SEROPREVALANCE OF COVID ANTIBODIES IN HEALTH CARE POPULATION OF GOVERNMENT RAJAJI HOSPITAL, MADURAI.

DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT FOR THE AWARD OF THE DEGREE OF

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BRANCH I - GENERAL MEDICINE

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MAY -2022

CERTIFICATE FROM THE DEAN

This is to certify that this dissertation **entitled "SEROPREVALANCE OF COVID ANTIBODIES IN HEALTH CARE POPULATION OF GOVERNMENT RAJAJI HOSPITAL, MADURAI."** is the bonafide work of **Dr. FATHHUR RABBANI S** in partial fulfilment of the university regulations of The Tamil Nadu Dr. M.G.R. Medical University, Chennai, for M.D General Medicine Branch I Examination to be held in MAY 2022.

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DECLARATION

I, **Dr. FATHHUR RABBANI S** solemnly declare that, this dissertation "**SEROPREVALANCE OF COVID ANTIBODIES IN HEALTH CARE POPULATION OF GOVERNMENT RAJAJI HOSPITAL, MADURAI."** is a bonafide record of work done by me at the Department of General Medicine, Government Rajaji Hospital,Madurai, under the guidance of Professor **Dr.M.NATARAJAN, MD.,** Department of General Medicine, Madurai Medical college, Madurai from (March 2021 to August 2021) I also declare that this bonafide work or a part of this work was not submitted by me or any others for any award, degree, diploma to any other University, Board either in India or abroad. This dissertation is submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfilment of the rules and regulations for the award of Degree of Doctor of Medicine (M.D.), General Medicine Branch-I-examination to be held in May 2022.

PLACE: MADURAI **Dr. FATHHUR RABBANI S**
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INTRODUCTION

COVID 19 IS A NOVEL CONTAGION FIRST IDENTIFIED IN WUHAN, HUBEII PROVINCE OF CHINA. Millions of cases have been diagnosed and notified across the world, over 180 countries with over 290,000 deaths , and following which WHO declared it a global pandemic.

As of the Indian scenario, the highest number of covid cases of around 6.3 million cases and total of 97000 deaths have been recorded around the month of September 2020.

Though the diagnostic gold standard of the infection is the reverse transcriptase – polymerase chain reaction of the nasal or throat swabs, asymptomatic infections have been documented where the clinical indications are not present to go for a nasal swab , the only way to determine the past episode of infection is to do a serological survey .

The serological test against covid, gives an estimate of the cumulative prevalence of COVID in a community, further estimating the prevalence of antibodies indicates the dynamics of the immune response , actual burden of the pandemic and the still vulnerable proportion of populations, and so the importance of vaccination is further reinforced .

This study about the seroprevalence of the antibodies in health care professionals of Government Rajaji Hospital , Madurai , is a pilot study that indicates the disease burden of the health care professionals

DETAILED STUDY PROPOSAL

TITLE :

SEROPREVALANCE OF COVID ANTIBODIES IN HEALTH CARE POPULATION OF GOVERNMENT RAJAJI HOSPITAL, MADURAI.

AIMS AND OBJECTIVES

1. To determine the positive prevalence of SarsCoV2 antibodies in the healthcare professionals of GRH Madurai over a time period of 6 months.
2. To make a subgroup analysis and estimate the age based and gender based workforce based prevalence of Sars CoV 2 in the health care population.

REVIEW OF LITERATURE

It was in the late December 2019, a pneumonia of mysterious origin which was later traced to the Hunan seafood wholesale, in china, wuhan, province of hubei **[1]** .Intial infection involved about 66% of the staff . The market was shut down and the local health alert was given on December 31, 2019 . **[2]** Later found to be cause of the outbereak was the novel beta corona virus, that was initially named as the 2019 - nCoV (novel corona virus), rekindled our memory of the bitter experiences with the Severe acute respiratory distress syndrome caused by one another beta corona virus , some 17 years past **[3]** .

