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Original Article

Microbiology Section

Dynamics of SARS-CoV-2 Antibody Response in a Longitudinal Cohort of Healthcare Workers from India

SONAM SINGLA[1], VYOMA SINGH[2], SMITA SARMA[3], CHARU YADAV[4], KAMINI VINAYAK[5], TEENA WADHWA[6], RENU SAXENA[7], YATIN MEHTA[8]

ABSTRACT

Introduction: Coronavirus Disease 2019 (COVID-19) pandemic has affected healthcare systems worldwide. Healthcare Workers (HCWs) form one of the most at-risk population groups for acquiring infection. Trend analysis of anti Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) antibody titres in vaccination naïve HCWs will give an insight into the role of natural protective immunity against reinfection.

Aim: To understand the dynamics of anti SARS-CoV-2 antibody response and its protective role against reinfection in a cohort of HCWs.

Materials and Methods: This observational longitudinal cohort study was conducted in a tertiary care hospital in Gurugram, Haryana, North India from June to December 2020. The study was approved by the Institutional Ethics Committee. Serum specimens from 230 HCWs were tested for anti-spike protein Immunogloublin G (IgG) antibodies by chemiluminescence immunoassay. The HCWs with positive antibody status and previous Polymerase Chain Reaction (PCR) confirmed infection (n=47) were followed-up over 180 days for serial antibody titres at four visits, each at a gap of 30-45 days. Participants were classified into asymptomatic (n=18), mild (n=17) and moderate (n=12) disease categories based on severity of previous COVID-19 illness. SPSS version 22.0 was used for statistical analysis. Intergroup comparison of means was done using Kruskal-Wallis test and Chi-square test. The $p<0.05$ was considered statistically significant.

Results: Positivity rate for anti SARS-CoV-2 IgG antibodies was 25.7%. Seroconversion rate was 90.74% in HCWs with history of previous Real Time-Polymerase Chain Reaction (RT-PCR) confirmed COVID-19 infection. Incidence of infection in seronegative group (n=171) was 12.96 per 10,000 person days while in seropositive group, it was 1.29 per 10,000 person days. Risk ratio for infection (baseline seronegative vs baseline seropositive) was determined to be 8.12 [95% Confidence Interval (CI) 1.068-61.755]. Incidence of PCR confirmed SARS-CoV-2 reinfection was inversely associated with antibody titres ($p=0.018$). Antibody response trend showed a peak in mean titres in the 46-90 days period followed by steep decline till 135 days and a gradual waning till 180 days.

Conclusion: Significant postinfection immunity is offered by even low to moderate amounts of antibodies and this occurs regardless of whether a seropositive HCW had previous asymptomatic or symptomatic infection. These findings have significant implications in establishing the protective role of anti-spike protein antibodies against subsequent infection.

Keywords: Coronavirus disease 2019, Immunity, Immunogobulin G, Severe acute respiratory syndrome coronavirus 2, Serial titres

INTRODUCTION

Coronaviruses are a group of enveloped positive-sense single stranded RNA viruses constituting the subfamily Orthocoronaviridae within the Nidovirales order. They are further divided into four genera: Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus [1]. In December 2019, a novel betacoronavirus causing severe acute respiratory syndrome emerged in Wuhan, China [2]. The virus, designated as SARS-CoV-2, unleashed a pandemic which has wreaked havoc on healthcare systems across the world. Globally, as of 9th August 2021, there have been 202,608,306 confirmed cases of COVID-19, including 4,293,591 deaths, reported by the World Health Organisation (WHO) [3].

The reasons for immunity to seasonal human coronaviruses being short in duration have not been fully elucidated. Reinfections have been observed with three of the four seasonal human coronaviruses (i.e., 229E, NL63, and OC43). Antibodies to SARS-CoV-2 and Middle East Respiratory Syndrome Coronavirus (MERS-CoV) were detectable until two years after infection but were reduced when re-testing was done within three years [4,5]. Large populations have demonstrated antibodies to the SARS-CoV-2 virus due to previous infection and speculations are that we are slowly inching towards 'herd immunity' [6-8]. With the advent of several subsequent waves of the pandemic and emergence of new variants of the virus in several countries, communities are not only at a greater risk of infection but also reinfections [9-13]. Despite there being widespread media reports and several observations made by clinicians with respect to a surge in reinfection cases in the past year, the number of published studies validating the same are far and few in between [14-17]. Existing knowledge on SARS-CoV-2 immune memory is limited [18,19]. Filling the gaps in our understanding of immune response has implications for the future course of the pandemic, the safety of frontline HCWs and the success of vaccination programs. Longitudinal studies to gain an insight into temporal kinetics of antibody responses in asymptomatic, mildly symptomatic, and severely ill populations are the need of the hour.

