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Concept of Serology, Serosurveillance and Herd Immunity in COVID 19

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HUMAN

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ABSTRACT

The new virus named SARS-CoV-2 appeared on this earth in 2019-2020. Today as of 14th June, globally there have been 7,895,352 confirmed cases of Coronavirus Disease 2019 (COVID-19), including 432,881 deaths, reported to WHO. India has 5,65,8614 number of cases with 1,51,432 number of deaths. The cases are still rising and main strategy which helped to contain the disease was to test, trace and isolate. India started testing very early in the course of the disease and geared up with supplementary tests from time to time. RT-PCR for viral RNA detection remains the gold standard for testing. For surveillance purposes in asymptomatic individuals and high risk contacts, serological testing was resorted to. There are number of tests made available for IgM and IgG determination. India also developed an indigenous kit for IgG detection. The kinetics and profile of antibodies in COVID 19 cases has been reviewed. The uses and limitations of serological tests have been discussed in details. Further clarification regarding some concepts of serosurveillance studies and role of herd immunity including re-infections has been reviewed.

INTRODUCTION

After starting of the reporting somewhere in January 2020, cases of Coronavirus Disease 2019 (COVID-19) showed rise till about first week of October when plateau was observed. Till then, globally there have been 7,895,352 confirmed cases of COVID-19, including 432,881 deaths, reported to World Health Organization (WHO).[1] India has 5,65,8614 number of cases with 1,51,432 number of deaths.[2] India had become second worst hit country in the World after USA. Out of the strategies which helped to contain the disease foremost was to test, trace and isolate apart from various preventive strategies like hand washing, social distancing, lockdowns etc. The concept behind this was that not only the symptomatic persons are transmitting the disease but along with them a huge burden of the disease transmission is due to asymptomatic/ pre-symptomatic cases. How to diagnose these? Is there any role of serology? What is the percentage of cases that develop antibodies and what level of protection is achieved by presence of antibodies are few questions which need explanation and lucidity.

The fast spread of COVID-19 has raised concern and panic around the world. The outbreak of COVID-19 first started in Wuhan of China.[3] With a dramatic increase in daily confirmed global cases, the WHO declared a global pandemic on 12th March, 2020.[4] WHO report for the first time, on 'unknown pneumonia patients' in Wuhan, China was flashed in media on 31st December 2019, though now it is being said that the disease could be present in China as early as August 2019. Subsequently, the Chinese Centre for Disease Control and Prevention confirmed a report of identification of a 'novel coronavirus' on 9th January 2020.[5] The detailed description of COVID-19 outbreaks in Wuhan, China was then published.[3] On 30th January 2020, the WHO declared COVID-19 as an international public health emergency.[6] Based on established practice, the new virus was named SARS-CoV-2 by the Coronavirus Study Group of the International Committee for the Taxonomy of Viruses, and the disease it causes as COVID-19 by WHO.[7,8] In India, initially, three cases of COVID-19 (having travel history from China) were reported from Kerala between 27th and 31st January 2020.[9]

VIROLOGY

Coronaviruses (CoVs) belong to the subfamily Orthocoronavirinae in the family Coronaviridae, Order Nidovirales. There are four genera within the subfamily Orthocoronavirinae, namely Alphacoronavirus (α-CoV), Betacoronavirus (β-CoV), Gammacoronavirus (γ-CoV) and Deltacoronavirus (δ-CoV).[10] SARS-CoV-2 is a single

stranded, positive sense RNA virus that belongs to the Coronaviridae family of the *β*-CoV genus. The SARS-CoV-2 genome (30kb in size) encodes a large, non-structural polyprotein (ORF1a/b) that is further proteolytically cleaved to generate 15/16 proteins, 4 structural proteins and 5 accessory proteins (ORF3a, ORF6, ORF7, ORF8 and ORF9).[11] The four main structural proteins are the spike (S), the envelope (E), the membrane (M), and the nucleocapsid (N) proteins.[12] Most antibody based tests are designed to capture antibodies, which recognize either the nucleocapsid (N) protein, the S1 subunit or the Receptor Biding Domain (RBD)of Spike (S) proteins. The N and S proteins are the two major coronavirus immunogens and many non peer reviewed studies have shown that RBD based tests show lower degree of response as S antigen shall possess larger number of epitopes.[13-16] By targeting S protein both cellular and humoral immunity can be developed by inducing neutralising antibodies and by developing protective cellular immunity.[17] Spike protein (S) helps SARS-CoV-2 in binding and entry into cells.[18] S1 subunit of S glycoprotein enables strong binding to the ACE2 receptor while S2 subunit helps in fusion with the host cell.[19] SARS coronavirus (SARS-CoV) and MERS coronavirus (MERS-CoV) are members of Beta coronaviruses.[20] Genome-wide phylogenetic analysis indicates that SARS-CoV-2 shares 79.5% and 50% sequence identity to SARS-CoV and MERS-CoV respectively.[21] However, there is 94.6% sequence identity between the seven conserved replicase domains in ORF1ab of SARS-CoV-2 and SARS-CoV, and less than 90% sequence identity between those of SARS-CoV-2 and other beta CoVs, implying that SARS-CoV-2 belongs to the lineage B (Sarbecovirus) of *β*-CoV .[22]