On January 13, 2020, a case in thailand was reported , which was documented as the first case outside china. spreading to more than 250 countries around the world since then , and by January 30 ,2020 , WHO declared the SARS CoV as the PUBLIC HEALTH EMERGENCY OF INTERNATIONAL CONCERN **[4]**

By 2003, a novel coronavirus, that caused a mysterious respiratory disorder, originated from southeast China, from the province of Guandong , and named as severe acute respiratory distress syndrome coronavirus fulfilling the postulates of Koch theory **[5]**. The mortality rate was around 10%–15%. Though medical facilities have improved there is no promising treatment or vaccine for SARS. Another outbreak occurred in the year of 2012 in the

middle east **[6]** . Though both are caused by the corona virus, the intermediate host for MERS was dromedary camel ,and the mortality rate was 37.5 % .

It was on 11 March 2020, WHO officially announced the COVID 19 outbreak as a global pandemic **[7]** . Following that ,though case in china have started coming down , case spikes in other regions of the world like america, asia and Europe has sharply increased and as of march 2021 ,253,370,319 total number of cases have been recorded and 5,107,390 death have been recorded globally **[8]**.

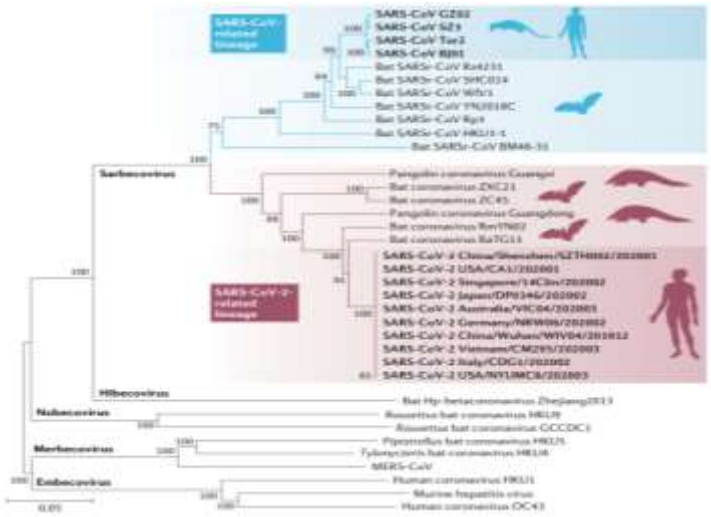
VIRIOLOGY :

In the order of Nidovirales and the suborder of Coronavirineae lies the family Coronaviridae. Further specified .into the subfamily of Ortho coronavirinae. Grouped into four genera –alpha coronavirus, beta coronavirus, gamma coronavirus, and delta coronavirus primarily infecting the birds and mammals, including humans and bats , corona virus are positive sense RNA viruses that are single stranded and enveloped [9] .

Gamma corona virus and Delta corona virus infect the birds , whereas the beta corona virus and alpha corona virus infect humans . Coronaviruses have been studied for decades using the model beta corona virus, murine hepatitis virus (MHV), and the human alpha corona virus HCoV-229E [10]. Human coronaviruses, widely circulate in population and causes seasonal outbreak of common cold and mild respiratory tract infections. But in striking

difference, severe acute respiratory syndrome coronavirus Middle East respiratory syndrome coronavirus (MERS-CoV) and SARS-CoV-2,that have come in the past 18 years are found to be more pathogenic . [11]

The phylogenetic analysis have shown that , the corona virus belong to a genus betacorona virus and subgenera Sabrecovirus [12]



VIRAL ENTRY :

SARS COV 2 virus particle is made up of 30-kb strand positive sense ribonucleic acid forming the genome this is coated with nucleocapsid(N) protein enclosed in a lipid bilayer containing four membrane proteins:

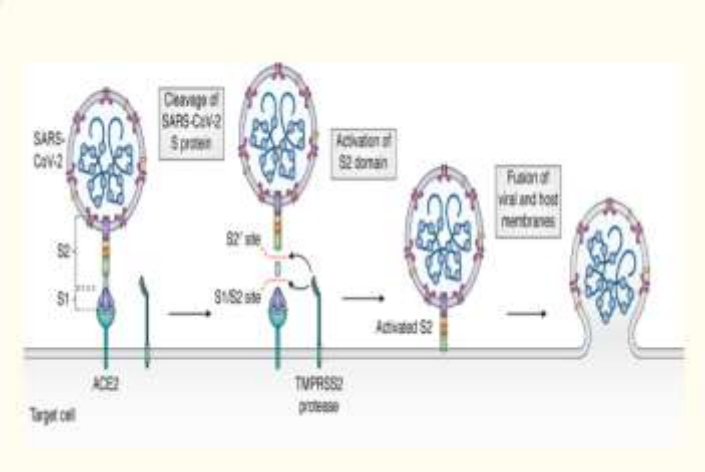
1. Spike (S)
2. Membrane (M),
3. Envelope (E)
4. Nucleocapsid(N) . [13]