There are hardly any cohort studies published from India that have investigated serial antibody titres in HCWs and their role in the protection against subsequent infections, although few have recently shed light on their duration and correlation with severity of infection [20]. The present study was conducted with the aim to perform a temporal analysis of anti SARS-CoV-2 IgG titres in a cohort of HCWs so as to understand the dynamics of antibody response, duration and magnitude of protection afforded, and association of disease severity with these parameters.

MATERIALS AND METHODS

An observational longitudinal cohort study was conducted to analyse the trend of antibody response in HCWs over a follow-up period of six months from June 2020-December 2020. The study was conducted at Medanta- The Medicity, Gurugram, Haryana, a 1250-bedded tertiary care centre in North India. There was no predefined sample size; participants volunteered for enrollment. Informed consent was obtained from all participants and Ethics Committee approval was duly taken {vide letter no. MICR-1136/2020 (Academic)}. The study was conducted in accordance with the Helsinki Declaration of 1975 and registered with the Clinical Trials Registry- India (CTRI/2020/07/026837).

Inclusion and Exclusion criteria: A total of 230 HCWs were included in the study. Serum specimens were collected from the study subjects and tested for antibodies to SARS-CoV-2. Furthermore, serial serum samples were collected only from individuals with previous RT-PCR confirmed SARS-CoV-2 infection (n=47) at four separate visits viz., first sample collected at (15-45) days after testing PCR positive (Day-0), second sample was collected between 46-90 days, third sample was collected between 91-135 days and fourth sample was collected between 136-180 days. Individuals who did not report having had PCR-confirmed infection were not included in the longitudinal cohort. However, they were tested for the presence of baseline antibodies.

Study Procedure

Antibody titres and the duration of their persistence were associated with severity of previous COVID-19 illness. The medical records of the participants were classified into asymptomatic (n=18), mild disease (n=17) and moderate disease (n=12) categories based on the severity of previous COVID-19 illness as per the Ministry of Health and Family Welfare- Government of India guidelines [21]. None of the HCWs reported symptoms which qualified them as having had severe disease.

The individuals who were categorised as having had asymptomatic infection, were high risk contacts of confirmed SARS-CoV-2 PCR positive cases and were tested as part of the institutional COVID-19 contact tracing protocol.

The LIAISON® Immunodiagnostic Anti-SARS-CoV-2 assay for IgG detection was used. The test was performed on LIAISON® XL Analyser (DiaSorin S.p.A., Saluggia, Italy). It uses a Chemi-Luminescence Immunoassay (CLIA) technology for the determination of anti-S1 spike protein and anti-S2 spike protein specific IgG antibodies to SARS-CoV-2 (further referred to as anti SARS-CoV-2 antibodies) in human serum or plasma samples. Strict adherence to the analyser operator's manual was ensured for proper assay performance. The analyser automatically calculates anti SARS-CoV-2 IgG antibody concentrations expressed as arbitrary units (AU/mL) and grades the results. Assay range is 3.8-400 AU/mL as per the manufacturer. Sample results were interpreted as follows: values of less than 12 AU/mL were negative, 12-15 AU/mL were considered equivocal and values greater than 15 AU/mL were positive.

In the following six months, disease outcome (COVID-19 infection) was documented whenever any HCW reported symptoms consistent with Influenza-Like Illness (ILI) and tested positive for SARS-CoV-2 by RT-PCR. Nasopharyngeal swabs were collected for this purpose and viral RNA extraction was done using Maxwell® RSC TNA kit on Maxwell® 16 instrument (Promega Corporation, Wisconsin, USA). For PCR, TaqPath TM COVID-19 CE-IVD RT-PCR Kit (Thermo Fisher Scientific Inc., Massachusetts, USA) was used with Quant StudioTM 5 Dx Real-Time PCR instrument (Applied Biosystems, California, USA) as per manufacturer's instructions.

