16% were children.

The mean age for anti-NMDAR encephalitis was 19.89 years in this series, comparable with the international literature. Most of the patients in the present study had neuropsychiatric symptoms (95%), which is almost the same as the large initial series by Dalamau et al., (100%) followed by seizures (87%), movement disorders, hallucination, and cognitive impairment which matches with the observation in most of the previous series. Ovarian teratoma is seen in 50% of anti-NMDAR patients, especially in Asian and African–American populations, but none of the patients in this series had ovarian teratoma on the CT abdomen.

In a number of case series from South Asia, the incidence of ovarian teratoma is only 8–9%. However the patient should be followed up for 2 years with tumor screening for ovarian teratoma. The EEG abnormalities reported are background slowing, epileptiform discharges, and delta brush as seen in 29% of the patient which matches with the world series.

The MRI abnormalities were seen in 50% of our patients most commonly as T2 weighted Fluid Attenuated Inversion Recovery hyperintensities in medial temporal lobes, insular cortex, and hippocampus which is almost the same as the large series by Dalamau et al., CSF was abnormal in 14 (71.4%) patients showing lymphocytic pleocytosis or raised protein which is more or less comparable to the previous study where CSF was abnormal in 80% patients. Intrathecal synthesis of anti-NMDAR antibody has also been reported. Around 76% of patients in this study were antibody positive (in serum and CSF) and the remaining were antibody negative. All the patients were treated with IVlg of which 83.33% of patients responded to IVlg with significant improvement in

![Fig. 5: Extreme delta brush in anti-NMDAR encephalitis](image-url)
cognition, behavioral disturbances, seizures, and movement disorders. All patients treated with IVlg were later put on a tapering dose of oral steroid or azathioprine. A patient who was diagnosed as SREAT received pulse IVIg followed by oral steroids. Two patients did not respond to IVlg and later received rituximab to which one showed significant improvement and the other was lost to follow-up. In the previous studies, significant improvement was documented in those with tumors with first-line therapy and tumor removal (75%). In this study none of the patients had tumors. Mortality in our study was 12% due to ventilator-associated pneumonia in contrast to international literature (4%). None of the patients had relapsed in the present study to date. Simultaneous infection with other neurotropic viruses is seen with NMDAR encephalitis. HSV encephalitis were noted in a total of four patients prior to the onset of present symptoms. These patients were initially diagnosed with HSV encephalitis on the basis of clinical features, MRI brain findings, and CSF, HSV, and PCR positivity. After receiving 14 days of IV acyclovir they had initial improvement and again relapse of their symptoms and this time had their CSF and serum anti-NMDAR positive. Reports showing HSV encephalitis acting as a trigger for NMDAR encephalitis were present where the seroconversion rate is 30%.

Anti-VGKC Complex-associated Encephalitis (Anti-LGI-1 and anti-CASPR2 Encephalitis)

It is a heterogeneous group of disorders with CNS and PNS involvement, affecting the elderly population and with antibodies against anti-LGI-1 or CASPR2. CASPR2-positive patients present with peripheral nerve hyperexcitability (neuromyotonia) and Morvan’s syndrome. Both are immunoresponsive. Relapse is seen in 31% of patients with anti-LGI1 encephalitis and 10% of those with anti-CASPR2 encephalitis.

Leucine-rich glioma inactivated-1 (LGI-1)—positive encephalitis represents 11.9% of all AIE. The patients are elderly and usually present with, psychiatric symptoms, impaired cognition, hyponatremia, and seizures. FDBS is the commonest form of seizure. In the present series, the mean age was 61 years which matches with the international literature. Cognitive impairment in the form of memory impairment, speech abnormality, and psychiatric disturbances is seen in all (100%) patients in this study. In previous studies, it was found that 89% of patients had memory disturbances. Psychiatric disturbances are common in LGI-1 encephalitis and are often treated by a psychiatrist initially before being referred to a neurologist. In the previous study, psychiatric disturbances were seen in 45% of patients in contrast to the present study having neuropsychiatric affection in 100% of patients which could be due to a small sample size. FDBS is a short episodic spasm of the upper limbs along with twitching of the ipsilateral face lasting for less than 3 seconds and occurring several times in 1 day, which was present in almost all the cases (100%) in contrary to the previous studies which showed FDBS in 20–40% patients with LGI-1 encephalitis. Intractable hyponatremia has been reported in 60% of patients with LGI-1 encephalitis owing to the inappropriate secretion of antidiuretic hormone due to the simultaneous expression of LGI-1 in the kidney and hypothalamus. In LGI-1 encephalitis abnormal signal intensities were found in the bilateral temporal lobe and basal ganglia. In this study, MRI Brain showed hyperintensities in the medial temporal lobe in four patients (80%). None of the patients had basal ganglia involvement. Long-term follow-up with MRI in the patients may show hippocampal atrophy without immunotherapy.

Anti-CASPR2-associated encephalitis is an amalgam of CNS and PNS involvement and affects the elderly male. Patients present with features of encephalopathy, cerebellar disturbances, peripheral nerve hyperexcitability (myokymia, fasciculations, cramps, and neuromyotonia), dysautonomia, neuropathic pain, and insomnia. Some patients develop Morvan’s syndrome. Tumor association is seen in 19% of patients. In this series two patients with CASPR2 antibody had neuropsychiatric presentation. The mean age of onset was 17 years in contrary to the previous literature. None of the patients had cerebellar dysfunction which also mismatches the previous large cohort series. Both the patients in the index study had myoclonic jerks. Though there are isolated case reports of myoclonus in CASPR2 encephalitis patient it is not reported in the large case series by van Sunderen et al. Immunotherapy is the treatment of choice. The response to immunotherapy in

Flowchart 1: Clinical algorithm for diagnosis of AIE; (AIE: autoimmune encephalitis, FBDS: facio-brachial dystonic seizures, IVMPS: intravenous methylprednisolone, IVlg: intravenous immunoglobulin, NDMARAnti-N-methyl-D-aspartate receptor encephalitis (anti encephalitis), LGI1: anti leucine-rich glioma inactivated (LGI-1), CASP2: contactin-associated protein 2 (CASPR-2), GABA: γ-aminobutyric acid, AMPAR: α-amino-3-hydroxy-5-methyl-4-isoxazolidinonic acid receptor, AB: antibody, MMF: mycophenolate mofetil)
the form of steroids and IVlg is 39% as seen in the literature. In our study, both patients responded to immunotherapy but later one was lost to follow-up.

Steroid-responsive Encephalopathy Associated with Autoimmune Thyroiditis (SREAT)

Steroid-responsive encephalopathy associated with autoimmune thyroiditis (SREAT) otherwise known as Hashimoto’s encephalopathy is an autoimmune neurological disorder characterized by encephalopathy in the form of abnormal behavior, altered sensorium, myoclonus, seizures, gait ataxia, and sleep abnormalities. It affects young females. TSH may or may not be normal but anti-TPO AB and anti-thyroglobulin AB are high. EEG may show background slowing, frontal intermittent rhythmic delta activity, triphasic waves, focal slowing, or epileptiform discharges. MRI Brain shows T2 hyperintensity in subcortical white matter or grey matter or grey–white junction. These patients respond well to high doses of steroids. The patient included in this study was an elderly female who had neuropsychiatric symptoms and myoclonus for 3 weeks with background slowing in EEG and raised anti-TPO AB in serum. MRI brain was normal. This patient responded well to a pulse dose of IVMPS followed by an oral steroid.

Limitations of the Study

In this study, about 23.8% of patients were antibody negative and diagnosed as “antibody-negative AIE.” We could only go for anti-NMDAR, anti-VGKC, AMPA1, AMPA2, anti-TPO, and anti-thyroglobulin antibody only. The vast array of antibodies for AIE is beyond the scope of our study.

Conclusion

Autoimmune encephalitis (AIE) is a potentially treatable immune-responsive entity with a plethora of clinical presentations corroborated with high titer of specific autoantibodies in serum, CSF, or both. The clinical finding is supported by some specific MRI and EEG features. A few distinct characteristics help to identify this potentially treatable condition early in the course. It may be an answer to a large number of cases having unexplained encephalitides (Flowchart 1).

Good clinical acumen is required for early diagnosis and treatment of this treatable condition. Literature on this subject is still expanding from our country in contrast to the plethora of studies from the West. The present study is aimed to highlight the clinic-investigational differences in AIE in India vis-à-vis Western literature.

The purpose of this study was to highlight the clinical profile of AIE from the Northwestern part of India. This is perhaps the largest study from this part of the country to the best of our knowledge.

References

**Introduction**
Coronary Heart Disease (CHD) is a multifactorial condition caused by atherosclerosis, hypertension, valvular heart disease, diabetes, smoking, and other factors. Previous epidemiological research found that hereditary disorders were more likely to impact younger people than older people. Premature coronary artery disease is related to a modest atherosclerotic load in the coronary arteries and a family history of CHD. CHD includes several pathogenic mechanisms, including the formation of atheromatous plaques, as a result of endothelial dysfunction. Nitric oxide (NO), which is produced from L-arginine by the endothelial NO synthase (eNOS) enzyme, operates as an endothelial function mediator and modulates cerebral blood flow and thrombogenesis. NO also protects against atherosclerosis by relaxing smooth muscle cells, decreasing platelet endothelial adhesion, vascular smooth muscle cell proliferation, and growth. Furthermore, NO aids in the prevention of atherogenic low-density lipoproteins (LDL) conversion to oxidized LDL. In various studies, the pleiotropic effects of NO have been linked to the connection between eNOS gene polymorphism and CHD. 

**Materials and Methods**
From January 2022 to May 2022, the SRM Medical College Hospital and Research Centre in Chennai, Tamil Nadu, India, conducted observational cross-sectional research. A total of 182 participants were had the same age and sex. As controls, 91 CHD patients and 91 healthy people were chosen (ECN: 1513/IEC/2018).

### Inclusion criteria
The CHD patients were chosen specifically because they had proven coronary angiography.

### Exclusion Criteria
Patients with psychiatric medicines, medications, thyroid, arthritis, rheumatoid arthritis, and acute or chronic infection were excluded.

Consent forms were obtained from all CHD participants and controls after permission from the Institutional Ethical Committee. Anthropometric measures were taken.

### Laboratory Measurements
Following an overnight fast, at the time of angiography, a blood sample (5 mL) was obtained from each patient in an aseptic vacutainer for the measurement of NO by the Griess method using enzyme-linked immunosorbbent assay (ELISA). In addition, whole blood was preserved in an ethylenediamine tetra acetic acid vacutainer for deoxyribonucleic acid (DNA) extraction before being transferred to Eppendorf tubes and stored at −20°C.

### Measurement of NO
In CHD patients and controls, serum NO was quantified as nitrite or nitrate using the Griess reagent. The inclusion of Griess reagents converts the nitrite into a rich purple azo molecule. This azo chromophore precisely regulates the quantity of nitrogen dioxide. At 540 nm, absorbance is measured.

### Molecular Analysis
**Deoxyribonucleic Acid Isolation**
In all CHD individuals, 1 mL peripheral blood samples were taken for DNA extraction from whole human blood, and controls were examined using the QIAGEN DNA extraction kit (catalog number—51104). The isolated DNA is measured in order to determine the purity of the DNA in the sample. The integrity of extracted DNA is validated using 2% agarose gel electrophoresis and ultraviolet spectroscopy before being stored at −20°C.

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Analysis of T-786C and G-894T eNOS Gene Polymorphism

The polymorphisms of the eNOS T-786C and G-894T genes were investigated using polymerase chain reaction (PCR) (Qiagen Rotor-Gene Q (Two-Plex) equipment) at the Department of Medical Research, with denaturation at 95°C for 2 minutes (30 cycles). Annealing at 58°C for 30 seconds, elongation at 72°C for 90 seconds, and final elongation at 70°C for 10 minutes.

Primer sequence for eNOS T-786C:
[5'-GTCTCTCAGCTTCCGTTTCTT-3'] forward primer
[5'-CCTTGAGTCTGACATTAGGGTATC-3'] reverse primer

Primer Sequence for eNOS G-894T
[5'-GAC CCT GGA GAT GAA GGC AGG AGA -3'] forward primer
[5'-ACC TCC AGG ATG TTG TAG CGG TGA -3'] reverse primer

Statistical Analysis

The Statistical Package for Social Sciences version 16 was used. To analyze the allele distribution in genotypes, the relationship between groups for qualitative factors was evaluated using the Chi-squared test. The p-values of <0.05 were deemed statistically significant.

RESULT

The majority of CHD and control patients are between the ages of 35 and 45. Cigarette smokers (57%, p = 0.028) and those with diabetes mellitus (p = 0.034) had a significant difference in the correlation between demographics and biochemical markers of CHD individuals (Figs 1 to 3).

The distribution of CHD patients according to the number of vessels injured comprises single vessel damage in 39 patients, double vessel damage in 32 patients, and triple vessel damage in 20 patients (Table 1).

Table 1: Demographics and biochemical variables subjects with CHD and healthy controls

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<th>Variables</th>
<th>Controls (n = 91)</th>
<th>CHD patient (n = 91)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>39.8 ± 2.7</td>
<td>40.3 ± 4.5</td>
<td>0.2138</td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>79 (86.8%)</td>
<td>84 (92.3%)</td>
<td>–</td>
</tr>
<tr>
<td>Female sex (%)</td>
<td>12 (13.1%)</td>
<td>7 (7.69%)</td>
<td>–</td>
</tr>
<tr>
<td>Height</td>
<td>170.5 ± 2.46</td>
<td>170.81 ± 3.44</td>
<td>0.4853</td>
</tr>
<tr>
<td>Weight</td>
<td>65.09 ± 3.26</td>
<td>71.75 ± 5.73</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI</td>
<td>21.91 ± 0.37</td>
<td>24.47 ± 1.61</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WC</td>
<td>84.77 ± 3.26</td>
<td>90.51 ± 4.45</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hip Circumference</td>
<td>99.54 ± 3.11</td>
<td>98.8 ± 5.23</td>
<td>0.2475</td>
</tr>
<tr>
<td>WHR</td>
<td>0.84 ± 0.02</td>
<td>0.90 ± 0.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SBP</td>
<td>117.5 ± 3.3</td>
<td>135.6 ± 4.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DBP</td>
<td>80 ± 1.2</td>
<td>74.3 ± 6.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>–</td>
<td>49 (57%)</td>
<td>–</td>
</tr>
<tr>
<td>Family history of CHD (%)</td>
<td>–</td>
<td>44 (37.6)</td>
<td>–</td>
</tr>
<tr>
<td>Number of diseased vessels</td>
<td>Single vessel damage</td>
<td>39 (42.85)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Double vessel damage</td>
<td>32 (35.16)</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Triple vessel damage</td>
<td>20 (21.97)</td>
<td>–</td>
</tr>
<tr>
<td>Fasting</td>
<td>90.24 ± 4.18</td>
<td>94.29 ± 6.98</td>
<td>NS</td>
</tr>
<tr>
<td>TC</td>
<td>168.8 ± 16.3</td>
<td>219 ± 41.42</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TG</td>
<td>84.6 ± 30.5</td>
<td>159.7 ± 69</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-C</td>
<td>46 ± 9</td>
<td>34 ± 7</td>
<td>NS</td>
</tr>
<tr>
<td>LDL-C</td>
<td>106.4 ± 12.59</td>
<td>189.4 ± 27.46</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VLDL</td>
<td>17.26 ± 8.77</td>
<td>28.06 ± 12.14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NO (µMol/L)</td>
<td>19.08 ± 4.74</td>
<td>12.97 ± 1.20</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

BMI, body mass index; DBP, diastolic blood pressure; FBG, fasting blood glucose; NS, not statistically significant; p-value of 0.05 is regarded as significant; SBP, systolic blood pressure; values presented as mean ± SD; WC, waist circumference; WHR, waist-hip ratio
All of the samples were amplified with a 458 kb eNOS T-786C gene product (Fig. 4). The eNOS T786C gene polymorphism differs significantly between CHD and control patients \((p = 0.05)\). The TT genotype had two fragments of 303 and 155 bp, whereas the TC genotype had four fragments of 303, 257, 155, and 46 bp, and the CC genotype had three fragments of 257, 155, and 46 bp (Fig. 5). The eNOS G-894T gene product was amplified to 517 bp (458 bp), and the eNOS G-894T gene polymorphism demonstrates a significant difference between CHD and control participants \((p < 0.05)\). The GG genotype had a single fragment of 517 bp, the GT genotype had three pieces of 517, 346, and 171 bp, and the TT genotype had two fragments of 346 and 171 bp (Fig. 6).

Table 2 shows the distribution of genotypes TT, TC, and CC, as well as the TC allele genotype consistency linked with the eNOS T786C mutation in control and CHD participants. The polymorphism of the eNOS T786C gene was shown to be significantly different between CHD and control patients \((p = 0.05)\), as shown in Table 2. Significant variations exist in the distributions of the TC genotype \((p = 0.017)\), CC genotype \((p = 0.011)\), and C-allele \((p = 0.001)\). The eNOS T786C polymorphism elevated the risk of CHD by 2.15-fold and 2.92-fold, respectively. C-allele carriers had 1.76 times the risk of CHD as T-allele carriers. In contrast, those with the CC genotype have a 2.842-fold \((p = 0.040)\) higher risk. Furthermore, the C-allele of the T786C polymorphism was substantially more common in coronary artery disease patients than in controls.12

The influence of TC genotype frequency of eNOS T-786C gene polymorphisms on demographic and biochemical parameters reveals that the mean amount of NO in TC-carrying participants was considerably lower than in TT-carrying people (Table 3). According to vessel damage, the genotype distribution of the eNOS T-786C gene polymorphism in CHD participants revealed that the TC genotype (71.8%) was more sensitive to double vessel damage than single vessel damage (69.2%) or triple vessel damage (55%) (Table 4).

Table 5 GT genotypes were shown to be 1.09-fold \((0.566-2.047)\) more likely to have CHD than TT genotypes \((0.303-2.295)\). We discovered that the G-894T eNOS gene polymorphism increases the risk of CHD by 0.864 times. The impact of eNOS G-894T gene polymorphisms on demographic and biochemical parameters—the mean amount of NO in GT participants was substantially lower than in GG subjects (Table 6).
Discussion

Coronary heart disease (CHD) is a leading source of morbidity and death across the world. A growing body of research suggests that various genetic variants are risk factors for the presence and progression of certain illnesses. Polymorphisms in eNOS, which is responsible for the synthesis of endothelial NO, a major artery vasodilator, might influence atherosclerosis pathophysiology and propensity. Endothelial-derived NO has various important roles in arteries, including platelet and leukocyte adhesion inhibition, endothelial vasodilation, and prevention of oxidized low-density lipoprotein production to avoid atherogenesis.13–15

The mean age of CHD patients was found to be older than that of controls (p = 0.2138). There was no significant difference between the patient and control groups in the mean values of fasting blood glucose, TC, high-density lipoprotein (HDL), LDL, very-LDL (VLDL), and thyroglobulin (TG) (Table 2).

The polymorphisms of the eNOS T-786C gene genotype frequencies, comprising TC, TT, and CC, were 64.8, 21.9, and 13.1% in CHD patients (Table 2). The mean age of CHD patients was found to be older than that of controls. There was no significant difference between the patient and control groups in the mean values of fasting blood glucose, TC, HDL, LDL, very-LDL (VLDL), and thyroglobulin (TG) (Table 2).

Table 2: The frequency of eNOS T-786C gene polymorphisms in control and CHD individuals

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Control n = 91 (%)</th>
<th>CHD subjects n = 91 (%)</th>
<th>OR</th>
<th>CI (95%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterozygous variant (TC)</td>
<td>34 (37.3%)</td>
<td>59 (64.8%)</td>
<td>2.4</td>
<td>1.13–3.80</td>
<td>0.017</td>
</tr>
<tr>
<td>Homozygous variant (CC)</td>
<td>5 (5.4%)</td>
<td>12 (13.1%)</td>
<td>2.92</td>
<td>1.34–10.44</td>
<td>0.011</td>
</tr>
<tr>
<td>Homozygous wild type (TT)</td>
<td>52 (57.1%)</td>
<td>20 (21.9%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 3: Effect of genotype frequency of eNOS T-786C gene polymorphisms on demographic and biochemical parameters

<table>
<thead>
<tr>
<th>Variables</th>
<th>TT (n = 20)</th>
<th>TC (n = 59)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>41.8 ± 9.7</td>
<td>42.3 ± 10.5</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.4 ± 1.3</td>
<td>25.56 ± 1.21</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>120.38 ± 16.57</td>
<td>129.01 ± 17.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>85.58 ± 11.57</td>
<td>81.22 ± 10.02</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>170.5 ± 31.87</td>
<td>191.9 ± 34.27</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>132.3 ± 29.26</td>
<td>157.54 ± 27.16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>37.41 ± 4.49</td>
<td>35.78 ± 4.35</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>168.15 ± 33.42</td>
<td>179.6 ± 37.18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NO (µmol/L)</td>
<td>12.97 ± 0.54</td>
<td>12.08 ± 0.19</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

NS, not significant; p-values of <0.05 are regarded statistically significant.

Table 4: eNOS T-786C gene genotype variation polymorphism in CHD patients based on vascular injury

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Single vessel damage (n = 39) %</th>
<th>Double vessel damage (n = 32) %</th>
<th>Triple vessel damage (n = 20) %</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterozygous variant (TC)</td>
<td>27 (69.2%)</td>
<td>23 (71.8%)</td>
<td>11 (55%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Homozygous variant (CC)</td>
<td>3 (7.69%)</td>
<td>4 (12.5%)</td>
<td>2 (10%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Homozygous wild type (TT)</td>
<td>9 (23%)</td>
<td>12 (37.5%)</td>
<td>7 (35%)</td>
<td>0.859</td>
</tr>
</tbody>
</table>

NS, not significant; p-values of < 0.05 are regarded statistically significant.

Table 5: The genotype frequency of eNOS G-894T gene polymorphisms differs between healthy and heart disease patients

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Control n = 91 (%)</th>
<th>CHD subjects n = 91 (%)</th>
<th>OR</th>
<th>CI (95%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterozygous variant (GT)</td>
<td>30 (32.9%)</td>
<td>47 (51.6%)</td>
<td>1.091</td>
<td>0.566–2.047</td>
<td>0.014</td>
</tr>
<tr>
<td>Homozygous variant (TT)</td>
<td>10 (10.9%)</td>
<td>8 (8.79%)</td>
<td>0.782</td>
<td>0.303–2.295</td>
<td>0.012</td>
</tr>
<tr>
<td>Homozygous wild type (GG)</td>
<td>51 (56%)</td>
<td>36 (39.5%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 6: Effect of eNOS G-894T gene polymorphism on clinical and biochemical characteristics

<table>
<thead>
<tr>
<th>Variables</th>
<th>GG (n = 36)</th>
<th>GT (n = 47)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>41.2 ± 10.1</td>
<td>42.8 ± 9.4</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.41 ± 1.22</td>
<td>24.52 ± 1.63</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>121.18 ± 15.23</td>
<td>131.07 ± 16.92</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>82.83 ± 10.97</td>
<td>81.52 ± 11.82</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>168.41 ± 39.46</td>
<td>194.31 ± 36.59</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>134.44 ± 25.63</td>
<td>150.14 ± 27.23</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>38.66 ± 4.37</td>
<td>36.5 ± 5.07</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>163.23 ± 24.58</td>
<td>169.75 ± 8.49</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NO (µmol/L)</td>
<td>12.89 ± 0.68</td>
<td>12.37 ± 0.54</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

NS, not significant; p-values of <0.05 are regarded statistically significant.
participants, and 37.3, 52.1, and 5.4% in the control group, respectively, in the current investigation. Reduced promoter activity of the eNOS gene triggered the formation of point mutations at nucleotides 5'-flanking region, which is strongly related to coronary spasm.16,17

The eNOS gene variant T-786C was connected to the severity of CHD.18

Kim et al. found a connection between T786C polymorphism and CHD and myocardial infarction (MI) in CHD and MI patients.19 When CHD patients were compared to controls, the frequency of the C-allele between the polymorphisms of the eNOS T786C gene was considerably greater.20

A massive meta-analysis of 69,235 participants discovered a link between gene polymorphisms and CHD.21 Libby and Kincel et al. found no significance between gene polymorphism and CHD.22,23

Similar to our work, a substantial difference in the frequency distribution of GG, GT, and TT genotypes between the CHD group and the control group.24 Variation in genotype distribution in the G894T gene between the CHD group and MI group discovered a link between gene polymorphism and CHD.21,22

Table 7: G-894T gene polymorphism genotype distribution in chd patients based on vessel damage

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Single vessel damage (n = 39)</th>
<th>Double vessel damage (n = 32)</th>
<th>Triple vessel damage (n = 20)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterozygous variant (GT)</td>
<td>25 (75.7%)</td>
<td>21 (65.6%)</td>
<td>13 (65%)</td>
<td>0.068</td>
</tr>
<tr>
<td>Homozygous variant (TT)</td>
<td>6 (15.3%)</td>
<td>5 (15.6%)</td>
<td>1 (5%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Homozygous wild type (GG)</td>
<td>8 (20.5%)</td>
<td>6 (18.7%)</td>
<td>5 (25%)</td>
<td>0.539</td>
</tr>
</tbody>
</table>

NS, not significant; p-values of <0.05 are regarded statistically significant

AUTHORS’ CONTRIBUTIONS
All the authors involved in the review of literature, collection of data, and preparation of the manuscript and also, and they were involved in reviewing and editing the manuscript.

REFERENCES
34. Afrasyap L, Ozturk G. NO level and endothelial NO synthase gene polymorphism (Glu298Asp) in the patients with coronary artery disease from the Turkish population. Acta Biochim Biophys Sin (Shanghai) 2004;36(10):661–666.
Abridged Prescribing Information

Active Ingredients: Metformin hydrochloride (as sustained release) and glimepiride tablets

Indication: For the management of patients with type 2 diabetes mellitus when diet, exercise and single agent (glimepiride or metformin alone) do not result in adequate glycemic control.

Dosage and Administration: The recommended dose is one tablet daily during breakfast or the first main meal. Each tablet contains a fixed dose of glimepiride and metformin hydrochloride. The highest recommended dose per day should be 8 mg of glimepiride and 2000 mg of metformin. Due to the prolonged release formulation, the tablet must be swallowed whole and not crushed or chewed.

Adverse Reactions:

For Glimepiride: hypoglycaemia may occur, which may sometimes be prolonged. Occasionally, gastrointestinal (GI) symptoms such as nausea, vomiting, sensations of pressure or fullness in the epigastrium, abdominal pain and diarrhea may occur. Hepatitis, elevation of liver enzymes, cholestasis and jaundice may occur. Allergic reactions or pseudo allergic reactions may occur occasionally. For Metformin: GI symptoms such as nausea, vomiting, diarrhea, abdominal pain, and loss of appetite are common during initiation of therapy and may resolve spontaneously in most cases. Metabolic acidosis, decrease in B12 absorption, very rarely lactic acidosis, hemolytic anemia, reduction of thyrotropin level in patients with hypothyroidism, hepatic insufficiency, photosensitivity, hepatobiliary disorders.

Warnings and Precautions:

For Glimepiride: Patient should be advised to report promptly exceptional stress situations (e.g., trauma, surgery, febrile infections), blood glucose regulation may deteriorate, and a temporary change to insulin may be necessary to maintain good metabolic control. Metformin Hydrochloride may lead to Lactic acidosis; in such cases metformin should be temporarily discontinued and contact with a healthcare professional is recommended. Sulfonylureas have an increased risk of hypoglycaemia. For Metformin: GI symptoms such as nausea, vomiting, diarrhea, abdominal pain, and loss of appetite are common during initiation of therapy and may resolve spontaneously in most cases. Metabolic acidosis, decrease in B12 absorption, very rarely lactic acidosis, hemolytic anemia, reduction of thyrotropin level in patients with hypothyroidism, hepatic insufficiency, photosensitivity, hepatobiliary disorders.

Contraindications:

Hypersensitivity to the active substance of glimepiride & Metformin or to any of the excipients listed. Any type of acute metabolic acidosis (such as lactic acidosis, diabetic ketoacidosis, diabetic pre-coma). Severe renal failure (GFR < 30 ml/min). In pregnant women. In lactating women. In patients with moderate or severe hepatic insufficiency. In patients with severe renal impairment. In patients with severe liver disease. In patients before undergoing surgery. In patients with a history of alcoholism. In patients with severe cardiac or respiratory failure. In patients with a history of severe urticaria or anaphylaxis.

Use in a special population:

Pregnant Women: Due to a lack of human data, drugs should not be used during pregnancy. Lactating Women: It should not be used during breastfeeding. Pediatric Patients: The safety and efficacy of drugs has not yet been established. Renal Impairment: A GFR should be assessed before initiation of treatment with metformin containing products and at least annually thereafter. In patients at increased risk of further progression of renal impairment and in the elderly, renal function should be assessed more frequently, e.g. every 3-6 months.

Additional information is available on request.