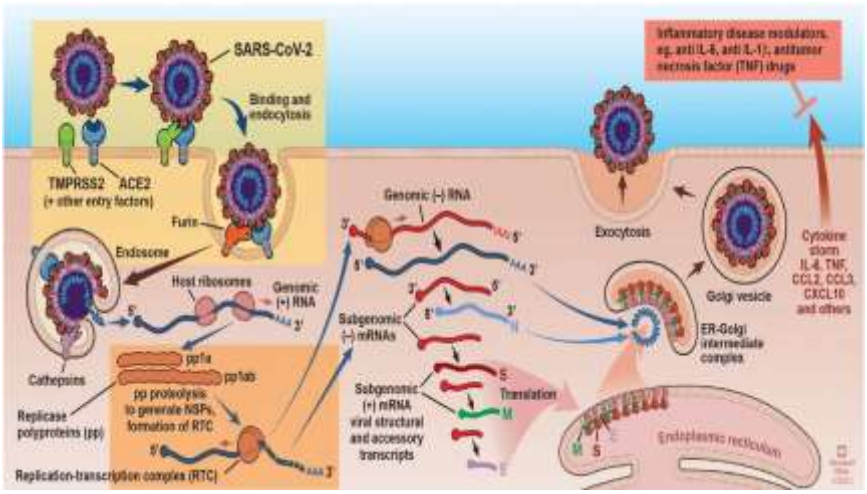
For all coronaviruses, the M protein is critical for organizing the new particles into the new virions and N protein associates with the viral genome and M to direct genome packaging into new viral particles. The E protein participates in the viral assembly by forming the ion channels for viral particles.

S protein protrudes from the surface of the viral membrane like a club , giving the virus its characteristic crown shaped appearance, which in latin is' CORONA' [14]. S protein binds with the target cell and helps in initiation of Fusion with the host cell membrane. The S protein consists of three subunits, since it is Homotrimeric. The three subunits are RECEPTOR BINDING DOMAIN (RBD),S1, S2. Each has a specific function of its own. The S1 subunit of coronavirus has been functionally divided into 2 domains, N terminal domain and the C terminal domain. The C terminal domain has the aminoacid sequence known as the Receptor Binding Domain. RBD is essential for the viral entry into the host cells S2 enabling the virus to enter the host cytoplasm following the process of membrane fusion [15].

For activation of the binding domains and ligands, certain sites should be cleaved off. The first cleavage site is at the S1/S2 boundary that can lead to certain structural changes in the S2 domain, making it in a state of perfusion Conformation. This process tends to separate the S1 from S2 site .The second cleavage occurs at the S2 cleaved site, that cause the viral and cell membrane fusion and release release the nuclear material in the host cytoplasm [16]

ACE2 receptor, is present in the cell surface of various Organs, with especially high abundance in the epithelial cells of the lung, small Intestine, pancreas, renal epithelial cells .Ace2 is a cell surface peptidase that can Cleave the angiotensin II. [17] Certain other proteases are also involved in the cleaving Of the binding domains, these include TRMPSS2, endosomal cathepsins and furin .Of these proteases, it is the TRMPSS2, that cleaves and activates the binding domains Of the SARS CoV 2. Protease expression also varies according to the tissues and location. After attaching to the host cell receptors, they undergo endocytosis and viral maturation and replication takes place with the help of N protein. [18]Inside the host cell, the single stranded positive RNA, forms a negative strand with The help of RNA polymerase. This can synthesize several other new positive strand RNA, Further with translation they create new proteins, that are enclosed in endoplasmic reticulum membrane, transported to golgi vesicle to the cellular membrane where there is exocytose [19].





