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PERSPECTIVE ARTICLE

SARS-CoV-2 antigenic diversity and role of passive surveillance in the control of COVID-19

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ABSTRACT
Many countries are engaged in making vaccine against COVID-19 as the world records more than 38 million SARS-CoV-2 infections and more than one million deaths. The infection raging through communities is expected to have evoked some degree of immunity in many asymptomatic and recovered individuals. However, the level of protective immunity and duration of such immunity have not been studied in depth. At the same time, spanning from the conventionalwhole virus vaccine to recombinant vaccines using Adenovirus vectors and first-of-its kind mRNA vaccines are in human trials. Before the effectiveness and safety of such vaccines are established billions of doses have been produced and stockpiled to save time in production and distribution. Antigenic diversity and the potential role of passive surveillance in COVID-19 regulation are explored in this report.
***Keywords**: COVID-19; Structural Proteins; SARS-CoV-2; Receptor Binding Domain.*

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INTRODUCTION

It is significant that RNA viruses evolve in infected hosts leading to a highly diverse pool of viral genome, and it poses challenge to control and eradication of the pathogen. Increasing detection of genomic divergence in circulating SARS-CoV-2 strains indicate possible existence/ generation of antigenic variants of different magnitude that may compromise efficacy of monovalent vaccines in controlling COVID-19. Further, differential virulence/ pathogenicity/ transmissibility is always a possibility as variations/ mutations in the nucleotide sequence of different structural and non-structural regions of SARS-CoV-2 strains are known to occur.

Ultimately, the pandemic and post-pandemic scenario will be influenced by the duration of immunity to COVID-19. After more than eight months into the pandemic, the level of protection following natural

infection is not known. Similarly, although different phases of clinical trials have generated data on neutralizing antibodies and protective immunity in different regimens and vaccines, we are far from giving a verdict on the length of protective immune response after such vaccinations in different geographies. In countries like India, the severity of disease and mortality has been attributed to non-specific and differential innate immunity in the population. It is expected that vaccine efficacy will be higher in those countries with high seroprevalence of virus- specific antibodies against structural proteins (SPs). These countries will also attain herd/ population immunity faster. After COVID-19 vaccination is introduced, the effect of vaccination needs to be assessed in terms of protective antibodies elicited, and to differentiate vaccination immunity from immunity due to natural infection in the target population. Therefore, appropriate companion diagnostics are required to monitor vaccinal immunity and virus transmission, and also to differentiate vaccinated people from those infected or superinfected.

CORONA VIRUSES AND SARS-CoV-2

Coronaviruses (CoVs) are known to cause disease in humans, animals and birds. In humans, CoVs cause respiratory tract infections. The zoonotic CoVs of SARS, MERS and COVID-19 belong to the genus β-CoV. The SARS-CoV-2 virion consists of spike (S) glycoprotein, envelope protein(E), dimeric hemagglutinin-esterase (HE) enzyme, membrane glycoprotein (M), nucleocapsid protein (N) and a positive sense single stranded RNA genome [1]. Antibodies directed against receptor binding domain (RBD) within the S1 subunit (of Spike glycoprotein) strongly correlate with virus neutralization [2, 3]. The positive sense and single stranded RNA genome of CoV that directly functions as mRNA results in rapid infection of the exposed host. There is lack of comprehensive information on how the CoVs shift host and cause zoonoses [4]. Natural adaptations involving viral receptors can alter host and tissue tropism[4]. Evolution of RNA viruses in infected hosts is well documented. SARS–CoV-2 genome in infected people could be highly diverse as observed in other RNA viruses [5]. Variation/mutations of SARS-CoV-2 following infection, may affect its virulence, infectivity, antigenicity and transmissibility [6, 7], though no hot spot genes have been detected for this [7].The mutation rate observed in SARS-CoV-2 is of the same magnitude as in SARS-CoV [8]. The high diversity in circulating virus population make it difficult for elimination [9]. In silico analysis for nucleotide variations in annealing sites of all available primer-probe sets targeting the E, N, and RdRp genes of South American SARS-CoV-2 sequences revealed that E gene is highly conserved compared to the other two [10]. Natural mutations in the S protein may result in increased infectibility of the virus during transmission [11]. In view of these, it is necessary to monitor SARS-CoV-2 evolution in real time by strengthening active surveillance in the population and recording associated clinical changes [7]. Regular passive surveillance targeting antibodies to structural proteins (SPs) and non-structural proteins (NSPs) of the virus is essential in monitoring virus spread/ elimination. Currently, 216 countries are affected with the pandemic, and there has been gradual increase in daily number of COVID-19 cases since April 2020. In India, a survey of 30,283 households estimated a cumulative 6,468,388 adult infections in India by the early May 2020 [12], that indicated low seroprevalence (< 1%) of SARS-CoV-2 infection among the adult population in India during that time. However, this situation must have changed as India on 13th October 2020 stands second in total number of COVID-19 cases (7,237,082) with 1,10617 deaths (https://www.worldometers.info/coronavirus/). Looking at the steady progress in the transmission of SARS-CoV-2 virus resulting in increased detection of human cases, the sero-prevalence of the disease is silently expanding day after day in affected countries. The level of convalescent and vaccinal immunity will determine extent of virus transmission in the population and clinical severity of COVID-19 [13]. Both IgM and IgG antibodies are detected five days after onset of clinical symptoms [14, 15]. Median seroconversions for IgM and IgG has been recorded between 10-14 days [16, 17]. There are many COVID-19 antibody assay kits in use [12, 18]. These tests use recombinant structural proteins of the virus, viz., RBD antigen and Nucleocapsid (N) antigen. An IgG capture ELISA using Vero CCL-81 cell antigen was developed in India for serological detection of anti-SARS-CoV-2 IgG in serum samples [19]. However, there is no assay system to monitor antibody response to non-structural proteins (NSPs) of the virus. Antibodies to NSPs indicate virus infection, and will be helpful in sero-surveillance of the virus in vaccinated areas. In view of current information on epidemiology of COVID-19 pandemic, the necessity of regular antigenic surveillance to maintain efficacy of the vaccine, and anti-NSP antibody surveillance to monitor elimination of virus from immunized/ vaccinated population is highlighted and discussed in this article.