DIAGNOSTIC TESTS

Human body responds to a viral infection immediately with a non-specific innate response followed by an adaptive response. This may be manifested as development of antibodies that specifically bind to the virus or/ and by making T-cells that recognize and eliminate other cells infected with the virus which is called cellular immunity. This combined adaptive response may clear the virus from the body. Serological antibody detection is the broad category of tests to diagnose COVID-19, and this method detects IgM, IgG or total antibodies against SARS-CoV-2. Serological testing is defined as an analysis of blood serum or plasma mainly but has been expanded to include testing of saliva, sputum, and other biological fluids for the presence of immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies.[23] Some workers have reported on the use of detection of immunoglobulin A (IgA) antibody in blood in acute phase diagnostics when Reverse Transcription Polymerase Chain Reaction

(RT-PCR) remains negative in some patients of COVID 19 with atypical symptoms.[24] IgA based immunoassays has been hypothesized to be less specific than IgG-based Enzyme-Linked Immunosorbent Assay (ELISA) due to cross-reactivity with serum samples from patients infected by other coronaviruses.[25]

Various techniques used for antibody detection include virus neutralization assay, ELISA, immunochromatographic assay, chemiluminescent immunoassay, etc.[13] Each of these formats brings advantages such as speed, multiplexing and automation along with disadvantages such as requirement of trained personnel, infrastructure and dedicated laboratories.[23] Complementary to these antibody-detecting methods are the rapid antigen tests wherein antibodies are used to detect the presence of viral antigen(s) in serological samples.[23] Recently Government of India (GOI) has approved Rapid antigen detection test (standard Q COVID19 Antigen) as point of care test in certain restricted settings.[10] Monoclonal antibodies are being explored for this purpose. The FDA granted EUA (Emergency use authorization) status to the first serology test, qSARS-CoV-2 IgG/ IgM Rapid Test, manufactured by Cellex Inc., on April 1, 2020, but continues to allow clinical laboratories and commercial manufacturers to launch serology tests without an EUA.[23]

Although real time RT-PCR (rRT-PCR) based viral RNA detection is the sensitive and accurate way to confirm the diagnosis of SARS-CoV-2 infection in practice, dozens of suspects with clinical symptoms failed to be diagnosed by rRT-PCR test. The risk of false-negative with rRT-PCR method may be due to several possible factors, such as quality of specimen collection, PCR reagents from different sources, multi-steps of RNA preparation and fluctuations of virus load in different phases of SARS-CoV-2 infection.[26] Further it has also been noted that a negative rRT PCR result does not mean that COVID-19 is absent since several factors can affect the results. Periodically sequencing the evolving viruses is also suggested to monitor any mutations in the regions targeted by the assays that might affect test performance.[27] Also, the presence of a non-SARS-CoV-2 pathogen does not preclude the possibility of COVID-19, approximately one-fifth of specimens positive for SARS-CoV-2 were positive for one or more additional common respiratory viruses.[28]

Types of serology assays [29]

a. Rapid Diagnostic Test (RDT): This is typically a qualitative (positive or negative) lateral flow assay that is small, portable, and can be used at point of care (POC). These tests may use blood samples from a finger prick, saliva samples, or nasal swab fluids.

b. Enzyme-Linked Immunosorbent Assay (ELISA): This test can be qualitative or quantitative and is generally a lab-based test. These tests usually use whole blood, plasma, or serum samples from patients. Many workers have studied various proteins of SARS CoV-2 in antibody detection based on ELISA technique, mainly N antigen, S antigen, RBP antigen. Recombinant variants of these antigens have also been used.[30-33]

c. Neutralization assay: This test relies on patient antibodies to prevent viral infection of cells in a lab setting. Neutralization assays can tell researchers if a patient has antibodies that are active and effective against the virus, even if they have already cleared the infection. These tests require whole blood, serum or plasma samples from the patient. Neutralization assays depend on cell culture, a lab-based method of culturing cells that allow SARS-CoV-2 growth (like VeroE6 cells). When virus and cells are grown with decreasing concentrations of patient antibodies, researchers can visualize and quantify how many antibodies in the patient serum are able to block virus replication. Neutralization tests measures the functional neutralizing antibodies (of any class) and is considered as a 'gold-standard' assay for assessing the serological correlates of protection. An application of SARS CoV-2 specific IgG ELISA and virus neutralization test coupled with epidemiological methods have documented virus transmission link to the local and imported COVID-19 cases from Singapore.[34]

d. Chemiluminescent immunoassay: This test is typically quantitative, lab-based, and uses whole blood, plasma, or serum samples from patients. A variation of this test can use magnetic, protein-coated microparticles, known as a chemiluminescent microparticle immunoassay.[35]