PATHOPHYSIOLOGY OF THE SARS COV 2 INFECTION :

Initially thought of a disorder of respiratory system, the furthering Research and ongoing works have shifted the covid from pneumonia pandemic to multisystem disorder. Due to the heterogeneity of receptor distribution and mechanism of action, various mode and route of injury and propagation are enumerated, the important among them are :

i. RAAS dysregulation caused by viral mediated down regulation

ii. Immune response dysregulation

iii. Injury to endothelium and thrombo-inflammation

iv. Cytotoxicity by virus mediated by ACE2 receptor

v. Tissue fibrosis

Having the largest surface area, the lung is the most frequent organ affected ,also due

To the inhalation mode of transmission . Rich vascularity also leads to rapid dissemination of the viral particles to other organs .Type II pneumocytes of the alveolar epithelium expresses majority of the ACE II receptors in the lung [20]. Following viral entry,the replication and production of viral particles and their release from the host cell Leads to the relaease of DAMPS (Damage Associated Molecular Patterns). This leads To the activation of the certain transcription factors naming few INTEFERRON REGULATORY FACTOR 3 (IRF 3) ,Nuclear Factor kappa beta (NFKB), which in Turn causes the release of pro-inflammatory mediators. The immune response varies between a immunocompetent and immunosuppresed host. In an immunocompetent host inflammatory signals recogonized present the processed viral particle through their APC(Antigen Presenting Cells) to the CD4 T cells, going on priming other cells :

CD8 T CELLS - Cytotoxic and kills the virus infected cells

B-CELL - producing neutralising antibodies against N and S protein

PHAGOCYTE - remove the dead cells and neutralized viruses .

Inadverent immune activation ,leads to intense inflammation with inefficient viral clearance , as well as overproduction of chemokines TNF-a ,IL-6,IL- b, and other pro-inflammatory cytotkines [21].This leads to a breach in the alveola –capillary barrier thus spreading the virus particles and inflammatory cytotkines to distant organs leading leading to multiorgan

dysfunction .The inflammation also gives rise to the thrombo-embolism due to the activation of coagulation cascade and neutrophill extracellular traps and suubendothelial fluid leakage .

MECHANISM INVOLVED IN LUNG INJURY :

Along the respiratory tract ACE2 receptor is expressed in various cell type, involving the nasal mucosa of the upper respiratory tract, bronchial, alveolar epithelial cells in the lower respiratory tract .The nasal expression of ACE2 was found to be high in adults compared to children, and therefore explaining the less incidence of infection in children compared to the adult counterpart . Individuals having COPD and smokers express ACE 2 in much higher concentration than normal, healthy individualsACE 2 is both protective and promoter of inflammation and their Consequences. Viral interaction leads to the ACE2 downregulation by clevage and Shredding ,but leads to accumulation of ANGII producing TNFa and IL-6.

But since Alveolar Type II epithelial cells are continuously self renewing, they are repeatedly Being targeted for viral entry and replication, leading to repeated cycles of injury and Repair, that ultimately leads to fibrosis of the lung tissue. The protective role is playedBy the ACE2/Angiotensin (1-7)/MAS axis, whose functions include reduction of Inflammation, fibrosis,pulmonary arterial hypertension, decreasing the rate of cancer growth ,decreasing metastatic potential of tumor.

In histopathological studies, there is inbcreased neutrophill Count, mononuclear cell count, with increased alveolar proteinaceous exudate and type II alveolar epithelial cell hyperplasia . When there is progression of lung injury, alveolar Septa becomes thickened, thrombus formation occurs, hyaline membrane formation Follows. Certain cases are also associated with multinucleated giant cells. There is also increased incidence of venous embolism leading to repiratory failure. There is consolidation with fibroblast proliferation along with fibrosis. [22]

PATHOPHYSIOLOGY OF ENDOTHELIAL INJURY :

ACE2 expression in the arterial /venous endothelium of various organs, this is proved by the microscopic evidence of SARS Cov virus particles in kidney, lung endothelial cells [23].Endothelial injury mediated by infection and associated endothelitis is found in various vascular bed , this triggers excessive thrombin production ,increased levels of VWF, activating the complement systems and inhibit fibrinolysis, ultimately leading to microvascular dysfuntion [24]. Cross communication occuring between neutrophill and platlets along with the activation of macrophages in the setting of endothelial inflammation can lead to exuberant release of proinflammatory cytokines, neutophill extracellular traps (NET) formation, fibrin andthrombi deposition. Formation of NET can lead to further damage of already inflammed endothelium and further activate the coagulation cascade both intrinsic and extrinsic.