STATISTICAL ANALYSIS

Statistical analysis was done using SPSS version 22.0. Descriptive analysis of categorical variables was depicted as frequency (percentage) and quantitative variables were described as mean±SD (parametric data) and median (interquartile range) for non parametric data. Normality was assessed using Shapiro-Wilk test. Intergroup comparison of means was done using Kruskal Wallis test (for non parametric data), one-way ANOVA (for parametric data) and Chi-square test (for categorical variables). $p<0.05$ was considered statistically significant. Incidence rate was calculated as number of PCR positive infection per at-risk day according to baseline antibody status.

RESULTS

A total of 230 healthcare workers were included in this longitudinal cohort study and underwent an assessment of baseline anti SARS-CoV-2 antibody titres. The gender-wise distribution of the study population comprised of 63.9% males (n=147) and 36.1% females (n=83). The number of participants who tested negative for baseline SARS-CoV-2 antibodies (henceforth, referred to as seronegative) was 171 (171/230=74.3%), while 59 (59/230=25.7%) were found to have antibodies above the cut-off value (henceforth referred to as seropositive).

Five HCWs had RT-PCR confirmed COVID-19 infection but did not have IgG antibodies even after 30 days had lapsed since the PCR test i.e., seroconversion did not occur. These individuals were not followed-up. Of the 59 baseline seropositive HCWs, 49 had previous history of COVID-19 infection in the one month preceding their enrollment in the study. Amongst these individuals, three were lost to follow-up. Ten seropositive HCWs (10/59=16.9%) had no history of ILI or PCR-confirmed SARS-CoV-2 infection. From these, one HCW was included in the follow-up group when he reported ILI symptoms three months later and was confirmed to have COVID-19 by RT-PCR. So, in essence, the longitudinal cohort (n=47) consisted of 46 seropositive HCWs who had a history of COVID-19, and one baseline seropositive HCW who was included midway into the study [Table/Fig-1].

Parameters	Baseline seropositive (n=59)	Baseline seronegative (n=171)	Total (n=230)
RT-PCR positive	49	5	54
RT-PCR negative	10	166	176
Total	59	171	230

[Table/Fig-1]: Baseline IgG antibody status amongst RT-PCR positive vs RT-PCR negative HCWs.
The Chi-square value was 156.75; p-value was <0.001*; Significant at p<0.05; RT-PCR: Real-time polymerase chain reaction; HCWs: Healthcare workers

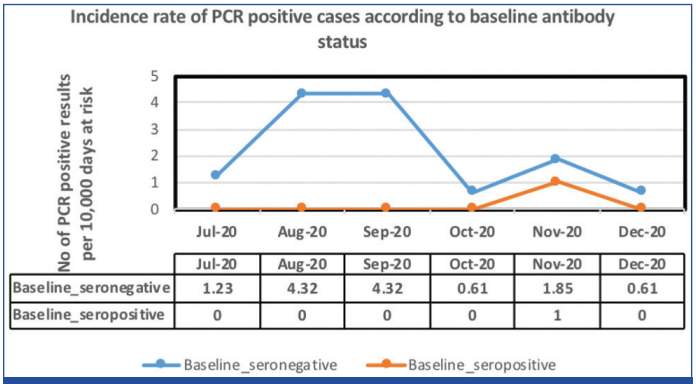
Rest of the baseline seropositive HCWs did not report any symptoms of COVID-19 or tested positive for SARS-CoV-2 RT-PCR at any point of time in the follow-up period. Seroconversion rate for anti SARS-CoV-2 IgG antibodies amongst participants with history of COVID-19 was 90.74% (49/54) after atleast 30 days of testing positive by RT-PCR. Of the 171, baseline seronegative HCWs, 21 became symptomatic and tested positive by RT-PCR on follow-up. As mentioned earlier, only one person from the seropositive group had a subsequent PCR-confirmed infection. Incidence of PCR positive SARS-CoV-2 reinfection cases were inversely associated with baseline seronegative status i.e., titres below the cut-off threshold (p<0.05) [Table/Fig-2].