There are several reports suggesting the evidence on antibody responses to SARS-CoV-2 infection.[13,14] Most of these studies show that people who have recovered from infection have antibodies to the virus. However, some of these people have very low levels of neutralizing antibodies in their blood suggesting that cellular immunity may also be critical for recovery.[28] The antibody-mediated humoral response is crucial for preventing viral infections. A subset of these antibodies, which reduce viral infectivity by binding to the surface epitopes of viral particles and thereby blocking the entry of the virus to an infected cell, are defined as neutralizing antibodies (NAbs).[36] Neutralization assays determine the ability of an antibody to inhibit virus infection of cultured cells and the resulting cytopathic effects of viral replication. For this assay, patient samples of whole blood, serum, or plasma are diluted and added at decreasing concentrations to the cell cultures. If neutralizing antibodies are present, their

levels can be measured by determining the threshold at which they are able to prevent viral replication in infected cell cultures. This type of testing requires cell culture facilities, and in the case of SARS CoV, Biosafety Level 3 (BSL3) laboratories. Despite these limitations, determination of neutralizing antibodies is important in the short term for the therapeutic application of convalescent plasma and in the long term for vaccine development.[23] There are few studies looking into whether neutralizing antibodies are produced in the individuals or not. A pseudotyped-lentiviral-vector-based neutralization assay to measure specific NAbs in plasma from recovered patients with SARS-CoV-2 showed variations in NAbs titers, approximately 30% of patients did not develop high NAbs titers after infection.[37] In the same study, the kinetics of SARS-CoV-2-specific NAbs development during the course of the disease in infected patients showed that the titres were low before day 10 post-disease onset and then increased, with a peak 10 to15 days after disease onset, remaining stable thereafter in all patients.[36]

In a study from recovered donors from COVID-19 infection, it was found that SARS-CoV-2 specific antibody titers were high and also NAbs titers were between 80 and 480.[38] The plasma obtained from the donors and transfused in the recipients on the same day lead to decrease in viral load. After transfusion, the titers of IgG and IgM in the recipients increased in a time-dependent manner. Moreover, presence of NAbs in the recipients played a vital role in the restriction of viral infection. [38]

SEROLOGY OF COVID 19

Due to COVID-19 pandemic, many manufacturers came up with a rapid and automated Point-of Care Tests (POCTs) for SARS-CoV-2. However, these kits have had reported varied sensitivity and specificity due to use of either ‘whole virus proteins’ or ‘virus derived recombinant proteins’ as a coating antigen. The performance of these tests is described by their "sensitivity," or their ability to identify those with antibodies to SARS-CoV-2 (true positive rate), and their "specificity," or their ability to identify those without antibodies to SARS-CoV-2 (true negative rate). A test's sensitivity can be estimated by determining whether or not it is able to detect antibodies in blood samples from patients who have been confirmed to have COVID-19 with a Nucleic Acid Amplification Test (NAAT). Further to know the specificity of the test estimation can be done by testing large numbers of samples collected and frozen before SARS-CoV-2 to demonstrate that the test does not produce positive results due to the presence of other causes of a respiratory infection, such as other

coronaviruses. These estimates of sensitivity and specificity are just that the estimates. The more number of samples used to validate a test, the smaller the confidence interval becomes, meaning that we can be more confident in the estimates of sensitivity and specificity provided.[39]

TESTING INTERPRETATION

Lots of literature is available regarding testing of antibodies both IgM and IgG in symptomatic cases of COVID-19. There is lots of variation regarding appearance of IgM and IgG antibodies after onset of disease. However, longitudinal profiling of both antibodies in various studies shows no specific chronological order.[12] Further, studies have been done on various simple and recombinant N and S proteins. It is said that analysis of the dynamics of Spike IgG antibodies may help to predict prognosis in COVID 19.[32,33,42-45] Regarding the interpretation of tests, it has been defined as a positive lgM, or convalescent sera with an increased lgG titer more than 4 times than that in the acute phase. Antibodies rise late in the course of illness, where the median duration of COVID-19 IgM antibody detection was found to be 5 days, while IgG detection around 14 days after symptom onset.[26]

Various reports are there in the literature regarding utilization of antibody tests in different phases of COVID 19 disease. Most of these are hospital based studies in symptomatic individuals.Serologic test is helpful for the diagnosis of SARS-CoV-2 infection in suspects and close contacts as well.[41] The highest concentration of IgM was detected on the ninth day after the onset of disease and class switching to IgG occurred in the second week.[42] The median duration of SARS-CoV-2 specific IgM antibody detection was 5 days of post onset with a positive rate of 85.4%. However, the positive detection rate was significantly increased (98.6%) when laboratory detection was performed collectively by IgM EIA and RT-PCR.[43]

Another hospital based study using an envelope and nucleocapsid proteins based EIAs showed IgM antibody detection within a week of Post Onset (P.O) and its detection up to 30 days and showed IgG antibodies after 10th day of infection and IgG antibodies remain in circulation for a longer time.[44] A study from Wuhan, China has reported a SARS CoV-2 RNA shedding for >30 days in 10% COVID-19 patients without any apparent symptoms and documented a higher levels of IgM antibodies at 9th week after disease onset.[45]

Though delayed, but robust antibody (IgM and IgG) response was observed to SARS-CoV-2 nucleocapsid (N) protein and spike (S) glycoprotein in critical patients between 17 to 23 days

after illness onset.[46] According to many workers, combination of nucleic acid and IgM-IgG antibody testing is a more sensitive and accurate approach for diagnosis and early treatment of suspected cases.[32,47,48] An ELISA to detect IgG and IgM antibodies to the RBD of the spike protein of SARS-CoV-2 was developed and its performance was evaluated using a panel of sera by microneutralization and PRNT tests.[49] Overall, SARS CoV- 2 specific IgM antibodies can be detected between 5 to 35 days of post onset using different type of coating antigens in ELISAs. More studies are required to understand the infectivity of SARS-CoV-2 amongst COVID-19 patients, viral RNA shedding and antibody appearance.