Hypoxia that have occurred as a consequence of the ARDS can lead to upregulation of the hypoxia inducible factor(HIF-1a), that causes hypoxia mediated hyperviscosity and lead to further aggravation of thrombi formation [25]. Though viral infection and inflammation can lead to damage of vascular endothelium, increasing the permeability and fluid leakage, ACE2/ANG (1-7)/MAS axis also have antiproliferative, antithrombotic effects. These are evidenced by the HPE section of the tissue from patients showing fibrinous exudates and microthrombi formation. There is also elevated levesls of thrombin, tissue factor V, factor VIII, fibrinogen. This forms the basis for using the D-DIMER levels, fibrinogen, platlet count prothrombin time in monitoring severe case of COVID pneumonia [26].

PATHOPHYSIOLOGY IN CARDIOVASCULAR SYSTEM :

Various tissues in the heart express ACE2 receptors in higher levels , including cardiac myocyte ,fibroblast, endothelial cells , smooth muscle cells [27]. The mechanism of cardiac damage is found to be direct viral mediated toxicity as evidenced by the extraction of SARS CoV2 RNA from the cardiac myocytes. Also the pericytes express high levels of ACE2 and since they are also targeted by SARS CoV2 ,this leads to endothelial dysfunction [28]. Increased levels of soluble ACE2 though not well expalinedare significantly increased in the presence of cardiovascular dysfunction and so can be serving as a potential biomarker. People who have preexisting cardiovascular disease

are also more prone to severe covid, due to high levels of ACE2 expression.

Elevated pulmonary venous pressure secondary to ARDS, pulmonary thromboembolism, virus mediated damage to vascular endothelium and smooth musce tissue dysfunction can lead to isolated right ventricular dysfunction. The cytokine storm resulting from the lung injury and pulmonary dysfunction can lead to myocardial injury.

Systemic cytokine storm also activate the endothelial cells and cause dysfunction of the coronary microvasculature causing ischemia and myocardial injury [29]. Hypotension ensuing from the sepsis, septic shock can lead to reduced myocardila oxygen supply. The resulting myocarditis can lead to failue of the myocardial pump , arrythmias such as ventricular fibrillation , ventricular tachycardia , atrial tachyarrythmias. [30]

One other presentation in cardiovascular system is the kawasaki like syndrome characterised by circulatory dysfunction and macrophage activaton syndrome, which are termed as multisystem inflammatory syndrome – adults (MIS-A). [31]

PATHOPHYSIOLOGY OF GASTROINTESTINAL AND LIVER INJURY :

As a co receptor for aminoacid uptake, ACE2 receptor are found abundant In the luminal surface of the intestinal epithelial scells. SARS CoV2 protein has been identified along multiple intestinal structures. Viral mediated direct injury is a plausible mechanism. There is live virus sheding from the

gastrointestinal system even after the Symptom resolution [32]. There is also evidence of diffuse inflammation of the endothelium Of the small intestinal submucosal vessels and evidence of mesentric ischemia. There is Also plasma cell and lymphocytic infiltration of the lamina propria alomng withintesrstial edema. Though not associated with the severe disease, presence of gastrointestinal manifestation, can lead to prolonged disease and one of the mechanism noted is alteration of the intestinal flora by the virus [33].

The inflammatory response can also pave the path for the malabsorption with enteric nervous system activation and increased intestinal secretion. This also leads to altered intestinal permeability. Covid induced gut dysbiosis as mentioned previously can increase the level of cytotokines, prolong the inflammatory response and can have exaggerated immune response. This also predicts the covid severity as most of the immunosuppresed individual, diabetics can have altered gut microbiome. A diet rich in fibre based on plant can improve the gut microbiome and improve the immunity. The gut microbiome can also improve the immunity. The gut microbiome also influence the susceptiblity to lung infection and can have a check over the dysregulated immune response. [34]

In the hepatobiliary system, there is evidence of direct damage to the biliary ductules due to expression of the ACE2 expression in the cholangiocyte. [35] Other mechanism of the liver damage are due to hypoxia induced metabolic derangement and hyperinflammation. Certain drug

treatment of covid that includes lopinavir,ritonavir , Remedesvir are also cause of the liver damage. The histo-pathological examination reveals kuppfer cell proliferation and chronic hepatic congestion but decreased frequency of hepatic cholestatic pattern, others include lymphocytic infiltrate ,portal fibrosis,ductular proliferation, lobular cholestasis, acute liver cell necrosis and central vein thrombosis. [36]