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THE VIRUS AND VARIATIONS IN STRUCTURAL PROTEINS (SPs)

The CoVs infecting mammals and avian species belong to family Coronaviridae (ICTV). The four genera are α-, β-, γ- and δ-CoV. The genera α- and β-CoV infect mammals, while the genera δ- and γ-CoV infect avian and certain mammals. The SARS-CoV-2 belong to the genus β-CoV and is zoonotic, causing respiratory disease (COVID-19) in humans [20]. The β-CoV genus has lineages A, B, C and D. The lineage B comprises of SARS-CoV and SARS-CoV-2, whereas MERS-CoV belongs to the lineage C. Human infectious CoVs belong to seven species, two α-CoV and five β-CoV. Genetically, SARS-CoV-2 is closely related (79% homology) to SARS-CoV of 2003, but distinct (50% homology) from MERS-CoV of 2012 [21]. The structure of SARS-CoV-2 includes four structural proteins viz., S, E, M, and N [22]. The HE (hemagglutinin esterase) glycoprotein is unique to *Embecovirus* subgenus of β-CoV [23].

The spike protein (S; ORF2) mediates receptor binding and membrane fusion and thus crucial for host tropism and virus transmission; and has two domains, S1 and S2. The S1 domain is involved in host cell receptor recognition and binding, whereas S2 domain is involved in triggering viral fusion with the host cell membrane. There is higher prevalence of SARS-CoV-2 strains (clade A2a) with substitution of aspartic acid (D) to Glycine (G) at the position 614 in the S protein, compared to the Wuhan strain[24], [25]. This clade of the virus is prevalent worldwide, and resistant to cleavage [24][4]. Presence of A2a clade that is characterized by $P^{323}L$ in RNA-dependent RNA polymerase and $D^{614}G$ in the S_D domain of the S glycoprotein has also been detected in India [26]. There were also novel variants in India having mutation of $G^{1124}V$ in the S2 gene [25, 26]. In the RBD, 11 non-synonymous mutations were observed adjacent to the ACE2 binding site. The Mab HA001 identified A^{475} and F^{486} in the SARS-CoV-2 RBD, representing new binding sites for neutralizing antibodies [11].