Last updated: March 13, 2023

* Data on File

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Abbreviations: HbA1c: Glycated hemoglobin; CV: Cardiovascular


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- Lack of Appetite
- Chronic Kidney Disease
Humoral Response After Two Doses of COVISHIELD™ Vaccine in Patients Undergoing Maintenance Hemodialysis

Manish R Balwani1*, Amit S Pasari2, Charulata Bawankule3, Amol Bhawane4, Priyanka Tolani5, Vijay M Katekhaye6

Received: 25 January 2023; Accepted: 03 April 2023

ABSTRACT

Objectives: Maintenance hemodialysis (MHD) patients are at increased risk of contracting coronavirus disease 2019 (COVID-19). Vaccine against COVID-19 offers the benefit of protection from severe illness. In this study, we assessed the humoral response after two doses of the COVISHIELD™ vaccine in MHD patients.

Materials and methods: In a prospective cohort study, the humoral response with two doses of the COVISHIELD™ vaccine was assessed after 14 ± 2 days of the second dose. The COVIPROTECT antibody titers against the spike protein were measured using the electrochemiluminescence immunoassay (ELECSYS, Roche Diagnostics International Ltd.). Data were analyzed to determine the predictors of antibody response.

Results: Between February and October 2021, 50 MHD patients were assessed. The mean age was 55.8 ± 10.8 years, and 72% were males. A total of 48 (96%) MHD patients have seropositivity. The median level of spike protein antibody was 579 U/mL [interquartile range (IQR25–75) 166–1852.75]. Compared to patients with no COVID-19 infection history, the median levels of antibodies were significantly higher in those with a history of COVID-19 (1047 vs 297 U/mL, p = 0.011). The antibody titers did not differ by age (p = 0.269), presence of comorbidities such as hypertension (p = 0.341), diabetes mellitus (p = 0.719) or ischemic heart disease (IHD) (p = 0.695), dialysis vintage (p = 0.660), and timing of diagnosis of COVID-19 in relation to vaccination (p = 0.261). Adverse events (AEs) occurred in one-third of patients that were mild and self-limiting. No serious AEs were observed in any patient.

Conclusion: In MHD patients, two doses of the COVISHIELD™ vaccine induced a substantial humoral response. Prior history of COVID-19 resulted in a higher antibody response. Thus, the COVISHIELD™ vaccine is efficacious and safe for use in patients with MHD.

INTRODUCTION

Maintenance hemodialysis (MHD) is a lifesaving alternative to kidney transplantation in patients with end-stage kidney disease. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic increased morbidity and mortality in MHD patients. With limited treatment options, the coronavirus disease 2019 (COVID-19) vaccine against SARS-CoV-2 perhaps offers the best hope of protection against the pandemic. In India, two vaccines, namely ChAdOx1 nCoV-19 coronavirus vaccine recombinant (COVISHIELD™) and whole-virus inactivated SARS-CoV-2 vaccine (COVAXIN™), were approved in January 2021 for use in adults. Both vaccines have demonstrated comparatively good efficacy in the general population. In MHD patients, the seroconversion by natural COVID-19 infection may be lower than in the non-MHD population. However, with most COVID-19 vaccines, including COVAXIN™ and COVISHIELD™, a good short-term humoral response has been demonstrated. Multiple factors, such as age, previous COVID-19 infection, immunosuppressive therapy, and the number of dosages, determine the vaccine response in MHD. From India, there is limited understanding of humoral response after COVISHIELD™ vaccination in MHD patients. In this study, we explored the antibody response to the spike protein of SARS-CoV-2 in MHD patients receiving two doses of the COVISHIELD™ vaccine.

MATERIALS AND METHODS

Design and Setting

In this prospective cohort study, we enrolled adult patients undergoing MHD who received two doses of recombinant ChAdOx1 nCoV-19 coronavirus vaccine (COVISHIELD™). This vaccine is manufactured by Serum Institute of India Pvt Ltd and AstraZeneca. This study was conducted at a center providing tertiary-level renal care and transplantation services. The center caters to the urban, semi-urban, and rural populations in India. We conducted the study according to the principles of the Declaration of Helsinki, good clinical practices, and applicable local regulatory guidelines. The study was approved by the local Institutional Ethical Committee. Informed consent was obtained from all the participants before enrolment into the study.

Population

In this study, we included adult patients (18 years and above) who are currently undergoing the MHD (frequency being twice or thrice a week) at our center and had completed the two doses of the COVISHIELD™ vaccine (1 or 3 months apart). In the initial period of the COVID-19 vaccination program, the second dose of the vaccine was administered 1 month apart, but later, a policy change was brought, and the second dose was administered after 3 months after the first dose. Hence, we included both groups of patients in our study. We excluded patients with an MHD of <3 months, patients taking a single dose of vaccine, and pregnant/lactating females.

Data Collection

We collected demographic data of patients such as age, gender, and comorbidities such as diabetes, hypertension, ischemic heart disease (IHD), and duration of dialysis vintage (months). History of COVID-19 infection was also captured along with the method of diagnosis (either by reverse transcriptase-polymerase chain reaction (RT-PCR)/spot antigen test or by seropositivity for antibodies). Based on the COVID-19 history, patients were divided into COVID-19 and non-COVID. All the patients who had completed two doses by the end of October 2021 were included in the study.

Antibody Assay

The antibody assay was conducted after 14 ± 2 days of the second dose of...
Humoral Response After Two Doses of COVISHIELD™ Vaccine

a vaccine. Under aseptic precautions, a venous blood sample was collected in sampling tubes containing Li-heparin, ethylenediaminetetraacetic acid, and sodium citrate. Using the electrochemiluminescence immunoassay (ELECSYS, Roche Diagnostics International Ltd.), the antibodies to the SARS-CoV-2 spike (S) protein receptor-binding domain (RBD) were assessed from the blood sample. The assay was performed on the COBAS® E immunoassay analyzer. In the first phase of incubation, a 20 µL of the sample, biotinylated SARS-CoV-2-S-RBD-specific recombinant antigen (<0.4 mg/L; 4-(2-hydroxyethyl)—piperazine)—ethane sulfonic acid buffer 50 mmol/L, pH 7.4) and SARS-CoV-2 S-RBD-specific recombinant antigen labeled with ruthenium complex (tris(2,2′-bipyridyl) ruthenium (II)—complex) forms a sandwich complex. In the second phase of incubation, with the addition of streptavidin-coated microparticles (0.72 mg/mL) complex becomes bound to the solid phase via the interaction of biotic and streptavidin. The reaction mixture was aspirated into the measuring cell, where the microparticles were magnetically captured onto the surface of the electrode. Unbound substances are removed with ProCell/ProCell M. Application of a voltage to the electrode induces chemiluminescent emission and is measured by a photomultiplier. If the concentration of spike protein antibodies was <0.80 U/mL, it was considered negative for anti-SARS-CoV-2-S antibodies, whereas levels of ≥ 0.80 U/mL were considered positive.

Outcomes

The primary outcome of our study was to determine the seroconversion of two doses of COVISHIELD™ vaccines with an assessment of antibody titers after 2 weeks of receiving the second dose. Secondary outcomes included assessing the antibody response in patients according to the history of COVID-19 infection and finding out the factors associated with improved antibody response.

Statistical Analysis

The data from the case record forms were entered into a Microsoft Excel sheet and was analyzed with Statistical Product and Service Solutions Software Version 15. The categorical variables were presented as frequency and percentages. The normality of the continuous variables was determined by plotting histograms and using the normality test of Kolmogorov–Smirnov. Normally distributed data were presented with mean and standard deviation, whereas nonnormally distributed data were presented as the median and interquartile range (IQR25–75). For statistical comparison of categorical variables, the Chi-squared test or Fischer’s exact test was applied. For normally and nonnormally distributed continuous variables, statistical comparisons were done with an independent sample t-test and Mann–Whitney U test, respectively. For all statistical comparisons, a p-value of <0.05 was considered statistically significant.

Results

Between February and October 2021, we recruited 50 dialysis patients who had completed two doses of COVISHIELD™ vaccines and were evaluated for anti-SARS-CoV-2-S antibodies after the second dose of a vaccine (Fig. 1). Table 1 enlists the baseline characteristics of the total population and COVID-19 and non-COVID-19 groups. The mean age was 55.8 ± 10.8 years, and 72% were males. The mean age was significantly higher in patients who had a history of COVID-19 (p = 0.015). The proportion of patients above the age of 60 years was also significantly higher in patients with a history of COVID-19 than in non-COVID-19 cases (p = 0.042). Among the comorbidities, hypertension, diabetes, and IHD were seen in 84, 52, and 18% of cases, respectively. The proportion of these comorbidities did not differ between the two groups. The median dialysis vintage was 14.5 months (IQR25–75 9.75–25.25) months. Though dialysis vintage was higher in the COVID-19 group, there was no significant difference compared to the non-COVID-19 group (median—21 vs 12 months, p = 0.099). Dialysis vintage of >12 months is seen in 56% of patients. The majority of patients with a history of COVID-19 had dialysis vintage >12 months as compared to the non-COVID-19 population (70.6 vs 48.5%, p = 0.136). Among COVID-19 patients, 64.7% were diagnosed with RT-PCR/antigen positivity.

Table 2 describes the distribution of anti-SARS-CoV-2-S antibodies in the study population. The median levels in the total population were 579 U/mL (IQR25–75 166 to

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**Table 1: Baseline characteristics**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Total (n = 50)</th>
<th>COVID-19 (n = 17)</th>
<th>Non-COVID-19 (n = 33)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>55.8 ± 10.8</td>
<td>60.9 ± 8.2</td>
<td>53.2 ± 11.1</td>
<td>0.015</td>
</tr>
<tr>
<td>Age groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤60</td>
<td>33 (66.0)</td>
<td>8 (47.1)</td>
<td>25 (75.8)</td>
<td>0.042</td>
</tr>
<tr>
<td>&gt;60</td>
<td>17 (34.0)</td>
<td>9 (52.9)</td>
<td>8 (24.2)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>36 (72.0)</td>
<td>10 (58.8)</td>
<td>26 (78.8)</td>
<td>0.136</td>
</tr>
<tr>
<td>Female</td>
<td>14 (28.0)</td>
<td>7 (41.2)</td>
<td>7 (21.2)</td>
<td></td>
</tr>
<tr>
<td>Comorbidities</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>26 (52.0)</td>
<td>9 (52.9)</td>
<td>17 (51.5)</td>
<td>0.924</td>
</tr>
<tr>
<td>Hypertension</td>
<td>42 (84.0)</td>
<td>15 (88.2)</td>
<td>27 (81.8)</td>
<td>0.558</td>
</tr>
<tr>
<td>IHD</td>
<td>9 (18.0)</td>
<td>5 (29.4)</td>
<td>4 (12.1)</td>
<td>0.242</td>
</tr>
<tr>
<td>Dialysis vintage (months)</td>
<td>14.5 (9.75–25.25)</td>
<td>21 (11.5–36)</td>
<td>12 (7–23.5)</td>
<td>0.099</td>
</tr>
<tr>
<td>≤12</td>
<td>22 (44.0)</td>
<td>5 (29.4)</td>
<td>17 (51.5)</td>
<td>0.136</td>
</tr>
<tr>
<td>&gt;12</td>
<td>28 (56.0)</td>
<td>12 (70.6)</td>
<td>16 (48.5)</td>
<td></td>
</tr>
<tr>
<td>Method of COVID-19</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antigen/RT-PCR positive</td>
<td>–</td>
<td>11 (64.7)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Asymptomatic seropositive</td>
<td>–</td>
<td>6 (35.3)</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>

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**Fig. 1: Patient flow diagram**
Humoral Response After Two Doses of COVISHIELD\textsuperscript{TM} Vaccine

1852.75. Of the total study population, two (4.0%) did not achieve the increase in the anti-SARS-CoV-2-S antibodies with levels remaining ≤1 U/mL. Both patients belonged to a non-COVID-19 group. Compared to the non-COVID-19 population, the median levels of anti-SARS-CoV-2-S antibodies were significantly higher in patients who had a history of COVID-19 (1047 vs 297 U/mL, \(p = 0.011\)). The rise of anti-SARS-CoV-2-S antibodies 2 to 100 U/mL was seen in the 15.2% non-COVID-19 population, with no patient in the COVID-19 group having antibodies in this range. Anti-SARS-CoV-2-S antibodies >500 U/mL were seen in a significantly higher proportion of COVID-19 patients (76.5 vs 36.4%; \(p = 0.040\)). By the method of diagnosis of COVID-19, the median antibody levels did not differ significantly, whether diagnosed by RT-PCR/antigen test or being an asymptomatic seropositive patient (1047 (IQR\textsubscript{25–75} 298–3452) vs 982 (IQR\textsubscript{25–75} 739–1881) respectively, \(p = 0.961\) (Fig. 2).

Table 3 shows the distribution of antibody levels by different subgroups in the total population and non-COVID-19 population. In the total population, the median levels of anti-SARS-CoV-2-S antibodies did not differ in patients aged above or below 60 years (\(p = 0.269\)). There was no significant association with age (\(r = 0.050, p = 0.731\)).

Adverse events (AEs) associated with vaccine administration are shown in Table 4. From a total of 32 patients’ data, 10 (31.3%) had AEs. Among AEs, fever (21.9%) was the most common, followed by body pain (9.4%). Headaches and loose stools were seen in one case each. The proportion of patients with AEs did not differ by subgroups. In the COVID-19 subgroup, the antibody titer did not differ by the timing of diagnosis of COVID-19 infection in relation to vaccination (\(p = 0.261\)).

Table 2: Antibody response in study participants

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Total (n = 50)</th>
<th>COVID-19 (n = 17)</th>
<th>Non-COVID-19 (n = 33)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median (IQR\textsubscript{25–75})</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibody groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤1</td>
<td>579 (166–1852.75)</td>
<td>1047 (579.5–2682.0)</td>
<td>297 (104–1585)</td>
<td>0.011</td>
</tr>
<tr>
<td>2–100</td>
<td>2 (4.0)</td>
<td>0</td>
<td>2 (6.1)</td>
<td>0.040</td>
</tr>
<tr>
<td>101–500</td>
<td>18 (36.0)</td>
<td>4 (23.5)</td>
<td>14 (42.2)</td>
<td></td>
</tr>
<tr>
<td>&gt;500</td>
<td>25 (50.0)</td>
<td>13 (76.5)</td>
<td>12 (36.4)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Association of different factors with antibody levels

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Overall population (n = 50)</th>
<th>Non-COVID-19 population (n = 33)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>n</td>
<td>Antibody levels</td>
</tr>
<tr>
<td>Age (years)</td>
<td>&gt;60</td>
<td>17</td>
<td>728 (216.72–3168.5)</td>
</tr>
<tr>
<td></td>
<td>≤60</td>
<td>33</td>
<td>368 (137.5–1634.5)</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>36</td>
<td>448.5 (160–1984.25)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>14</td>
<td>735.5 (163–1614.5)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>Yes</td>
<td>26</td>
<td>579.5 (181.33–1571.75)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>24</td>
<td>620 (144.25–2452.0)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Yes</td>
<td>42</td>
<td>735.5 (181.33–2358.25)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>8</td>
<td>249 (95.5–1479.0)</td>
</tr>
<tr>
<td>IHD</td>
<td>Yes</td>
<td>9</td>
<td>728.0 (129.5–1322.5)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>41</td>
<td>466 (175.5–2178.0)</td>
</tr>
<tr>
<td>Dialysis vintage (months)</td>
<td>&gt;12</td>
<td>28</td>
<td>735.5 (163.25–2297.75)</td>
</tr>
<tr>
<td></td>
<td>≤12</td>
<td>22</td>
<td>448.5 (160.75–1852.75)</td>
</tr>
<tr>
<td>History of COVID-19</td>
<td>Yes</td>
<td>17</td>
<td>1047 (579.5–2682.0)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>33</td>
<td>297 (104.0–1585.0)</td>
</tr>
<tr>
<td>COVID-19 diagnosis</td>
<td>Before vaccine</td>
<td>10</td>
<td>2080.5 (739.3–2946.5)</td>
</tr>
<tr>
<td></td>
<td>After vaccine</td>
<td>7</td>
<td>774 (298–1150)</td>
</tr>
<tr>
<td>Vaccine dosing interval</td>
<td>≤1.5 months</td>
<td>11</td>
<td>815 (139–1646)</td>
</tr>
<tr>
<td></td>
<td>&gt;1.5 months</td>
<td>39</td>
<td>466 (182–2050)</td>
</tr>
</tbody>
</table>
Humoral Response After Two Doses of COVISHIELD™ Vaccine

Fig. 3: Scatter plot of the association between age (A) and dialysis vintage (B) with antibody levels

Table 4: Adverse effects after vaccination

<table>
<thead>
<tr>
<th>Adverse effects</th>
<th>Total (n = 32)</th>
<th>COVID-19 (n = 12)</th>
<th>Non-COVID-19 (n = 20)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of patients with AEs</td>
<td>10 (31.3)</td>
<td>4 (33.3)</td>
<td>6 (30.0)</td>
<td>0.844</td>
</tr>
<tr>
<td>Fever</td>
<td>7 (21.9)</td>
<td>2 (16.7)</td>
<td>5 (25.0)</td>
<td>–</td>
</tr>
<tr>
<td>Body pain</td>
<td>3 (9.4)</td>
<td>1 (8.3)</td>
<td>2 (10.0)</td>
<td>–</td>
</tr>
<tr>
<td>Hand pain</td>
<td>1 (3.1)</td>
<td>1 (8.3)</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Loose stools</td>
<td>1 (3.1)</td>
<td>0</td>
<td>1 (5.0)</td>
<td>–</td>
</tr>
</tbody>
</table>

did not differ in the two groups (p = 0.844). No patients had developed any thrombotic complications or any bleeding manifestations.

Discussion

We observed that among patients undergoing MHD, we observed seroconversion in 96% of patients. Previous infection with COVID-19 was associated with significantly increased antibody titer for SARS-CoV-2 spike protein from the COVISHIELD™ vaccine. Other factors such as age, diabetes, and dialysis vintage did not affect the antibody response. During the pandemic of COVID-19, MHD patients were at the highest risk of contracting the disease and had increased morbidity and mortality. The efficacy of the COVISHIELD™ vaccine has been proven in healthcare workers in India. However, compared to healthy individuals, patients undergoing MHD have variable immune states. The expected immune response after two doses of vaccine can be affected by various factors. Evidence suggests that compared to healthy controls, chronic kidney disease patients have a variable response in the form of the lower and delayed rise of antibodies after COVID-19 vaccination. In our study, all but two (4%) patients developed detectable antibody titers 14 days after the second dose of the COVISHIELD™ vaccine. In a study Saudi Arabian cohort, significant antibody response was reported with Pfizer/BioNTech or AstraZeneca/Oxford vaccine. The measured time of antibody titer was after 89.2 ± 25.7 days of the second dose of the vaccine. A study from Taiwan involving 436 MHD patients reported a 56% rate of seropositivity assessed after a mean time of 22 days from ChAdOx1 nCoV-19 vaccination. Older age was negatively associated with antibody response. Another study from India reported an 88.9% (64 cases) response rate for positive antibody response among 72 studied MHD patients after two doses of the COVISHIELD™ vaccine. The study observed old age to be associated with nonresponders. Compared to these data, the antibody response in our study was better with the COVISHIELD™ vaccine. Our findings are supported by observation from Bruminhent et al., who reported a 100% response after two doses of the COVISHIELD™ vaccine. Variable responses in different studies could be attributed to different factors such as age, nutritional status, dialysis vintage, presence of comorbidities, etc. Though we did not find the impact of any of such major factors on seropositivity after vaccination, younger age, better dialysis adequacy, and higher albumin levels are reported to be positively correlated with better antibody response. We found previous COVID-19 infection as an important trigger for eliciting a significantly better humoral response. It is an expected response as the immune system is primed with prior infection. Similar findings have been reported in the general population as well, where prior COVID-19 infection was associated with higher anti-SARS-CoV-2-5 antibodies than those without such infection receiving the vaccine alone. Thus, a prior COVID-19 infection boosts the immune response induced by COVISHIELD™.

Coronavirus disease 2019 (COVID-19) vaccination is also associated with some adverse effects. Fever and body pain were common findings in our study. Evidence from Indian studies indicates AEs following immunization (AEFI) varied from nearly 30–67%. A systematic review of COVID-19 vaccine trials observed that fever, fatigue, myalgia, and headache were common AEFI. These were mild, transient, and reverted within a few days with supportive treatment. Serious AEs like vaccine-induced immune thrombotic thrombocytopenia and cerebral venous sinus thrombosis have been reported with COVID-19 vaccines. We did not observe any thrombotic or bleeding complications in any of the participants. A report from the AEFI committee under the Ministry of Health and Family Welfare of India showed the rate of potential thromboembolic events to be 0.61 cases/million doses with the use of the COVISHIELD™ vaccine. In another analysis of 54,571 adverse reaction reports from the EudraVigilance database, only 28 were thrombotic adverse reactions after Oxford/AstraZeneca vaccine. Despite MHD patients being at high risk for thromboembolic complications, there were no such complications in our cohort. These pieces of evidence indicate the risk of thromboembolic events is minimal, and the benefits outweigh the risks with the use COVISHIELD™ vaccine, which is safe for use in MHD patients.

Our study highlights that a good humoral response can be obtained with the two doses of the vaccine, even in MHD patients. However, there are certain limitations to our study. The sample size was small, which might limit the generalizability. In the initial part of the study, patients received the second dose after 4 weeks, and later the gap between the two doses was 84 days. We did not analyze these patients separately as the numbers would be smaller to conclude. The humoral response after a vaccine can be affected by numerous factors, such as nutritional level and physical activity, that were not assessed in our analysis. We did not compare the response after two doses of vaccine in MHD patients to the general population, which would have provided greater insights into the immunological response in MHD cases. Also, a prospective follow-up and reassessment after a certain period was not done due to financial constraints, which might suggest the persistence of the response.

Conclusion

In a cohort of MHD patients, two doses of the COVISHIELD™ vaccine elicit significant anti-SARS-CoV-2-5 antibodies response
measured after 2 weeks of the second dose. Prior COVID-19 infection is associated with a better antibody response than those without it. A watchfulness over antibody titers over a longer period may be necessary to ascertain the protection from COVID-19 in these susceptible populations.

**Compliance with Ethical Standards**

We conducted the study according to the principles of the Declaration of Helsinki, good clinical practices, and applicable local regulatory guidelines. The study was approved by the institutional ethical committee (DMIMS(DU)/IEC/2021022/450) at JNMC, Wardha, India. Informed consent was obtained from all the participants before enrolment into the study.

**Authors’ Contributions**

All authors have contributed equally to this research and manuscript.

**Data Availability**

The data underlying this article will be shared on reasonable request to the corresponding author.

**Acknowledgments**

We thank our hospital staff for their support team in the smooth conduct of the study. We also thank Ms Simran Bhanushali and Ms Nikita Thakre for their contribution to data entry and compilation.

**References**

Glycemic Control and Mucormycosis during COVID-19 Pandemic in India: A Study of Stage and Outcome

Vagisha Sharma¹, Kushal Kriplani², Isha P Tuli³*, Shilpam Sharma⁴, Anurag Narula⁵

Received: 12 April 2023; Accepted: 29 April 2023

ABSTRACT

Introduction: Mucormycosis mostly targets immunocompromised patients or those with uncontrolled diabetes but is now an important complication after coronavirus disease 2019 (COVID-19) infection. The stage of mucormycosis at presentation greatly impacts its treatment course and prognosis. Prior research has failed to evaluate an association of patient factors, especially glycated hemoglobin (HbA1c) levels and random blood sugar (RBS) at presentation, with the stage and outcome of mucormycosis.

Objectives: To investigate the relationship between glycemic control and the stage of mucormycosis at presentation and to investigate various factors affecting mucormycosis.

Materials and methods: All patients with clinicopathologically confirmed mucormycosis presenting to Safdarjung Hospital, New Delhi, during the COVID-19 pandemic were enrolled in the study. A questionnaire consisting of demographic information, comorbidities, history, and treatment of COVID-19 and diabetes was filled out at the time of admission. Blood glucose and HbA1c levels at presentation were noted. The above-noted parameters were compared with the stage and outcome of mucormycosis. The data obtained was analyzed.

Results: A total of 75 mucormycosis patients were enrolled in the study. The mean age of the participants was 45.17 years, and 85.33% of them survived the disease course. There was no statistically significant difference between the survivors and nonsurvivors concerning mean HbA1c and RBS levels. But there was a statistically significant correlation such that the stage of mucormycosis increased with progressive worsening glycemic control markers HbA1c and RBS. The most common comorbid condition was diabetes mellitus (72%); however, only coronary artery disease in the patient was significantly correlated with mortality. A history of COVID-19 infection was reported in 67.6% of the patients, but this was not significantly associated with mortality outcomes. Patients without a history of COVID-19 reported significantly higher RBS and HbA1c levels at presentation than the COVID-19-associated mucormycosis group.

Conclusion: The study showed a positive correlation between the stage of mucormycosis and serum glycemic control markers at presentation. Clinicians must order blood sugar and HbA1c levels at presentation as cues for a better understanding of disease severity. A thorough clinical examination and history taking for patient risk factors predisposing to mucormycosis are also crucial since the presence of proptosis and coronary artery disease are significantly correlated with the mortality outcomes. The extent of poor glycemic control at presentation was not associated with mortality outcomes.

INTRODUCTION

Mucormycosis is one of the most aggressive opportunistic fungal infections, conventionally limited to the severely immunocompromised and those with uncontrolled hyperglycemia and ketoacidosis with electrolyte imbalance. It has recently been highlighted as a complication of coronavirus disease 2019 (COVID-19).¹ Anecdotal case reports of orbital cellulitis and sinonasal involvement of the nasal mucosa, the paranasal sinuses, the orbit, and the brain, proposed by Honavar⁴ The stage of mucormycosis at presentation critically influences its clinical outcome and treatment options.⁵ The development or worsening of diabetes mellitus is one of the repercussions of COVID-19 infection, and further research suggests an association between worse COVID-19 outcomes and poor glycemic control in patients irrespective of their prior diabetic status.

It is proposed that the diabetogenic and ketogenic state associated with COVID-19 infection can cause an increased incidence of mucormycosis.⁶ Despite this, no prior research evaluates an association of glycemic control at presentation with the stage of mucormycosis. We assessed the blood sugar and glycated hemoglobin (HbA1c) levels at the patient’s presentation—to determine its association with the stage of mucormycosis at presentation and any relation with the outcome of the disease.

MATERIALS AND METHODS

Study Design, Duration, Setting, and Procedure

A retrospective observational study was conducted among patients admitted with clinicopathologically confirmed diagnoses of rhino-orbital cerebral mucormycosis (ROCM) to Safdarjung Hospital, New Delhi, between March 2020 and June 2022. Safdarjung Hospital is one of India’s main centers for education and treatment of otorhinolaryngology and one of the referral centers for COVID-19 patients.

In this study, demographic data, comorbidities, antecedent COVID-19, history of steroid use, disease characteristics, and outcomes were retrospectively reviewed from patient records. The data obtained was analyzed to find any possible correlation between the mucormycosis stage and outcome with patient variables.

The study was approved by the Institutional Ethics Committee of Safdarjung Hospital, New Delhi. Informed consent was obtained from all the subjects.

1,2Intern Doctor; 3Professor; 4Senior Resident, Department of ENT; 5Senior Specialist, Department of Ophthalmology, Vardhaman Mahaveer Medical College and Safdarjung Hospital, Delhi, India; *Corresponding Author

We found that orbital or facial edema was the most common primary symptom observed (34 patients, 45.33%) (Fig. 1A). Other notable symptoms included nasal discharge, nasal blockage, and lid swelling, reported in 29.33, 29.33, and 26.66% patients, respectively. Symptoms such as altered sensorium, eyelid erythema, face and teeth numbness, and dental pain were almost exclusively reported in patients in stage 4 of mucormycosis (Table 2 and Fig. 1B). The presence of proptosis was significantly associated with an increased risk of mortality in the patient (\(p\)-value = 0.0152) (Table 1 and Fig. 1C). Other symptoms like eyelid edema, mandible ulcer, and facial discoloration were also reported (Figs 1D to F).

Among all the ROCM patients, the most common comorbid condition was diabetes (72%); of this, 17.33% of patients had new-onset overt diabetes. Another 17.33% of the patients had prediabetes. There was no statistically significant difference between the survivors and nonsurvivors in relation to mean HbA1c values and mean random blood sugar (RBS) levels (Table 1). But there was a statistically significant association between increased HbA1c and higher staging of ROCM (\(p\)-value of <0.001) (Fig. 2). Similarly, there was a statistically significant association between

**Study Population**

Based on results reported by Soman and Sunavala, and using the appropriate statistical formula for sample size calculation, and considering a lower margin of error (2%) due to the large number of mucormycosis cases following the second and third wave of the COVID-19 pandemic in India, a desired minimum sample size of 48 participants was obtained. We could study 75 participants after applying the inclusion and exclusion criteria detailed below.

**Inclusion Criteria**

Patients with a clinicopathological diagnosis of mucormycosis (presenting within 4 weeks of the start of symptoms).

**Exclusion Criteria**

- Patients with complicated sinusitis without microbiological/pathological/radiological evidence of acute invasive fungal rhinosinusitis.
- Patients with acute invasive fungal rhinosinusitis (caused by a fungus other than mucor).
- Patients with a diagnosis of allergic rhinosinusitis/chronic fungal rhinosinusitis.