PATHOPHYSIOLOGY OF UROGENITAL INVOLVEMENT :

The proximal tubular cells of kidney which are brush border cells areSusceptible to injury by SARS CoV2 infection as ACE2 is expressed here [37]. There is tight regulation of balance between ACE2/Ang(1-7) and Ang II for sustaining normal Kidney function. Demonstratiom of viral inclusion particular within the tubular epithelium, podocytes, endothelial cells of glomerular capillary loops, lymphocytic endothelialitis within the kidney in glomerular capillary endothelial cells also suggest that microvascular dysfunction could be due to secondary endothelial damage. There is also possibility of underlying sepsis and sepsis associated AKI. Immune complex mediated glomerulopathy due to viral protein or viral meadiated effector mechanism can occur, these are suggested by formation of collapsing focal segmental glomerulosclerosis. The most commom abnormality seen is mild to moderate proteinuria. Certain patients can have heamturia, elevated blood urea nitrogen, these proteinuria could be due to direct podocyte injury,

epithelial dysfunction ,a defect in receptor mediated endocytosis of proximal tubular epithelial cells. Other causes of kidney injury found are volume depletion and interstitial nephritis . [38] [39]

PATHOPHYSIOLOGY IN ENDOCRINE SYSTEM :

With severe disease course there is worsening hyperglycemia ,along with ketoacidosis in patient with diabetes. ACE2 expression is documented in endocrine pancreas. Binding of SARSCoV 2 to ACE2 receptors in pancreas can lead to direct damage and decrease in pancreatic insulin release and hyperglycemia. Another mechanism proposed is increased rate of fat breakdown. Increased hepatic glucose production,decreased insulin secretion with increased insulin resistance and increased ketogenesis due to counter regulatory hormone synthesis. Even in patient without preexisting diabetes,covid can cause hyperglycemia due to ACE2 mediated damage. [40]

Risk factor for more severe illness include obesity,which can affect pulmonary function by reducing the lung volume and compliance ,increase in airway resistance and associated with diabetes. The excess cytokine, adipokine, chemokine,proinflammatory cytokines like TNF a, IL6,IL18, adipokine, leptin ,all increase the inflammatory response. [41]

Molecular mimicry to ACTH by SARSCoV 2 and direct antibody against ACTH, is the key stratergy of the virus by reducing the cortisol level, producing cortisol insufficiency. Degeneration have been noted in the adrenal

gland along with necrosis. This leads to altered response to cortisol secretionin SARS patients. Since ACE2 is also expressed in both hypothalamus ans pituitary tissue, there is viral tropism and there is hypophysitis due to direct viral mediated hypothalamic damage . [42]

PATHOPHYSIOLOGY IN NERVOUS SYSTEM :

Olfactory bulb ,astrocye ,oligodendrocyte neurons all contain ACE2 receptor. So when the olfactory epithelium is infected ,the other areas of the brain can also become infected due to rapid dissemination. The cerebral vascular endothelium is also affected by the SARS COV2 and can also reach the brain through the infected leucocytes by crossing the blood brain barrier. [43]

Neurovirulence of the covid also induce the proinflammatory prothrombotic cascade with the cytokine storm. Encephalitis due to direct virus mediated damage, cytokine mediated damage can also cause acute necrotizing encephalopathy, following recovery from acute infection, there is a possibility of indirect injury in the form of guillian barre syndrome, transverse myelitis. [44]

Cerebrovascular disease occurrence is also seen with severe covid 19, since covid 19 is a state of hypercoagulability,it can preciptate both venous and arterial thrombosis. Dysregulated immune system and excess inflammatory response ,autoimmune mechanism triggers the coagulation cascade, reflected

by the elevated prothrombin, fibrinogen and D-DIMER level, low anti thrombin level, thrombocytopenia and DIC. With such an imbalance of the coagulation system microvascular thrombosis starts and propagates. Cardiac arrythmias due to hypoxia can also lead to cardioembolism, fluctutation in blood pressure can lead to cerebro vascular accidents both ischaemic and hemorrhagic stroke. [45]

