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Research Article

Seroprevalence of COVID-19 Antibodies among Patients with Haemophilia

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I N F O

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A B S T R A C T

Introduction: COVID-19 has taken over the world like wildfire and has become one of the leading causes of death among infectious diseases. COVID-19 patients have one or more of the following symptoms: high-grade fever, dry cough, muscle ache, eneralized fatigue, difficulty breathing, and loss of taste and smell. Age of the patient along with the comorbidities affecting the patient determine the severity.

Method: The place of study was Lok Nayak Hospital located in New Delhi. The type of study was prospective observational. The incorporation of 112 subjects was done for the study on the basis of inclusion criteria. Informed consent was obtained from the patients after which they underwent rigorous history taking, general and systemic examination and relevant investigations. Interpretation and compilation were done with MS Excel (R) Office 365, GraphPad Prism 8.4.2 and Statistical Package for Social Sciences version 25. Seroprevalence was estimated by measuring the COVID-19 antibodies in these patients' serum with an ELISA kit.

Results: Seroprevalence in the current study came out to be 13.39%, which was significantly lower as compared to the overall pooled seroprevalence of India. This was due to better knowledge, awareness and care taken by these patients towards the disease during the pandemic times. COVID-19 antibody index was in low ranges in patients with haemophilia (PWH) and further exploration and subsequent seroprevalence studies are needed to monitor the antibody index.

Conclusion: Many factors contribute to the seroprevalence of COVID-19, including demographic profile, occupation, socio-economic status of the country, and personal protective measures taken. Seroprevalence studies are important in making healthcare policies and prioritisation in the early rollout of vaccination for susceptible groups of people and their protection.

Keywords: COVID-19, Seroprevalence, Haemophilia

Introduction

COVID-19 had a massive toll on humanity in recent years. Though most of the patients have mild symptoms, some can progress rapidly to interstitial pneumonia and severe respiratory distress which need hospitalisation and even ventilatory support with intensive care in some cases. Various serosurveys were done to monitor the combined seroprevalence of SARS-CoV virus infection in the populace and the possibility of herd immunity in that population. Systematic review and meta-analysis of peer-reviewed journals and pre-print servers were also done for publications from Jan 1, 2020, till Mar 30, 2021. 241 eligible studies were taken into account comprising 6.3 million subjects from 60 countries with a total prevalence of antibodies being 9.47% (95% CI 8.99-9.95%) but with heterogeneity.[1]

Among the patients who had a history of COVID-19 infection, almost one-third had low antibody titres with low neutralising activity. This was especially associated with mild or asymptomatic disease. Wide variability is seen in antibody response because of the wide spectrum of disease with higher antibody levels correlating directly with higher antigen burden and severity of the disease.[2]

The need for prevention of clinical infection and reduction of severity of the disease has led to the production of vaccines which generate an immune response to the spike protein with repeat doses increasing the titres to a level comparable to or greater than those of the patients. The adequacy of circulating antibody levels is paramount and therefore vaccine boosters may be needed to maintain the levels of neutralising antibodies.[3]

A coagulation cascade is a complex process with the involvement of multiple clotting factors. Genetic mutations can impair one or many factors often resulting in disorders causing recurrent and major bleeds. Haemophilia is one such disorder in which there is a deficiency of clotting factor VIII and factor IX based on which it is called haemophilia A or B respectively. Being X-linked recessive diseases, both of them are very rare in females. Both of these diseases have a similar presentation but bleeds associated with haemophilia A are more frequent and severe.[4]

Patients with haemophilia (PWH) require multiple hospital visits for coagulation factors due to frequent bleeds at various sites in the body. The study aimed to assess the seroprevalence among these PWH.

Material and Methods

This prospective observational study was undertaken at Haemophilia Day Care Center of Lok Nayak Hospital with the involvement of 112 subjects attending the hospital between November 2020 and March 2021. Subjects age more than 13 years diagnosed with haemophilia were taken into the study with no exclusion criteria. Ethical approval and informed consents from subjects was taken beforehand after explaining the nature of the study and possible outcomes.

Demographic and clinical characteristics of patients were recorded including age, gender, occupation, symptoms of COVID-19, COVID-19 status, history of hospitalisation, and number of visits per month to the hospital. A case record form was used for compiling the details of subjects including demographic features and hospitalisation history. All records were compiled by trained personnel.

Qualitative detection of total antibodies for the SARS-COV-2 virus was done in the serum sample of the subjects using WANTAI SARS-CoV-2 Ab ELISA kit. This is a sandwich enzyme immunoassay kit with a two-step incubation process. It uses micro wells made of polystyrene which are pre-coated with recombinant viral antigen. In the first step, the wells are filled with the serum of the patient during which any antibody if present in the serum gets bound to the recombinant antigen coated on the wall. These are then washed to remove any unbound antibodies. Following this, for the second incubation, another recombinant antigen is added which has been conjugated with horseradish peroxidase. This conjugated antigen then binds to the antibody on the wells and forms an antigen-antibody-antigen sandwich. The well is again washed and chromogens are now added. Chromogens are colourless compounds which are hydrolysed by the horseradish peroxidase bound to the "sandwich" resulting in the formation of a blue-coloured product. Sulphuric acid is added to this to stop the reaction which, in turn, also converts the blue colour to a yellow colour. The intensity of this yellow colour is measured and it is proportional to the amount of antibody present in the serum. Samples negative for any antibody to the virus will remain colourless.