**Statistical Analysis**

Statistical analysis was performed using IBM Statistical Package for the Social Sciences statistics software (version 29). Quantitative data were presented as mean and standard deviation and qualitative data were presented as percentages and frequencies. The comparisons of the quantitative data were statistically evaluated using the two independent sample t-tests, according to the normal distribution assessed by the Shapiro–Wilk test. The comparisons of qualitative data were evaluated using the Chi-squared test. If the data was not normally distributed, the modified Kruskal–Wallis test was used for the analysis of quantitative data. The level of statistical significance was set at 5% (\(p\) < 0.05).

**Results**

We studied a total of 75 patients (mean age 45.17 ± 14.77) with mucormycosis, of which 64 survived (mean age 40.80 ± 15.74), and 11 patients did not survive (mean age 48.83 ± 21.33). Our patients had the ROCM clinical stages (Honavar classification) 4 from 1 to 4d. There were two patients with stage 1, 23 patients with stage 2, 28 patients with stage 3, and 22 patients with stage 4 of mucormycosis (Table 1).
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Categories</th>
<th>Overall (percentage %)</th>
<th>Survivors (percentage %)</th>
<th>Nonsurvivors (percentage %)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>45.17 ± 14.77</td>
<td>40.80 ± 15.74</td>
<td>48.83 ± 21.33</td>
<td>0.2151</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>48 (64%)</td>
<td>41 (64.06)</td>
<td>7 (63.64)</td>
<td>0.8342</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>27 (36%)</td>
<td>23 (35.94)</td>
<td>4 (36.36)</td>
<td></td>
</tr>
<tr>
<td>History of COVID-19</td>
<td>Yes</td>
<td>48 (67.6%)</td>
<td>39 (65.0%)</td>
<td>09 (81.82%)</td>
<td>0.1826</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>23 (30.6%)</td>
<td>21 (35.0%)</td>
<td>02 (18.18%)</td>
<td></td>
</tr>
<tr>
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<td>21</td>
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<td>Nasal discharge</td>
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<td>21</td>
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<td>0.1104</td>
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<td>1</td>
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<tr>
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<td>Loss of vision/dimunition</td>
<td>6</td>
<td>6</td>
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<tr>
<td></td>
<td>Palatal ulcer</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0.6764</td>
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<tr>
<td></td>
<td>Face/teeth numbness</td>
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<td>3</td>
<td>2</td>
<td>0.0974</td>
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<td>Lid swelling</td>
<td>20</td>
<td>17</td>
<td>3</td>
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<tr>
<td></td>
<td>Orbital/facial discoloration</td>
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<td>6</td>
<td>0</td>
<td>0.2897</td>
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<tr>
<td></td>
<td>Ptosis</td>
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<td>1</td>
<td>0.7479</td>
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<tr>
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<td>Redness around the eye</td>
<td>8</td>
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<td>0</td>
<td>0.2147</td>
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<tr>
<td></td>
<td>Nasal ulcer/crusts</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0.6764</td>
</tr>
<tr>
<td></td>
<td>Diplopia</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0.6764</td>
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<td>Face deviation</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0.6764</td>
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<td>Altered sensorium</td>
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<td>0</td>
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<td></td>
<td>Paraplegia</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0.6764</td>
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<tr>
<td></td>
<td>Dental pain</td>
<td>7</td>
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<td>0</td>
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<td></td>
<td>Abnormal face movements</td>
<td>2</td>
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<td>1</td>
<td>2 (2.67)</td>
<td>2 (3.13)</td>
<td>(0)</td>
<td>0.5552</td>
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<tr>
<td></td>
<td>2a</td>
<td>5 (6.67)</td>
<td>5 (7.81)</td>
<td>(0)</td>
<td>0.3373</td>
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<tr>
<td></td>
<td>2b</td>
<td>8 (10.67)</td>
<td>8 (12.5)</td>
<td>(0)</td>
<td>0.2147</td>
</tr>
<tr>
<td></td>
<td>2c</td>
<td>5 (6.67)</td>
<td>5 (7.81)</td>
<td>(0)</td>
<td>0.3373</td>
</tr>
<tr>
<td></td>
<td>2d</td>
<td>5 (6.67)</td>
<td>5 (7.81)</td>
<td>(0)</td>
<td>0.3373</td>
</tr>
<tr>
<td></td>
<td>3a</td>
<td>4 (5.33)</td>
<td>2 (3.13)</td>
<td>2 (18.18)</td>
<td>0.0401*</td>
</tr>
<tr>
<td></td>
<td>3b</td>
<td>9 (12)</td>
<td>6 (9.38)</td>
<td>3 (27.27)</td>
<td>0.0915</td>
</tr>
<tr>
<td></td>
<td>3c</td>
<td>13 (17.33)</td>
<td>10 (15.63)</td>
<td>3 (27.27)</td>
<td>0.3458</td>
</tr>
<tr>
<td></td>
<td>3d</td>
<td>2 (2.67)</td>
<td>1 (1.56)</td>
<td>1 (9.09)</td>
<td>0.1522</td>
</tr>
<tr>
<td></td>
<td>4a</td>
<td>11 (14.67)</td>
<td>10 (15.63)</td>
<td>1 (9.09)</td>
<td>0.5715</td>
</tr>
<tr>
<td></td>
<td>4b</td>
<td>3 (4)</td>
<td>3 (4.69)</td>
<td>(0)</td>
<td>0.4636</td>
</tr>
<tr>
<td></td>
<td>4c</td>
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<td>4 (6.25)</td>
<td>1 (9.09)</td>
<td>0.7271</td>
</tr>
<tr>
<td></td>
<td>4d</td>
<td>3 (4)</td>
<td>3 (4.69)</td>
<td>0 (0)</td>
<td>0.4636</td>
</tr>
<tr>
<td>Comorbidities</td>
<td>DM on OHA</td>
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<td>9 (14.06)</td>
<td>2 (18.18)</td>
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</tr>
<tr>
<td></td>
<td>DM on insulin</td>
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<td>9 (14.06)</td>
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<tr>
<td></td>
<td>DM—untreated</td>
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<td>16 (25)</td>
<td>4 (36.36)</td>
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<tr>
<td></td>
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<td>13 (17.33)</td>
<td>10 (15.63)</td>
<td>3 (27.27)</td>
<td>0.3458</td>
</tr>
</tbody>
</table>

Contd…
Mucormycosis in the COVID-19 Pandemic

In our study, a majority (67.6%) of mucormycosis patients had a proven history of COVID-19 infection. The mechanism and risk factors through which COVID-19 can increase the risk of mucormycosis are manifold, most importantly being COVID-19-associated hyperglycemia. Other factors responsible are immune dysfunction, altered mucosal clearance, and local immunity. COVID-19 can also indirectly put the patient at risk of infection due to the increased use of immunomodulators, corticosteroids, tocilizumab, etc.9

The history of COVID-19 did not significantly affect mortality in our study (p-value of >0.05). Contrastingly, a study by Sebastian et al. showed higher mortality in a group of CAM (67.57%) compared to mucormycosis without a history of COVID-19 (61.90%).10 This finding could be explained by the fact that our study population had patients who had recovered from COVID-19 and thus did not have any added mortality attributable to COVID-related complications, unlike in the study by Sebastian et al., where patients had COVID-19 infections and mortality attributable to its complications. There was no significant association between a higher stage of mucormycosis and antecedent COVID-19 infection (p-value of >0.05) (Table 1).

Glycemic control markers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Overall (percentage %)</th>
<th>Survivors (percentage %)</th>
<th>Nonsurvivors (percentage %)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>New-onset prediabetes</td>
<td>13 (17.33)</td>
<td>12 (18.75)</td>
<td>1 (9.09)</td>
<td>0.4343</td>
</tr>
<tr>
<td>Acute lymphoblastic leukemia</td>
<td>3 (4)</td>
<td>3 (4.69)</td>
<td>0 (0)</td>
<td>0.4636</td>
</tr>
<tr>
<td>No comorbidity</td>
<td>1 (1.33)</td>
<td>1 (1.56)</td>
<td>0 (0)</td>
<td>0.6764</td>
</tr>
<tr>
<td>HTN</td>
<td>9 (12)</td>
<td>8 (12.5)</td>
<td>1 (9.09)</td>
<td>0.7479</td>
</tr>
<tr>
<td>DKA</td>
<td>2 (2.67)</td>
<td>1 (1.56)</td>
<td>1 (9.09)</td>
<td>0.1522</td>
</tr>
<tr>
<td>On immunosuppressive medications</td>
<td>1 (1.33)</td>
<td>1 (1.56)</td>
<td>0 (0)</td>
<td>0.6764</td>
</tr>
<tr>
<td>Chronic corticosteroid use</td>
<td>2 (2.67)</td>
<td>2 (3.13)</td>
<td>0 (0)</td>
<td>0.5523</td>
</tr>
<tr>
<td>Hepatitis C</td>
<td>2 (2.67)</td>
<td>2 (3.13)</td>
<td>0 (0)</td>
<td>0.5523</td>
</tr>
<tr>
<td>H/O ear, nose, and throat surgery</td>
<td>2 (2.67)</td>
<td>2 (3.13)</td>
<td>0 (0)</td>
<td>0.5523</td>
</tr>
<tr>
<td>Varicose vein</td>
<td>1 (1.33)</td>
<td>1 (1.56)</td>
<td>0 (0)</td>
<td>0.6764</td>
</tr>
<tr>
<td>Cerebrovascular accident</td>
<td>2 (2.67)</td>
<td>1 (1.56)</td>
<td>1 (9.09)</td>
<td>0.6764</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>1 (1.33)</td>
<td>0 (0)</td>
<td>1 (9.09)</td>
<td>0.0152*</td>
</tr>
<tr>
<td>Osteomyelitis</td>
<td>1 (1.33)</td>
<td>1 (1.56)</td>
<td>0 (0)</td>
<td>0.6764</td>
</tr>
<tr>
<td>Smokers and/or alcoholics</td>
<td>1 (1.33)</td>
<td>1 (1.56)</td>
<td>0 (0)</td>
<td>0.6764</td>
</tr>
<tr>
<td>Hypothyroid</td>
<td>2 (2.67)</td>
<td>2 (3.13)</td>
<td>0 (0)</td>
<td>0.5523</td>
</tr>
</tbody>
</table>

* p < 0.05 signifies a significant value; DKA, Dibetic Ketoacidosis; DM, Diabetes Mellitus; H/O, History of; HTN, Hypertension; OHA, Oral Hypoglycemic Agents

**DISCUSSION**

Mucormycosis in the COVID-19 Pandemic

In our study, a majority (67.6%) of mucormycosis patients had a proven history of COVID-19 infection. The mechanism and risk factors through which COVID-19 can increase the risk of mucormycosis are manifold, most importantly being COVID-19-associated hyperglycemia. Other factors responsible are immune dysfunction, altered mucosal clearance, and local immunity. COVID-19 can also indirectly put the patient at risk of infection due to the increased use of immunomodulators, corticosteroids, tocilizumab, etc.9

The history of COVID-19 did not significantly affect mortality in our study (p-value of >0.05). Contrastingly, a study by Sebastian et al. showed higher mortality in a group of CAM (67.57%) compared to mucormycosis without a history of COVID-19 (61.90%).10 This finding could be explained by the fact that our study population had patients who had recovered from COVID-19 and thus did not have any added mortality attributable to COVID-related complications, unlike in the study by Sebastian et al., where patients had COVID-19 infections and mortality attributable to its complications. There was no significant association between a higher stage of mucormycosis and antecedent COVID-19 infection (p-value of >0.05) (Table 1).
COVID-19. This suggests that even though patients with COVID-19 may be at risk of developing mucormycosis—the progression and worsening of the disease stage are determined by patients' own immune system defenses and risk factors.

**COVID-19 and Hyperglycemia**

Diabetes mellitus was the most common comorbid condition in our study, reported in 72% (54 out of 75) of the patients. While 17.33% of the study population had new-onset overt diabetes, another 17.33% had new-onset prediabetes. Among those with new-onset diabetes and prediabetes, 69.2% (18 out of 26) had a history of COVID-19. In the past couple of years following the onset of the pandemic, studies have revealed that COVID-19 can induce new-onset diabetes in certain individuals, manifesting as acute hyperglycemia in patients without prior diabetes history either during active disease or following recovery. The specific factors that mediate the development of diabetes in some COVID-19 patients include challenges with glucose utilization, disruptions in insulin production, stress-related high blood sugar, preexisting diabetes, and diabetes caused by taking steroid medications. On a molecular level, SARS-CoV-2 infection negatively impacts the insulin/insulin-like growth factor signaling pathway in various cells and tissues involved in respiratory, metabolic, and endocrine functions. The binding of the virus to angiotensin-converting enzyme 2 receptors in the pancreatic β-islet cells leads to an array of changes, including islet inflammation, amyloid deposition, β-cell dysfunction, and/or apoptosis which mediate the development of type 1 and type 2 diabetes mellitus.

In our study, the mean values of HbA1c and RBS at presentation in those without a history of COVID-19 (10.2, 259.8) were higher than those with CAM (8.8, 224.8), and the difference was statistically significant ($p$-value of <0.05). This is in concordance with similar findings reported by Sebastian et al., who reported significantly higher levels of HbA1c and RBS at presentation in the non-COVID-19-associated mucormycosis group. This suggests that those who developed COVID-19 better monitored their overall health and, thus, had better glycemic control than those who did not develop COVID-19. This less severely deranged glycemic control in the CAM group suggests other factors, like the effect of COVID-19 on immunity and mucosal defenses, play a major role in mucormycosis pathogenesis in this group. The non-COVID-19 group had worse glycemic control; a higher level of HbA1c also suggests prolonged uncontrolled hyperglycemia, which would be the likely driver of mucormycosis in this group.

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Stage of ROCM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 (n = 2)</td>
</tr>
<tr>
<td>Nasal block</td>
<td>11 (47.83%)</td>
</tr>
<tr>
<td>Nasal discharge</td>
<td>14 (60.87%)</td>
</tr>
<tr>
<td>Orbital/facial pain</td>
<td>3 (13.04%)</td>
</tr>
<tr>
<td>Orbital/facial edema</td>
<td>8 (34.78%)</td>
</tr>
<tr>
<td>Proptosis</td>
<td>2 (7.14%)</td>
</tr>
<tr>
<td>Loss/diminution of vision</td>
<td>2 (8.70%)</td>
</tr>
<tr>
<td>Palatal ulcer</td>
<td>2 (8.70%)</td>
</tr>
<tr>
<td>Face/teeth numbness</td>
<td>3 (13.04%)</td>
</tr>
<tr>
<td>Lid swelling</td>
<td>1 (4.55%)</td>
</tr>
<tr>
<td>Orbital/facial discoloration</td>
<td>1 (3.57%)</td>
</tr>
<tr>
<td>Ptosis</td>
<td>1 (4.35%)</td>
</tr>
<tr>
<td>Eyelid erythema</td>
<td></td>
</tr>
<tr>
<td>Nasal ulcers/crusts</td>
<td>2 (8.70%)</td>
</tr>
<tr>
<td>Diplopia</td>
<td>1 (3.57%)</td>
</tr>
<tr>
<td>Face deviation</td>
<td>2 (8.70%)</td>
</tr>
<tr>
<td>Altered sensorium</td>
<td></td>
</tr>
<tr>
<td>Paraplegia</td>
<td>1 (3.57%)</td>
</tr>
<tr>
<td>Dental pain</td>
<td></td>
</tr>
<tr>
<td>Abnormal facial movements</td>
<td>1 (3.57%)</td>
</tr>
</tbody>
</table>

**Fig. 4:** Summary of the interpretation of key results from this study
Glycemic Control and Mucormycosis during COVID-19 Pandemic in India

Mucormycosis Staging and HbA1c and RBS at Presentation

In our study, we found a statistically significant association between increased HbA1c and higher staging of ROCM at presentation (p-value of <0.001) (Fig. 2). Similarly, there was a statistically significant association between increased RBS levels and higher staging of ROCM at presentation (Fig. 3). Therefore, we conclude that a discussion of the severity of mucormycosis staging is incomplete without addressing the glycemic control at presentation. We also advocate for a need to assess blood glucose levels and HbA1c at presentation as cues to a better picture of mucormycosis extensiveness.

A study by Rao and R, found a significant association between the higher clinical stage of mucormycosis and increasing serum iron and inflammatory markers levels.16 Another study by V and St. found that the staging of mucormycosis positively correlated with the serum lipid profile levels.17 Another study by Wahid et al. has correlated a higher neutrophil-lymphocyte ratio with the higher stages of mucormycosis.18 However, to the best of our knowledge, no study to date has tried to investigate the staging of mucormycosis with the level of glycemic control at presentation.

The key associations obtained in our study concerning glycemic control at presentation, COVID-19 history and mucormycosis stage and outcome are summarized below (Fig. 4).

Prognostic Factors of Mucormycosis Outcomes

Gender: We found that males made up the majority of mucormycosis patients (64%), but there was no significant difference in mortality between males (14.58%) and females (14.83%). This is in concordance with the results reported by Riad et al., and Ostovan et al.19,20 The reason for male susceptibility to mucormycosis are not yet understood, but estrogen's protective role in paracoccidioidomycosis may offer insight. The exact effect of estrogen on the incidence of mucormycosis remains unexplored.21

Corticosteroid use: Several studies in recent years have established a correlation between corticosteroid use and the development of mucormycosis. However, reviewing past literature found limited research on the relationship between corticosteroid use and mucormycosis-related mortality. Sebastian et al. reported a higher concomitant steroid use in COVID-19-associated mucormycosis.10 Our study findings support the conclusion that steroid use does not significantly increase the risk of CAM-related mortality. This is also in concordance with the study published by Riad et al.19 Thus, we recommend that the use of steroids in the management of serious COVID-19 patients should not be discontinued fearing the risk of mucormycosis.

Diabetes: Our study found that a history of diabetes associated with a higher incidence of ROCM was not significantly associated with mortality. This is in concordance with the studies published by Riad et al. and Ostovan et al.19,20

Coronary artery disease: Our study demonstrates a statistically significant correlation between mucormycosis mortality and coronary artery disease (p = 0.0152). This association may be partially attributed to the known detrimental effect of coronary artery disease on outcomes in patients with COVID-19.22 Our results were in stark contrast to a study by Mishra et al., who did not find any statistically significant association between coronary artery disease and CAM mortality.23

Symptoms: Our study demonstrated a statistically significant correlation between mortality in mucormycosis patients and the presence of proptosis (p = 0.0152). No other symptoms at presentation were significantly associated with the overall outcome of COVID-19-associated mucormycosis. Therefore, patients with proptosis should be prognosticated early and managed in a high-dependency unit setting. A study by Mishra et al. did not find any statistically significant association between symptoms at the presentation of disease and CAM mortality.23

CONCLUSION

Worse glycemic control at presentation is associated with a higher stage of mucormycosis at presentation but has no impact on mortality. It’s crucial to order blood glucose and HbA1c levels in all COVID-19-associated and non-COVID-19-associated mucormycosis patients—to screen for new-onset diabetes and measure the extent of progression of the mucormycosis stage before diagnostic tests and imaging have been ordered. A history of other factors predisposing to immunocompromise is also crucial. Other novel findings include an association of increased mortality outcomes in those with proptosis as the primary symptom and those presenting with comorbid coronary artery disease. We recommend more vigilant care of patients having either of these, perhaps with an early transfer to a high-dependency unit. We also found that the use of corticosteroids for COVID-19 treatment was not associated with the development of mucormycosis, and hence, the threat of mucormycosis is not a reason to discontinue steroid use for COVID-19 treatment.

REFERENCES

In hypertensive patients with CAD

Initiate with **Tazloc-Beta 25**
Telmisartan 40 mg + Metoprolol Succinate 25 mg PR

**Assured Control on Sympathetic Over Activity**

74% HCPs Prefer the Combination of Telmisartan + Metoprolol

Control over Heart Rate

24 Hour BP Control

1. Data on file

In hypertensive patients with CAD

**Tazloc-Beta 50**
Telmisartan 40 mg + Metoprolol Succinate 50 mg PR

**Superior Cardiovascular Protection**

In Post PCI

METOPROLOL™ & TELMISARTAN™

Reduces risk of

MACE
Recurrent MI

In patients with hypertension & diabetes,

Tazloc®-AM
Telmisartan 40/80 mg + Amlodipine 5 mg

The Complete Protection

Amlodipine offers a significant reduction of

57%

in Coronary Revascularisation and Progression of Carotid Artery Atherosclerosis

Reference: J Assoc Physicians India. 2018;Mar;66(S):64-9
Comparison of Guillain-Barre Syndrome Cases during and Prior to the COVID-19 Pandemic: A Multicentric Study

Praveen Panicker1*, Dileep R2, Abdul Gafoor V3, Prasanth S R4, Thomas Iype5, James Jose6, Antony Stanley7

Received: 15 February 2023; Accepted: 31 March 2023

ABSTRACT

Background: Guillain-Barre syndrome (GBS) is one of the most common neurological manifestations associated with severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection. Although data for a strong causal association is lacking, anecdotal reports, case series and systematic reviews linking the two have emerged in the literature. This prompted us to compare the clinical features, electrophysiology, and outcomes of GBS cases presenting during the pandemic with cases reported during a similar time period prior to the pandemic.

Materials and methods: Prospective data of GBS cases diagnosed as per the National Institute of Neurological Disorders and Stroke (NINDS) criteria was collected for a 6-month period (July–December 2021) at three tertiary care teaching hospitals during the coronavirus pandemic and compared with retrospective records-based data of cases prior to the pandemic (January–July 2019).

Results: A total of 40 cases were included in the cases, out of which 17 were in the prepandemic and 23 in the postpandemic period. A total of three cases temporally related to coronavirus disease 2019 (COVID-19) infection and four cases following COVID-19 vaccination were seen in the pandemic cohort. The clinical features, electrodiagnostic features, and outcomes were comparable during both periods. A slightly higher rate of in-hospital complications and single mortality was reported in the postpandemic period.

Discussion: The number of GBS hospital admissions, clinical presentation, electrodiagnostic features, and short-term outcomes did not differ significantly between the prepandemic and postpandemic periods; a slightly higher incidence of in-hospital complications was observed during the postpandemic period.

INTRODUCTION

Although primarily affecting the respiratory system, various neurological manifestations have been associated with the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection affecting both the peripheral and central nervous systems.1–3 Various case reports, case series and systematic reviews suggest an association between SARS-CoV-2 infection and Guillain-Barre syndrome (GBS).4–7 However, strong epidemiological evidence has been lacking to definitively associate this viral infection with the occurrence of GBS.8

The control measures, including social distancing measures and widespread lockdowns to curb the spread of the pandemic, have affected hospital admission patterns, time delay to hospital admission following symptom onset, and receiving timely treatment, including immunotherapy and intensive care, including ventilation. It is intuitive that this will have an impact on the outcomes and recovery of incident cases.

This prompted us to compile data from tertiary care teaching hospitals catering to >10 districts in our state. We attempted to compare the clinical features, treatment response, complications, electrophysiology, and investigation findings among GBS admissions in a 6-month period during the coronavirus disease 2019 (COVID-19) pandemic with cases presenting prior to the pandemic during a similar period.

MATERIALS AND METHODS

Data Collection and Selection of Patients

All patients presenting with clinical history and examination findings consistent with GBS satisfying the National Institute of Neurological Disorders and Stroke (NINDS) criteria were included. Relevant investigations to rule out GBS mimics were done. Written informed consent was obtained from all patients during the prospective data collection period. Institutional research and ethical committee approvals were obtained from all three participating centers prior to data collection.

Demographic data, clinical details, including presenting symptoms, time from onset to hospital admission, treatment received and response to treatment-related fluctuations, and outcome as gauged by GBS outcome score and modified Rankin score at discharge, were collected using a structured proforma. Along with this, electrophysiology findings, cerebrospinal fluid study and other ancillary lab data were compared between the two groups.

Statistical Analysis

Demographic data, clinical characteristics, antecedent events, characteristics of subtypes, and electrophysiology are represented in numbers and percentages. Means with standard deviation (SD) or median values with minimum and maximum values were presented as appropriate. Chi-squared tests were used to analyze categorical variables. Continuous variables were compared using the student’s t-test for data with normal distribution and Mann–Whitney U test for data that were not. Any p-values <0.05 were considered statistically significant. All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) version 24.0 (SPSS, Chicago, Illinois, United States of America).

RESULTS

A total of 40 GBS cases were included in the data analysis. A total of 17 incident cases were during the prepandemic period vs 23 cases in the postpandemic period.

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Comparison of Guillain-Barre Syndrome Cases

**Table 1:** Comparison of clinical features and antecedent events between prepandemic and pandemic group

<table>
<thead>
<tr>
<th>Factors</th>
<th>Frequency (prepandemic) n = 17</th>
<th>Frequency (pandemic) n = 23</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical Features</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quadripareis</td>
<td>13 (76.5%)</td>
<td>17 (73.91%)</td>
<td>0.85</td>
</tr>
<tr>
<td>Paraparesis</td>
<td>2 (11.8%)</td>
<td>6 (26.09%)</td>
<td>0.26</td>
</tr>
<tr>
<td>No limb weakness</td>
<td>2 (11.8%)</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Sensory symptoms</td>
<td>14 (82.4%)</td>
<td>20 (87%)</td>
<td>0.69</td>
</tr>
<tr>
<td>Areflexia</td>
<td>13 (76.5%)</td>
<td>12 (52.2%)</td>
<td>0.25</td>
</tr>
<tr>
<td>Hyporeflexia</td>
<td>4 (23.5%)</td>
<td>10 (43.5%)</td>
<td>0.25</td>
</tr>
<tr>
<td>Normal deep tendon reflexes</td>
<td>–</td>
<td>1 (4.5%)</td>
<td>–</td>
</tr>
<tr>
<td>Mean time from onset of weakness to admission (days)</td>
<td>8.18 (SD: 4.95)</td>
<td>8.91 (SD: 6.76)</td>
<td>–</td>
</tr>
<tr>
<td>Facial weakness</td>
<td>4 (23.5%)</td>
<td>10 (43.5%)</td>
<td>0.19</td>
</tr>
<tr>
<td>Bulbar weakness</td>
<td>2 (11.8%)</td>
<td>5 (21.8%)</td>
<td>0.41</td>
</tr>
<tr>
<td>Ataxia</td>
<td>5 (29.4%)</td>
<td>7 (30.4%)</td>
<td>0.94</td>
</tr>
<tr>
<td>Pain</td>
<td>3 (17.6%)</td>
<td>7 (30.4%)</td>
<td>0.36</td>
</tr>
<tr>
<td><strong>Antecedent event</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gastroenteritis</td>
<td>2 (11.8%)</td>
<td>2 (8.7%)</td>
<td>0.75</td>
</tr>
<tr>
<td>None</td>
<td>10 (58.8%)</td>
<td>10 (43.5%)</td>
<td>0.34</td>
</tr>
<tr>
<td>Upper respiratory infection</td>
<td>5 (29.4%)</td>
<td>2 (8.7%)</td>
<td>0.09</td>
</tr>
<tr>
<td>COVID-19 infection</td>
<td>–</td>
<td>3 (13.04%)</td>
<td>–</td>
</tr>
<tr>
<td>COVID-19 vaccination</td>
<td>–</td>
<td>4 (17.39%)</td>
<td>–</td>
</tr>
<tr>
<td>Dengue infection</td>
<td>–</td>
<td>1 (4.35%)</td>
<td>–</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>–</td>
<td>1 (4.35%)</td>
<td>–</td>
</tr>
</tbody>
</table>

**Table 2:** Guillain-Barre syndrome (GBS) clinical subtype and electrophysiology between prepandemic and pandemic group

<table>
<thead>
<tr>
<th>GBS clinical subtype</th>
<th>Frequency (prepandemic) n = 17</th>
<th>Frequency (pandemic) n = 23</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensorimotor</td>
<td>10 (58.82%)</td>
<td>12 (52.17%)</td>
<td>0.68</td>
</tr>
<tr>
<td>Pure motor</td>
<td>5 (29.41%)</td>
<td>11 (47.83%)</td>
<td>0.24</td>
</tr>
<tr>
<td>Sensory</td>
<td>2 (11.76%)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Electrophysiology</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Axonal</td>
<td>6 (35.30%)</td>
<td>4 (17.4%)</td>
<td>0.196</td>
</tr>
<tr>
<td>Demyelinating</td>
<td>11 (64.70%)</td>
<td>19 (82.6%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Motor</td>
<td>7 (41.2%)</td>
<td>14 (60.9%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Sensorimotor</td>
<td>10 (58.8%)</td>
<td>9 (39.1%)</td>
<td>–</td>
</tr>
</tbody>
</table>

**Demographic and Clinical Features**

The median age at presentation was 48 years (minimum: 13, maximum: 70) in the pandemic period vs 49 years (minimum: 17, maximum: 73) in the postpandemic period. Male-to-female ratios were 1.24 and 1.87:1 in the two respective periods. A summary of the comparison of various clinical findings is given in Table 1.

**DISCUSSION**

We observed that the number of hospital admissions of GBS cases was comparable during both periods. During the early days of the COVID-19 pandemic, there was some early evidence of increased incidence of GBS cases from hotspot regions in Italy, however; subsequent studies have failed to demonstrate a definitive epidemiologic link as was evident in the Zika virus infection-related outbreaks of GBS cases. Moreover, hospital admissions are not reflective of the incidence in the community, which can only be gleaned from large-scale epidemiological studies covering large populations. A cohort study from India which included data from multiple tertiary care centers in India found a reduction in the number of GBS cases during the government-mandated lockdown period. This could be attributed to the decrease in transmission of infectious diseases, especially those transmitted by droplet spread (e.g., influenza), because of the restrictions on movement and enhanced hygiene measures.