Apart from the potential use of serology assays in contact screening, detection of antibodies has been shown to improve diagnosis of positive cases. Antibody kinetics data from China showed that positive detection rate increased significantly, when PCR is used in combination with IgM ELISA assay (98.6%) compared to PCR alone (51.9%), and using antibody detection can improve diagnosis of COVID-19 including subclinical cases. Compared to PCR, the IgM detection rate was reported to be lower in the first 5 days post symptom onset (100% for PCR vs.71.4% for IgM), but was higher afterwards (44.3% for PCR vs 87.9% for IgM).[43]

In India, we have also started with serology testing in contacts of symptomatic patients.[50] A similar approach is used in Italy, where viral clearance is indicated by negative PCR accompanied by specific IgG detection.[51] In India, interpretation of testing is being suggested based on the following corollaries. (Table 1)

Testing strategy (WHO)

Ideally, the strategy should be to conduct two tests as in low prevalence populations, which will be much of the asymptomatic general population, the result of a single antibody test is not likely to be sufficiently accurate to make an informed decision regarding whether or not an individual has had a prior infection or truly has antibodies to the virus. A second test, typically one assessing for the presence of antibodies to a different viral protein, generally would be needed to increase the accuracy of the overall testing results.[33] Inaccurate immunodiagnostic tests may falsely categorize people in two ways. The first is that they may falsely label people who have been infected as negative like in the early part of the infection and the second is that people who have not been infected are falsely labeled as positive due to cross reactivity with known set of six human CoVs.

Availability of tests

During the COVID-19 pandemic, the FDA has worked with more than 400 test developers who have already submitted or said they will be submitting, EUA requests to the FDA for tests that detect the virus or antibodies to the virus. FDA has authorized 120 tests under EUAs, which include 104 molecular tests, 15 antibody tests, and 1 antigen test.[52]

Although the Government of India - ICMR started working on diagnosis and treatment/ prevention in the early part of the pandemic. For testing of COVID 19 outbreak and unprecedented disease, in order to limit the spread as well to treat those who have moderate/ severe disease, laboratories need to gear up to diagnose the COVID 19 cases. For the COVID 19 disease diagnosis, the gold standard is RT- PCR based viral RNA detection methods.[50] However to monitor the disease stages and to identify past infection and immunity, serology based tests are to be used. Viral cultures are not recommended for diagnosis. At first, the Indian Council of Medical Research (ICMR) established screening as well as confirmatory assays for the SARS-CoV-2 at National Institute of Virology (NIV) Pune and afterwards, E gene based real-time RT-PCR kits were distributed to 13 Virus Research and Diagnostic Laboratories (VRDLs) situated in different parts of the country.[53] There was sudden emergence and spread of SARS-CoV-2 which was an insurmountable challenge to the public health system of India. However, intensive and timely efforts of various arms of the Government of India resulted in a well-coordinated action. India has successfully demonstrated its ability to establish quick diagnosis of SARS-CoV-2 at NIV, Pune, and the testing VRDLs. Further, the genetic characterization of SARS CoV-2 strains was essential to track transmission pathways. The full genomes (n=21) study from India revealed 99.97% identity to SARS-CoV-2 that was detected from the Wuhan city, China.[54]

India has also started with testing for IgG antibodies for sero surveillance studies. Scientists at ICMR-NIV, Pune have developed and validated an indigenous IgG ELISA test for antibody detection for SARS-CoV-2. The test has undergone intense validation in three stages and has been found to have high sensitivity and specificity. IgG antibodies generally start appearing after two weeks of onset of infection, once the individual has recovered after infection and last for several months. Therefore, the IgG test is not useful for detecting acute infection but indicates episode of SARS-CoV-2 infection in the past. However, detection of IgG antibodies is useful in the following situations: i.) Sero-surveys help to understand the

proportion of population exposed to SARS-CoV-2 infection including asymptomatic individuals. ii.) Survey in high risk or vulnerable populations.

WHO supports these studies, as they are critical for understanding the extent and risk factors associated with infection. These studies will provide data on the percentage of people with detectable COVID-19 antibodies, but most are not designed to determine whether those people are immune to secondary infections.[55] Initially, RT-PCR kits were distributed to all the VRDL's to confirm the diagnosis of COVID-19. Later on as per letter dated 30th May 2020, IgG ELISAs for serosurveys were put into use to understand the proportion of population exposed to SARS CoV-2 infection including asymptomatic individuals. Indigenous IgG ELISA kit "COVID KAVACH ELISA" for antibody detection for COVID-19 developed by NIV Pune- ICMR.[56] The test is not useful to detect acute infection but indicates episodes of SARS CoV- 2 infections in the past. ICMR has given list of the various validated ELISA kits for use along with the company name and batch number as well.[57] Recently as on 15th June, an indigenous antigen detection kit has also been approved for testing in India.[50]

Uses of antibody detection

1.Serology tests are comparatively easier to perform, requiring less technical expertise and equipment compared to nucleic acid detection.

2.Samples are blood that is collected in tubes, which pose less potential risk to the staff handling the samples as compared to naso-oral secretions. Samples are easier to obtain compared to respiratory samples, involving less risk to the operator. It can be performed in a basic clinical laboratory and smaller community settings (POCT), therefore reaching a wider application.

3.Incorporation of serology assays in diagnostic algorithms and discharge criteria may ease the burden or divert the workload from nucleic acid detection, which is applicable for some clinical situations.