It was reported that the receptor-binding motif (RBM) is the main functional region in RBD that binds to human ACE2 receptor [11]. The authors demonstrated that binding of RBM motif of SARS-CoV-2 with human ACE2 receptor was positively influenced by amino acid residues of P, Q, F, A and L at the positions 499, 493, 486, 475 and 455, respectively. The RBD induces clade- specific virus neutralizing antibodies, and there is weaker cross neutralization between SARS-CoV and SARS-CoV-2.Data indicated that six non-synonymous mutations in the RBD of SARS-CoV-2, viz., N^{439}/R^{426}, L^{452}/K^{439}, T^{470}/N^{457}, E^{484}/P^{470}, Q^{498}/Y^{484} and N^{501}/T^{487}, led to enhanced binding affinity of the virus to human ACE2 receptor. These natural mutations in the S protein are helpful in monitoring the increased infectibility of the virus during transmission [11]. Several mutations in the Spike protein have been detected globally [27]**.** Novel variants having $R^{203}K$, and $G^{204}R$ in SR-rich region of the nucleocapsid (N) gene, involved in the viral morphogenesis, have been reported in India [26]. The N (nucleocapsid) protein is also immunogenic, and induces antibodies earlier than S glycoprotein during infection, making it suitable for diagnostic assay [28]. Nucleotide sequence homology of SARS-CoV-1 at the N and S genes with other Betacoronaviruses (MERS and COVID-19) is 33-47% and 29%, respectively, while sequence homology to Alphacoronaviruses is much lower (25-29% homology at N and 23-25% for S). SARS-CoV-2 is much similar to SARS-CoV-1 at the N and S genes, having sequence homology of 90% and 76%, respectively, compared to MERS-CoV (48% and 35%, respectively [29]. Therefore, these 2 proteins when used in ELISA for SARS-CoV-2 antibody assay, are likely to have some level of cross reactivity [13, 30, 31].

NON-STRUCTURAL PROTEINS (NSPs) OF SARS-CoV-2

NSPs coded by viruses helps in their replication. These proteins are immunogenic and produced before the synthesis of structural proteins. The RNA genome codes for the polyproteins 1a and 1ab that act as replicase. The viral proteases generate up to 16 non-structural proteins (NSPs), NSP1 to NSP16, from ORF1ab [32]. NSP1 degrades cellular mRNAs and inhibit host protein synthesis. NSP2 recognizes prohibitin. NSP3 is a transmembrane protein and has Papain- like protease activity. NSP4 is a transmembrane scaffold protein. NSP5 carries a 3- Chymotrypsin- like (3CL) protease domain required for polyprotein processing. NSP6 is also a transmembrane scaffold protein. The NSP7 and NSP8 proteins help in RNA polymerisation as a primase enzyme. NSP9 is an RNA-binding protein that activates exoribonuclease and 2-O-methyltransferase activity. NSP10 forms heterodimer with NSP16 and NSP14. NSP12 functions as RNA polymerase (RdRp), whereas NSP13 has RNA helicase and triphosphatase activities. NSP14 functions as N7 methyltransferase and exoribonuclease. NSP15 acts as a endoribonuclease. NSP16 has also 2-O- methyltransferase activity. Viral exoribonuclease provides proofreading function in SARS–CoV and SARS-CoV-2 [7, 33, 34]. In silico analysis of the viral polymerase and 3-chymotrypsin-like cysteine protease (3CL-Pro) that play key role in pathogenicity of SARS-CoV-2 revealed that Wuhan SARS-CoV-2 genome shares > 96% and 86% identity with bat-CoV and pangolin-CoV, respectively. This analysis revealed novel mutations in South American and African SARS-CoV-2, and differentiated them [35]. A 382-nucleotides deletion in ORF8 (deletion mutant) was found in a virus

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who had a travel history to Wuhan [36]. The polyprotein 1ab (pp1ab) gene located at the 5' end of the SARS-CoV-2 genome is a virulence factor and inhibits host gene expresion [37]. Comparison of 144 SARS-CoV-2 isolates around the world, revealed no change in the viral pp1ab in most of the isolates throughout the outbreak time; however, a deletion of 8 amino acids (32 to 39 aa;32GDSVEEVL 39; GISAID database) was found in the virulence factor NSP1 of a virus isolated from a patient without critical symptoms. Compared to other β -coronaviruses, a 42 amino acid signature of "DSQQTVGQQDGSEDNQTTTIQTIVEVQPQLEMELTPVVQTIE" was present only in SARS-CoV-2. This signature is located between the amino acids 983 and 1024 of pp1ab that corresponds to the N-terminal of papain-like protease. Seven mutations in RdRp (RNA dependent RNA polymerase enzyme) of Indian isolates of SARS-CoV-2 have been recorded [38]. It is proposed that these mutations might have functional consequences. Antibodies elicited in the body by the NSPs can be targeted to differentiate between immunized and naturally infected humans. In Foot and Mouth Disease (FMD), a disease of livestock (bovines, sheep, goats, pigs) caused by a Picornavirus with an RNA genome sense similar to SARS-CoV-2, individual NSPs, such as 3D, 2C and 2B, as well as polypeptides, such as 3AB and 3ABC have been used for differentiation of infected and vaccinated animals (DIVA) [39-42]. Currently, available commercial COVID-19 kits detect antibodies to the structural proteins of the virus, viz., RBD and Nucleocapsid protein [18]. Monitoring of antibodies to some of the major NSPs, viz., NSP3 (Papain- like protease), NSP 5 (3-Chymotrypsin- like protease), NSP 12 (RdRp), and NSP 13 (Helicase), will be helpful in assessing degree of virus elimination from immunized population. Possible super infection of immunized population can be identified by presence of anti-NSP antibodies. These antibodies can also be targeted to identify asymptomatic individuals. The rate of asymptomatic infection with COVID-19 has been reported from 4% to 80% across different populations and exposure scenarios, and therefore, seroprevalence studies will help in establishing a more accurate estimate of the number of infected individuals at regional, national and global level [32, 43-45], identified 116 unique variants/ mutations; 8782C > T in ORF1ab gene, ^{28144}T > C in ORF8 gene and ^{29095}C > T in the N gene, and many noncoding mutations in 5′ UTR or 3′ UTR regions of the virus. The NSP3 region had more variants in the analysed samples. The NSP3 is a papain-like protease associated withCoV infection [46, 47]. Application of anti-NSP antibody detection assays will help in assessing and monitoring the effect of vaccination in disease control.