There was no significant difference in the clinical presentation during the two periods, including cranial nerve involvement, bulbar weakness, ataxia, involvement of deep tendon reflexes, and severity of the disease. GBS disability scores and modified Rankin scores at discharge did not show statistically significant differences between the two groups. There was a single mortality in the pandemic period which is much less compared to the reports in the literature (International GBS Outcome Study, 7%).

Electrophysiology-wise, the demyelinating variant was more frequent during both periods. A slight increase in the proportion of this variant was seen in the pandemic period. However, there was no statistically significant difference between the two groups (Table 2). Three cases of post-COVID-19 infection (all demyelinating) and four cases of post-COVID-19 vaccination (two axonal and two demyelinating) GBS cases were seen in the pandemic cohort. Uncini et al., in their systematic review of COVID-19 infection-related GBS cases, reported that 80.5% of all cases were of the demyelinating variety; however, individual case reports have reported both axonal and demyelinating patterns. Similarly, there have been various case series and reports of GBS documenting post-COVID-19 vaccination GBS cases especially following live viral vector platform-based vaccines. A recent systematic review including 88 post-COVID-19 vaccination GBS cases reported a favorable outcome in most cases; however, a severe disease requiring mechanical ventilation was seen in 14.7% of
cases. The bifacial paresis variant was found to be much more frequent compared to seasonal GBS cases (15.9 vs <5%).

No significant delay was observed from onset to presentation and initiation of treatment during the pandemic period compared to the prepandemic phase. Higher proportions of patients receiving plasma exchange compared to intravenous immunoglobulin are attributable to individual center preferences and economic considerations.

A slightly higher rate of complications, including aspiration pneumonia and mortality seen during the pandemic period (Tables 3 and 4), could possibly be attributed to the redistribution of ICU facilities and manpower for COVID-19 care, which might have affected the neurology intensive care of critically ill GBS patients.

## References

ORIGINAL ARTICLE

Efficacy and Tolerability of Tenofovir/Lamivudine/Dolutegravir among Antiretroviral Therapy Naive Human Immunodeficiency Virus Infected Patients of a Tertiary Care Center in Eastern India

Debroop Sengupta1, Sandip Ghosh2*, Shantasil Pain3, Nandini Chatterjee4

Received: 25 March 2023; Accepted: 25 April 2023

ABSTRACT

Background: Although many drug regimens have been used in the treatment of human immunodeficiency virus (HIV) infection, the National AIDS Control Organization (NACO) of India recommends the use of a fixed-dose combination of tenofovir/lamivudine/dolutegravir (TLD) as a first-line regimen since 2020. In spite of much global data on the use of this combination, experience in the Indian population is still limited. We aim to find out the efficacy and tolerability of this novel regimen, in a tertiary care center of Eastern India.

Materials and methods: A descriptive observational study, longitudinal in design performed in the antiretroviral therapy (ART) center of a tertiary care hospital in Kolkata, West Bengal, India. All patients who attended the ART center from April 2021 to October 2022 were enrolled in the study following inclusion and exclusion criteria. A detailed history, clinical examination, necessary biochemical tests, and CD4 count of all patients were done at baseline. Subsequently, they were followed up for 6 months with monthly visits when they were enquired about any adverse effects requiring therapy interruptions. At the end of 6 months, CD4 count and viral load were measured.

Results: Out of a total sample of 249 patients, the TLD regimen was efficacious in 99.2% (n = 247) in whom viral load was suppressed to <1,000 copies/mL after 6 months of treatment. The regimen had to be temporarily discontinued in 6% of patients (n = 15). The most common cause of treatment interruption was hepatic dysfunction (3.2%) followed by cutaneous manifestation (2.4%). In 14 out of 15 patients, the regimen could be reintroduced and was safely tolerated afterward. Only one patient had to be shifted to an alternative regimen due to tenofovir-induced nephrotoxicity. Thus TLD was tolerated in 99.6% (n = 248) patients.

Conclusion: The fixed-dose combination of TLD is a highly efficacious and well-tolerated first-line regimen for ART naïve patients with HIV infection having >95% adherence.

Journal of the Association of Physicians of India (2023): 10.59556/japi.71.0311

INTRODUCTION

Human immunodeficiency virus (HIV) is a global pandemic with 37.6 million people living with HIV all over the world.1 India is a high HIV burden country with a prevalence of 0.21% among the general population.2 Many drug regimens have been tried over the past in HIV treatment. Currently, Integrase Strand Transfer Inhibitor (INSTI) containing regimen is the first-line treatment for HIV infected patients all over the world since 2016. The National AIDS Control Organization (NACO) in India recommends using the fixed-dose combination of tenofovir/lamivudine/dolutegravir (TLD) as the first-line regimen for antiretroviral therapy (ART) naïve HIV infected patients since 2020.3 We aim to find out the efficacy and tolerability of the TLD regimen among patients attending the ART center of a tertiary care hospital in Eastern India.

MATERIALS AND METHODS

Our study was a descriptive observational one, longitudinal in design. All patients who had attended the ART center of a tertiary care hospital in Kolkata, West Bengal, India, within a span of 1.5 years from April 2021 to October 2022 were enrolled in the study following inclusion and exclusion criteria.

Inclusion Criteria

Adults greater than 16 years of age, who were newly diagnosed with HIV-1 infection and were naïve to any antiretroviral therapy and subsequently started on a TLD regimen were included after informed consent.

Exclusion Criteria

Patients with chronic kidney disease (eGFR <60 mL/minute/1.73 m²), decompensated chronic liver disease, known connective tissue disease, and chronic alcoholics were excluded from the study.

Results

Baseline Characteristics

A total of 376 patients were enrolled in the study. Of them, 64 patients were subsequently transferred out to other ART centers, 23 were lost to follow-up, and 40 patients were eliminated due to poor adherence to ART (average adherence <95% in first 6 months). None of these adherence issues were related to the adverse effects of the drugs. No death was reported among the patients.

Data were collected on the clinico-biochemical profile of the sample population at the initiation and after 6 months of starting ART. A detailed history of all chronic drug therapies due to any other coexisting disease was also noted. CD4 count testing was done at 0 and 6 months. Viral load testing was done at 6 months as per the national guidelines. On each monthly visit, adherence was calculated using the pill count method and patients were enquired about any adverse reactions.

Case Definitions

Efficacy was defined as the proportion of patients having viral load <1,000 copies/mL of blood after 6 months of treatment. Tolerability was defined as the proportion of patients in whom the TLD regimen could be continued for at least 6 months without changing to an alternate regimen because of any adverse effect.

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out of eight (75%) patients who developed hepatic dysfunction had a history of intake of both antitubercular drugs and ART. ART and Anti-tubercular drugs were withheld in all six of them. After the subsidence of liver injury, they were later sequentially restarted on a modified anti-tubercular regimen with levofloxacin-streptomycin-ethambutol followed by ART with TLD. TLD was well tolerated afterward. Of the remaining two patients with hepatic dysfunction, one had coinfection with the hepatitis C virus, and the other was diagnosed with Ebstein–Barr virus infection. ART was temporarily stopped in both of them and reintroduced after the subsidence of liver enzymes. Both of them tolerated the regimen.

Among the six patients having skin manifestation, five patients developed symptoms within 2 weeks of initiation of ART. Three of them developed a minor maculopapular erythematous rash, and two of them had itching. All of them were

### Tolerability

Only 6% (n = 15) of patients had major adverse effects requiring ART interruption during the first 6 months of treatment. Of them, 3.2% (n = 8) had hepatic dysfunction, 2.4% (n = 6) had cutaneous manifestations, and only 0.4% (n = 1) had renal dysfunction (Fig. 3). Six

### Efficacy

After 6 months of treatment, virological failure (viral load ≥1000 copies/mL of blood after 6 months of treatment with adherence >95%) occurred in two patients (0.8%) (Fig. 2). In the first patient, the viral load was 1,269 copies/mL and in the second patient, it was 66,601 copies/mL. Although average adherence in both patients was >95% in the last 6 months, step-up adherence counseling was done as per NACO protocol, drug interactions were reevaluated and both patients were referred to State AIDS Clinical Expert Panel. As per expert opinion, both of them were continued on the TLD regimen. The viral load of both, after 3 more months of ART with TLD was below the detection level indicating the effectiveness of the regimen.

### Table 1: Baseline characteristics of the study population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N (%)</th>
<th>Total (n = 249)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>37 (± 11.4)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>182 (73.1)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>66 (26.5)</td>
<td></td>
</tr>
<tr>
<td>TG/TS</td>
<td>1 (0.4)</td>
<td></td>
</tr>
<tr>
<td>WHO clinical stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage I</td>
<td>146 (58.6)</td>
<td></td>
</tr>
<tr>
<td>Stage II</td>
<td>34 (13.7)</td>
<td></td>
</tr>
<tr>
<td>Stage III</td>
<td>39 (15.7)</td>
<td></td>
</tr>
<tr>
<td>Stage IV</td>
<td>30 (12.0%)</td>
<td></td>
</tr>
<tr>
<td>Baseline CD4 count (cells/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;200</td>
<td>90 (36.1)</td>
<td></td>
</tr>
<tr>
<td>200–349</td>
<td>60 (24.1)</td>
<td></td>
</tr>
<tr>
<td>349–499</td>
<td>48 (19.2)</td>
<td></td>
</tr>
<tr>
<td>≥500</td>
<td>51 (20.5)</td>
<td></td>
</tr>
<tr>
<td>Co-treatment with antitubercular drugs</td>
<td>50 (20.1)</td>
<td></td>
</tr>
<tr>
<td>Cotrimoxazole prophylaxis therapy</td>
<td>173 (69.5)</td>
<td></td>
</tr>
<tr>
<td>Viral hepatitis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>13 (15.2)</td>
<td></td>
</tr>
<tr>
<td>Hepatitis C</td>
<td>7 (2.8)</td>
<td></td>
</tr>
</tbody>
</table>

TG/TS Transgender/Transsexual

**Fig. 1:** Timeline of study and number of patients

**Fig. 2:** Pie diagram showing distribution according to viral load status after 6 months of ART
managed with antihistamines and did not require interruption of ART for >1 week and all of them tolerated ART and cotrimoxazole afterward. In one patient there was extensive skin reaction in the form of toxic epidermal necrolysis, but the reaction developed after 4 months of treatment with ART and occurred 3 days after taking azithromycin tablet for upper airway infection. Hence the reaction could not be directly attributed to the ART regimen. Her ART was temporarily withheld and later restarted after 2 weeks when the lesions subsided.

One patient who had baseline creatinine 1.41 (eGFR = 62 mL/minute/1.73 m^2) at the time of initiation of ART with TLD, had a rise in creatinine up to 2.1 mg/dL and had to be shifted to an alternative regimen without tenofovir.

Thus, among all patients, only 0.4% (n = 1) could not tolerate the TLD regimen and was changed to an alternative regimen.

**Discussion**

There have been many studies on the efficacy and tolerability of Dolutegravir (DTG)-containing regimens like TLD in the treatment of ART naïve HIV infected patients. While many trials like the SINGLE trial, a trial by Meireles et al., NAMSAL ANRS 12313 Study Group, et al., clearly depicted the superiority of dolutegravir over efavirenz, in terms of safety and efficacy endpoints, trials like “NADIA” compared DTG to protease inhibitors like darunavir and found it to be noninferior. A systematic literature review and meta-analysis of 68 studies by Kanters et al. concluded that DTG can be considered safe, effective, and tolerable as a first-line regimen in combination with lamivudine/emtricitabine and tenofovir disoproxil fumarate. The superiority of DTG was further emphasized when the PADDLE study and GEMINI trial suggested that dual therapy with DTG and Lamivudine could be an alternative first-line treatment option, especially in resource-poor settings.

In spite of much evidence of the superiority of the TLD regimen, there is a dearth of data on the Indian subcontinent. We found only two studies as of March 2022 on the efficacy and tolerability of TLD in the Indian population. One of them was conducted in Chennai, Tamil Nadu, India, on 564 patients which showed 100% efficacy and tolerability among patients treated with DTG-based regimens. Sleep disturbances and neuropsychiatric symptoms were not reported in this cohort although these were relatively common in the European cohort. Another Indian study on TLD done on 288 patients, concluded that although 14.4% adverse effects had occurred related to the study treatment, only 0.4% had treatment discontinuation due to treatment-related adverse effects. Around 86.8% of patients developed viral load suppression within 6 months of treatment but the suppression cut-off in his study was kept at HIV-1 RNA levels ≤50 copies/mL.

Our results also demonstrated that TLD has excellent efficacy among the study population with virological failure in only 0.8% (n = 2) of patients who also had virological suppression later after step-up adherence counseling. It was also a well-tolerable regimen with 6% developing significant side effects to treatment which required treatment interruption during the first 6 months. Among them, only 0.4% needed a change in regimen due to tenofovir-induced nephrotoxicity. Similar to the Indian study by N. Kumarsamy et al, neuropsychiatric side effects were relatively rare in our cohort.

**Study Limitations**

The study was a single-center study. Viral load estimation at baseline could not be done for all patients as during the study period there was no provision for the same under the national guidelines.

**Conclusion**

The fixed-dose combination of TLD is a highly efficacious and well-tolerated first-line regimen for ART naïve patients with HIV infection having >95% adherence.

**Acknowledgments**

Special thanks to all members of the ART center, IPGME & SSKMH, Kolkata, West Bengal, India.

**References**

Dysbiosis in Irritable Bowel Syndrome

Philip Abraham1*, Nitesh Pratap2

Received: 26 May 2022; Accepted: 29 June 2023

ABSTRACT

The human gut microbiota fosters the development of a dynamic group of microorganisms impacted by diverse variables that include genetics, diet, infection, stress, ingested drugs, such as antibiotics and small intestine bacterial overgrowth (SIBO) as well as the gut microbiota itself. These factors may influence the change in microbial composition, which results in dysbiosis (microbial imbalance) and exposes the gut to pathogenic insults. Dysbiosis is incidental to the pathogenesis of inflammatory diseases such as irritable bowel syndrome (IBS) and metabolic diseases, including type 2 diabetes and obesity. IBS exhibits different symptoms like abdominal pain or discomfort, distention/bloating, and flatulence. To treat IBS, modifications of dysregulated gut microbiota can be done using treatment strategies like a low-fodmap diet, antibiotics that cannot be absorbed like rifaximin and neomycin, probiotics and prebiotics, and fecal microbiota transplantation (FMT).

INTRODUCTION

In healthy people, there is a homeostatic balance of microorganisms in the gastrointestinal (GI) tract to ensure that it remains healthy and is devoid of excess pathogenic bacteria. A healthy gut microbiome prevents the inhabitation of the gut by pathogenic bacteria by hindering their adherence to the GI tract walls. A compositional alteration of microbes causing an imbalance (“dysbiosis”) can make the gut prone to pathogenic insults.1 Dysbiosis is a loss of bacterial flora in the gut that is linked to various health problems.2,3 It interferes with the immune system’s and mucosal barrier’s ability to maintain homeostasis, which promotes invasion and proliferation of the pathogenic species along with their ability to adhere to the gut wall. Dysbiosis is also closely related to systemic inflammation.1,4-5

The etiology of irritable bowel syndrome (IBS) has been postulated with a decline in bacterial activity, however, some specific microbes distinguishing these patients are still not well known.6 Understanding the role of the intestinal microbiota is crucial when developing future approaches to the treatment of IBS.7 In accordance with the amount and uniformity of the stools, the subgroups of IBS can be distinguished by the Rome IV criteria for diagnosis (i.e., loose/watery and hard/lumpy)—IBS-diarrhea, IBS-constipation, a combination of both irritable bowel syndrome-mixed variety (IBS-M), and unspecified (IBS-U).8 This disorder affects up to 20% of the population worldwide, with conventional indications like abdominal pain, distention/bloating, flatulence, and discomfort in the abdominal part in addition to altered bowel patterns.6 People with IBS tend to have a lower quality of life (QOL) and increased levels of anxiety.9 They are more susceptible to disorders like migraine, fibromyalgia, and depression, also known as “effective spectrum disorders” or “functional somatic syndromes.”10 IBS is a huge burden on healthcare costs and work absenteeism.11

In this review, we will delve into the variables that contribute to the etiology of IBS, primarily the involvement of dysbiosis, and the relevant therapeutic modalities. Using search engines like Pubmed and Google Scholar, we did an extensive literature search of published medical reports in the English language from 2010 to 2021 for the review. Abstracts were identified using keywords like IBS, complications, gut microbiota, dysbiosis, treatment, probiotics, and fecal microbial transplantation (FMT). The computer-processed search was supported by manual searches of references and review papers. Only the full-text articles along with those describing human-subject results were considered.

Pathophysiology of IBS

The pathophysiology of IBS is not completely perceived. Genetic predisposition, food intolerance, visceral hypersensitivity, altered gut–brain and brain–gut axis, dysbiosis, and disruption of innate immunity are conducive to this condition.1,2,13

Genetic Factors

Several studies on IBS have explored the influence of genetics on its pathophysiology.14 Genetic risk ranges from rare single-gene aberrations to complex polygenic scenarios with mixtures of typical variants. In several investigations, polymorphisms in genes linked to the pathophysiology of IBS have been observed.12 Other epigenetic factors, like DNA methylation, may contribute to IBS.15 The ongoing search for candidate genes is fueled by the fundamental notion that, in the case of a genetically predisposed individual, environmental circumstances are likely to have a significant impact on pathogenesis.16

Dietary Factors

The pathogenesis of IBS is profoundly impacted by diet.17,18 Many of the IBS patients (between 65 and 90%) have noticed that certain foods, like milk, wheat/grains, vegetables, diets with abundant fats, spicy foods, coffee, the use of alcohol, and trigger...
their symptoms. Also, excess fat is linked to an amplified colonic motor response and visceral sensitivity is increased in individuals with IBS. Duodenal lipids infusions increase the transition time of the small intestine. Unabsorbed carbohydrates lead to flatulence and luminal distension, especially in individuals with dysbiosis, high visceral sensitivity, and abnormal gas handling.

Thus, a diet that corrects microbial dysbiosis or restricts the ingestion of “offending” foods that stimulate the aberrations may be beneficial in managing IBS symptoms.12

Gut Microbiota

The intestinal epithelium's integrity is maintained by eubiosis; it strengthens the intestinal barrier, which defends against pathogens; and it helps with nutritional absorption and vitamin synthesis.20 Dysbiosis is viewed in the majority of patients as a crucial player in the onset and persistence of IBS.7,22 Bellini et al. found compromised lactobacilli and bifidobacteria activities in IBS patients; other researchers have observed a link between dysbiosis and the pathophysiology of IBS.13,21 Inflammatory mediators, severe inflammation, intense immune reactions, and pathogens cause changes in the environment of the intestine and a break in the intestinal barrier. This alters the structural integrity of the gut; disrupts the gut–brain axis and gut neuromuscular junction.12

Table 1 summarizes some of the microorganisms that are altered in IBS.

### Infection

Postinfectious IBS (PI-IBS) succeeds as an enteric infection; it accounts for 3–35% of the 10–15% IBS prevalence in North America and Europe. PI-IBS patients have a distinctive composition of gut bacteria in comparison to healthy people and IBS patients with no previous infection. This is again due to a decrease in diversity.1,10 PI-IBS patients may have a genetic predisposition in the form of abnormalities in genes encoding for bacterial recognition, cytokine secretion, and mucosal integrity.23 The focus should be placed on host-microbe interactions in PI-IBS treatments, such as by carefully modifying the microbial ecosystem of the intestine or using local antiinflammatory drugs.22

### Stress

Psychological stress is a significant element of risk for IBS development. Stress alters the microbial composition of the gut, its motor apparatus, and its immunological reactivity.24,25 There are several mechanisms through which stress may influence the ecology of the gut microbiota, including direct changes brought on by stress, the release of stress hormones like noradrenaline,26 and changes in the microbial niche that affect interbacterial signaling and growth.23 Knowles et al. found a decrease in the levels of health-promoting bacteria in university students who were under stress.27 A study by Lutgendorff et al. revealed that excessive stress can affect colonic motor activity, which can lead to dysbiosis and reduce the number of beneficial lactobacilli, which may eventually cause IBS.28 The pattern of dysbiosis under stress is unpredictable and this increases the risk of autoimmune disease and infections.29

### Antibiotics

Antibiotics reduce the number of bacteria as well as bring about a shift in the overall composition of the gut microbiome. This may alter the metabolites and microenvironment in the intestine and thus have an impact on the growth of other commensals.30 Studies report that individuals who have been on antibiotic therapy for non-GI-related indications might have an increased chance of the occurrence of new or recurrence of existing IBS symptoms and an increased likelihood of developing long-term PI-IBS.32 Human studies have reported that variation in the gut microbiota and gene expressions can persist for up to 2 years after discontinuation of antibiotics.33

### Small Intestine Bacterial Overgrowth (SIBO)

This is a qualitative as well as quantitative change in the intestinal gut microbiome; it is diagnosed by jejunal culture (>10^5 CFU/mL).34 Various intrinsic and extrinsic factors regulate and impede the excessive growth of small intestinal bacteria. Intrinsic factors include gastric acid and bile acid secretion, gut defense mechanisms, peristaltic movement, gut antibacterial peptides, production of mucin, and prevention of bacterial retrograde translocation via the ileocaecal valve from the lower gut to the upper gut. Extrinsic factors include nutrient intake and diet, medications altering motility (prokinetics), bacterial and viral infection, and drugs modulating the gut microbiota (pre and probiotics, proton pump inhibitors, H2 receptor blockers, and antibiotics).34,35 A defect in these factors can lead to dysbiosis and disproportionate colonization of bacteria.36

A meta-analysis revealed an elevated risk of developing SIBO in IBS patients when compared with controls.37 On the contrary, Walters and Vanner studied patients with IBS using lactulose and D-xylose breath tests and showed that the number of IBS patients who were reported positive for SIBO was similar to the control group.38

### Treatment of IBS-targeting Dysbiosis

#### Dietary Interventions

Diet has a considerable influence on the pathogenesis of IBS, as food and its byproducts may impact the permeability and motility of the intestinal tract, the microbiome, visceral sensation, gut–brain interactions, Immune system regulation, and neuroendocrine function.39

#### Low-FODMAP Diet (LFD)

Recently, there has been increasing evidence suggesting that bloating, pain, and other symptoms associated with IBS appear to be caused by fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs) in 70% of patients.7 Due to dietary interventions, the average daily intake of FODMAPs in patients with IBS was lowered from 15–30 gm/day to 5–18 gm/day.39 Because of a LFD, the severity and QOL scores improved in those patients suffering from IBS, according to Marsh et al.’s meta-analysis.37 Despite its advantages, there are drawbacks, such as a reduction in the intake of the prebiotic fructans and galactooligosaccharides (GOS) from the diet by up to 50%, thus reducing the carbohydrate substrate available for colonic fermentation.79 Nonetheless, a study conducted in the United Kindom found that a diet low in FODMAP can be nutritionally beneficial and sufficient for up to 18 months after the first dietitian-led instruction.7 Foods that are fatty, spicy, or contain alcohol or caffeine might trigger GI symptoms. The effects of gluten generally include alterations in intestinal permeability along with stimulation of the enteric and autonomous neuronal systems.72

### Nonabsorbable Antibiotics

Considering the significant role that bacteria play in the GI system in the etiology of the
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...disease, nonabsorbable antibiotics have been identified as an efficient treatment option for IBS. Rifaximin, a semisynthetic derivative of rifamycin and a nonabsorbable antibiotic, acts by inhibiting RNA synthesis, that is, inhibiting the initiation of chain formation by binding to the β-subunit of the bacterial DNA-dependent RNA polymerase enzyme, which eventually stops translocation and terminates transcription. Rifaximin is effective in managing IBS patients suffering from intestinal symptoms with little to no side effects, and the onset of resistant bacterial strains is nearly nonexistent. When administered orally, <0.5% of rifaximine is absorbed, thus leading to no significant adverse events, drug interactions, or toxicity.1

Targets 1 and 2 are randomized controlled trials that investigated the potential benefits of rifaximin in alleviating symptoms in IBS patients without constipation. Rifaximin considerably reduced general symptoms and bloating when given at a dose of 550 mg three times per day for 2 weeks as opposed to a placebo. Patients who merely suffer from IBS-D responded to a 2-week course of rifaximin but relapsed throughout the post-treatment observational phase were enrolled in the Target 3 study, where patients were randomized to an additional 2 weeks of rifaximin or placebo treatment. Patients on rifaximin treatment responded more to treatment as compared to placebo. Additionally, lower rates of adverse events were found, demonstrating that rifaximin repeat therapy has a favorable response and can be taken into consideration for significantly easing symptoms in IBS-D patients. A double-blind study reported that patients on rifaximin treatment had low methane levels on the on-spot test; colon transit time was also improved.

Rifaximin is not generally prescribed to patients suffering from constipation-predominant IBS. However, a study showed that a 4-week combination treatment with rifaximin and neomycin as compared to neomycin alone was found to be superior in ameliorating the indications of straining and bloating in IBS-C patients; the constipation severity score was much lower in the combination group when compared with neomycin alone. Furthermore, a combination of rifaximin and neomycin has shown to be one of the most effective combinations in treating IBS patients producing methane on lactulose breath tests.

**Probiotics and Prebiotics**

**Probiotics**

Probiotics are GI-friendly living or attenuated microbes that change the microbiota in the gut to improve the host’s health. They help exert an antiinflammatory effect and reduce visceral hypersensitivity. Studies suggest that probiotic strains not only modulate gut inflammation but also yield antimicrobial peptides that aid in the eradication of harmful bacteria, resulting in enhanced mucosal barrier performance. Guyonnet et al. suggested that probiotics have a positive effect on discomfort, health-associated QOL score, bloating in constipation-predominant IBS, and stool frequency in subjects with <3 stools/week. The safety profile of probiotics is very good, although their effects may be modest and the mechanism of their function is relatively unknown. Despite a large number of studies, the scientific evidence has substantial constraints, including the use of highly variable microbial strains or combinations of microorganisms and ambiguity regarding the sustainability and components of commercial products due to the absence of guidelines to guarantee product quality.

Various nonpathogenic bacilli, such as streptococcus, bifidobacteria, and lactobacilli, as well as Escherichia coli Nisle 1917, and yeasts such as Saccharomyces boulardii (S. boulardii) have been used as effective probiotics. A few of the most widely used probiotics are Lactobacillus rhamnosus LGG, Lactobacillus plantarum 299v, Lactobacillus reuteri, Lactobacillus casei, Lactobacillus acidophilus, and Bifidobacterium infantis, brevis, or lactis. Irrational use of antibiotics may lead to undesirable consequences. According to Rosania et al., rifaximin and a probiotic like Lactobacillus casei together significantly reduced SIRO symptoms compared to the antibiotic alone. Recent research conducted by Liu et al. reported that Lactobacillus plantarum CCFM6810 significantly alleviated the clinical symptoms and prevented gut microbial dysbiosis in IBS-D patients. Furthermore, after 8 weeks of treatment with Bifidobacterium bifidum, Gl pain, and the severity of IBS can be reduced. Martoni et al. observed that on 4 weeks of treatment with acidophilus, considerable progress was seen in the bloating symptoms and bowel habits of IBS patients.

Yeast probiotics share the same intrinsic antibiotic resistance as bacterial probiotics. However, due to their fungal origin, yeasts are innately resistant to Gl and bile acids, can survive at body temperature, and hinder the growth of pathogens. They can alter humoral and natural immunity as well as the ecology of the gut microbiome in healthy individuals. In a randomized double-blind trial, IBS patients who were treated with S. boulardii reported reduced incidence and severity of pain, diarrhea, flatulence, eructation, and gurgling, thereby improving QOL. A retrospective analysis by Guslandi et al. recommended that S. boulardii should be used in conjunction with mebeverine as an add-on therapy for treating IBS.

**Prebiotics**

Oligosaccharides and polysaccharides like fructooligosaccharides or GOS, that are indigestible and help in the promotion of the growth and/or activity of bacteria that are beneficial to the host are known as prebiotics. Prebiotics have a short lifespan, necessitating frequent doses, which is a constraint. One of the earliest synthetic prebiotics that has been established to increase gut bacteria is lactulose. Paineau et al. and Silk et al. reported that prebiotics tend to mitigate dysbiosis by fostering favorable shifts in the gut microbiome. They facilitate the replication of gut bacteria, such as Bifidobacterium. Patients receiving active treatment reported less abdominal pain and flatulence as well as an enhancement in their QOL. The probiotic treatment promotes beneficial microbiota changes and reduces IBS symptoms without causing more severe ailments. Therefore, it can be advantageous to thoroughly understand the duration and dosage.

**Fecal Microbiota Transplantation (FMT)**

The FMT is the infusion of distal fecal material from a healthy donor into the GI tract of a recipient to reestablish healthy intestinal flora. In a systematic review by Halkjaer et al., FMT treatment had beneficial outcomes for 28 out of 48 IBS patients (58%) with no major adverse events. The effect of FMT on moderate-to-severe IBS-D and IBS-M patients was evaluated through a randomized, double-blind, placebo-controlled trial. After 3 months, 65% of patients receiving active treatment responded well to it and had fewer severe IBS symptoms than 43% of patients receiving a placebo. However, there was no reduction in severity after 12 months. Before FMT can be regarded as a standard remedy for IBS, more research must be carried out regarding its mechanism of action, appropriate donor choice, route of administration, longevity with constancy of response, and short and long-term safety.