An Antibody Index (AI), which is a semi-quantitative estimation of the antibody titre, was calculated as the ratio of sample absorbance (A) and cut-off (C.O.). The range of AI through this kit is 1-19.14.

Negative Results (A/CO < 1, AI < 1): Specimens giving absorbance less than the cut-off value are negative for this assay, which indicates that no SARS-CoV-2 antibodies have been detected with WANTAI SARS-CoV-2 Antibody ELISA kit, therefore there are no serological indications for current or past coronavirus disease COVID-19.

Reactivity is considered when the absorbance is at least equal to or greater than the cut-off value that is the ratio is more than or equal to 1.

The data of this study were analysed using descriptive and inferential statistics on Statistical Package for the Social

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Sciences software, for Windows (SPSS version 25). Mean and standard deviation were computed for continuous variables and the statistical differences between categorical variables and continuous variables were calculated using chi-square ($\chi 2$) test and t test respectively. Statistical significance was considered when the p value was less than 0.05.

Result

112 subjects with a mean age of 25.23±10.33 years were included in the study. Out of these 112 cases, 96 cases (85.71%) were of haemophilia A and 16 (14.28%) were of haemophilia B.

72 (64.28%) subjects were either unemployed or students. The mean number of visits to the hospital for infusion of coagulation factor was 10.86±7.31 during the pandemic time. The majority of the study population faced difficulty in travelling (94, 83.92%) across the city to reach the hospital, while 89 (79.46%) people had a fear of going out and contracting the virus.

All the cases took personal protective awareness measures like wearing masks in public places, following hand hygiene, and taking social distancing measures.

Out of 112 cases, 20 cases got tested for COVID-19 by RT-PCR and all cases were reported negative. Only 2 people out of these 20 had COVID-19-related symptoms, the rest were tested due to some other reasons (travel permit, routine test at bus stations, etc.).

None of the cases was vaccinated by the time we enrolled them in the current study (by March 2021). All the study population was vaccinated in due course of time.

A seroprevalence of 13.39% was estimated with a mean AI of 1.68 in the seropositive population. All 15 seropositive patients had a COVID-19 AI below 5 (low antibody index). A meta-analysis conducted by Rostami et al.[1] having outcomes from 241 studies comprising a total of 275 datasets from 60 different countries showed the pooled global seroprevalence to be 9.47% (8.99%-9.95%) and the seroprevalence in India based on 13 studies to be 23.38% (18.55%-28.22%).

A seroprevalence of 15.2% and 10% was estimated in unemployed and employed subjects respectively.

Out of the total 112 cases, 13 (11.6%) cases were symptomatic for COVID-19 in the last 3 months with fever (69.23%) and cough (38.46%) being the major symptoms. 4 (26.66%) were symptomatic and 11 (73.34%) were asymptomatic in seropositive patients. Out of 96 seronegative cases, 8 (8.33%) were symptomatic and 88 (91.67%) were asymptomatic.

Discussion

A defect in the coagulation cascade of a person is seen in haemophilia. This defect is caused due to an X-linked recessive genetic mutation which results in decreased formation of coagulation factors VIII and IX. Depending on where the mutation occurs, the amount of coagulation factor in the patient's blood varies, based on which haemophilia is classified into three grades: mild (> 5%), moderate (1% to 5%) and severe (< 1%). A deficiency of these specific coagulation factors causes the patient to bleed frequently. Patients present with severe bleeding manifestations which causes long-term disability in them.[5]

COVID-19 disease spectrum ranged from an asymptomatic state to severe multiorgan dysfunction. Many patients who contracted the virus and remained asymptomatic, developed the antibodies in their blood, which could be detected using an antibody assay for SARS-CoV-2.[6]

Though it is difficult to predict the future of any disease, a prediction can be made by studying the past of that disease which can be provided by the presence of seroprevalence in the population. Herd immunity levels in healthy and asymptomatic populations can also be extrapolated. As the infection occurs in a waxing and waning pattern, monitoring of seroprevalence data can also help in understanding the natural transmission history of this new infectious disease.[1,7,8]

Table 1.SARS-CoV-2 Seroprevalence Estimates, and Estimated Numbers of SARS-CoV-2-infected People (for which Multiple Datasets were Available from January 2020 to March 2021)[1]

Sustainable Development Goal Regions (Number of Datasets Available for a Particular Region)	Number of People Screened (Total)	Number of Seropositive People	Pooled Seroprevalence, % (95% CI)	Estimated Population Size (2020)
Global (275)	6,367,734	519,407	9.47 (8.99-9.95)	7,794,798,739
Central and Southern Asia (20)	171,519	34,841	22.91 (19.11-26.72)	1,940,369,612
Pakistan (3)	3595	836	28.88 (2.24-55.52)	220,892,340
China (20)	329,900	7026	1.73 (1.33-2.14)	1,439,323,776
India (13)	151,235	31,800	23.38 (18.55-28.22)	1,380,004,385
Current Study	112	15	13.39	347,026

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In the current study, the age of the test population ranged from 13 years to 61 years with a mean age of 25.23 ± 10.33 years, with 31.25% of the population below the age of 19 years and the majority of the study population lying in the range of 19 years to 29 years enrolled in the Haemophilia Day Care Centre in Maulana Azad Medical College and associated Lok Nayak Hospital.