CONCLUSIONS AND PREPAREDNESS

Vaccination strategy for disease control depends on extent of prevalence of the infection in the susceptible population, and antigenic nature of circulating virus strains. There are more than 10 genetic types of the virus identified in the World. The incubation period of COVID-19 varies from 2 – 14 days, more commonly 4-5 days after virus exposure. Affinity of antibodies to antigens increases over time since sensitization. High-affinity antibodies cause virus neutralization by binding to specific epitopes.Antibodies against both S and N proteins appear as early as day 9 onwards and correlate with virus neutralizing response, suggesting seroconversion may lead to protection for at least sometime. The duration and nature of immunity against SARS-CoV-2 infection is not yet known. Other human β- CoVs have immunity lasting for only one year, and there could be some amount of cross-reactivity/ cross immunity between different human CoVs. Both IgM and IgG have been detected as early as 5 days post clinical symptom. Median seroconversion for IgM and IgG has been recorded between 10- 14 days post sickness. Vaccine efficacy is likely to be higher in countries with high seroprevalence, leading to faster herd immunity. As the disease has emerged since only less than one year, there has been limited information on its seroprevalence. In India, number of cases are higher in states with higher population density. The R0 value has been between 1.55 to 3.0, with mean incubation period of 5.2 days. Looking at the gradual increase in number of new cases, we may reach a point soon where sero- prevalence crosses 50% mark. Extent of the infection and rate of its spread can be better estimated, compared to nucleic acid tests and antigen tests, by assays targeted at antibodies against both SPs and NSPs of the virus. There is also a need for continuous vigilance for the emergence of new variants/ strains. Also, there is a need to develop appropriate serological assays to monitor virus activity in the population. Regular serological assays targeted at antibodies against SPs and NSPs of SARS-CoV-2, can identify individuals infected by the virus in the past, and differentiate from those vaccinated, to assess the extent of transmission of the virus in a population, duration of vaccinal immunity, identify super infection, and monitor possible antigenic variations. It is not premature to consider non-specific antibody response to BCG (and trained immunity) , prior exposures to Malaria, and helminth infections in countries such as India and those in South Asia and Sub-Saharan Africa. Such antibodies may already provide a degree of protection against SARS-CoV-2. To the contrary, existing antibodies against other circulating Corona viruses and asymptomatic

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individuals recently infected with Cov-2 raise serious red flags in the context of antibody mediated enhancement of disease and hypersensitivity reactions. SARS and MERS Corona viruses primarily infect respiratory parenchyma not macrophages, and hence, reduce the chance of antibody dependent enhancement of disease as seen in Dengue virus. However, challenge with live virus post vaccination has resulted in vaccine hypersensitivity reactions (VAH) in animals, similar to those in humans given inactivated vaccines respiratory syncytial virus or measles vaccines. Such reactions should be taken seriously when considering whole virus vaccines alongside of RNA, DNA-based and recombinant adenovirus-vectored vaccines. Collection of robust longitudinal sero-epidemiological data and long term evaluation of adverse events after introduction of vaccines are essential in deciding safe and cost-effective modalities for control of COVID-19.

DISCLOSURE

Authors declare that they do not have any conflict of interest.

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