**Newer Therapies: SBI**

Serum-derived bovine immunoglobulin (SBI)/protein isolate comprises a mixture of immunoglobulins in different concentrations, such as >50% immunoglobulin G (IgG), 5% IgM, and 1% IgA. They assist in the attachment and neutralization of endotoxins along with other microbes, preserving a...
Table 2: Different treatments for the management of dysbiosis in IBS

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Reference/author-name</th>
<th>Study type</th>
<th>Treatment group</th>
<th>Study population/cell lines</th>
<th>Dose</th>
<th>Duration</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probiotic</td>
<td>Choi et al.³³</td>
<td>Randomized, double-blind, placebo-controlled multicenter trial</td>
<td>Patients treated with either <em>S. boulardii</em> (n = 34), or placebo (n = 33)</td>
<td>Patients with IBS-D and IBS-M</td>
<td><em>S. boulardii</em> at 2 × 10⁶ live cells as a daily dose</td>
<td>4 weeks</td>
<td><em>S. boulardii</em> had a positive impact on QOL and symptoms in patients with diarrhea-predominant IBS or mixed-type IBS. IBS-related symptoms like bowel movement frequency and stool consistency also improved. Overall improvement in IBS-QOL was greater in <em>S. boulardii</em> group than on placebo (15.4 vs 7.0%; p &lt; 0.05)</td>
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<tr>
<td></td>
<td>Mupas et al.²⁴</td>
<td>Meta-analysis (double-blind)</td>
<td>Two groups—<em>S. cerevisiae boulardii</em> and placebo</td>
<td>Patients suffering from IBS-D</td>
<td>9 × 10⁶ CFU/day</td>
<td>4 weeks</td>
<td>Improvement of symptoms observed. Decrease in the number of stools (p &lt; 0.05) and improvement in consistency (p &lt; 0.05)</td>
</tr>
<tr>
<td>Prebiotic</td>
<td>Silk et al.³⁹</td>
<td>Single-center, parallel, patient-blinded, randomized crossover, and controlled trial</td>
<td>Prebiotic vs placebo</td>
<td>Patients with Rome II-positive IBS</td>
<td>Prebiotic—3.5 or 7 g/day</td>
<td>12 weeks</td>
<td>Prebiotics significantly enhanced fecal bifidobacteria (3.5 g/day p &lt; 0.005; 7 g/day p &lt; 0.001). Prebiotics at 3.5 g/day significantly changed stool consistency, improved flatulence, bloating, a composite score of symptoms, and SGA (p &lt; 0.05). Prebiotic at 7 g/day significantly improved SGA (p &lt; 0.05) and anxiety scores (p &lt; 0.05)</td>
</tr>
<tr>
<td>Prebiotic</td>
<td>Alexea et al.⁶⁰</td>
<td>Randomized, placebo-controlled, double-blind, parallel-group, and multicenter clinical trial</td>
<td>Tablets containing a mixture of vegetable oligo and polysaccharides, reticulated protein, and excipients croscarmellose sodium and magnesium stearate vs placebo (tablets containing corn starch, croscarmellose sodium, and magnesium stearate)</td>
<td>Diarrhea-predominant IBS</td>
<td>Oligo and polysaccharides—750 mg; reticulated protein, 250 mg; and excipients croscarmellose sodium—133 mg and magnesium stearate—17 mg</td>
<td>7 weeks</td>
<td>Significant improvement in symptoms observed in patients treated with oligo and polysaccharides and reticulated protein between visits two and three in abdominal pain (p = 0.0167) and flatulence (p = 0.0373). QOL of patients receiving active treatment increased from baseline to visit three (p &lt; 0.0001)</td>
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<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>FMT</td>
<td>Johnsen et al.63</td>
<td>Double-blind, randomized, placebo-controlled, parallel-group, and single-center study</td>
<td>Active treatment vs placebo</td>
<td>Patients aged 18-75 years, with IBS with diarrhea or with diarrhea and constipation (excluding dominating constipation) defined by Rome III criteria, scored as moderate to severe according to the IBS severity scoring system; a score of ≥175</td>
<td>Transplant—50–80 gm of feces mixed with 200 mL isotonic saline and 50 mL 85% glycerol</td>
<td>–</td>
<td>Around 65% of participants receiving active treatment vs 43% receiving placebo showed a response at 3 months (p = 0.049). FMT produced significant symptom relief in IBS patients</td>
</tr>
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<td></td>
<td>Holvoet et al.75</td>
<td>Double-blind, placebo controlled clinical trial</td>
<td>Fresh donor stool vs placebo</td>
<td>Patients with refractory IBS symptoms, aged 18–75 years and without constipation</td>
<td>–</td>
<td>–</td>
<td>Acceptable relief of IBS and bloating symptoms at 12 weeks (49 vs 29%, p = 0.004)</td>
</tr>
<tr>
<td>Dietary interventions</td>
<td>Staudacher et al.76</td>
<td>Randomized controlled trial</td>
<td>Sham diet/placebo Sham diet/probiotic LFD/placebo LFD/probiotic</td>
<td>Patients with IBS (18–65 years old), based on Rome III criteria</td>
<td>–</td>
<td>4 weeks</td>
<td>LFD is associated with adequate symptom relief and significantly reduced symptom scores compared with a placebo. Coadministration of multistrain probiotic increased numbers of <em>Bifidobacterium</em> species, compared with placebo, and might be given to restore these bacteria to patients on a LFD.</td>
</tr>
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<td></td>
<td>Krieger-Grubel et al.77</td>
<td>Randomized, crossover study</td>
<td>LFD vs low-lactose diet (LLD)</td>
<td>Patients with functional bowel symptoms according to Rome IV criteria</td>
<td>–</td>
<td>3 weeks</td>
<td>LFD and LLD effectively improved overall GI symptoms. LFD tended to be more effective than LLD in reducing GI symptoms like abdominal pain and bloating.</td>
</tr>
<tr>
<td>Antibiotic interventions</td>
<td>Pimentel et al.46</td>
<td>Double-blind, randomized, placebo-controlled trial</td>
<td>Either neomycin and placebo or neomycin twice daily and rifaximin</td>
<td>IBS-C</td>
<td>Neomycin—500 mg twice daily Rifaximin—550 mg three times daily</td>
<td>14 days’ treatment followed by 4 weeks of follow-up</td>
<td>A combination of neomycin and rifaximin superior to neomycin alone in improving multiple symptoms</td>
</tr>
<tr>
<td>SBI</td>
<td>Good et al.67</td>
<td>Retrospective chart analysis</td>
<td>SBI/protein isolate added to patients’ current standard care</td>
<td>14 IBS patients with differing forms—7 IBS-D, 2 IBS-C, 2 IBS-M, and 3 IBS-bloating</td>
<td>5 or 10 gm/day</td>
<td>Up to 17–32 weeks</td>
<td>Overall improvement in symptoms with better stool consistency, decreased frequency as well as reductions in abdominal pain, bloating, distention, and incontinence</td>
</tr>
</tbody>
</table>

SGA, subjective global assessment
They function by attaching to foreign toxic substances or antigens, prohibiting the movement of those materials through the epithelial cell layer, leading to a decrease in the number of antigens penetrating the lamina propria to activate an immune response. This leads to an increase in antiinflammatory cytokine transforming growth factor-β (TGF-β) expression, which maintains the GI immune homeostasis. Toxic substances or antigens, prohibiting the movement of those materials through the gut barrier, are known to aid in the pathophysiology, current research and future approaches for dysbiosis in irritable bowel syndrome: a review on the gut microbiota and therapeutic interventions for irritable bowel syndrome with constipation: a 26-week, randomized, double-blind, placebo-controlled trial to evaluate efficacy and safety. Am J Gastroenterol 2012;107(11):1702–1712.


Dysbiosis in Irritable Bowel Syndrome

Long COVID-19: A Systematic Review

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Received: 08 March 2023; Accepted: 15 June 2023

Abstract
Coronavirus disease 2019’s (COVID-19) wide dissemination casts long-term health jeopardy for millions. Long COVID-19, a lingering multisystem malady, weaves a complex array of symptoms. Understanding its full impact requires extensive research over months or years. The pace of recovery remains uncertain, challenging healthcare systems. An evidence-based symphony of medical care and support is urgently needed for long haulers. Understanding long COVID’s genesis and advocating for patients is vital. Our comprehension remains limited, prompting a systematic scoping study to explore the existing knowledge and pave the way for future research, illuminating the enigma of “long COVID” and guiding the path towards understanding this relentless condition.

Introduction
Behold coronavirus disease 2019 (COVID-19) that dread disease unleashed upon the world by the malevolent severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus, a devastating pandemic that has cast a shadow of sorrow, claiming countless lives and inflicting untold suffering upon the global stage. The majority, a staggering 80% of hapless souls, bore the weight of mild to moderate afflictions, but alas, a mere handful, a mere 5%, succumbed to the grave complications that imperiled their very existence.1 And yet, even for those who emerged from the clutches of COVID-19, a rare few still grapple with new or lingering maladies that persist, a specter haunting them for weeks or months. This condition, this relentless specter, is known by many names: “long COVID,” “long haulers,” or the ominous “post-COVID syndrome.”2

“Long COVID,” a mysterious ailment, marks the enduring presence of diverse symptoms, an unwelcome companion that lingers on, regardless of the viral status, be it a negative PCR result or otherwise. Like an enigmatic dance, this expression encapsulates the realm of “post-COVID syndrome,” a domain of manifestations that may come and go, wax and wane.3 Some symptoms persist from the acute phase, while others emerge anew, weaving a tale of complexity and uncertainty. Biochemical and radiological signs of recovery may emerge in these protracted COVID patients, yet the journey is far from over, extending into the period between microbiological and clinical recovery.4 It is a divide marked by time, dividing the realm of postacute COVID, lasting over 3 weeks but <12 weeks, from the realm of chronic COVID, stretching beyond the 12-week threshold (Fig. 1 and Table 3).5

Long COVID, an enigma that defies the early formal reports from distant Wuhan, portrays a more protracted and intricate course of the disease.6 Here, we recount the how and why, the genesis of long COVID, and delve into the world of long-haulers, illuminating the pressing need for patient advocacy and informed policymaking during pandemics. Though initially labeled as “mild” COVID-19 patients, many traversed a treacherous path, facing life-threatening manifestations and traumatic experiences, often without the comforting touch of healthcare professionals. In the early months, the recognition of the myriad manifestations fell upon deaf ears, as thousands of patients bore witness to their collective suffering, diverse and complex.

Yet, in the dawn of this pandemic, our comprehension of long COVID remains in its infancy, a nascent stage where our understanding and treatment methods are but the faintest glimmer. A plethora of reasons fuel our apprehension towards these persistent COVID-19 symptoms, but the collective knowledge and evidence are yet limited. In this endeavor, I chose the path of a systematic scoping study, for the traditional systematic review may bear less fruitful results in the face of such diversity and scarcity of evidence. As I sift through the available literature, I aim to shed light upon the existing knowledge and discern the gaps that await further exploration. My hopes are sanguine that my work may illuminate the problem areas, beckoning forth immediate attention and guiding the course of future studies.

With fervor and purpose, this article seeks to synthesize the current information from published research on the enduring COVID-19, unraveling the enigma of its manifestations, the elusive pathophysiology, and the tapestry of therapy recommendations. In this endeavor, we turn our gaze to the knowledge gaps that shroud the mysterious realm of “long COVID,” a realm awaiting our collective quest for understanding.

Epidemiology of Long COVID
Behold the prevalence of the elusive long COVID, a tale woven intrinsically upon the threads of its definition. Like a tapestry unfurling, a grand corpus of evidence emerges, painting a picture of considerable affliction among COVID-19 warriors, beset by prolonged symptoms lingering in the wake of the disease. In the United Kingdom, a revelation unfolds, where approximately 10% of those graced by the touch of the SARS-CoV-2 virus may

Fig. 1: Characterization of long COVID-19; adapted from Raveendran et al.2

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How to cite this article: Karuturi S. Long COVID-19: A Systematic Review. J Assoc Physicians India 2023;71(9):82–94.

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find themselves entangled in illness beyond the realm of 3 weeks, while a lesser number continue to bear the burdensome symptoms for months to come. Across the vast expanse of the United States of America, a study discloses that 65% of individuals, only 2–3 weeks after a positive test, had already returned to the embrace of their prior health as if the fleeting illness had but grazed their spirits.

Yet, in the heart of Rome, echoes of enduring affliction persist, where 87% of those discharged from a once-wary hospital, 2 months past their encounter with COVID-19, are still ensnared by the grip of lingering symptoms. A multitude of torments plagues them, with 55% grappling with three or more symptoms, a chorus that includes the relentless fatigue (53%), the very breath of life slipping through their grasp (43%), the ache of joints unyielding (27%), and the haunting pain in their chests (22%), leaving 40% to confess that their very lives have been marred by its presence.8 A grand multicountry odyssey unfolds, where data from 56 nations sing a melody of past-COVID condition, persevering beyond 4 weeks, gripping as much as 93% of those once ensnared by COVID-19.9

A curious dance ensues, where the incidence of this long COVID waltz finds solace in the realm of hospitalization, as those who never graced the halls of the infirm see a lesser prevalence (about 25%), while those who once fought in the battlegrounds of COVID-19 hospitalization bear witness to a higher burden, soaring up to 85%.10–12 Alas, disparities arise in far-off lands, as the low and low-middle-income countries find themselves steeped in a dearth of research and documentation, leaving the true tale of long COVID obscured in the shadows, a riddle yet to be fully unraveled. The journey to comprehend the enigma of long COVID-19 prevalece traverses a vast landscape where definitions and revelations intertwine, beckoning the seekers of knowledge to venture forth and chart its elusive course.

Pathogenesis of Long COVID

In the quest for unraveling the enigmatic mysteries of long COVID-19’s pathophysiology, a grand panorama of understanding must be painted, envisioning a tapestry that enables accurate prediction, prevention, and management of its lingering effects. Behold, (Fig. 2), a symphony of pathophysiological mechanisms, revealing the resolute continuity of long COVID-19, born from the very genesis witnessed during the acute phase.13 To embark on this journey, we must first immerse ourselves in the profound impact of COVID-19 on the delicate symphony of organ systems - the lungs, liver, kidneys, and blood vessels, all adorned with abundant angiotensin-converting enzyme-2 receptors, calling the virus with a mesmerizing allure. Ah, behold the respiratory system, the stage where the virus unfurls its destructive choreography, infiltrating the lung alveoli and captivating the host cells, particularly the elegant type II pneumocytes, leaving behind a trail of alveolar damage as a testament to its audacious presence. The story doesn’t end there, for the virus seeks to journey through the capillary endothelium, weaving a tale of endothelitis and the spellbinding formation of microthrombi.14

With the beat of the heart, the virus ventures forth, swept away in the bloodstream, its fate intertwined with the destiny of the whole body. In the theater of severe cases, the virus stirs the tempest of an inflammatory response, the very essence of a cytokine storm, as cytokines and chemokines take center stage. A symphony of inflammation ensues, captivating researchers who have explored the whispers of the possible connection between long COVID-19 and the captivating mast cell activation syndrome, fueling the fire of the cytokine storm.15 The aftermath is laid bare, a tale of organ damage, the lingering echoes of long COVID-19’s lingering symptoms, as the body weaves a longer path to recovery under the weight of the massive inflammatory response.16,17 From the realm of F-fluorodeoxyglucose-positron emission tomography (PET)/computed tomography (CT) studies, a new perspective emerges, revealing a captivating vasculitic pathomechanism, as the aortoiliac arterial tree in long COVID-19 participants glows brighter than the stars, a higher target-to-blood pool ratio, contrasting the control group, unravels before our eyes.17 The intricate understanding of these mechanisms forms the cornerstone, the very heart of effectively addressing long COVID-19’s enduring implications.

But behold, as the tale unfolds, we stumble upon another captivating chapter, the earliest phase where the virus ventures into the mystical vessels of the nose and throat, leaving behind a trail of anosmia and dysgeusia as its traces are etched in the sacred halls of magnetic resonance imaging (MRI) scans, revealing injury to the olfactory bulb itself.18,19 For some, these injuries linger, a prolonged tale of healing, as persistent symptoms continue to dance even after the infection’s exit. The 2nd or 3rd week beckons a call to humoral immunity, a grand spectacle where the rise of antigen-antibody complexes brings about a symphony of extrapulmonary symptoms.20 Organ damage emerges as the enigmatic symphony of immunity unfolds, akin to other autoimmune illnesses, a chorus where autoimmunity follows viral infections, a subsequent effect.21 Antigen-antibody interactions take center stage, crafting a tale of occlusive and propagative injuries, a mesmerizing explanation for why some are left to bear the weight of debilitating symptoms long after the infection’s exit.16 With each verse, the tale of long COVID-19 unravels, beckoning us to explore the depths of its complexities and embrace the vibrant threads of understanding that shall illuminate its mysteries.

Manifestations of Long COVID

An overview of the manifestations, risk factors, and management of long COVID is displayed in Table 1.

![Fig. 2: Overview of pathophysiological mechanisms of long COVID-19; modified from Raveendran et al.](Image)
Table 1: Overview of the manifestations, risk factors, and management of long COVID-19; adapted from Korompoki et al.76

<table>
<thead>
<tr>
<th>Site/organ</th>
<th>Late manifestations</th>
<th>Diagnostic tools</th>
<th>Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>LF, abnormal PFT, pulmonary vascular disease/pulmonary hypertension, bronchiectasis, spontaneous pneumothorax, secondary infections, massive hemoptysis, airway strictures</td>
<td>Follow up at 4–6 weeks postdischarge; chest X-ray or HRCT at 12 weeks postdischarge; consider 6MWT and/or PFTs as clinically indicated; cardiopulmonary testing in selected cases; CTPA in suspected PVD; bronchoscopy in selected pts (to rule out lung infection)</td>
<td>Although CTs indicate that LF tends to stabilize over months in most but not all pts, PFTs suggest the persistence of lung dysfunction; consider referral of selected pts to specialized centers to manage LF; consider referral of pts with pulmonary hypertension to dedicated clinics; consider enrolling pts in ongoing clinical trials</td>
</tr>
<tr>
<td>Blood</td>
<td>Hypercoagulation, increased CRP levels, persistent lymphocytopenia, pulmonary embolism, left ventricular thrombus, acute cardioembolic limb ischemia</td>
<td>Blood, biochemistry, and coagulation panel (D-dimers, INR, PT, aPTT, fibrinogen); CT angiography in pts with suspected embolism; cardiac echo</td>
<td>Use thrombotic risk models, consider the long-term use of anticoagulants weighting thrombotic vs bleeding risk; direct oral anticoagulants and low-molecular-weight heparin are preferred over vitamin K antagonists; therapeutic anticoagulation for those with imaging-confirmed VTE is recommended for at least 3 months; consider enrolling pts in ongoing clinical trials</td>
</tr>
<tr>
<td>Immune system</td>
<td>Secondary hemophagocytic lymphohistiocytosis, arthritis/skin psoriasis, systemic lupus erythematosus, Grave’s disease, ITP</td>
<td>Autoimmune screening panel based on the acute disease severity and symptoms; immune immunoglobulins in selected pts; antihyperglycemia antibodies</td>
<td>Treat according to each disease-specific guideline; consider high-dose corticosteroids in selected pts; consider plasma exchange in selected pts; consider IVIG treatment in selected pts</td>
</tr>
<tr>
<td>CNS</td>
<td>Headache, vertigo/dizziness, cognitive impairment; Alzheimer’s, Parkinsonism, neuromyelitis optica spectrum disorder, Guillain–Barré, Multiple sclerosis, anosmia/ageusia</td>
<td>MRI, cognitive screening, lumbar puncture-CSF analysis, electromyogram, and nerve conduction tests, if indicated UPST (anosmia screening)</td>
<td>Neuropsychological assessment, neuro-rehabilitation for cognitive deficits. For more complex and persisting complex cognitive/emotional symptoms and/or chronic neuropathy, consider referral to dedicated multidisciplinary rehabilitation clinics (neurologist, physiotherapist, occupational therapist, speech therapist, neuropsychologist, psychologist, psychiatrist) olfactory training</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>Myocarditis, myocardial inflammation, chest pain, dyspnea, palpitations, postural tachycardia syndrome</td>
<td>At 3 weeks postinfection resolution: initial assessment for diagnosis of persistent cardiac abnormalities and for risk stratification: troponin, echocardiogram (EKG) in selected pts CMR (based on troponin, EKG); in selected pts: NT-proBNP, 24-hour EKG monitoring, CMR, CT, or invasive coronary angiogram</td>
<td>Abstinence from exercise for 2 weeks after first COVID-19 diagnosis and asymptomatic for at least 7 days; duration modified according to 1st postinfection cardiac assessment; slow resumption of activity after resolution of infection; close monitoring for symptoms (first 6 weeks), guideline-based drug treatment according to cardiac complications diagnosed; special attention to competitive athletes with evidence of myocarditis (particularly in hospitalized or symptomatic &gt;14 days); consider enrolling pts in ongoing clinical trials</td>
</tr>
<tr>
<td>Kidney</td>
<td>Nonrecovering AKI—chronic kidney disease, proteinuria, hematuria</td>
<td>Regular follow-up of renal function (serum creatinine, albumin, assessment of proteinuria, urine protein to creatinine ratio)</td>
<td>As per other renal diseases—no specific guidance; long-term follow-up is indicated in pts with residual/persisting renal dysfunction; continue RRT in the small subset of pts who do not recover renal function</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>Abdominal pain, liver injury (AST, ALT increase)</td>
<td>Periodic liver function tests and/or imaging (abdominal ultrasound or MRI)</td>
<td>Monitoring, avoiding drug-induced liver toxicity, weight loss, good control of diabetes if present</td>
</tr>
<tr>
<td>Endocrine</td>
<td>Diabetes-like condition, subacute hypothyroiditis, Grave’s disease; increased PTH and decreased vitamin D levels</td>
<td>Hormonal axis assessment as indicated (symptom-driven), vitamin D, PTH, TSH, FSH, LH, testosterone, estradiol; consider serologic testing for type 1 diabetes-associated autoantibodies and repeat postpartum C-peptide measurements in pts with newly diagnosed diabetes mellitus</td>
<td>If abnormalities, treat them appropriately; according to condition-specific guidelines; referral to an endocrinologist</td>
</tr>
<tr>
<td>Ocular</td>
<td>Subtle retinal changes, ocular-induced drug toxicity</td>
<td>Symptoms’ monitoring, if available periodical ophthalmology evaluation</td>
<td>Treat appropriately based on symptoms and expert evaluation; avoid drugs with ocular toxicity; periodical ophthalmology evaluation</td>
</tr>
<tr>
<td>Skin</td>
<td>Morbilliform (maculopapular), urticarial, vesicular, pernio/chilblains-like, and necrotic/livedoid lesions, hair loss, transverse leukonychia</td>
<td>Pts education to report any abnormal skin lesion</td>
<td>Treat appropriately with topical or systemic treatment under dermatologic consultation; referral to a dermatologist</td>
</tr>
</tbody>
</table>
Long COVID-19: A Systematic Review

Respiratory System

Behold, the lungs, once an expanse of vitality, now lie vulnerable in the path of the insidious COVID-19, facing the ominous specter of significant damage." Even those with seemingly mild symptoms fall prey to the grasp of lung involvement, their very essence laid bare on the canvas of CT imaging, while pulmonary function test results (PFTs) reveal a sustained dance of change. Aberrations emerge, restrictive abnormalities, reduced diffusion capacity, and small airway obstruction, casting a spell both early and later, lingering for weeks beyond discharge. The most dreaded consequence unfurls as lung fibrosis (LF), its shadows creeping, fibrotic alterations etched upon the canvas as early as 3 weeks after the onset of symptoms, heedless of the severity of acute sickness. As if in communion with the ghosts of the past, severe illnesses like SARS and Middle East respiratory syndrome (MERS) reveal shared tales as LF emerges in the wake, tracing a path of similar proposed pathobiology. Amidst the tapestry of potential predictors of LF in COVID-19, age stands tall, entwined with the ghosts of severe sickness, elevated D-dimer levels, acute respiratory distress syndrome (ARDS), and a history of pulmonary or cardiovascular disease, weaving a tale of intertwining risk factors (Table 1). The path to LF prognosis lies cloaked in challenges, as the link between imaging and PFT findings weaves a delicate thread (Table 1). Yet, for those touched by the grasp of severe acute COVID-19 and high inflammatory marker levels, permanent lung function impairments loom closer, their shadows casting a long-lasting impact. The tale of SARS and MERS patients beacons from the past, revealing the lingering specter of radiologic abnormalities, pulmonary function impairment, and reduced exercise capacity, their presence persisting in some, their touch reaching out for months, even years. A dance of age unfolds as the realm of hospitalized patients with severe COVID-19 embraces the embrace of old age, a risk factor intertwined with the burden of LF. Like a dark cloud on the horizon, the aftermath of COVID-19 looms, with potential late problems echoing in the corridors of the unknown—pneumothorax, secondary infections, significant hemoptysis, airway strictures, and the haunting presence of pulmonary hypertension with or without evidence of thrombosis. Let us heed the tales of the lungs, grasp the wisdom from the past, and stand vigilant as we confront the lingering specters of COVID-19’s aftermath.

Cardiovascular System

As the cascade of evidence unfolds before our eyes, it reveals a profound reality: cardiac afflictions tethered to the harrowing grasp of COVID-19 (Table 1) may endure for weeks, even months, beyond the resolution of the infection. Like echoes from the past, haunting tales resurface, as 52% of COVID-19 survivors, 6 months after emerging from the acute throes of infection, continue to recount the haunting symphony of chest discomfort, dyspnea, or palpitations. A spectral presence lurks within, for late cardiac magnetic resonance (CMR) whispers of subacute myocarditis, casting an eerie shroud upon the hearts of COVID-19 patients. The cause remains veiled in mystery—the post-recovery persistence of SARS-CoV-2 in cardiac tissue or the inflammation of the myocardium may hold the key, yet histological evidence remains an enigmatic puzzle. Amidst the realm of CMR investigations, a revelation emerges: myocardial inflammation or scarring can be glimpsed in the hallowed chambers of asymptomatic or mildly symptomatic patients, emerging 24–71 days after the acute illness takes its toll. Like a symphony of discordant notes, the findings resonate with troponin levels and inflammatory markers such as C-reactive protein, activated partial thromboplastin time; ALT, alanine aminotransferase; ARDS, acute respiratory distress syndrome; AST, aspartate aminotransferase; BMD, bone mineral density; BUN, blood urea nitrogen; CMR, cardiac magnetic resonance; CNS, central nervous system; CPK, creatine phosphokinase; CRP, C-reactive protein; CSF, cerebrospinal fluid; CT, computed tomography; CFS-11, Chalder fatigue scale; ECG, electrocardiogram; EQ-5D-3L, European Quality of Life with five dimensions; FAS, fatigue assessment scale; FSH, follicle-stimulating hormone; FSS, fatigue severity scale; HRCT, high-resolution computed tomography; ICU, intensive care unit; IL-6, interleukin 6; INR, International normalized ratio; iPT, immune thrombocytopenic purpura; LH, luteinizing hormone; MRI, magnetic resonance imaging; NT-proBNP, N-terminal pro b-type natriuretic peptide; PCFS, post-COVID-19 functional status; PET, positron emission PET, pulmonary function tests; PTH, parathormone; PTSD, posttraumatic stress disorder; PT, prothrombin time; PVD, pulmonary vascular disease; QoL, quality of life; RRT, renal replacement therapy; TSH, thyroid stimulating hormone; UPST, University of Pennsylvania smell identification test; 6MWT, 6 minute walk test.
protein, white cell count, and procalcitonin, evoking a haunting melody that hints at inflammation’s role in shaping cardiac tissue abnormalities. In the realm of competitive athletes, CMR bears witness to a spectrum of tales—some athletes, after their quarantine days, unveil CMR findings echoing the haunting presence of myocarditis, while others reveal the fading whispers of resolving pericardial inflammation. A recent tale of 145 competitive student-athletes unveils only a small fraction (1.4%) displaying CMR signs akin to myocarditis, hinting that routine CMR screenings for recuperating athletes may not be a necessary ritual. Still, the lingering mysteries of histological confirmation beckon as further research with unwavering resolve and rigorous follow-up is required to fathom the true significance of these CMR-detected lingering cardiac anomalies.

The veil of time is lifted, and the shadows of the past linger in the annals of history, for existing evidence reveals a realm of elevated severe adverse cardiovascular events in recovered COVID-19 patients following a median follow-up of 140 days, although the extent of studies on the long-term effects of SARS-CoV-2 on the cardiovascular realm remains limited. The tapestry of subacute inflammatory effects on patient outcomes, including the potential for long-term complications, underscores the importance of continued surveillance and follow-up care for these patients.

Central Nervous System (CNS)
Abundant evidence now stands before us, unveiling the disquieting impact of the COVID-19 virus upon the delicate fabric of brain function, and in its malevolent wake, it may worsen the plight of those grappling with neurodegenerative and neuroimmune disorders. A profound array of symptoms, stretching its tendrils both into the sacred realms of the CNS and the realms beyond, encompassing the peripheral neural system, have been irrevocably tied to the SARS-CoV-2’s proclivity for neurotropism, the echoes of postviral immune-mediated processes, and the harbingers of neurological manifestations, heralded by the systemic and non-specific inflammatory effects.