Viral tropism to olfactory nerve and other nervous tissue followed by thalamus,brainstem and temporal lobe, amygdala, limbic system leads to gustatory and olfactory dysfunctionin the patients. Since the ACE2 receptors varies among various ethinic groups the manifestation also varied . [46] [47]

IMMUNE MEDIATED MANIFESTATION:

Certain patients present with immune medaited manifestation pertaining to certain organs and systems. Various immune mediated manifestaion are present to name a few chillblains,eryhtema multiforme, ITP,hemolytic anemia, GBS, enchepalitis,myelitis,myocarditis, pericardiaal tamponade, glomerulonephritis, thyroiditis, pancreatitis. These can mimic various autoimmune disease. [48]

Chilblains is a rare inflammatory condition that affects the extremity after exposure to cold, which can lead to erythema, itching, painful, violaceous, lesions. These patients contain virus particles in the biopsy from the skin , negative results of PCR testing for SARS CoV 2. Erythema

multiforme which is an inflammatory condition. There is an age wise variation in the pathophysiology. Childrens have these lesions associated with chillblains, adults these lesions are associated with drug use like azithromycin, hydroxychloroquine. Various other manifestations are Erythema nodosum, generalised pustular figurate erythema, periorbital erythema, puffiness, retiform purpura, sweet syndrome. [49]

Peripheral smear shows lymphopenia, which is a apparent feature ,also it is relevant with prognosis linking it with ARDS, poor survival. [50] Thrombocytopenia and anemia are also reported, symptomatically these can present with TTP/HUS. Patients presenting with TTP/ITP are much older and platlet count falls approximately after two weeks of the onset of Covid 19 symptoms. Autoimmune hemolytic anemia is also diagnosed in the older patients following covid one to two weeks later.

SYSTEMIC IMMUNE MEDIATED MANIFESTAITON :

CYTOTKINE RELEASE SYNDROME:

Release of large amount of cytokine can lead to hyperinflammatory phenotype. There is dysregulated immune response with release of cytokine and leading to multiorgan failure and high mortality. It is characterised by symptoms such as fever, tachypnoea, tachycardia, hypovolemia and SIRS. IL 6 is a key factor and a pro inflammatory mediator that causes hyperferritenemia, elevated CRP and hypercoagulable state. The systemic

involvement can overlap with the syndromes like kawasaki disease in children and HLH,APLA , systemic vasculitis in adults.

MULTIINFLAMMATORY SYNDROME OF CHILDREN:

Follwing infection with SARSCoV2, a systemic inflammtary syndrome acquired in children, that was similar to that of kawasaki syndrome and it wastermed as PIMS – paediatric inflammatory multisystem syndrome also known as MISC- multisystem inflammatory syndrome in children. It involves children of age more than 5 years who are non white and involving the gastrointestinal, cardiovascular, mucocutaneous, respiratory system. Kawasaki syndrome typically affects less than 5 years asians and lower rate of ICU admission and death than MIS-C. coronary arteries are involved int the similar frequency in adults.

HAEMOPHAGOCYTIC LYMPHO HISTIOCYTOSIS:

There are hyperferritenemia , hyperinflammatory syndrome have different pathogenesis but with virus as a main external trigger. Though covid related HLH is a rare complications, since the number of case increase in pandemic, the HLH are also reported. The main diagnostic features of HLH are fever, splenomegaly, hypofibrinogenemia, hyperferritenemia, high serum concentration of IL2 receptor (sCD25). Low activity of NK cell/ hemophagocytosis in bone marrow.

ANTI PHOSPHOLIPID SYNDROME :

Though SARS Cov2 can cause coagulopathy as it is a procoagulant state, there is increasing evidence of autoimmunity where lupus anticoagulant is positive in 90% cases. In most studies, LA postivity is detected in half of tested patients. But since LAC can be elevated in various intesnse inflammatory and infective state. LAC positivity does not equal with APS and the use of heparin for thromboprophylaxis also intereferes with the assay for lupus anticoagulant. But anticardiolipin antibodies are detected at a less positive rate. Although it is possible that the patient infected with COVID can have APL related thrombosis, the viral trigger of such an event is not accepted avidly.