4.The advantage of cheap, rapid tests for healthcare workers would allow them to be cleared and return to work. The concept of immunity passports/ shield immunity has been discussed wherein; recovered individuals who are seropositive are assumed to be immune to re-infection and are thus allowed travel/ routine work.

5.Furthermore, the availability and use of automated ELISA platforms in future has the potential of high testing capacity compared to PCR assays.

6.Serology assays may be a tool in studying the sero epidemiology of COVID-19. Availability of tests with good performance will give a more accurate picture of the overall spread of COVID-19.

7.Individuals who have mounted stronger antibody response (assumed protective titres) may be considered as donors for plasma therapy.

Limitations

1.The performance issues of rapid tests in general.

2.As antibodies appear later during the disease course, so lag period may be there before the test becomes positive. So, sensitivity of test depends on time of testing or duration of illness.

3.Serology-based tests are not currently recommended for diagnosis.

4.Absence of antibodies in patient's sera does not rule out infectious status.

5.Presence of antibodies does not confirm the immune status.

6.Possibility of cross-reactivity with other non-SARS-CoV-2 coronavirus strains, such as coronavirus HKU1, NL63, OC43, or 229E.

7.Availability of validated test kits and cost effectiveness.

Serological interpretation of Covid-19 testing (Table 1)

Based on five corollaries:

1.IgM appears and disappear early and IgG appears late and remains for longer time.

2.Presence of antibody does not label a patient to be immune.

3.Absence of antibody does not rule out infectiousness of the patient.

4.Presence of antibody is not only due to COVID-19 but due to cross reactivity to other coronaviruses.

5.IgG antibodies must have appeared by 14 days of onset of symptoms.

Symptomatology	Antibodies		Interpretation
	IgM	IgG	
Asymptomatic	-ve	-ve	❖ No Abs produced ❖ May be recent exposure
	-ve	+ve	❖ Prior infection ❖ Unlikely virus spreader*
	+ve	-ve	❖ Recent infection ❖ Repeat for IgG Ab after 14-21 days*
	+ve	+ve	❖ Prior infection > 14 days ago ❖ Repeat testing to confirm IgG only Ab status after 7-14 days*
Symptomatic	-ve	-ve	❖ <7 days: RT-PCR test to be done ❖ >7 days: unlikely to be COVID-19
	-ve	+ve	❖ Prior infection ❖ Capable of virus spreading ❖ Quarantine for 14days
	+ve	-ve	❖ Recent infection ❖ Repeat for IgG Ab after 14-21 days*
	+ve	+ve	❖ Prior infection > 14 days ago ❖ Repeat testing to confirm IgG only Ab status after 7-14 days*

* Molecular test should be performed to assess viral shedding status.

SERO SURVEILLANCE STUDIES

The diagnosis and sero-surveillance of COVID-19 can be challenging in a country like India with high population to be covered. Nucleic acid-based assays though offer high accuracy in the diagnosis of SARS-CoV-2, but for serosurveys conventional serological assays, like ELISA, that are specific to COVID-19 IgM and IgG antibodies are used as a high-throughput alternative.[58] Several serological tests have been developed to detect immunoglobulins (IgG & IgM) against viral proteins.[14,59] A study by Ozturk, *et al*, provided an overview of IgM and IgG profiles in COVID-19 relative to time since symptom onset. They compared performance characteristics between assays in symptomatic and recovered patient groups. The combination of IgM levels in two groups showed similar sensitivity for COVID-19 as

IgG but greater specificity and identified 4/10 people (vs. 3/10 by IgG) with prior symptoms and negative molecular testing to have had COVID 19. Therefore, disease severity and timing both influence levels of IgM and IgG against SARS-CoV-2, with IgG better for early detection of severe cases but IgM more suited for early detection of milder cases.[60]

Antibody detection has been conducted in some surveys. Healthcare professionals survey results has been published from Germany (n=316), Czech Republic (n=269) that used commercial IgG ELISAs for serology.[61,62] The overall SARS-CoV-2 seroprevalence in healthcare workers was very low i.e. 1.6 % in Germany and 1.8% in Czech Republic. A largest seroprevalence study conducted using a lateral flow immunoassay from Santa Clara County, USA showed the prevalence of 1.5% and after weighting for population demographics, the estimated prevalence was 2.8%.[63] Similarly, a community based seroprevalence study that used a lateral flow immunoassay showed 4.65% seropositivity in adults aged 35-54 years from the USA.[64] Another study performed at hospital emergency department staff from the USA showed 5.9% seropositivity with 5.6% indeterminate results by commercial semiquantitative Anti-SARS-CoV-2 IgG kit.[65] A study conducted from Switzerland using a commercial IgG EIA estimated seroprevalence of 3.1%, 6.1% and 9.7% in the 1st, 2nd and 3rd week, respectively.[66] Using different types of assay systems, SARS-CoV-2 seroprevalence studies was studied in various countries and these studies are helpful to know the estimates of infection so that mitigation measures can be implemented or reviewed.