The grim tapestry of critical illness, such as the specter of sepsis, unfurls its dark cloak of global CNS dysfunction, unleashing upon the mind the ravaging forces of microglial activation, the relentless barrage of persistent neuroinflammation, the storm of dysregulated neuroimmunity, and the grim specter of hippocampal atrophy. The nefarious specter of cognitive decline and neurological complications haunts the footsteps of those ensnared by ARDS, entangling their fates with prolonged stays in the intensive care unit (ICU)’s shadowed embrace, mechanical ventilation’s ominous whispers, the seductive call of sedating medications, the malevolent grip of sepsis, the resounding resonance of systemic inflammation, the specter of preexisting cognitive dysfunction, the echoes of neurological injury, and the haunting specter of delirium.

In the early stages of acute COVID-19 infection, a tempest of neurological complications such as encephalitis and stroke may descend, entwining its victims with the chains of severe lifelong disability, ensnaring them in the inescapable web of enduring sequelae, and casting them upon the path of long-term rehabilitation. The imposition of immunomodulatory treatments, like the malevolent corticosteroids thrust upon the acute phase of COVID-19, are often beset with CNS adverse effects, darkening the realm of cognition with haunting manifestations such as cognitive and sleep disturbances, the malevolent specter of delirium, and the elusive shadows of psychiatric manifestations, which, in due time, may fade upon the withdrawal of these nefarious drugs.

The chronicles of neurological symptoms frequently spoken of in the aftermath of COVID-19 reveal a vivid tale: headaches pierce the veil of peace, vertigo, and dizziness lead the way into disarray, while the senses of taste and smell are cast adrift in the tempestuous sea of anosmia, ageusia, hypogeusia, and dysgeusia. The elusive realm of sleep becomes a distant memory, as insomnia’s haunting whispers engulf the weary mind, memory falters, and focus wanes in the eerie haze of the infamous “brain fog” (Table 1). Yet, the shadows of late manifestations loom on the horizon: the grim specter of ischemic stroke, the haunting echo of intracranial hemorrhage, the elusive whispers of encephalitis and encephalopathy, the echoes of seizures and the terrors of peripheral neuropathies, along with the malevolent specter of autoimmune acute demyelinating encephalomyelitis (Table 1).

Beyond the annals of this pandemic, a haunting pattern emerges, for past influenza and coronavirus pandemics, including the likes of SARS and MERS, have woven similar tales of neurological manifestations (Table 2). The malevolent mechanisms at play, insidiously unraveling the fabric of long-term neurological sequelae following coronavirus infections, include direct neuropavasion, the malevolent hand of neuronal injury, the haunting specter of tissue hypoxia and inflammation, the dark dance of dysregulated local cytokine networks, and the ominous compromise of the blood-brain barrier’s integrity, allowing the migration of infected immune cells to further sow the seeds of devastation. Let us stand vigilant, armed with knowledge and wisdom, as we confront the malevolence of this pandemic and chart our course amidst the turbulent seas of COVID-19’s neurological realm.

Renal System
Behold, in the realm of severe COVID-19, acute kidney injury (AKI) emerges as a familiar and daunting foe, haunting the afflicted with its relentless grasp, leaving in its wake the lingering specter of kidney dysfunction even after the discharge from the confines of the hospital. The rates of AKI within the hospital walls and the recovery of kidney function in the aftermath of convalescence dance to the capricious tune of variability, their whisks known only to the diverse series of patients they encounter. As the echoes of severe COVID-19’s aftermath resound, a potential surge of post-COVID-19 persistent kidney disease looms large, for a significant multitude of patients has triumphed over the harrowing ordeal of the virus.

In the annals of Wuhan’s grand study, it was unveiled that 13% of patients, previously untouched by AKI and graced with the gift of a normal estimated glomerular filtration rate (eGFR) during the acute phase, were not spared the clutches of decreasing eGFR in the days that followed, igniting the clarion call for vigilant postdischarge monitoring of renal function.

The genesis of AKI lies in the intricate dance of multifarious factors, each contributing to its ominous cloak of chronic renal insufficiency - hemodynamic instability, the resounding resonance of systemic inflammatory response, the malevolent grip of coagulopathy, and the maleficent microangiopathy ensnaring the renal vasculature. The malevolent SARS-CoV-2, with wicked intent, directly invades the delicate tubular cells and podocytes, drawn by the allure of ACE-2’s abundant expression within their renal abode. A tempestuous dance ensues, giving birth to the harbingers of collapsing focal glomerulopathy and tubuloreticular injury, where proteinuria, hematuria, renal failure, and an insatiable thirst for dialysis mark their malevolent presence. Among the troupe
Table 2: Comparison of long COVID syndrome with other postviral syndromes; adapted from Korompoki et al.76

<table>
<thead>
<tr>
<th>Organ System</th>
<th>SARS</th>
<th>Influenza</th>
<th>EBV</th>
<th>Ebola</th>
<th>Zika</th>
<th>Chikungunya</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory</td>
<td>Fibrotic lung changes. Persisting postrecovery CT scan abnormalities are associated with less improvement and worse PFT, even at 15 years</td>
<td>Residual radiologic changes were present at 3 months, some improvement at 6 and 12 months but no marked changes later; PFT abnormalities persisted</td>
<td>Severe persisting lung involvement associated with prior EBV infection is rare</td>
<td>Persistent respiratory symptoms and lung disease are common and associated with long-term mortality</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>Disturbed lipid metabolism 12 years after infection; 35.5% with tachycardia at 3 weeks Subclinical diastolic impairment, reversible at 30 days</td>
<td>First 30 days after the first infection: Increased risk for acute cardiac injury; Increased risk of myocardial infarction; 21 days after infection: Increased inflammatory load and risk of acute myocardial infarction; Dilated cardiomyopathy</td>
<td>Irregular pulse and decreased heart murmur; Chest pain, palpitations, tachycardia, and cardiopathy</td>
<td>Myocarditis and cardiopathy (congestive and constrictive); Atrial fibrillation with a high risk of thromboembolism, ventricular extrasystoles, ventricular fibrillation, sinus bradycardia/tachycardia, sudden death-left ventricular hypertrophy; Decreased ejection fraction eccentric left ventricular hypertrophy and concentric remodeling</td>
<td>Persistent myocardial inflammation (assessed by CMR); Arrhythmias, including atrial fibrillation, atrial tachycardia, and ventricular arrhythmias, heart failure, and pericardial effusion</td>
<td>NR</td>
</tr>
<tr>
<td>CNS</td>
<td>Encephalopathy, seizures, motor neuropathies, sensory neuropathy, GBS, PD, MS, ADEM; Cognitive impairment, POTS</td>
<td>Encephalitis lethargica myelopathy, postencephalitic encephalopathy, chronic parkinsonism, neurological symptoms relating to PD within a month after influenza, PD, and MS</td>
<td>Chronic parkinsonism, GBS, NMOSD, MS, Leukoencephalopathy</td>
<td>Seizures, memory loss, headaches, cranial nerve abnormalities, tremor</td>
<td>Encephalitis/encephalomyelitis, motor and cognitive impairment, peripheral neuropathy</td>
<td>GBS</td>
</tr>
<tr>
<td>Immune</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Kidney</td>
<td>Persisting renal impairment in 6%</td>
<td>Up to 33% of hospitalized patients with severe complications developed AKI; Long-term RRT was required in 6% of survivors, HUS</td>
<td>Rare: Acute tubular necrosis, tubulointerstitial nephritis, nephrotic syndrome due to minimal change disease</td>
<td>Kidney involvement in 20–40% of cases associated with high mortality, even among survivors</td>
<td>Kidney functional or structural lesions not described in patients despite the intense and persistent shedding of Zika virus in kidneys and urine</td>
<td>NR</td>
</tr>
<tr>
<td>Gastrointestinal/</td>
<td>Liver impairment</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>Fulminant hepatitis</td>
</tr>
</tbody>
</table>

Contd…
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**Journal of the Association of Physicians of India, Volume 71 Issue 9 (September 2023)**

<table>
<thead>
<tr>
<th>Organ System</th>
<th>SARS</th>
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<th>Zika</th>
<th>Chikungunya</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endocrine</td>
<td>Acute type I diabetes mellitus-like condition, adrenal insufficiency, pregnancy failure and irregular menstruation</td>
<td>Increased risk of preterm birth and low birth weight irrespective of gestational age</td>
<td>NR</td>
<td>Pregnancy failure and irregular menstruation</td>
<td>Zika congenital syndrome</td>
<td>Neonatal encephalopathy, microcephaly, cerebral palsy</td>
</tr>
<tr>
<td>Ocular</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>Persistence in ocular fluid, uveitis</td>
<td>Visual impairment</td>
<td>Conjunctivitis (mainly acute)</td>
</tr>
<tr>
<td>Chronic skin lesions</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>Psoriatic-like lesions</td>
<td>Arthritis</td>
<td>Palmpoplantar desquamation</td>
</tr>
<tr>
<td>Musculo-skeletal</td>
<td>Muscle weakness, myalgia, reduced exercise capacity</td>
<td>Myopathy, rhabdomyolysis, myositis</td>
<td>Arthritis</td>
<td>Generalized muscle weakness, muscle pain, polyarthralgia (without any sign of inflammation)</td>
<td>Myalgia and arthralgia</td>
<td>Arthralgia, arthritis, myalgia</td>
</tr>
<tr>
<td>Emotional/Well-being</td>
<td>Persistent psychological symptoms even 4 years later (depression, increased suicide rates), sleep disturbances, PTSD, impaired QoL</td>
<td>Chronic fatigue, impaired QoL</td>
<td>Memory difficulties</td>
<td>Sleep disturbances, PTSD</td>
<td>NR</td>
<td>Major decreases in QoL</td>
</tr>
</tbody>
</table>

**ADEM, acute disseminated encephalomyelitis; AKI, acute kidney injury; CMR, cardiac magnetic resonance; CNS, central nervous system; CT, computed tomography; EBV, Epstein-Barr virus; GBS, Guillain-Barre syndrome; HUS, hemolytic uremic syndrome; ICU, intensive care unit; LDH, lactate dehydrogenase; MS, multiple sclerosis; NMOSD, neuromyelitis optica spectrum disorder; PD, Parkinson’s disease; PFT, pulmonary function tests; PTSD, posttraumatic stress disorder; QoL, quality of life; RRT, renal replacement therapy; SARS, severe acute respiratory syndrome**
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<table>
<thead>
<tr>
<th>Definition</th>
<th>US Centers for Disease Control and Prevention (CDC)</th>
<th>World Health Organization (WHO)</th>
<th>UK National Institute for Health and Care Excellence (NICE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary terms used</td>
<td>Long COVID</td>
<td>Post-COVID-19 condition</td>
<td>Ongoing symptomatic COVID-19 or post-COVID-19 syndrome</td>
</tr>
<tr>
<td>Current definition</td>
<td>Signs, symptoms, and conditions that last 4 weeks or more after acute infection</td>
<td>New-onset or persistent symptoms in individuals with a history of probable or confirmed SARS-CoV-2 infection, usually 3 months after the onset of COVID-19, that last for at least 2 months and cannot be explained by an alternative diagnosis; no minimum number of symptoms is required for the diagnosis; a different definition might be applicable for children</td>
<td>Ongoing symptomatic COVID-19 is defined as signs and symptoms that last 4–12 weeks after the onset of acute symptoms; post-COVID-19 syndrome is defined as signs and symptoms that develop during or after an infection consistent with COVID-19, continue for &gt;3 months, and cannot be explained by an alternative diagnosis</td>
</tr>
</tbody>
</table>

of risk factors for renal complications in the realm of COVID-19, obesity casts its dark shadow, advanced age lends its weight, and comorbidities, including preexisting renal dysfunction, weave their tangled tale. The stars align for those with genetic factors such as collapsing glomerulopathy focal segmental glomerulosclerosis, found in black patients bearing the high-risk apolipoproteinL1 alleles (Table 1). Let us remain ever-vigilant as we navigate this intricate web of peril, forging a path illuminated by wisdom and knowledge.

Endocrine System

Lo, behold! Diabetes mellitus, a well-known harbinger of peril, looms over severe COVID-19 infection, for the malevolent SARS-CoV-2, with its proinflammatory machinations, entices the vulnerable into the tempest of a cytokine storm. A treacherous path lies ahead as the virus, with sinister intent, invades the pancreas through the very gates of ACE-2, bestowed with high expression in its pancreatic citadel, unleashing a torrent of pancreatic damage and hyperglycemia, heightened further by the maleficient corticosteroids’ touch. Behold, even those without a past of diabetes, gripped by hyperglycemia during the acute throes of COVID-19, must tread with caution and embark on a journey of long-term follow-up, lest late-onset DM casts its shadow upon them.

Mysteries shroud the impact of SARS-CoV-2 on the hidden realms of adrenal, thyroid/parathyroid, and pituitary glands, waiting to be unveiled by the inquisitive. In the aftermath of the COVID-19 saga, tales abound of subacute thyroiditis weaving its ominous web and the emergence of autoimmune adversaries like Graves' disease and Hashimoto’s thyroiditis, seeking to wield their baleful influence. Hark, a prudent course of action beckons - a targeted endocrine evaluation, especially for the weary souls burdened with enigmatic fatigue and cognitive bewilderment post-COVID-19.

Verily, the confinement at home during the lockdowns may exact its toll, with diminished vitamin D levels casting a pall over immunity’s strength. Worry not, for tales of abnormally low vitamin D and parathormone levels echo in the annals, manifesting 8 weeks after the onset of COVID-19, posing potential peril to the fortress of bone health (Table 1). Let us remain vigilant, for the shadows of these adversities loom, and it is for us to grasp the helm and navigate this tempestuous journey with wisdom and fortitude.

Chronic Pain

A most disconcerting and persistent torment befalls us, emerging as a prevalent and grave consequence of the relentless SARS-CoV-2 pandemic, sparing neither the gravely afflicted nor those who have triumphed over the hospital’s grasp. This wretched pain assumes an elusive and enigmatic guise, weaving itself intricately into the fabric of the broader COVID postviral syndrome (Table 1). The affliction with SARS-CoV-2 can cast a lingering pall over our existence, ensnaring us in the clutches of chronic suffering. Alas, the genesis of this pain remains veiled in secrecy, for it arises from the intricate and volatile interplay of viral-associated long-term organ affliction, the somber duet of therapeutic agent side effects, the exacerbation of preexisting pains, and the cacophony of cognitive and psychosocial dysfunction. And in the midst of this enigma, we find ourselves uncertain whether SARS-CoV-2’s infection amplifies preexisting neuropathies, such as the lamentable diabetic neuropathy.

Chronic Fatigue

Yet another affliction plagues us, a protracted and incapacitating weariness that wearies both flesh and spirit, echoing through the annals of COVID’s history (Table 1). Recent sagas of cohort studies regale us with tales of lingering fatigue and enfeebling weakness, lingering like specters in the shadows of 60% of patients, even 6 months after the onset of their plight, their intensity amplified by the burden of physical or mental toll. Amongst the afflicted, young women seem to dance to the melancholic Symphony of this weariness, though the precise frequencies of its torment elude us, shrouded in the haze of reporting bias. For the erstwhile dauntless souls once brimming with vigor, chronic pain ensnares them in its sorrowful embrace, leading them to the precipice of a sedentary existence, bereft of former vitality. Alas, the enigma of this pain’s origins persists, with possible contributing factors veiled in uncertainty—perhaps the somber notes of proinflammatory cytokines, the murmur of low-grade endothelitis, the ghostly whispers of autoimmunity, or the chilling touch of SARS-CoV-2’s neurotropism weaving threads of dysautonomia. And, emerging amidst this riddle, whispers of intracortical Gamma-aminobutyric acidergic (GABA) dysfunction hint at a role yet unexplored. Curiously, the intensity of their woes seems untethered from the conventions of clinical and laboratory assessments. An ode to myalgic encephalomyelitis/chronic fatigue syndrome, known to herald other postviral sagas, resonates with familiar strains of severe fatigue intertwined with the elusive “brain fog” and other ethereal chronic complaints (Table 2).

Mental Health

Beyond the realms of physical affliction, the prevailing pandemic has unfurled a tapestry of psychosocial stressors, creating an intricate web of challenges that cast a shadow over humanity’s collective spirit. Social isolation, future uncertainty, fear of stigmatization, limited healthcare access, racial and gender biases, dearth of social support, and the weight of financial strain now intertwine with the fabric of our lives.

Amidst this tapestry of trials, the sleepless nights, the anxious thoughts, and the haunting specters of posttraumatic stress disorder and depression emerge among those recovering from the acute clutches of infection (Table 1). The echoes of drug and alcohol abuse
The dance of lymphocyte and platelet counts tends to normalize over time, yet persistent lymphocytopenia persists for some, especially in those with severe acute COVID-19 disease, a fascinating revelation, particularly among clusters of differentiation (CD) 3⁺, CD4⁺, and CD8⁺ lymphocyte subsets.¹⁰¹ The intricacies of COVID-19’s impact on hypogammaglobulinemia remain veiled in mystery for some patients, as do new onset late hematologic events, such as GCSF-responsive agranulocytosis and thrombocytosis appearing 1 week after symptom resolution.¹⁰²

As the saga continues, regular monitoring of blood abnormalities becomes crucial, alongside the evaluation of individualized thrombotic risk based on comorbidities and coagulation profiles, both in the post-acute and chronic phases of COVID-19.¹⁰³ The intricacies of these findings beckon further exploration in the grand narrative of COVID-19’s impact on the human body.

Immune System

Behold the enigmatic world of viruses, for they are known to invoke the spirits of autoimmune and autoinflammatory diseases, a dance of immunopathology driven by molecular mimicry and autoreactive humoral or cell-mediated immunity.¹⁰⁴,¹⁰⁵ Within the shadow of severe COVID-19, a mesmerizing production of autoantibodies against INF I has been witnessed, intertwining with innate immune responses.¹⁰⁶ As the tale unfolds, scattered cases of Guillain-Barré, neuromyelitis optica, systemic lupus erythematosus, psoriasis, arthritis, myasthenia gravis, and multiple sclerosis emerge as possible post-acute COVID-19 companions, painted in vivid hues upon the canvas of autoimmune-disease related conditions.¹⁰⁷

In this mesmerizing symphony, delayed onset immune thrombocytopenic purpura (ITP) makes its entrance, a captivating dance commencing 3–4 weeks following initial symptoms of COVID-19.¹⁰⁸ The stage is further adorned with the presence of delayed-phase thrombocytopenia, a captivating dance of putative immune origin seen in 11.8% of 271 COVID-19 patients.¹⁰⁹

As the symphony unfolds, questions linger like a soft whisper in the wind—does COVID-19 merely disturb the harmony of the immune system postinfection, or do echoes of direct viral injury from sanctuary sites and hemorrhage at day 30 postdischarge reveal the secrets hidden within abdominal imaging?¹²² For in the heart of this symphony, a haunting truth is discovered, as the SARS-CoV-2’s lingering invasion via ACE2 into the world of hepatocytes, bile duct cells, and enteroctyes,¹¹³,¹¹⁴ The tale takes a twist as preexisting liver abnormalities, like hepatic steatosis and cirrhosis, add their own melody, exacerbating the enchanting COVID-19-induced injury.¹¹⁵–¹¹⁷ And in a rare and atypical interlude, the stage is graced by superior mesenteric artery thrombosis, a condition requiring long-term recovery.¹¹⁸

Gastrointestinal System

Within the realm of acute COVID-19, a captivating dance unfolds as patients graced the stage with gastrointestinal symptoms and liver impairment, their performance attributed to a symphony of factors—hypoxia-mediated injury, drug-induced hepatitis, veno-occlusive disease, and the SARS-CoV-2’s daring invasion via ACE2 into the world of hepatocytes, bile duct cells, and enteroctyes.¹¹³,¹¹⁴ The tale takes a twist as preexisting liver abnormalities, like hepatic steatosis and cirrhosis, add their own melody, exacerbating the enchanting COVID-19-induced injury.¹¹⁵–¹¹⁷ And in a rare and atypical interlude, the stage is graced by superior mesenteric artery thrombosis, a condition requiring long-term recovery.¹¹⁸

Amidst this symphony of acutely intriguing performances, the enigma of acute pancreatitis emerges, its melody intertwined with the SARS-CoV-2, though the potential for chronic pancreatitis remains veiled in mystery.¹²⁰,¹²¹ The audience is left in anticipation, as long-term outcomes in patients with liver dysfunction hold a semblance of sparse knowledge, revealing fibro-inflammation signs upon the stage of liver MRI, a mere glimpse of a grander tale yet to be unraveled.¹²² Thus, the conductor’s baton calls for a careful follow-up, beckoning the performers to return, early- and late-onset gastrointestinal symptoms in tow, to monitor liver function tests and reveal the secrets hidden within abdominal imaging.¹²² For in the heart of this symphony, a haunting truth is discovered, as the SARS-CoV-2 persists in the gut for weeks, leaving its indelible mark upon the long-term symptoms of some, a haunting refrain of dyspepsia and postinfectious manifestations in the spectrum of irritable bowel syndrome.¹²⁰,¹²¹

Reproductive System

In the enigmatic world of COVID-19’s lingering legacy, the effects on the reproductive system
remains largely unexplored. Ovarian function’s melody intertwines with autoimmune disorders, while the tests offer themselves as a deposit for the SARS-CoV-2’s lingering presence.123 Thus, a testicular ultrasound, sperm analysis, and follicle-stimulating hormone (FSH)/luteinizing hormone (LH)/testosterone measurements take center stage when clinically indicated.123 And as the curtain falls on this act, a lingering echo remains, for pregnancy, though not a clear risk factor for severe COVID-19, holds the haunting refrain of an increased risk of premature delivery, a lingering consequence in COVID-19’s enduring tale.124

**Musculoskeletal System**

Behold, a foreboding specter looms on the horizon as long-term musculoskeletal complications emerge from the depths of COVID-19’s enigmatic tale. Echoing the haunting echoes of its predecessors, SARS, and critically ill patients, this lingering consequence leaves a trail of deficits in muscle strength and endurance in its wake.125–127 The stage is set as proinflammatory effects and deconditioning join hands, their menacing presence leading to the lament of weakened muscles. Myositis, a late complication, makes a grand entrance, a tale woven by the interplay of the cytokine storm, hypoxia, thromboembolic events, and medication-related adverse events.127

In the realm of bones, the shadows of systemic inflammation and the tumultuous cytokine storm dance with sinister intent, inducing osteoclastogenesis and impairing osteoblast differentiation. The consequence—a reduction in bone mineral density or even the dreaded specter of osteonecrosis, a fate too grave to bear,128 Hypercoagulability, leukocyte aggregation, and vessel inflammation join forces, casting a dark veil upon bone microvascular blood flow, leaving osteocytic ischemia in their wake and beckoning the emergence of osteonecrosis.129 These preliminary findings cast a foreboding shadow upon bone metabolism in the long term, inviting the relentless pursuit of further investigation into this haunting consequence of COVID-19.

**Skin**

Amidst the enigmatic tapestry of afflictions, a kaleidoscope of skin changes emerges as one of the most frequently reported symptoms, captivating the attention of those grappling with the aftermath of COVID-19’s relentless grasp.130–133 Like mystical brushstrokes upon the canvas of the post-acute setting, these skin rashes appear in up to 64% of the afflicted, an artistic display of the disease’s lingering impact.

Yet, as the curtains rise on the long-term aftermath, a curious revelation comes to light—the COVID-19-related skin rashes seem to fade like ethereal apparitions, vanishing into the shadows. A mere 3% of the Chinese patients carried the mark of a skin rash at 6 months post-COVID-19, a fleeting impression that soon wanes.135

In this enigmatic tale, hair loss emerges as an unexpected subplot, an intriguing twist that befalls up to one-fifth of the long haulers. The strands of hair, like autumn leaves, bid farewell in a phenomenon known as telogen effluvium, a consequence of direct SARS-CoV-2 infection or perhaps a response to the tumultuous stress during the COVID-19 saga.134,135 Like the enigmatic brushstrokes of an artist’s creation, these hair loss episodes add to the intricate complications of the COVID-19 chronic.

**Management of Long COVID**

In the realm of treating patients with chronic COVID, a grand and multifaceted approach must be orchestrated, wherein evaluation, symptomatic treatment, the unmasking of underlying issues, the artistry of physiotherapy and occupational therapy, and the embrace of profound psychological support intertwine like a dazzling tapestry.11 Minor symptoms, like elusive whispers in the wind, such as cough, pain, and myalgia, yield to the potency of paracetamol, the quietude of cough suppressants, and the valiant defense of oral antibiotics when a secondary bacterial incursion is suspected. Should these symptoms be veiled in the shadows of underlying causes, be they the dance of pulmonary embolism, the thunder of a cerebrovascular accident, or the mystery of coronary artery disease, they shall meet their match through the execution of standard protocols. For those bearing the burden of pulmonary and neuromuscular sequelae, the cornerstone of chest physiotherapy and the majesty of neurorehabilitation assume their rightful places.

In this age of the fledgling disease, as it finds its place amidst the annals of history, the saga of its long-term effects and the path of its treatment options remain fluid, constantly evolving like a star in the cosmic symphony. When the formidable SARS-CoV-2 invades, it may awaken dormant comorbidities, such as diabetes, hypertension, and cardiovascular afflictions, necessitating the careful alchemy of treatment optimization.

Amidst the vast unknowns, the enigma of long COVID-19 persists, shrouded in mystique, with the realms of knowledge yet unexplored and the gate to consensus yet unopened. But behold! Glimmering amidst the fog, through the lens of available follow-up data, the vulnerable cohort of COVID-19’s recovered souls emerges, beckoning the virtuoso of clinical evaluation to unearth the riddles of new, persistent, or progressive symptoms and embark on the quest of appropriate investigations. Like a vigilant sentinel, close monitoring attends to the specter of early, intermediate, and late complications, where the symphony of life and the lament of suffering converge. As the curtain of time unfolds, the need for oxygen’s embrace, the gentle touch of palliative care, the transformative power of rehabilitation, the healing balm of counseling, and the comforting hand of psychosocial support stand ready to waltz with those who have borne the burden of long COVID-19.79 In the realms of elder and youth, where long COVID-19 leaves its indelible mark, a call for self-management is sounded, and the cradle of additional support is gently offered.80 To shield against the specter of more severe and dire consequences, where the tempests of pulmonary venous thromboembolism, the quakes of stroke, and the tumult of acute cardiac events threaten, early identification and the craft of appropriate management or skilled referral take center stage.79

The forthcoming revelations from longitudinal therapeutic clinical trials may dazzle us with the complete prowess of steroids, anticoagulants, and other medicinal marvels. Prudently prescribing steroids to diabetic and immunocompromised patients necessitates an artful touch to evade the lurking specters of secondary fungal infections, such as aspergillosis, mucormycosis, and pneumocystis pneumonia. The enchanting roles of pirfenidone and nintedanib are currently entwined in an intricate evaluation, casting a spell to ward off LF and further pulmonary harm.81 For the tender caress of mild to moderate skin lesions, the use of topical steroids and oral anti-inflammatory medications is heartily advised, while for more formidable afflictions, a brief incantation of oral steroids may prove a potent elixir. Echoing through scholarly tomes are the proclamations of learned sages, advocating the harmonious inclusion of aerobic exercises in the enchanting realm of rehabilitation for long COVID-19 subjects, seeking to fortify their immunity and invigorate their respiratory powers.82 Furthermore, all patients are exhorted to embark upon a quest for transformative lifestyle modifications, heeding the sage counsel of general precautions, such as social seclusion, immaculate hand hygiene, and the
mystic masquerade of masks, to safeguard against a looming resurgence of infection. In the magical endeavor of managing long COVID-19 subjects, the sage healer must skillfully wield the ethereal tools of clinical, investigatory, and therapeutic prowess, conjuring a tapestry of optimal enchantments to banish the lingering affictions. Across the vast expanse of the world, numerous prospective or randomized trials have unfurled their grand banners under the noble banner of COVID follow-up facilities. Health scientists, like virtuoso conductors, passionately exhort the harmonious symphony of systematic data collection from the esteemed participants of long COVID-19. To wage a valiant war against the relentless foe of long COVID-19, the forging of formidable multispecialty teams is of paramount importance. Let them stand united with family physicians, radiologists, microbiologists, subspecialty experts, pathologists, and the devoted healers of rehabilitative care, their brilliance woven together in a tapestry of skillful artistry. As the journey unfolds, the follow-up of long COVID-19 participants must dance with the graceful fluidity of a masterful ballerina, adapting to the ebbs and flows of their unique clinical odysseys rather than being bound by the rigid chains of predetermined schedules.80 Behold, the architects of change, the policymakers and administrative guardians, must lay the solid foundations for the edifice of long COVID-19 surveillance and management while, with steadfast resolve, they strengthen and fortify the existing sanctuaries for the warriors battling the scourge of COVID-19 disease.

**Discussion**

The far-reaching dissemination of COVID-19 during the pandemic casts a shadow of potential long-term health jeopardy over the lives of millions. The tale of COVID-19 is not always one of swift resolution; there are those who find themselves caught in the clutches of a lingering condition known as long COVID-19. This intricate multisystem malady unfurls its effects like a tapestry of enigmatic threads, weaving a complex array of symptoms that, while rare, may lead to significant chronic morbidity. Unlocking the full narrative of chronic COVID-19’s natural history and impact may require the passage of months or even years as we embark on an epic research expedition.