In comparison to the global seroprevalence of 9.47% (95% CI 8.99-9.95%), the seroprevalence of SARS-CoV-2 in the current study came out to be 13.39% in patients with haemophilia. Overcrowded living conditions, higher co-morbidities and lack of access/ inadequate medical care services in developing countries increase the vulnerability towards SARS-CoV-2 and other respiratory infections.[1] Also, poor infrastructure and poverty in developing countries make detection, quarantine, and reduction in daytime use of public transport very challenging. All these findings could have contributed to higher seroprevalence in the current study.[9,10]

In the same study, the pooled seroprevalence of 23.38% (95% CI 18.55-28.22%) was estimated in India around the time of May 2021, shortly after which we began enrolling current cases for the study.[1] In comparison to it, the current study had a lower seroprevalence, owing to the nature of the bleeding disorder and people following the guidelines issued by the World Federation of Hemophilia (WFH) for patients, including hygienic measures and information to help them reduce their SARS-CoV-2 exposure and associated risks.[11] Haemophilia Day Care Centers were in regular contact with the patients, guiding and counselling them over the telephone about the nature of this new disease and measures to help them reduce their exposure to the novel virus.

In accordance with the global studies indicating lower seroprevalence estimates for people of age less than 19 years, the current study showed that 40% of reactive cases were of age less than 19 years and 53.33% of reactive cases belonged to the age group of 20-29 years. Current results suggest that seroprevalence rates are higher in adults due to the fact that adults have to get involved in community activities and have to go out of the house more often.[12,13]

The seroprevalence of SARS-CoV-2 was found to be less statistically significant based on the occupation of the study population. Employed individuals had a seroprevalence of 10% while unemployed including students had a combined seroprevalence of 15.2% in the current study. Seroprevalence in unemployed with school-going cases was higher compared to the current overall seroprevalence of 13.39%. These seroprevalence data are lower than the general population seroprevalence of 23.38%.[1] The adolescents and children in the survey showed higher antibody positivity. There were stringent lockdown of schools and other educational institutions placed by the government, indicating that the transmission through adults who were going out of house, was an important source of transmission. The period from August 2020 saw a wide reversal of the lockdown and laxity on the part of the populace to follow precautionary measures, especially in cramped households contributing to high rates of household infection.

In the current study, 17.85% of people got tested for COVID RT-PCR in the last 3 months for various reasons (travel permit, routine test at bus stations, etc.) and all of them tested negative for SARS-CoV-2. 26.66% of reactive cases had COVID-related symptoms in the last 3 months as compared to the 8.33% of non-reactive cases who had these symptoms. This result was statistically significant due to the fact that the seropositive patients likely got remotely infected by COVID-19, and had very minor symptoms of the disease.

Seropositive patients who were symptomatic in the last 3 months developed a better antibody response of 1.92 ±1.38 as compared to those who were asymptomatic (1.60 ±0.95), implying that the patients who developed COVID-related symptoms were mounting a better response of antibodies as compared to their asymptomatic counterparts.

The COVID-19 antibody index was in the lower range of the antibody index (range = 1-19.14). This correlation needs further evaluation and warrants subsequent seroprevalence studies to monitor antibody response among PWH in future.

Limitations of the Study

- The sample size of the current study was very small and hence the study was not powered enough to achieve statistically significant correlations.
- The estimated seroprevalence levels are likely to be an underestimation as there is a regression of antibodies following COVID-19 infection.
- Vaccination was not introduced in India while recruiting the study population, thus post-vaccination status of COVID-19 antibodies could not be estimated in the study population.

Conclusion

The seroprevalence of COVID-19 antibodies in patients living with haemophilia is 13.39% in the current study conducted from November 2020 to March 2021, which is significantly lower as compared to the seroprevalence in India at that point in time. Patients with haemophilia were counselled telephonically through Haemophilia Day Care Center throughout the pandemic and were encouraged not

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to step out of their houses, to wear face masks whenever stepping out of the house, and use sanitiser. They were explained the importance of hand washing and of following the measures of social distancing in public places.

Due to the nature of their illness, they develop spontaneous bleeding even on trivial trauma. For early healing of haematoma, they need to visit the nearest haemophilia centre. These patients took utmost care while travelling in public transport to reach the hospital and get coagulation factors. The mass awareness programmes run by the Government for COVID-19 prevention helped these patients from contracting this deadly virus. Seroprevalence studies are important in making healthcare policies and prioritisation in the early rollout of vaccination for the susceptible group of people and their protection.

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Conflicts of Interest: None

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