Seroprevalence surveys are of utmost importance to assess the proportion of the population that has already developed antibodies against the virus and might potentially be protected against subsequent infection.[67] In a study by Stringhini, *et al*, they assessed anti-SARS-CoV-2 IgG antibodies using a commercially available ELISA, targeting the S1 domain of the spike protein of SARS-CoV-2; over the course of the 5 study weeks. They observed an increase in seroprevalence from about 5% to about 11%, which is to be expected considering time to seroconversion after symptoms (median 10·4 days) and that the peak of the epidemic was reached the week before the start of their survey.[68] So, population-based serosurveys measuring anti-severe acute respiratory syndrome coronavirus 2 (anti-SARS-CoV-2) antibodies also provides one method for estimating infection rates and monitoring the progression of the epidemic.[68] They found that young children (5–9 years) and older people (≥65 years) had significantly lower seroprevalence than the other age groups. However, more

studies are needed to better understand infection and antibody dynamics among young children.[68]

Gudbjartsson, *et al* assessed SARSCoV-2 seroprevalence in the population of Iceland and assessed longitudinal changes in antibody levels within the first 4 months after SARS-CoV-2 infection and their correlation with sex, age, existing phenotypes and Covid-19 symptoms.[69] The humoral immune response is critical for the clearance of cytopathic viruses and is generally important for the prevention of viral reinfection.[70] A relationship between a humoral immune response to SARS-CoV-2 infection and protection against reinfection by this virus has been shown in rhesus macaques but has yet to be established in humans.[71] Their results indicate that antiviral antibodies against SARS-CoV-2 did not decline within 4 months after diagnosis.

Well-validated serologic assays for SARS-CoV-2 are urgently needed. Several small comparative studies of commercial SARS-CoV-2 antibody assays have been published.[72-75] A highly specific assay is required for screening populations with a low seroprevalence, such as that in Iceland. In a recent study from Faroe Island, 0.6% seroprevalence of severe acute respiratory syndrome coronavirus 2 infection was found.[76]

The efficient control of an outbreak depends on the rapid diagnosis of the disease. According to a study by Dhama, *et al*, the IgM levels last more than 1 month, indicating a prolonged stage of virus replication in SARS-CoV-2-infected patients.[77] The IgG levels were found to increase only in the later stages of the disease, indicating that the specific antibody profiles of SARS-CoV-2 and SARS-CoV were similar.[78] These findings can be utilized for the development of specific diagnostic tests against COVID-19 and can be used for rapid screening.[77]

A large number of serologic tests, platforms and methodologies are being employed to determine seroprevalence in populations to select convalescent plasmas for therapeutic trials and to guide policies about reopening. However, tests have substantial variability in sensitivity and specificity, and their ability to quantitatively predict levels of nAb is unknown.[79] A study by Luchsinger, *et al*, measured levels of antibodies in convalescent plasma using commercially available SARS-CoV-2 detection tests and in-house ELISA assays and correlated serological measurements with nAb activity measured using pseudotyped virus particles, which offer the most informative assessment of antiviral activity of patient sera against viral infection.[79] According to them, a large proportion of convalescent

plasma samples have modest antibody levels and that commercially available tests have varying degrees of accuracy in predicting nAb activity. They stated the Ortho Anti-SARS-CoV-2 Total Ig and IgG high Throughput Serological Assays (HTSAs), as well as the Abbott SARS-CoV-2 IgG assay, quantify levels of antibodies that strongly correlate with nAb assays and are consistent with gold-standard ELISA assay results. HTSAs are more suitable for clinical laboratories and offer limited antigen diversity but allow high-throughput and sensitive, semi-quantitative results These findings provide immediate clinical relevance to serology results that can be equated to nAb activity and could serve as a valuable 'roadmap' to guide the choice and interpretation of serological tests for SARS-CoV-2.[79]

The findings of ICMR serosurvey indicated that 0.73% of adults in India were exposed to SARS-CoV-2 infection, amounting to 6.4 million infections in total by early May 2020.[80] The survey was conducted from May 11 to June 4 and covered 28,000 individuals whose blood samples were tested for IgG antibodies using COVID Kavach ELISA kit. The survey results showed that the seropositivity rate was highest in rural areas (villages) at 69.4%, while it was recorded at 15.9% in urban slums and 14.6% in urban non-slums. Seropositivity was highest in the age group of 18-45 years (43.3), followed by those aged 46-60 years (39.5) and lowest seropositivity among those above 60.[80] The result of a serosurvey in Andhra Pradesh has shown that 19.7% of people have developed antibodies to COVID-19. It also revealed that a high percentage of people who had contracted the coronavirus were asymptomatic. [80]

The Union Ministry of Health and Family Welfare also shared the sero-surveillance study report in Delhi, conducted from June 27 to July 10 in all 11 districts of the national capital. Researchers took blood samples from select citizens after their consent and tested the samples for lgG antibodies and COVID-19 infection using COVID KAVACH ELISA, as approved by the ICMR. Nearly six months into the epidemic, only 23.48% of the people are affected in Delhi, which has several pockets of dense population. [81]

As the world develops plans to find a new balance between minimizing the direct impacts of COVID-19 on those infected and the indirect effects on all of society, serological studies such as this are crucial for providing new insights about transmission and the otherwise hidden immunological state of the population.

Currently, the exact estimates of the prevalence of SARS-CoV-2 antibody positive individuals in the population is not known, and prevalence may change based on the duration the virus is in the country and the effectiveness of mitigation measures. Moreover, prevalence

may vary widely between locations and between different groups of people, such as health care workers, due to different rates of infection. Laboratory tests that detect antibodies to SARS-CoV-2 need further validation to determine their accuracy and reliability.