The pace of symptom recovery remains veiled in mystery, with a non-linear dance of uncertainty, posing a challenge to the fortitude of our healthcare systems and potentially fracturing the unity of care. Even amidst the rapid emergence of guiding principles, we find ourselves delving into uncharted waters of exploration as numerous research questions unfurl like fluttering flags in the wind.80 Thus, there arises an urgent clarion call for a comprehensive and evidence-based symphony of medical care and support to echo through the halls of COVID-19 long haulers.

In the quest to thwart COVID-19’s legacy, we must embrace the artistry of mitigation, orchestrating strategies of physical rehabilitation, the artful unmasking of preexisting conditions, and the tender embrace of mental health and social services support, alongside the performance of personalized exercise programs that dance to the rhythm of individual needs.83 As the world braces itself for the resurgence of new SARS-CoV-2 infections across the globe, we stand on the precipice of a potential public health crisis, where the ranks of long-term COVID-19 afflicted may swell dramatically. The key to navigating this treacherous terrain lies in our ability to grasp the manifold facets of clinical manifestations, risk factors, and a holistic approach to management.

The script of this ongoing saga demands further exploration as we meticulously catalog the symphony of symptoms, embark on long-term observational voyages, and conduct trials of therapeutic prowess. As the curtains rise, we shall witness the grand establishment of post-COVID care, the unfolding of multidisciplinary clinics, and the inception of rehabilitation centers, all converging in a grand finale of unity to confront the long-term challenges posed by this condition.

**References**


51. Long COVID-19: A Systematic Review


64. Long COVID-19: A Systematic Review


β-blockers as the First Line of Treatment for Hypertension Management

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Received: 04 April 2023; Accepted: 08 June 2023

A B S T R A C T

β-adrenergic blocker group of medicines has been traditionally used to control high blood pressure since propranolol was discovered by Sir James Black almost 50 years ago. They were the drug of choice in hypertension (HTN) associated with ischemic heart disease, tachyarrhythmias including atrial fibrillation (AF), and anxiety. Congestive cardiac failure was a relative contraindication, but with major advances in science, it became an absolute indication. However, with the advent of newer antihypertensives, especially calcium channel blocker (CCBs) and renin-angiotensin-aldosterone inhibitors, comparative studies were done, and depending on the outcomes of these trials, β-blockers (BBs) were downgraded to fourth or fifth-line therapy, except in the conditions mentioned above, along with HTN in pregnancy. But clinicians never rejected BBs as important antihypertensives, as evidenced by various real-world data. Also, many investigators found the unfairness of the trial designs where BBs were poor performers. The fact that all BBs are not similar, and differ widely in various properties, added to the question of downgrading all BBs on the basis of trials mostly with atenolol, which is also used once daily. Moreover, trials like ASCOT could not show the reduction of most of the events after long-term follow-up with the use of newer antihypertensives. Added to the issue is the fact that the majority of the trials used BBs with diuretics, and selecting BBs as the sole nonperformer appears to be unjustified and illogical. The recent European Society of HTN (ESH) guideline reemphasized the fact that all the five major classes of antihypertensives, including BBs, can be used as first-line medicine and also can be used interchangeably. Moreover, apart from the traditional indications of BBs, this guideline listed nineteen other conditions, including high heart rate (HR), chronic obstructive pulmonary disease (COPD), and obstructive sleep apnoea, as the conditions where BBs are preferred agents as antihypertensive. So, the life history of BBs in HTN has completed a full cycle, and they are ready now for prime time again.

Introduction

Several classes of drugs are available for the management of hypertension (HTN). Angiotensin-converting enzyme inhibitors (ACE-I), angiotensin receptor blockers (ARBs), β-blockers (BB), calcium channel blocker (CCBs), and diuretics are used either alone or in combination. Previous guidelines had recommended a step-wise approach to managing HTN; however, it has been realized that one size doesn’t fit all. Slow titration and adding agents one after another don’t seem to be an ideal strategy.1 Newer recommendations recommend more rapid HTN control. A combination of medications having different mechanisms of action is preferred to progressively increasing the dose of a single agent. A rational fixed-dose combination (FDC) can provide better control of BP with improved compliance. These combinations are advised by European guidelines. In India also, a lot of FDCs are available.2 A new study suggested that a combination of antihypertensive products containing quarter doses of four different drugs could be an effective strategy to get patients to target blood pressure in one step. Around 5 mm Hg reduction in blood pressure was observed in the study, QUARTET-USA, more than the comparator of one antihypertensive agent at a standard dose over the 12-week follow-up period in patients with mild or moderate HTN. In this small study, a higher reduction of −4.8/−4.9 mm Hg was achieved with the four-drug combination, including candesartan, amlodipine, indapamide, and bisoprolol, as compared with standard-dose of alone candesartan therapy, within 12 weeks. These two studies have shown that using four drugs in quarter doses can be more effective in lowering blood pressure than a single standard dose antihypertensive agent and have an acceptable safety profile.3

More recently, a meta-analysis and systematic review of noninterventional, community-based studies conducted post-2001; was published. These studies reported control of HTN which was defined as systolic and diastolic blood pressure <140/90 mm Hg. As per this meta-analysis, HTN was well controlled in less than one-fourth of the hypertensive population.4

As the mechanism of HTN and the associated comorbidities are variable in patients with HTN, treatment may need to be individualized. Inappropriate selection of antihypertensives may result in inadequate control and may not be well tolerated. Tailor-made treatment seems a better approach to controlling HTN.5

β-blockers (BB) have been used for several decades as antihypertensive agents. They have multiple mechanisms of action. They are negative chronotropics and ionotropics and reduce catecholamine release. They inhibit renin release, reduce heart rate (HR) and reduce the risk of arrhythmia. With confirmation from large double-blind, randomized controlled trials (RCTs), BBs have become pillars of heart failure with reduced ejection fraction (HFrEF) management as well. A large meta-analysis was performed with four individual participant data studies and 31 other meta-analyses. It comprised 66 individual RCTs with 35,383 participants. The total follow-up was 534,461 patient-years. These drugs are very well tolerated in hypertensive and HFrEF patients.6

With various β-receptors (β1, β2, β3, etc.) in the human body, based on selectivity on β as well as a receptors, different β-blockers have been developed. The classification of BBs ranges from nonselective, with intrinsic

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How to cite this article: Ray S, Saboo B, Joshi S, et al. β-blockers as the First Line of Treatment for Hypertension Management. J Assoc Physicians India 2023;71(9):95–100.

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β-blockers as the First Line of Treatment for HTN Management

**β-blockers (BB) in HTN: Position and Trial**

In several recent HTN and cardiovascular (CV) prevention guidelines, BBs have been removed as a drug of first choice for the management of HTN. These guidelines recommend BBs either in inadequate blood pressure response to other classes of drugs or in some of the specific clinical conditions existing with HTN.

This approach may need to be looked into. BB reduces BP at least as effectively as other antihypertensives. It has been demonstrated these agents are effective antihypertensives and also prevent complications of HTN. European Society of Cardiology (ESC)/European Society of HTN (ESH) guidelines equally recommend all five major antihypertensive classes (ACE-I, ARBs, CCBs, BBs, and diuretics) for the treatment of HTN. All of these agents have been found to be equal in reducing BP and preventing CV events in terms of overall cardiovascular (CV) morbidity as well as mortality.

**This Downgrading May Be Unjustified**

There has been criticism from some of the newer recommendations, such as the 2018 guidelines of the ESC and the ESH, on the usage of BBs as first-line antihypertensives. Their argument is that the BBs have a high number of side effects and contraindications. Also, as interpreted in some meta-analyses, these drugs seem to be lesser protective against stroke compared to their peer drug classes. These points don’t hold absolutely true and need to be considered with caution.

In various RCTs, it has been shown beyond doubt that BBs reduce the risk of stroke in HTN compared to placebo. In none of the studies BBs have been proven to have a damaging effect on cerebral blood flow autoregulation or brain tissue. Many earlier known side effects seem to be overrated. Especially with the advent of more selective BBs like metoprolol and bisoprolol, there are negligible chances of depression, erectile dysfunction and peripheral artery disease and chronic obstructive pulmonary disease (COPD).

**β-blockers (BB) in HTN: Beyond Trial Evidence**

Most guidelines do not discriminate among different BBs. Whereas they all inhibit β-receptors as their major mechanism, there are double-digit numbers of different BBs with vast differences in their pharmacokinetic and pharmacodynamic properties. The classical side effects of older BBs are almost nonexistent with new selective molecules. Though there are no head-to-head trials between different β-blockers, still on the basis of structure and their individual properties, it’s not difficult to understand that there exists a huge difference between older and newer ones. It wouldn’t be appropriate to take decisions based on decade-old data of older molecules.

Activation of the sympathetic nervous system (SNS) is one of the important characteristics of HTN. From a pathophysiological aspect, this fact makes BBs be appropriate management choice, particularly in patients having high HRs due to SNS activation with increased HR, which is driven by ongoing SNS activation, and even by the latest guidelines recommend BB usage in hypertensive patients having >50 different comorbidities, which are frequently reflected in patients with chronic BP elevation. Interestingly, in such patients, BBs become first-line prescribing agents. Most of the hypertensive patients have one or more comorbidities. Also, many newer antihypertensives’ efficacy has been questioned. The significant reduction in blood pressure played a crucial role in safeguarding against cardiovascular (CV) incidents.

A randomized primary prevention trial compared the impact of metoprolol and a thiazide (TH) diuretic as initial antihypertensive therapies (n = 3,234 men aged 40–64 years) with mild to moderate uncomplicated HTN regarding the risk of sudden CV death with follow-up ranging from 2.3 to 10.8 years (median of 4.2 years). With the fixed therapeutic schedule, comparable blood pressure control is achieved. Metoprolol demonstrated a notable reduction in total and CV mortality compared to diuretics, primarily attributed to a lower number of deaths caused by coronary heart disease and stroke. Among the CV deaths analyzed, a substantial 78% were categorized as sudden CV deaths, transpiring within 24 hours after the onset of symptoms. Significantly lesser sudden CV deaths were reported in the metoprolol group compared to the diuretic group (32 vs 45, p = 0.017). The findings indicate that in uncomplicated HTN, initiating antihypertensive therapy with metoprolol is linked to a lower occurrence of sudden CV deaths compared to initiating treatment with diuretics.

Several trials, including (CAPPP, NORDIL, and STOP-HTN2), were carried out to compare the efficacy of BBs and diuretics with ACE-I or CCBs. However, these trials did not reveal any significant difference in their antihypertensive efficacy. In contrast, the Losartan Intervention for Endpoint Reduction (LIFE) trial demonstrated a significant treatment effect when using losartan. Losartan exhibited a greater reduction in the combined endpoint of death, myocardial infarction (MI), and stroke compared to the BB atenolol, despite achieving a similar reduction in blood pressure over a reported 25% reduction in new-onset diabetes. Additionally, there was 25% reduction in new-onset diabetes, which is attributed to the effect on insulin resistance. However, the trial was specifically designed to evaluate the effectiveness of losartan in individuals with high-risk HTN who exhibited signs of left ventricular (LV) hypertrophy. It would not be appropriate to generalize the results to hypertensive patients with lower risk levels. Furthermore, no conclusions can be drawn regarding the comparative efficacy of losartan versus a diuretic based on these findings.

The ASCOT-BPLA study demonstrated that among patients with moderate risk of CV events, initiating an antihypertensive drug regimen with a combination of CCB and ACE-I was more effective than starting with a BB and a diuretic. This approach significantly reduced the occurrence of various CV events, all-cause mortality, and the risk of developing new-onset diabetes. While diuretics have gained wide acceptance as a first-line antihypertensive treatment, BBs are often reserved for later stages in the management of HTN. The lack of equal consideration for β-blockers does not appear to have a valid justification.

As per the algorithm of major recommendations, a renin-angiotensin system (RAS) blocker (an ACE-I or angiotensin receptor blocker (ARB)), with a CCB or TH/TH-like diuretic is preferred as initial therapy. In case there is a need for three drugs, a combination of an ACEI or ARB with a CCB and a TH-diuretic are considered the right choice, preferably in a single pill combination.

β-blockers (BBs) are typically recommended for specific indications, including angina, post-MI, HF, EF, or HR control in arrhythmias. In fact, when any of the aforementioned comorbidities are present, it is advisable to combine BBs with one of the other primary antihypertensive drug classes.

β-blockers (BBs) have a similar efficacy as that of other first-line antihypertensives...
and have solid evidence of preventing CV complications. Therefore, it is difficult to justify why several HTN guidelines have removed this remarkable class of antihypertensives from their previous position as one of the first-choice drugs for HTN treatment. The downgrading of BBs raises questions. The presumed drawbacks of BBs, such as an increased risk of erectile dysfunction or depression, may have been exaggerated or given undue emphasis. Also, exaggeration of COPD or peripheral artery disease is not a problem with metoprolol or bisoprolol. Guidelines typically restrict their recommendations regarding BB usage to cardiac conditions such as angina pectoris, post-MI, or HF while providing limited or no mention of other CV or non-CV conditions in which these medications may be necessary or preferred. There are various conditions that frequently accompany HTN, and BBs can be antihypertensives of choice in these conditions. It is crucial to prioritize BBs with established efficacy in preventing and treating diseases as the first-choice treatment according to guidelines.18

Indians stand to benefit immensely if early and appropriate intervention is instituted to control blood pressure in young adults. In the absence of guidelines and good-quality studies, Indian clinicians will have to lead the war against HTN in young adults based on their clinical experience. The current study is based on the experience of Indian clinicians to lead the way for the effective management of HTN in young adults in India. One of the classes of drugs not accorded due respect is the BBs. In fact, BBs have the potential to have a special place in the management of HTN in young adults in India because sympathetic over-activity is one of the factors implicated in the development of HTN (Table 1).19

### Recent Recommendation of Guideline

The recommended approach is to combine a RAS blocker (such as an ACE-I or ARB) with a CCB or diuretic as the preferred treatment combination. However, alternative combinations involving any of the five major drug classes (ACE-I, ARB, BB, CCB, TH/TH-like diuretic) can also be utilized.20 Class 1A Recommendation ESC 2021

In view of the latest pieces of evidence and recommendations with major concomitant conditions, the use of BBs is warranted as a first-line medication, along with other drugs, if needed.

### Heart Rate (HR) Control

Prevalence of sympathetic hyperactivity was found in both males and females, although the magnitude of prevalence is more pronounced among females.

Elevated sympathetic nerve traffic has been observed in hypertensive individuals across different age groups, including young, middle-aged, and elderly populations. This increase in sympathetic activity has also been documented in conditions such as pregnancy-induced HTN and in individuals with systolic HTN or isolated systolic blood pressure.21 Moreover, higher HRs have been observed in patients with both high blood pressure and those presenting metabolic risk factors such as obesity, metabolic syndrome, or diabetes mellitus.

These collective observations support the notion that sympathetic hyperactivity is a widespread phenomenon in hypertensive patients, independent of the diverse clinical manifestations associated with the condition. Studies have shown that sympathetic activity is notably higher in obese individuals with HTN compared to lean individuals. Additionally, the sympathetic overdrive observed in HTN is not a stable condition but rather follows the increase in blood pressure, progressing from uncomplicated stages to more complicated stages that may occur over the course of the disease.

Compared to normotensive individuals, hypertensive conditions are characterized by a progressive increase in the number of neural bursts. Higher activation of the sympathetic system was found to be more pronounced in complicated than uncomplicated stages of HTN. Interestingly, sympathetic nerve firing was not observed to differ between hypertensive patients with a blunted nocturnal blood pressure fall (non-dippers) and those with a normal nocturnal blood pressure fall (dippers), despite the former group being known to exhibit a higher severity of CV risk compared to the latter group.22

There are two major requirements of SNS which act as an amplifier of blood pressure elevation. The initial evidence suggests that sympathetic neural factors play a role in the early stages of blood pressure elevation. In young hypertensive patients, the rise in circulating plasma norepinephrine levels corresponds to the simultaneous increase in HR. This observation indicates the loss of inhibitory influence exerted by vagal tone on the sinus node while the excitatory influences become enhanced.23 Second evidence indicates that mild and borderline hypertensive patients already have a high adrenergic drive which is indicative of an activated central sympathetic outflow. The norepinephrine spillover technique has shown similar sympathetic activation in essential forms of HTN patients also.

Converging data from various studies suggests that HTN is a dynamic process occurring at different stages within the chain of events leading to an initial elevation in blood pressure, eventually progressing to a complicated hypertensive state.24

### Sympathetic Nervous System (SNS) is Elevated in HTN Patients; Once Detected, How to Treat These Patients?

Nonpharmacological interventions, such as low-calorie dietary interventions and regular physical exercise programs, have also exhibited remarkable sympathomodulatory effects. This phenomenon can be attributed to the marked dietary sodium restriction, which triggers hyperinsulinemia and renin-angiotensin stimulation. These two effects promote sympathoexcitation and hinder the baroreflex control of both vagal and sympathetic drive.

Among the antihypertensive drug treatment, some of the pharmacological antihypertensive drugs (such as BBs) may elicit profound marked sympathoinhibitory effects. Whereas the classes like long-acting calcium antagonists leave no sympathoinhibitory effects, classes like diuretics and short-

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**Table 1:** Effects of different antihypertensive drug classes on peripheral and cardiac sympathetic drive

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Effects on peripheral SNS</th>
<th>Effects on cardiac SNS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central sympatholytics</td>
<td>Significant decrease</td>
<td>decrease</td>
</tr>
<tr>
<td>α-blockers</td>
<td>Significant decrease</td>
<td>No change</td>
</tr>
<tr>
<td>TH diuretics</td>
<td>Significant decrease</td>
<td>No change</td>
</tr>
<tr>
<td>Anti-aldosterone agents</td>
<td>Decrease</td>
<td>No change</td>
</tr>
<tr>
<td>BBs</td>
<td>Decrease</td>
<td>Marked decrease</td>
</tr>
<tr>
<td>Short-acting CA</td>
<td>Significant increase</td>
<td>Significant increase</td>
</tr>
<tr>
<td>Long-acting CA</td>
<td>Reduction, no change</td>
<td>No change, increase</td>
</tr>
<tr>
<td>ACE-Is</td>
<td>Reduction, no change</td>
<td>No change</td>
</tr>
<tr>
<td>Angiotensin II receptor blockers</td>
<td>Reduction, no change</td>
<td>No change</td>
</tr>
</tbody>
</table>

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**Journal of the Association of Physicians of India, Volume 71 Issue 9 (September 2023)**
β-blockers as the First Line of Treatment for HTN Management

acting calcium antagonists increase the sympathoinhibitory effects.

Currently, there is a scarcity of information regarding the effects of various antihypertensive drug combinations on autonomic CV function. The available evidence is primarily based on indirect markers of sympathetic drive, such as plasma norepinephrine, which are considered less sensitive in assessing these effects.25

**Heart Rate (HR) is a Marker, a Target, and a Prognostic Factor in HTN**

The CV continuum is one of the leading causes of premature death and disability in our country; however, its progression is far from inevitable. Recent years have witnessed significant progress in understanding the series of events that lead to coronary artery disease, accompanied by therapeutic advancements. As a result, interventions can now be implemented at various stages of the CV continuum, spanning from asymptomatic individuals with atherosclerosis risk factors to patients approaching end-stage congestive HF (CHF). Numerous interventions, including lifestyle modifications and pharmacological therapies such as BBs, ACE-Is, and statins, are available. These interventions play a crucial role in preventing or slowing down the development of symptomatic heart disease, ultimately extending life expectancy.

Within the entire CV continuum, BBs have the potential to intervene at multiple points, leading to a deceleration or even interruption of its progression. Over the past two decades, research has unveiled new and sometimes unexpected advantages of β-blockers, expanding their application to novel indications such as CHF and prevention of cardiac events in noncardiac surgeries. Additionally, these findings have reinforced the established role of BBs in conditions like HTN and post-MI for secondary prevention (Fig. 1).26

Higher values of HR were found to be strongly associated with an increased risk of developing atrial fibrillation (AF). The LIFE study revealed that every 10 beats/minute (bpm) increase in HR was associated with a 15% higher risk of developing new AF.27 The study demonstrates that individuals with a persistent HR of 84 or higher have a significantly greater risk of developing AF compared to those with an HR below 84. Furthermore, after a 4-year follow-up, there is an estimated 6.0% higher absolute incidence of AF in individuals who maintain or develop an HR of 84 or higher (Figs 2 and 3).28

The mechanism linking increased HR with mortality involves several factors. Firstly, a higher HR serves as a marker of increased sympathetic tone, which leads to increased myocardial ischemia. Additionally, it acts as a promoter of atherosclerosis, potentially contributing to plaque disruption in the coronary arteries and other circulatory pathways. Moreover, a higher HR may indicate subclinically decreased LV systolic function. Furthermore, a higher HR is known to be associated with an overactive sympathetic tone, which increases susceptibility to ventricular arrhythmias. In patients with atherosclerosis, a higher HR is associated with increased myocardial ischemia due to elevated myocardial oxygen demand and coronary vasoconstriction, leading to decreased myocardial blood flow.

Increasing development of atherosclerosis was observed with increasing HR. Also, in young post-MI patients, there was a two-fold increase in coronary artery stenosis scores. In patients undergoing serial angiography, a higher risk of disruption of coronary plaque was observed. An increase in HR is also shown to be associated with increasing atherosclerosis in experiments. The explanation for the phenomena could be exposure to coronary endothelium because of increased oscillatory shear stress.29

According to the study, the occurrence of CV death and all-cause mortality varied based on the HR of 84 bpm throughout the
duration of the study. The findings indicate that the persistence or development of an HR of 84 bpm or higher was associated with a greater risk of CV death and all-cause mortality compared to individuals with an HR below 84 bpm. Specifically, the persistence or development of an HR of 84 bpm or higher was linked to an estimated 2.2% higher absolute incidence of CV death and a 4.3% higher incidence of all-cause mortality after a 4-year follow-up period.\textsuperscript{29}

Evaluation of participants in the VALUE trials has revealed that an elevated HR serves as a long-term predictor of CV events in patients with high-risk HTN. Importantly, this effect remains consistent and is not modified by achieving good blood pressure control. Thus, baseline tachycardia was a true long-term predictor of adverse outcomes in HTN.\textsuperscript{30}

**Heart Failure (HF) with Reduced Ejection Fraction (HFrEF) Patients**

The use of BBs has been associated with a mortality benefit as evidenced by studies such as MERIT-HF, Cardiac Insufficiency Bisoprolol Study II (CIBIS II), and COPERNICUS. These trials, along with a clear understanding of the underlying mechanisms of action, provide a strong therapeutic rationale for the use of BBs. BEST trial\textsuperscript{31} has shown a clear trend toward survival benefit with bucindolol, but as compared to other studies, it didn’t provide a convincing demonstration of BB effectiveness in the treatment of HF.

As mortality benefits have been demonstrated by CIBIS-II trials, it was stopped early. In the trial, bisoprolol demonstrated a lower rate of mortality compared to the placebo group, with 156 (11.8%) deaths in the bisoprolol group compared to 228 (17.3%) deaths in the placebo group. The hazard ratio was calculated to be 0.66 [95% confidence interval (CI) 0.54–0.81, \(p < 0.0001\)], indicating a significant reduction in the risk of death with bisoprolol. Notably, there was also a notable decrease in the occurrence of sudden deaths among patients receiving bisoprolol, with a hazard ratio of 0.56 (95% CI 0.39–0.80, \(p = 0.0011\)).\textsuperscript{32}

In smaller randomized placebo-controlled studies focusing on HF patients where mortality benefits were not the primary endpoints, BBs have demonstrated a substantial reduction in total mortality by approximately 30–35%. Only two studies have assessed survival as the predefined endpoint in HF–MERIT-HF study\textsuperscript{33} and the Cardiac Insufficiency Bisoprolol Study II (CIBIS II).\textsuperscript{34} Both BBs studied, CIBIS and MERIT-HF, are of a lipophilic nature. CIBIS enrolled patients in NYHA functional class III-IV with an ejection fraction of 0.35 or less, while MERIT-HF included patients in class II with an ejection fraction up to 0.40. Both studies demonstrated similar survival benefits for patients in NYHA classes III and IV. In MERIT-HF, there was a 38% reduction in mortality (95% CI 0.48–0.79), and in CIBIS II, there was a 34% reduction (95% CI 0.54–0.81). Additionally, both studies showed comparable decreases in sudden death: 41% in MERIT-HF and 44% in CIBIS II. Another study reported a 49% reduction in death from worsening HF (95% CI 0.33–0.79), while in CIBIS II, the reduction was 26% (95% CI 0.48–1.14). Although the outcome was not statistically significant in CIBIS II, when considering both studies together, it becomes evident that β1 blockade also has a clinically important impact on reducing deaths from worsening HF.

COPERNICUS trial with carvedilol results have also demonstrated long-term treatment substantial benefits in patients with severe chronic HF. When carvedilol was added to conventional therapy for an average duration of 10.4 months, it resulted in a significant reduction of 35% in the rate of death and a 24% decrease in the rate of death or hospitalization.\textsuperscript{35} In trials involving timolol administration to patients aged 65–75 years who survived MI, significant reductions were observed compared to placebo in overall mortality (\(p < 0.05\)), total cardiac death (\(p < 0.01\)), sudden death (\(p < 0.05\)), and reinfection (\(p < 0.01\)).\textsuperscript{36}

**Hyperkinetic Heart Syndrome**

Sympathetic activation is believed to play a role in the development and persistence of HTN in numerous patients. The newer generation of centrally acting sympatholytics may prove particularly beneficial compared to the older generation of nonselective sympatholytics. With no effect on α2 receptors, moxonidine has superior tolerability over clonidine. Moxonidine has little or no sedation or dry mouth. Clinical trials have shown that, along with its superior efficacy, moxonidine is as effective as ACE-I, BBs, CCBs, and diuretics in lowering blood pressure. As a result, the central modulation of the SNS has regained attention as a promising approach to reducing blood pressure.

There is an urgent need to generate data on the prevalence and management of sympathetic overactivity (SO) in newly diagnosed Indian hypertensive patients. With >25% prevalence of HTN in Indian urban, it’s imperative to address the need for the right antihypertensive based on the underlying pathophysiology. It can help not only control blood pressure but also prevent and minimize CV risk. The prevalence of SO in newly diagnosed hypertensive patients in India has been discovered to be as high as 62.42%\textsuperscript{37}

Among the study population, the most frequently prescribed antihypertensive drugs were metoprolol alone, followed by combinations of metoprolol with amiodipine, amiodipine alone, olmesartan alone, and combinations of metoprolol with olmesartan. With metoprolol, the average reduction in BP was 24.61 and 13.99 mm Hg in systolic and diastolic blood pressure, respectively. Also, the heartbeat was reduced by an average of 14.53 bpm. No serious adverse event was reported in this study.\textsuperscript{38}

In a combined analysis of five retrospective cohort studies, it was observed that the average HR in patients with COPD who were treated with BBs was lower than that in patients with COPD who were treated without BBs, in addition to receiving standard care in both groups.\textsuperscript{39}

With newer cardioselective BBs, apprehension of respiratory side effects is not much now. The utilization of BBs in patients...
with COPD not only proves to be safe but also reduces their overall mortality and in-hospital mortality. Additionally, cardioselective β-blockers may potentially decrease exacerbations in COPD patients. Importantly, cardioselective β-blockers do not interfere with the action of bronchodilators used in COPD treatment. Secondly, and of greater significance, β-blockers can mitigate the HR acceleration induced by bronchodilators. Therefore, when appropriate, β-blockers should be prescribed more liberally for patients with COPD who also have heart disease.

Psychiatric adverse events, notably depression, are believed to occur relatively frequently during BB therapy. Contrary to concerns, BB therapy was not found to be associated with an increased risk of depression or most other psychiatric adverse events when compared to placebo. Therefore, the apprehension regarding the association between BB usage and depression appears to be overemphasized.

Discussion

Like in most of the therapies, the step-up approach for the management of HTN is based more on the length of experience with the molecule(s) rather than the evidence. With targets getting stricter, the step-up approach itself is not mandated currently. There is no convincing evidence why a molecule with one mechanism should be preferred over another. BBs, having strong evidence in the management of HTN and having proven benefits in patients with higher CV risks, should be considered among the first line of choice in HTN management. Most HTN patients have one or more comorbidities; so is much more common. Due to nonselectivity in the first and second generations of BBs, there used to be certain unacceptable adverse events associated. However, with the advent of the newer generation of highly selective BBs like metoprolol and bisoprolol, most of these adverse events are not seen frequently. These molecules have a well-established role across the CV continuum. Sooner or later, a hypertensive patient might develop such CV comorbidities ranging from coronary artery disease to HF. Newer BBs are among first-line therapy in all of these conditions. Looking at this, there seems no reason why these BBs shouldn’t be considered as a first-line medication for HTN management.