Baseline antibody status	Presence of ILI and PCR positive outcome in follow-up period	Absence of ILI and/or PCR negative outcome in follow-up period	Total number of cases
Seropositive	1 (1.6%)	58 (98.4%)	59
Seronegative	21 (12.3%)	150 (87.7%)	171
Total number of cases	22	208	230

[Table/Fig-2]: Association of baseline antibody status with PCR outcome on follow-up of six months.
Chi-square value=5.682, p=0.018*

- Incidence in seronegative group: 21/16195 cases per person days=12.96 per 10,000 person days
- Incidence in seropositive group: =1/7743 cases per person days=1.29 per 10,000 person days
- Incidence rate ratio=Incidence in seropositive/Incidence in seronegative=1.29/12.96=0.099

Risk ratio for infection based on antibody status (baseline seronegative vs baseline seropositive) was determined to be 8.12 (95% CI 1.068-61.755). For PCR positive individuals it was 0.138 (95% confidence interval 0.019-1.004) while for those who were COVID-19 naïve, it was 1.121 (95% CI 1.05-1.196). This implies that seronegative HCWs were at eight time higher risk of getting infected with SARS-CoV-2 as compared to seropositive individuals. Incidence varied by calendar time and reflected a consistently higher incidence in the seronegative HCWs. A peak in cases was noted during August-September 2020 and expectedly coincided with the first wave of the pandemic in India which occurred post the relaxation of lockdown regulations from June 2020 onwards [Table/Fig-3].



	Jul-20	Aug-20	Sep-20	Oct-20	Nov-20	Dec-20
Baseline_seronegative	1.23	4.32	4.32	0.61	1.85	0.61
Baseline_seropositive	0	0	0	0	1	0

[Table/Fig-3]: Graphical representation of incidence of PCR positive cases in HCW over a follow-up period of six months.

The follow-up cohort of 47 HCWs were tested for anti SARS-CoV-2 IgG antibody titre at four follow-up visits viz., 1st sample was collected 15-45 days after testing PCR positive (day-0), 2nd sample was collected between 46-90 days, 3rd sample was collected between 91-135 days and 4th sample was collected between 136-180 days for only 14 HCWs as the rest were lost to follow-up for reasons like refusal to give further samples, job attrition etc. Mean age of this set of participants was 37±11 years. Mean follow-up duration was 124±31 days. The gender distribution consisted of 26 males and 21 females.

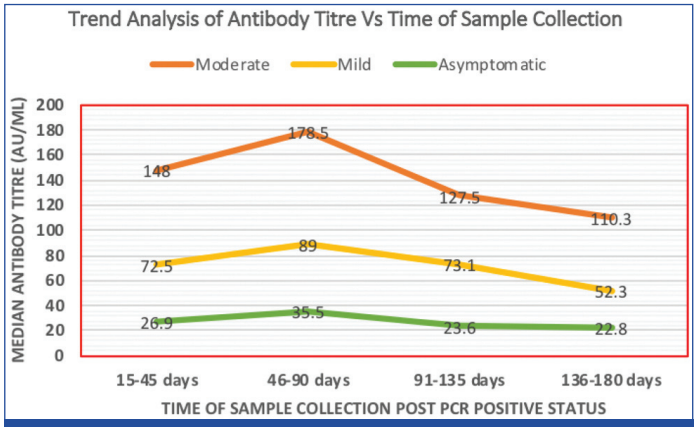
We compared antibody titres against the severity of past infection to assess the differences in immune responses in each category. We observed a positive relation between increases in the geometric mean titres of IgG for all four serial samples versus the severity of previous infection i.e., more was the severity of symptoms more was the antibody response [Table/Fig-4]. Antibody response trend showed a peak in the mean titres in the 46-90 days period followed by a steep decline till 135 days and a gradual waning thereafter [Table/Fig-5]. Notably, only two HCWs (from the asymptomatic HCW category) demonstrated complete seroconversion below the cut-off value of 15 AU/mL over the course of 180 days of follow-up.

DISCUSSION

The SARS-CoV-2, a human betacoronavirus, first reported from Wuhan, China in December 2019, has emerged as the latest viral pneumonia pandemic engulfing more than 200 countries. The HCWs are a particularly high-risk group for infection due to constant exposure to the pathogen. A recent meta-analysis of 11 studies found that the proportion of HCWs who were SARS-CoV-2 positive among all COVID-19 patients were 10.1% [22]. In India, a case-control study conducted by the Indian Council of Medical Research COVID-19 team analysed data of over 23,000 symptomatic HCWs and found the SARS-CoV-2 infection prevalence rate of 5% [23].