Regarding the validation studies, clinical implementation urgently requires validation of these new assays. Since real-life performance data are scarce, the COVID-19 pandemic has been marked by an inspiring level of inter-laboratory collaboration. Based on the testing done to determine whether an individual is immune to SARS CoV-2, we must know the pre-test probability in the specific population being tested, as well as the sensitivity and specificity for protective antibodies of the assay.

A significant challenge is that, to date, serological data are largely limited to hospitalized, ill patients. There is reason to suspect that serological findings in asymptomatic or mildly symptomatic exposures may not correlate as well as in hospitalized patients, particularly as anecdotal evidence suggests individuals with low viral loads produce lower antibody titers(unpublished). A recent report on 11.06.2020 of sero surveillance in India found only 0.76% positivity, which is very low in community and also in Punjab < 1 % (ICMR unpublished report).

HERD IMMUNITY IN COVID 19

The term herd immunity was first used in 1923 by Topley and Wilson.[82] Fox pointed out four conditions under which such collective/group immunity can occur.[83] The infectious pathogen must be found and restricted to a single host. As the second condition, the transmission must occur primarily through direct contact. It has been established that the transmission of SARS-CoV-2 occurs by direct, person-to-person contact (coughing, sneezing, and inhalation of droplets) and contact transmission. Third, the infection must induce solid, long-lasting immunity. In this regard the immune response induced by SARS-Cov-2 in humans, it has not been possible to establish the mechanism by which the immune system generates a long-term response that could combat the disease and prevent reinfection Finally, collective or group immunity is maximized if the population possesses a random mixing pattern that will depend on the preventive measures implemented worldwide e.g., quarantine, isolation, lockdowns, and social distancing. Theoretically, it is possible to achieve group immunity under the aforementioned assumptions.

If a large group of people – the herd – is immune to a virus, then an individual in the middle of this group is unlikely to become infected. The virus has a very hard time getting through the herd. Herd immunity, then, happens when people in a community are protected from a virus and its associated disease to a degree that people who are not immune are still protected because of the high population immunity. Herd immunity can slow the spread of a contagious virus. Herd immunity can be alternatively achieved by vaccinating people if and when there is an available vaccine.

Herd immunity stems from the effects of individual immunity scaled to the level of the population. The point at which the proportion of susceptible individuals falls below the threshold needed for transmission is known as the herd immunity threshold.[84] Above this level of immunity, herd immunity begins to take effect, and susceptible individuals benefit from indirect protection from infection.

Mathematical modeling has been at the forefront to study the response to this ongoing pandemic in an attempt to contain its impact and limit further transmission. These modeling-based approaches are being used to guide decision making and inform the public health response.[85] Under the simplest model, the herd immunity threshold depends on a single parameter known as Reproductive Number (R0) or the basic reproduction number. R0 refers to the average number of secondary infections caused by a single infectious individual introduced into a completely susceptible population.[84] If we consider a hypothetical pathogen with an R0 of 4, this means that, on average, one infected host will infect four others during the infectious period, assuming no immunity exists in the population. Mathematically, the herd immunity threshold is defined by 1 – 1/R0 (e.g., if R0 = 4, the corresponding herd immunity threshold is 0.75).[84] Therefore, the more communicable a pathogen, the greater its associated R0 and the greater the proportion of the population that must be immune to block sustained transmission.[84] A similar parameter important for understanding population-level immunity is the effective reproduction number (Re or Rt). Re is defined as the average number of secondary cases generated by a single index case over an infectious period in a partially immune population.[86]

Unlike R0, Re does not assume a completely susceptible population and consequently, will vary depending on a population's current immune state, which will change dynamically as an outbreak event or vaccination campaign unfolds. Ultimately, the goal of vaccination programs is to bring the value of Re below 1. Establishing a "critical" population immunity

percentage to curb the expansion of COVID-19 is, with current scientific knowledge, purely speculative. Hence, if the R0 of SARS-Cov-2 fluctuates between 2 or 3, herd immunization would require infection of about 50% to 70% of the world's population to keep the disease under control (or close to 80% if the R0 is higher). In addition, the mass vaccination of billions of people could be one of the most important global challenges of the 21st century.[87]

Currently, more that100 vaccines are being developed to combat SARS-CoV-2. The COVID-19 pandemic seems likely to come to an end only when an effective vaccine is created and applied, and herd immunization is acquired. Various studies suggest that protection against reinfection with coronavirus species tends to diminish given sufficient time, although longitudinal serological studies are needed to assess the duration of SARS-CoV-2 immunity. If this proves to also be true for SARS-CoV-2, persistent herd immunity may never be attained in the absence of recurrent vaccination. Thus, by vaccinating certain groups of the population, the spread and R ratios of the virus will go down. In the absence of a vaccine, building herd immunity against SARS-Cov-2 through natural infection is theoretically possible. However, there is no ethical path to reach this goal, as the social consequences of natural exposure may be devastating. There is a concept of Laissez-Faire Attitudes or Natural Herd Immunity which is a type of mitigation measure that can be found in epidemiological books and has been successfully applied in cases such as the 1918 H1N1 influenza pandemic because of nonavailability of vaccine.[88]