References

3. Presented by Dr Mark D. Huffman at the American Heart Association Scientific Sessions, Chicago, IL, November 6, 2022.
Early Gestational Diabetes Mellitus: An Update

Wesely Hannah1, Rajendra Pradeepa2, Ranjit Mohan Anjana3, Ram Uma4, Mangesh Tiwaskar5, Viswanathan Mohan6

Received: 22 June 2023; Accepted: 30 June 2023

ABSTRACT

Hyperglycemia occurring in pregnancy is a growing burden worldwide. It is now standard of care to screen all women during pregnancy, both to detect preexisting diabetes as well as gestational diabetes mellitus (GDM). Traditionally, GDM was diagnosed at 24–28 weeks. However, with many international bodies recommending screening at first contact or booking, we are now diagnosing GDM earlier on in pregnancy. Based on the time of gestation at which it is diagnosed, GDM can be classified as conventional gestational diabetes mellitus (cGDM) or early gestational diabetes mellitus (eGDM). The cGDM is diagnosed between 24 and 28 weeks of gestation while eGDM is diagnosed in early pregnancy (<20 weeks). Till recently, there was little and conflicting evidence, on whether diagnosing and treating eGDM was beneficial or safe. The recent Treatment of BoOking Gestational diabetes Mellitus (ToBOGM) study, was a randomized control trial, showing clear benefits of diagnosing and treating eGDM. ToBOGM also showed that the best results were seen in those screened before 14 weeks of pregnancy and those in the higher band of glucose levels (FPG 95–109 mg/dL, 1-hour >191 mg/dL, and 2-hour glucose 162–199 mg/dL). In India, where the burden of hyperglycemia in pregnancy is high, the findings from the ToBOGM study further emphasize the need for screening for GDM at the time of first booking of the pregnancy followed by appropriate treatment for those detected to have eGDM.

INTRODUCTION

The prevalence of gestational diabetes mellitus (GDM) is increasing worldwide. GDM is associated with an increased risk of adverse pregnancy outcomes which can impact both the mother and the child.1 Appropriate treatment can significantly decrease the adverse effects of GDM on maternal and fetal outcomes.2,3 Traditionally, screening for GDM is advocated between 24 and 28 weeks of gestation. Till recently, early pregnancy blood glucose screening, that is, during the first trimester or at the first prenatal visit (booking visit) had been advised mainly to identify undiagnosed type 2 diabetes or prediabetes in women entering pregnancy. However, such screening has also led to the identification of milder degrees of hyperglycemia which does not satisfy the criteria of overt diabetes or even prediabetes. This condition has been referred to as early gestational diabetes mellitus (eGDM). However, no specific diagnostic tests or thresholds are currently recommended for diagnosing eGDM. Hence, researchers have adopted various diagnostic criteria and thresholds to report on eGDM.4–6 There is evidence to show that eGDM is associated with the risk of adverse pregnancy outcomes.4–6 The benefits of screening and treatment of eGDM however became clear after the results of the Treatment of BoOking Gestational diabetes Mellitus (ToBOGM) study were recently published.7 This article is to emphasize the importance of eGDM based on the ToBOGM study results.

DEFINITIONS

Gestational diabetes mellitus (GDM)—GDM is defined as any degree of glucose intolerance with onset or first recognition during pregnancy.8 This is classified into two types:

Conventional gestational diabetes mellitus (cGDM)—GDM diagnosed between 24 and 28 weeks of gestation is called “cGDM.”

Early gestational diabetes mellitus (eGDM)—intermediate hyperglycemia diagnosed earlier in pregnancy (i.e., <20 weeks of gestation) that doesn’t satisfy the criteria for overt diabetes or prediabetes but satisfies the criteria for GDM, is referred to as eGDM.

Recommendations on eGDM Screening

Risk-based screening for overt diabetes during the first prenatal visit is endorsed by the American Diabetes Association but no specific recommendations are available for eGDM screening.9 The International Association of Diabetes and Pregnancy Study Groups (IADPSG) recommends early pregnancy screening for GDM using fasting plasma glucose (FPG) >92 mg/dL (5.1 mmol/L) but <126 mg/dL (7.0 mmol/L). However, this threshold has not been validated for this purpose.10,11 In 2015, the National Institute for Health and Care Excellence advised risk-based early screening for GDM.12 Risk-based early screening is endorsed by the Australasian Diabetes in Pregnancy Society.13

The International Federation of Gynaecology and Obstetrics has advised universal early pregnancy screening for eGDM.14 The Diabetes in Pregnancy Study Group of India was one of the first to recommend universal screening of all pregnant women in the first trimester of pregnancy.15 The advent of early pregnancy screening has led to the identification of hyperglycemia below the threshold of overt diabetes or prediabetes, which could be labeled as eGDM. However, the potential benefits or the possible harm of diagnosing and treating eGDM was not known, till recently, as there was no randomized controlled trial (RCT) conducted on eGDM, and the available evidence has been conflicting.

Current Evidence on eGDM

In a systematic review, conducted by our group, we reported that the prevalence of eGDM ranged from 0.7 to 36.8% based on the findings of 41 studies from across the globe.4 Maternal characteristics such as older age, higher body mass index, previous history of GDM, family history of diabetes, and multiparity were reported as risk factors for eGDM.4,16 Some studies reported that women diagnosed with eGDM were prone to adverse pregnancy outcomes such as cesarean delivery, induction of labor, macrosomia, preterm delivery, and shoulder dystocia.4,17 Findings from 13 cohort studies pooled in a meta-analysis, showed increased risk of perinatal mortality, neonatal hypoglycemia, and higher insulin use in women diagnosed with eGDM compared to cGDM.4 A meta-analysis of RCTs comparing early screening and treatment with standard care showed...
Early Gestational Diabetes Mellitus: An update

reduced risk of large for gestational age, in studies that screened and treated early in the first trimester.18 Studies also showed that the occurrence of postpartum dysglycemia (diabetes or prediabetes) and the need for insulin use were higher in the eGDM group compared with the cGDM group.4

Benefits of Early Treatment

The ToBOGM study—the ToBOGM study (Australian New Zealand Clinical Trials Registry number, ACTRN12616000924459) aimed to clarify whether early treatment of women diagnosed with hyperglycemia before 20 weeks of gestation, improved obstetric outcomes.6,219

The study was carried out in 17 sites in Australia and also in Sweden, Austria, and India (the Madras Diabetes Research Foundation represented India in this trial). As per protocol, 4,000 women had to be recruited and risk-based screening using an early oral glucose tolerance test (OGTT) by IADPSG criteria was proposed. A total of 43,721 women were approached, of whom 4,537 women were recruited and 3,681 women underwent an OGTT before 20 weeks of gestation. A total of 802 women had eGDM of whom 406 women were randomized to immediate treatment (intervention group) while 396 women served as controls. Follow-up data was available in 98.6% (n = 793) participants. The mean gestational age at early OGTT screening was 15.6 ± 2.5 weeks (range: 4–19 weeks and 6 days).

Women who fulfilled any of these OGTT thresholds—fasting glucose level of ≥92 mg/dL (≥5.1 mmol/L), 1-hour glucose ≤190 mg/dL (10.0–10.5 mmol/L) or a 2-hour glucose level of ≥153 mg/dL (≥8.5 mmol/L) were randomized into four groups:

Group I—intervention group (n = 400): These women had abnormal early OGTT and were randomized to early GDM management and were thus the “immediate treatment participants” as described in the protocol.19 They were provided with dietary advice and instructions on self-monitoring of capillary blood glucose. These women were treated with insulin or metformin as per the local guidelines.

Group II—control participants (n = 400): These women had eGDM but were randomized to “no treatment group” or the control group but they were screened again between 24 and 28 weeks to rule out cGDM.

Group III—decoy participants (n = 800): These women had normal early OGTT and were screened again at 24–28 weeks but were included in the study as “decoys” as they would help as a point of reference while comparing outcomes.

Group IV—nonactive participants (n = 2400): These women had normal early OGTT and their medical case reports were reviewed but they did not actively participate in the trial.

What Did the Results Show?

The composite primary outcome of adverse neonatal outcomes included—birth at <37 weeks’ gestation, birth trauma, birth weight of ≥4500 g, respiratory distress, phototherapy, stillbirth or neonatal death, or shoulder dystocia. The other primary outcomes were—pregnancy-related hypertension (preeclampsia, eclampsia, or gestational hypertension) and neonatal lean body mass. The secondary outcomes in the mother were total gestational weight gain, cesarean delivery, induction of labor, perinatal injury, quality of life, and maternal hypoglycemia. The secondary outcomes in infants were birth weight, large-for-gestational-age, small for gestational age (SGA), mean upper-arm circumference, neonatal fat mass, severe neonatal hypoglycemia, and number of days in the neonatal intensive care unit.

The composite primary neonatal outcome was seen in 24.9% (94/378) in the intervention group and 30.5% (113/370) in the control group, with a significant adjusted risk difference (−5.6% age point, 95% CI: −10.1 to −1.2, p = 0.02). Thus a clear beneficial effect was observed in the intervention group. However, other primary outcomes like pregnancy-related hypertensive and neonatal lean mass did not show any significant difference between intervention and control groups. Further analysis of this data showed the greater benefit of early treatment in terms of these composite adverse neonatal outcomes in women who were in the higher glycemic band in the OGTT, that is, FPG 95–109 mg/dL (5.3–6.0 mmol/L), 1-hour >191 mg/dL (≥10.6 mmol/L) and 2-hour glucose 162–199 mg/dL (9.0–11.0 mmol/L) than those in the lower glycemic value, that is, FPG 92–94 mg/dL (5.1–5.2 mmol/L), 1-hour ≤190 mg/dL (10.0–10.5 mmol/L), and 2-hour 153–161 mg/dL (8.5–8.9 mmol/L). Similar findings were reported earlier in cGDM by Crowther et al.20 and Bhavadharni et al.21 who also reported that GDM women with higher glycemic values tend to have better response to treatment. ToBOGM also showed that women identified and treated before 14 weeks of gestation showed the greatest benefits of early treatment. ToBOGM also showed that treating women in the lower glycemic band increased the risk of SGA babies.7

CONCLUSION

The entity called eGDM has now been established well in the literature. The benefits of early identification and treatment of eGDM have been clearly demonstrated in the ToBOGM study. ToBOGM also showed that the benefit of early treatment is more pronounced in women with higher glycemic values and in those diagnosed before 14 weeks of gestation. Future work on eGDM will throw more light on the diagnostic criteria for eGDM, that is, whether it should be based on FPG, an OGTT, or an hemoglobin A1C test. Despite these unanswered questions, the value of screening for GDM at the time of first booking of pregnancy is rapidly gaining ground. As eGDM has worse outcomes than cGDM and the prevalence of GDM is very high in India,22,23 these findings are even more relevant to our country.

REFERENCES

Early Gestational Diabetes Mellitus: An update


BOOK REVIEW

Medical Understanding of Yoga

by Dr. Prakash C. Malshe

Yoga is an ancient way of life in India and it has been followed by a large number of people across the Globe as well. Importance of Yoga for spiritual pursuit has attracted a lot of attention from various people across the World and India has historically been the epicenter of it. Yoga encompasses physical exercises, dietary discipline/restrictions, apart from the ways to increase control of impulses/anger/envy/jealousy/lust/addictions and to a broader way of spiritual activities. Application of Yoga for medical ailments (both for prevention and treatment) has been the core of this book and the author has explained the importance of breath control, Aum recital, periodic fasting, yogasanas and SuryaNamaskar in details and readers (in this case the medical doctors) can find it fairly clear to perform these activities, if interested. The author has put in a lot of effort in not only lucidly writing about yogasanas with photographs, but he has been at length to corroborate the effects of yogasanas on circulatory-respiratory-gastrointestinal-endocrine and nervous systems. He has gone into details of breathing techniques and their applications to resolve medical complaints like nasal sinus congestion, viral/allergic respiratory complaints, digestive complaints and specific non communicable diseases like obesity, diabetes, hypertension, ischemic heart disease. The intention of the author (himself a medical doctor with special interest in yogic sciences) to inform medical doctors and to orient them to the interphase of human physiology and effects of yoga on the same. The author has gone on to explaining the concept of voluntary control of involuntary phenomena (like stimulating sympathetic or parasympathetic activity or influencing the appetite center towards satiation through different physical maneuvers) and further aspects like samadhi, human hibernation and hatha yoga as manipulating nature.

Some ideas like drinking air either through perforated straw or while exercising different SuryaNamaskaras and then filling the stomach with that air and later on through Sheershahasana (head down legs up position), allowing that air to travel up the intestines so as to improve satiety (or reduce eating) are interesting. Similarly, breath holding (at deep exhalation) to create hypoxia (and increase blood CO₂) is thought provoking.

It is necessary to think whether yoga, yogasanas and SuryaNamaskar are meant for health attainment/maintenance and preventive aim OR for therapeutic applications. We live in evidence based scientifically rigorous medical World where this clarity will be needed. The different yogasanas need to be learnt by the person preferably under an expert yoga teacher so as to reap benefits (only if performed on a daily basis for long periods of time). Intuitively, the person needs to be physically fit to attain proficiency in yogasanas and train the body systems in a way to be capable to execute them the right way and then over a period of time be able to drink air to fill stomach and intestines. The author can be more explicit to scientifically show the preventive benefits of them. The yoga for treatment aspect has to be accompanied by well conducted clinical studies. Untrained or uninitiated person (especially if obese, hypertensive, diabetic, IHD) being expected for breath holding exercises or Sheershahasanas needs practical feasibility outlook.

All hypotheses (creating hypoxia to improve coronary collaterals, drinking air to fill in stomach to treat Helicobacter pylori infection or filling air in intestines to augment satiety and reduce body weight) would need substantiation by robust (hard) evidences to be implementable.

On a scale of 1–10 (ascending order), I would rate the book at 6 and give compliments to author for his work.

Dr. Suhas Erande MD
Milk-Alkali Syndrome: A Century-old Cause of Hypercalcemia Requires the Addition of Venous Blood Gas in Hypercalcemia Workup

Utsav Sahu1*, Tamal Trivedi2, Rajesh Gupta3

Received: 20 April 2023; Accepted: 09 May 2023

ABSTRACT
The Milk-Alkali syndrome (MAS) is identified by the triad of high serum levels of calcium, metabolic alkalosis, and acute kidney injury, usually caused by consuming excessive amounts of calcium and absorbable alkali. If not treated promptly, the syndrome can result in rapid hypercalcemia, acute renal failure, and metastatic calcification. Notably, an increasing number of cases of MAS have been observed, potentially due to the rampant use of calcium-based over-the-counter supplements for the prevention and treatment of osteoporosis in postmenopausal women. Herein, we report a case of severe hypercalcemia due to prolonged intake of calcium carbonate supplements in the absence of any alkali. The case report highlights the importance of including venous blood gas (VBG) analysis as a part of the workup for hypercalcemia, as metabolic alkalosis can help clinch the diagnosis of MAS in the setting of severe hypercalcemia.

INTRODUCTION
The Milk-Alkali syndrome (MAS) consists of the triad of hypercalcemia, metabolic alkalosis, and various degrees of renal failure associated with the ingestion of large amounts of calcium and absorbable alkali. This syndrome was discovered in the 1930s after treatment of peptic ulcer disease with milk and sodium bicarbonate had become common.1 Once a classic cause of hypercalcemia, the MAS virtually disappeared with the advent of new therapies for peptic ulcer disease and, by 1985, was considered the cause of <1% of cases of hypercalcemia.2

In recent times, there has been a notable rise in the occurrence of MAS. This surge can be attributed to the widespread usage of over-the-counter calcium preparations among postmenopausal women as a means of preventing and treating osteoporosis. Moreover, healthcare professionals often prescribe calcium carbonate to individuals with chronic kidney disease to prevent the development of secondary hyperparathyroidism. In light of the evolving research, some experts have proposed renaming the syndrome as a calcium-alkali syndrome.3 MAS now accounts for >10% of the cases of hypercalcemia and is believed to be the third most common cause of in-hospital hypercalcemia after hyperparathyroidism and malignant neoplasms.4

CASE DESCRIPTION
A 71-year-old normotensive, nondiabetic, and euthyroid female presented with confusion, irrelevant talks, and urinary incontinence. Her blood pressure was 160/84 mm Hg, and random blood glucose was found to be 125 mg/dL. Routine blood investigations revealed hemoglobin: 13.2 gm/dL (normal range: 12–15), blood urea: 103 mg/dL (normal range: 20–40), serum creatinine: 4.2 mg/dL (normal range: 0.7–1.2), total calcium: 14.9 mg/dL (normal range: 8.5–10.5), phosphorous: 4.2 mg/dL (normal range: 3.5–4.5), albumin: 3.9 gm/dL (normal range: 3.4–4.6), her venous blood gas (VBG) confirmed metabolic alkalosis, with a pH of 7.540, partial pressure of carbon dioxide 52 mm Hg, partial pressure of oxygen 51 mm Hg, and hydrogen carbonate 42mmol/L. She gave a history of taking analgesics for joint pains and supplements of calcium carbonate for osteoporosis for the last 20 years.

Other investigations revealed serum angiotensin-converting enzyme of 36 U/L (normal range: 8–52), thyroid stimulating hormone of 4.2 mIU/mL (normal range: 0.5–5.0), 25-hydroxy (OH) —vitamin-D of 40.4 ng/mL (normal range: 30–100), and normal serum protein electrophoresis. Despite high serum calcium level, her parathyroid hormone (PTH) was not suppressed, and her intact PTH level was found to be 46.1 pg/mL. Technetium Sestamibi scan did not localize any parathyroid adenoma, and the positron emission tomographic scan ruled out the possibility of any paraneoplastic syndrome. On the basis of metabolic alkalosis, even in the setting of severe renal dysfunction, and excluding other causes of hypercalcemia, MAS was diagnosed. On further questioning, the patient admitted to perhaps taking more calcium than she should have. But there was no history of any antacid intake.

She was treated with saline diuresis and an injection of calcitonin 200 units, given subcutaneously thrice daily for 2 days, and her symptoms and renal functions started improving. Within 1 week after admission, her renal functions improved significantly, her blood urea: 50 mg/dL, serum creatinine: 1.8 mg/dL, and her total calcium came down to 8.5 mg/dL, and she was discharged with clear instructions to avoid any calcium carbonate supplements.

DISCUSSION
Despite considerable clinical experience, there is a lack of substantial data regarding the underlying causes of MAS. Over the years, various factors have been suggested as potential contributors, such as gastric juice depletion, preexisting renal disease, inadequate chloride intake, hemorrhage, anemia, and impaired liver function. However, the precise pathogenesis remains poorly understood. To diagnose MAS, it is essential to observe the ingestion of excessive amounts of both calcium and absorbable alkali. What constitutes “excessive” is unclear but generally indicates at least 4–5 gm of calcium carbonate daily.5

The development of hypercalcemia requires not only excessive calcium intake but also the inability to effectively excrete the excess calcium. This is because the skeletal system has a limited capacity to buffer calcium. To maintain appropriate serum calcium levels, precise regulation of calcium absorption from the small intestine and excretion by the kidneys is crucial.

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Milk-Alkali Syndrome

Furthermore, individual variations in the buffering capacity of bone may influence an individual's susceptibility to developing hypercalcemia. When calcium intake increases, it results in a reduction in the kidneys' ability to convert vitamin D into its active form, known as 25-hydroxylation. Consequently, there is a significant decrease in the fractional absorption of calcium in the small intestine. In certain individuals, despite ongoing calcium ingestion and suppressed levels of 1,25-OH vitamin D, there is persistent high urinary calcium excretion, indicating elevated intestinal absorption. Under normal circumstances, renal calcium excretion closely reflects calcium absorption. However, if substantial amounts of calcium are consistently ingested, and the kidneys are unable to excrete it effectively, hypercalcemia becomes a foreseeable outcome. In some cases, the failure to adequately suppress calcitriol levels may contribute to the development of MAS in individuals with high oral calcium intake.6

The PTH level should be suppressed by the high serum calcium level in patients with MAS. But in the setting of severe renal dysfunction, the PTH level is raised. Renal dysfunction is also associated with metabolic acidosis. But in the setting of MAS, metabolic alkalosis is the essential feature. Differentiating MAS from other causes of hypercalcemia is important as treatment is supportive. Adequate hydration to correct hypovolemia, along with loop diuretics, like furosemide, to increase urinary calcium excretion, may be sufficient to correct hypercalcemia. Patients diagnosed with MAS should generally avoid the use of bisphosphonates due to their potential to induce prolonged hypocalcemia.7

CONCLUSION
The recent resurgence of MAS can be attributed to the increased awareness of osteoporosis and the widespread use of calcium carbonate supplements for preventive purposes. It is crucial to educate the public about the potential adverse effects of exceeding the recommended dosage of calcium supplementation. It is generally considered safe to have a daily intake of elemental calcium of no >2 gm.8 However, even doses lower than 2 gm per day may lead to MAS if other predisposing factors are present.

The exact mechanism of how MAS develops is still not fully understood. However, there appears to be a unique interplay between hypercalcemia and alkalosis within the kidneys. This interplay creates a self-reinforcing cycle, contributing to the clinical manifestation of MAS, VBG analysis should be included in the workup of hypercalcemia as the presence of metabolic alkalosis can help clinch the diagnosis of MAS.

In general, MAS has a good prognosis if adequately managed, even complicated MAS cases associated with posterior reversible encephalopathy syndrome may also have a favourable outcome.10 The general public, as well as healthcare professionals, should be aware of the potential negative consequences of consuming excessive quantities of calcium carbonate.

LEARNING POINTS
- Healthcare providers and the general public, at large, need to be educated about calcium supplementation and their potential negative effects if the recommended dosage is exceeded.
- Metabolic alkalosis in the setting of hypercalcemia-induced acute kidney injury should alert the physician about MAS, the third most common cause of hypercalcemia in hospitalized patients.
- Venous blood gas (VBG) analysis is a simple and inexpensive test that should be included as part of the workup for hypercalcemia, as metabolic alkalosis can help clinch the diagnosis of MAS before moving to expensive nuclear imaging studies.

ACKNOWLEDGMENTS
We would like to acknowledge the guidance of our mentor, Dr Sanjay Kalra, who contributed to the development of this case report.

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REFERENCES
Rita Levi and Nerve Growth Factor

Jayant V Pai-Dhungat

Rita Levi-Montalcini (1909–2012) was born in Turin, Italy, to a wealthy Jewish family. Levi-Montalcini studied medicine at Turin University. After graduation, she worked as a developmental biologist. Her research there was about the effects peripheral tissues have on nerve cell growth. Because of her Jewish ancestry, she was forced into hiding in Florence during the German occupation of Italy (1943–45) and was able to resume her research at Turin only after the war.

In 1946, Rita Levi-Montalcini was invited to work at Washington University in St. Louis, United States of America, with zoologist Viktor Hamburger who was studying the growth of nerve tissue in chick embryos. Here Rita Levi and Hamburger found that when tumors from mice were transplanted to chick embryos, they induced potent growth of the chick embryo nervous system, specifically sensory and sympathetic nerves. Since this outgrowth did not require direct contact between the tumor and the chick embryo, Rita Levi-Montalcini concluded that the tumor released a nerve growth-promoting factor that had a selective action on certain types of nerves. Following this discovery, Rita turned to a more sensitive cell culture system in order to measure NGF activity in various extracts. NGF proved to be an extremely potent biological substance. Levy remained in the United States for 30 years before finally returning to Italy, where she lived in Rome. She eventually held dual citizenship in Italy and the United States.

Biochemist Stanley Cohen (1917) joined the research group in St. Louis in 1953. A total of 3 years later, they had purified a nerve growth-promoting extract from a mouse tumor. During this research, Stanley Cohen discovered another growth factor in the salivary gland in the male mouse. Cohen termed this substance epidermal growth factor (EGF) because it could stimulate the proliferation of epithelial cells in the skin and cornea.

During the last decade, several growth factors have been isolated, like EGF, platelet-derived GF, and fibroblast GF.

An innovative Cuban product containing recombinant human EGF has been developed for peri and intralesional infiltration in the diabetic foot. It has been approved for marketing and is undergoing trials. Rita Montalcini Levi retired as director of the Laboratory for Cell Biology, Rome in 1979. She shared the 1986 Nobel Prize in Medicine or Physiology with Stanley Cohen “For their discoveries of growth factors.”

She died aged 103 years, thereby becoming the longest-living Nobel laureate. This remarkable woman endured a staggering amount of obstacles in order to become a doctor and scientist.
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Avior
A Case Study of Scrub Typhus at Khamasom Phungdhar, Chingai Block, Ukhrul, Manipur

Dhananjoy Singh Chingangbam1, Brahmacarimayum Hrishikesh Sharma2, Pamreila Grace KAS3, Thotreichon J4

With or without eschar (Fig. 1), body aches, muscle pain, etc. as usual. The most important point to remember here is early diagnosis and treatment of the patients with effective drugs in time to save the lives of innocent people in the districts. In this regard, the medical department started field detection with rapid kits for early detection in remote areas. In order to identify the mites from the prone areas, some mites were collected and identified as Laelaps piloscutuli (courtesy of Zoological Survey of India, Kolkata)1 from the infested dead rat that might be Rattus nitidus. If anyone knows of such species of mite, we would appreciate the information. There is a need for the identification of the mice and rats from the districts to find out the preferred host or any other hosts of the mites from the locality of the positive cases. The recommendations are early diagnosis, prevention from mites, healthy habits, intense work on ecology, the biology of mites, and routes for the disease to prevent the repetition of an unfortunate loss of lives in the future.

Sir,

Much has been said about infection prevention and control (IPC) implementation and training among Doctor of Medicine in Microbiology candidates. IPC has been hotly debated during and post coronavirus disease 2019 (COVID-19).1 The biggest challenge that arose during the second COVID-19 wave was when new nursing staff were recruited to care for COVID-19 patients. Non-COVID-19 hospitals were facing challenges as nursing staff resigned due to fear of contracting COVID-19, and COVID-19 hospitals were facing the challenges of new staff joining the hospital to attend to COVID-19 patients.2

Infection control is a field focusing on preventing nosocomial and healthcare-associated infections (HAI). HAI is considered one of the most critical issues in healthcare.

The problem was encountered in both COVID-19 and non-COVID-19 hospitals, that is, new nurses with less training in patient care, especially when it came to IPC.

In the pre-COVID-19 era, the infection control nurse (ICN) was used to make rounds and train healthcare workers (HCWs). In COVID-19 times, where social distancing, restricted availability of personal protective equipment, and fear of contracting COVID-19 are prevalent, it has been difficult to send ICNs to train HCWs, especially in COVID-19 wards.

Excellent nursing staff with good knowledge and skills are the backbone of any hospital. The high attrition rate among nursing staff and the booming healthcare industry post-COVID-19 have left the industry with no options but to hire untrained or less-trained staff. Given the challenges facing COVID-19 and non-COVID-19 hospitals, it is now necessary to incorporate IPC training in the 4th year of the nursing curriculum. This will help nurses in training to inculcate best practices and expose them to IPC strategies.3

Figs 1A to C: The mite (A)—Dorsal; (B)—Ventral recovered from Khamasom Phungdhar infesting a dead rat, identified as Laelaps piloscutuli Hunter,1961. Bar represents 10 μm. Eschar (C) from a patient.
Infection prevention and control (IPC) is a very critical topic for providing patient safety, and both undergraduate and graduate nursing students should be competent in IPC. A study done by Rawhia Dogham showed that the current curriculum has few topics pertaining to infection control but that exhaustive knowledge and its importance during patient care is required. In 2011, the WHO reported that 7% of patients in developed countries and 15% in low and middle-income countries have a HAI at any given point in time. These HAI are also associated with a considerable mortality rate. Furthermore, HAI also pose an occupational risk to HCWs. For example, the prevalence of hepatitis B surface antigen, human immunodeficiency virus (HIV), and hepatitis C virus (HCV) was reported to be 1.56, 0.26, and 1.05%, respectively, among HCWs. The WHO reports 3 million cases exposed to blood-borne pathogens each year. These exposures lead to 15,000 HCV, 70,000 HBV, and 500 HIV infections among 35 million HCWs internationally. The majority of infections are reported in developing countries. Training in IPC decreases these incidences. Infection control guidelines involve the most common principle of standard precautions.

Nurses are the backbone of the healthcare system. The implementation of any policy relies on them. To comply with the policies and protocols, it becomes important to raise their awareness with respect to IPC, not only once they have started practicing their profession but also especially by incorporating IPC training into their nursing studies. As a part of their curriculum, nursing students are placed in various intensive care units and other wards. They are also involved in patient care during their clinical postings. They can be a major source of decreased compliance with any hospital’s IPC-related policies or protocols.

Corporate hospitals have dedicated infection control doctors and ICNs. These hospitals have trained personnel, or they have been trained on the job in due course. With the advent of National Accreditation Board for Hospitals and Healthcare Providers (NABH), it has become mandatory to have infection control, which is responsible for meeting the needs and requirements of IPC practices. Dealing with infection control is challenging. The medical colleges going for NABH also need to have a dedicated IPC program. As a result, they have started implementing IPC, but it has become difficult in terms of HAI surveillance, data capturing, and auditing. Therefore, state nursing faculties must now include an IPC program. Instructors and trainers must be from those hospitals which have dedicated departments and trained personnel. Moreover, for practical knowledge, these nursing colleges can connect with these hospitals. Remuneration can be given to the trainers. After joining any hospital, an induction training of nurses which includes basic IPC, can be included in the hospital policy. Going further, undergraduate nursing curricula can include an IPC program which addresses these issues. This will lead to enhanced IPC implementation at the individual HCW level. The challenges faced during this pandemic will then be overcome.

References

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