Parameters	Asymptomatic (n=18)	Mild disease (n=17)	Moderate disease (n=12)	*p-value
Follow-up duration (days)	112±27	129±30	134±33	0.221
Age (years)	35±9	38±8	38±16	0.711
Mean antibody titre (AU/mL) First visit (15-45 days)	26.9 (18.6-34.2)	72.5 (60.6-80.1)	148 (127-193.5)	<0.001
Mean antibody titre (AU/mL) Second visit (46-90 days)	35.5 (28.6-41)	89 (80.2-109.5)	178.5 (156-206)	<0.001
Mean antibody titre (AU/mL) Third visit (91-135 days)	23.6 (19.2-31.3)	73.1 (38.1-86.4)	127.5 (99-55.7)	<0.001
Mean antibody titre (AU/mL) Fourth visit (136-180 days)	22.8 (20-23.5)	52.3 (25.4-83.7)	110.3 (94.6-36)	<0.001

[Table/Fig-4]: Intergroup comparison of median antibody titre.
*p-value <0.05 statistically significant using Kruskal Wallis test of significance



[Table/Fig-5]: Longitudinal trend of anti-SARS-Cov-2 IgG antibody titres.

Another report from Mumbai found the prevalence of COVID-19 amongst asymptomatic and previously symptomatic HCWs to be 4.3% and 70%, respectively [24]. Most studies from India have focused solely on examining sero-prevalence of SARS-CoV-2 and epidemiological risk factors for infection in HCWs. A cohort study published from Chennai, India demonstrated an average duration of 104 days for persistence of IgG antibodies and a positive correlation with presence of symptoms. However, antibody levels of study subjects were tested on only one occasion [20].

A priori, we wanted to further investigate the role of protective immunity in a hospital setting. Hence, a longitudinal study to map the SARS-CoV-2 anti S1/S2 IgG antibody levels of study subjects at multiple occasions and their durability was planned in a cohort of HCWs. The observed seroconversion rate for IgG antibodies was expectedly above 90% after about 30 days of diagnosis of infection. In a report from China, serial samples from 63 patients were assessed, out of whom 97% sero converted for both IgM and IgG antibodies with median days of seroconversion after symptom onset being 13 days for both antibodies [25].

In another case-control report examining 77 samples to compare immune responses to the Receptor-Binding Domain (RBD) of the spike protein it was found that nine days after symptom onset, 98% of patients had a positive IgG response with a specificity of 100% [26]. Zhao J et al., reported a sero conversion rate of 93.1% and 64.7% for IgM and IgG antibodies respectively. Seroconversion for IgG occurred after 14 days [27]. In the present study, 45 HCWs of the follow-up cohort had consistently high values above the cut-off threshold till at least 180 days from day-0, while seroreversion occurred in only two individuals.

Similar observations were made in a multicentric longitudinal study conducted in India where no fall in anti-RBD IgG titers was reported after at least 10 weeks of follow-up. This is an ongoing project where more prospective data is yet to be published [28]. In stark contrast to our data, a multicentric report from USA found that out of 156 HCWs who were tested for serial titers of SARS-CoV-2 antibodies at an interval of approximately 60 days, 146 (93.6%) had a decline in antibody levels, and 44 (28.2%) had a decline to levels below the threshold for positivity, thus showing complete seroreversion [29].

In the present cohort, mean antibody titres peaked between 46-90 days and there was biphasic decay thereafter. The antibody levels declined steeply during the time-gap between second and third samples followed by a slower rate of decay. A mathematical model has been proposed for analysing antibody kinetics by the utilisation of prior data from other coronaviruses. There searchers predict that antibody responses peak within 2-4 weeks after symptom onset, followed by rapid decay in the first 3-6 months and an estimated one-year period for 60% decay from the peak response [30]. A comprehensive report on immune memory against SARS-CoV-2 was published where the dynamics of memory B cells, CD 8+ cells and CD 4+ cells was analysed over eight months. It was observed that immune memory persisted in most subjects for longer than five months after infection. The investigators also observed persistent anti-spike IgG antibodies from 20-240 days post symptom onset and a half-life of 140 days [31]. In another report from China, it was found that anti nucleoprotein antibodies persisted till 194 days in in-patients [32]. These findings are contrary to one of the reports on antibody durability, where half-life was found to be only 36 days in a cohort of community patients [33].

The present study reported that antibody titres increased proportionately with severity of previous infection. Similar findings have been reported in a cohort of 56 HCWs by Birch T et al., [34]. Another study from Bangladesh reported that mildly symptomatic patients developed IgM and IgA responses by day 14 in 72% and 83% of individuals, respectively, while 95% of individuals developed IgG response, and rose to 100% by day 30. However, asymptomatic infected individuals with SARS-CoV-2 developed antibody responses significantly less frequently, with only 20% positive for IgA and 22% positive for IgM by day 14, and 45% positive for IgG by day 30 after infection [35].