Besides providing individual protection, vaccination programs also aim for so-called population or herd immunity. For example, global immunization coverage of more than 80% against smallpox virus has reduced the transmission rates to uninfected individuals to such low levels that the virus has been eradicated and for measles, 91-94% of a population must be vaccinated to achieve herd immunity and prevent new measles outbreaks. These examples illustrate well that the threshold for vaccination induced herd immunity is pathogen specific. A threshold value of ~67% is estimated to be sufficient for achieving herd immunity against SARS-CoV-2, assuming that the basic R0 of the virus is three, i.e., one infected individual infects three new individuals.[89]

The specifics about coronavirus and herd immunity are not yet fully characterized. Those individuals who are immune will be able to get back to work and be protected from reinfection and, probably, not transmit the virus or disease. But recently there have been reports of reinfections with Covid 19 in the World.[90] About five days after the first case of

confirmed reinfection by novel coronavirus (SARS-CoV-2) 142 days after the first symptomatic episode in a 33-year-old adult was first reported by researchers at the University of Hong Kong, a second such case has now been reported in the U.S. Like in the first reported case of reinfection in Hong Kong, the second case of reinfection by SARS-CoV-2 virus in Nevada, U.S. was confirmed through genetic sequencing.

While the Hong Kong adult exhibited overt symptoms when infected for the first time in March but was only asymptomatic during the second infection in mid-August, the adult in Nevada had overt symptoms during both infections. In fact, the second infection caused severe symptoms, including hypoxia (lack of oxygen in the body) and breathlessness. The Nevada adult first tested positive for the virus on March 25 and needed hospitalization. Symptoms included sore throat, cough, headache, nausea, and diarrhea. The symptoms resolved on April 27 and the patient twice tested negative for the virus by RT-PCR on May 9 and May 26. But one month after recovering, the patient once again exhibited symptoms (fevers, headache, dizziness, cough, nausea, and diarrhea) and sought care on May 31 and subsequently hospitalized on June 5 when found to be hypoxic. The patient required ongoing oxygen support and had symptoms such as myalgia, cough and shortness of breath, the researchers write. The patient tested positive for SARS-CoV-2.

Researchers at the Reno School of Medicine, University of Nevada sequenced the genome of the samples collected during the first and second infections and found them to be distinctly different. Though the genome sequences from both infections belonged to the same clade (20C), there are distinct differences in mutations between the two sequences. While there were five single point mutations compared to the reference genome in the sequence from the first infection, the sequence data from the second infection showed six additional point mutations and one multi-nucleotide variant. The authors rule out the possibility of the virus experiencing mutations to become the virus that caused the second infection within the body of the patient. For the virus in first infection to experience mutations to become the virus in the second infection, the "virus would have had to exhibit a rate of 83.64 substitutions per year, a rate that markedly exceeds that of 23.12, currently observed", they write. They thus conclude that the "odds of this occurring are vanishingly remote and virtually assure that these are two distinct viral infection events".[91]

There's been a lot of talk of herd immunity (again) in recent days, especially with the release of the loftily named Great Barrington Declaration (https://gbdeclaration.org/) which basically

calls for building herd immunity.[92] "The most compassionate approach that balances the risks and benefits of reaching herd immunity is to allow those who are at minimal risk of death to live their lives normally to build up immunity to the virus through natural infection, while better protecting those who are at highest risk. We call this Focused Protection," it says.

Lately, it has been said that the herd immunity level may be just around 40% instead of the widely believed 60%, according to mathematicians at the University of Stockholm and the University of Nottingham; and only around 11% of people exposed to an infected person are likely to contract Covid-19, according to a team led by Ramanan Laxminarayan of the Centre for Disease Dynamics, Economics & Policy. It still does not make sense to pursue an expose-and-infect strategy. That's because some of those infected tend to be superspreaders (8% infect 60%, according to the second study cited above); we do not know enough about the long-term impact of even mild infections; and the mathematicians themselves, in the first study cited above, caveat that the 40% number is not even directional but merely to show how "population heterogeneity affects herd immunity". So still, serosurveys are important not only for assessing when a country is likely to achieve herd immunity but to measure prevalence of the infection and the proportion of the population that has been exposed to it. A city or state with a high exposure rate (as measured by the serosurvey), can safely assume that with stringent enforcement of mask discipline, social distancing, hand hygiene and rules on public gatherings, it can open up just about anything (including schools) with a lower risk than a city or a state with a lower exposure rate. That's good enough reason for the Union government as well as states to get serious about serosurveys, just as it is good enough reason for them to get serious about the enforcement of Covid-safe behavior.[93]

Finally, mass serological testing is now needed to determine how many individuals have been infected, how many individuals are immune, and how far we are from reaching the herd immunity threshold. That said theoretically even if re-infection can occur after sterilizing immunity wanes, enduring memory cells of the adaptive immune system would likely facilitate immune control of the virus and limit disease pathology, which would hopefully decrease the clinical severity of subsequent infections. Lastly, urgent steps should be taken to design and develop safe and efficacious vaccines to prevent further spread of COVID-19 and establish vaccine induced herd immunity.

FUTURE

Till date, the use of antibody-detecting rapid diagnostic tests for patient care have not been recommended but the continuation of work to establish their usefulness in disease surveillance and epidemiologic research should be carried on.[94] Improved performance of serological testing may provide information for public as well as healthcare workers assessment and monitoring. Assessment of immunity in population, particularly in areas identified as hotspots will help to inform further response and strategies in future waves of the pandemic.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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