Tan W et al., have also demonstrated that in a longitudinal cohort of 67 patients, IgM and IgG titres were significantly higher in patients with severe symptoms than those who had milder symptoms. Also, patients with weak IgG antibody response had a faster viral clearance versus patients with strong antibody response who had delayed viral clearance [36]. In a bid to explain this phenomenon, researchers analysed anti-RBD antibodies of severely ill COVID-19 patients and demonstrated a unique serologic signature where increased IgG1 antibodies were seen with distinct post-translational modification (i.e., reduced fucosylation). This enhances Fc receptor (fragment crystallisable) binding and in turn augments effector functions of innate immunity including inflammatory cytokine production [37]. Five HCWs who had prior history of COVID-19 but tested negative for baseline IgG antibodies may have seroconverted later. However, they were not followed-up for their subsequent antibody status. Interestingly, all of them had had an asymptomatic infection. Even low to moderate amounts of anti-spike protein antibodies seemed to offer significant postinfection immunity. The present study found the seronegative cohort to be at eight times higher risk for acquiring infection than those who had IgG antibodies. Furthermore, whether the HCW had a previous asymptomatic or symptomatic infection, it had no bearing on protective immunity as only one subsequent symptomatic reinfection was seen in the seropositive group. This finding is corroborated by very similar observations made by the researchers of Oxford University Hospitals. The study cohort comprised of 11,364 HCWs who tested negative for baseline antibody results and 1265 who tested positive for baseline antibody results. The latter group included 88 HCWs who seroconverted in the follow-up period. The incidence of SARS-CoV-2 infection was 1.09 per 10,000 days at risk amongst seronegative HCWs versus 0.13 per 10,000 days at risk for the seropositive group (adjusted incidence rate ratio, 0.11; 95% confidence interval, 0.03 to 0.44; p=0.002) [38].

Limitation(s)

A drawback in the evaluation of possible reinfections in the present study could have been that it was based solely on self-reporting of ILI symptoms by the HCWs and not screening by regular PCR tests. Nonetheless, the difference in number is gross and cannot be attributed to asymptomatic reinfections alone. Other limitations in the report may be that some individuals in the baseline seronegative group with previous COVID-19 illness may have had antibody levels that might still have been rising and hence, missed the signal threshold limit. The study cohort was derived from a convenience sample that consisted only of a limited number of HCWs, which might result in non representativeness. The follow-up duration was also limited to six months.

CONCLUSION(S)

Data from reports including the present study are encouraging and indicate that immune memory has a role in preventing transmission of infection. Mass vaccination (and its success) remains a distant dream for several poor nations and large swathes of vulnerable populations across the world. As such, studies that analyse natural humoral and cellular immunity especially in high-risk groups like HCWs are imperative to assess the magnitude and duration of protection from reinfection, differences in asymptomatic and symptomatic disease, and the effect of herd immunity on disease transmission. Such sero-epidemiological research can be extrapolated to larger population groups and will go a long way in instituting robust public health interventions.

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PARTICULARS OF CONTRIBUTORS:
1. Senior Resident, Department of Microbiology, Medanta- The Medicity, Gurugram, Haryana, India.
2. Associate Consultant, Department of Microbiology, Medanta- The Medicity, Gurugram, Haryana, India.
3. Associate Director and Head, Department of Microbiology, Medanta- The Medicity, Gurugram, Haryana, India.
4. Attending Consultant, Department of Biochemistry, Medanta- The Medicity, Gurugram, Haryana, India.
5. Lab Manager, Department of Biochemistry, Medanta- The Medicity, Gurugram, Haryana, India.
6. Senior Consultant, Department of Microbiology, Medanta- The Medicity, Gurugram, Haryana, India.
7. Director, Department of Lab Medicine, Medanta- The Medicity, Gurugram, Haryana, India.
8. Chairman, Department of Anaesthesia and Critical Care, Medanta- The Medicity, Gurugram, Haryana, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Smita Sarma,
Associate Director and Head, Department of Microbiology, Medanta- The Medicity, Sector 38, Gurugram, Haryana, India.
E-mail: sarma.smita@rediffmail.com

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