SYSTEMIC VASCULITIS IN COVID :

There is a increasing evidence to link the SARS CoV 2 and systemic vasculitis includes kawasaki like othe diseases. The organ commonly involved are CNS, skin, gastrointestinal system following 2 weeks after symptom of SARS Cov infection.

The histopathological examination is mostly consistent with the leucocytoclastic vasculitis and other vasculitis are cutaneous , retinal, CNS vasculitis, but their incidence is very much lower .

MYOSITIS:

Substantial amount of infected people have muscular inflammation. Though myositis has been reported at around 10 – 11 %, frequency of rhabdomyolysis is low. Most cases are reported in adult males and mostly in first week of symptom onset. The immune mediated muscular damage coulld be due to and also involve critical illness myopathy and superimposed steroid myopathy. Viral mediated direct cytolysis and damage due to elevated cytokine could be causing the immune related muscular damage, along with necrotizing autoimmune myopathy, manifesting as increasing muscular weakness and inflammatory marker and high CK levels.

SYSTEMIC AUTOIMMUNE DISEASE :

Immune syndrome triggered by Covid 19 include SLE, Sjogren syndrome, sarcoidosis, but all these syndromes are reported at a incidence of very low numbers.

TRANSMISSION:

Main mode of transmission is by aerosol from infected person and droplets are also produced during procedures like surgery from running tap water, toilet flush, which can generate aerosol. Most virus infects the respiratory system by aerosol and they include the respiratory syncytial virus, parainfluenza, corona, rhinovirus. Three modes of transmission are documented and they are aerosol, self-inoculation of nasal mucosa and droplet

transmission. Two types of air transmission include airborne transmission which means spread by small droplet and droplet nuclei which is droplet transmission by large droplet aerosol.

Direct transmission occurs by sneezing, coughing, during which droplets are sprayed directly into the mucosa or conjunctiva of the host. Contact transmission occurs when infected secretion are deposited on fomite and host has direct physical contact with it.

One of the prime modality of transmission is by fomite and by self-inoculation from such contaminated fomite. These are due to the poor hand hygiene and lack of following the proper disease control measures and etiquette. The major route of transmission is between people through respiratory droplets and contact routes. There are also evidence mounting to the fecal contamination caused by the person infected and covid 19 could also be transmitted by the fecal route. SARS Cov 2 can survive in the air for many hours. Certain medical and life saving procedures can generate aerosols like endotracheal intubation, bronchoscopy, manual ventilation, nebulization, prone ventilation, tracheostomy, CPR. There are reports of sample taken from airvent and exhaust from patients room testing positive for covid 19, due to the deposition of aerosol particles.

SOURCE AND SIZE OF THE PARTICLES:

The source of SARSCov2 transmission is when a symptomatic person sneezes, cough, talks, exhales. Large droplets which are heavy fall to the nearby floor, near to the source. They contaminate the nearby fomite that could lead to the transmission when susceptible person gets in contact with it. Smaller droplets that are ejected gets converted to aerosol or bioaerosol that have smaller diameter, that become airborne and can spread to larger distance and contribute to spread. Sometimes larger droplets get evaporated and they become smaller called as droplet nuclei. Bioaerosols are aerosols that encapsulate the virus particle. Normal breathing through nose can produce few aerosols and more droplets that account for upto 95 % and talking, coughing, sneezing produced more aerosols than droplets.

FATE OF THE EXHALED DROPLETS:

The exhaled particle can undergo evaporation, interact with other types of particle, and removed from the surrounding by deposition. There are various motions of particles that can influence the particle ,these include inertia force, turbulent diffusion, Brownian movement, gravity, thermal gradient, electromagnetic radiation, electrostatic forces. If the size is less than 1 micrometer, then diffusion plays an important mechanism of transmission. When larger than 1 micrometer gravity becomes important factor, when 1-100 micrometer, they evaporated and evaporated droplets containing the virus

becomes bioaerosols. Droplets of size 0.5-20 micrometer will get retained in respiratory tract and can cause infection. While coughing and sneezing, the turbulent flow of cloud of gas is suspended as droplets of various sizes.

The 2mm safe exclusion zone to prevent possible droplet transmission from infected person to susceptible host is accepted. The larger droplets which are >100 um diameter will fall to the floor with a horizontal distance of 2 mm from source. In a scenario of sneezing the larger droplets are carried away upto 6 mm of horizontal distance with a velocity of 50 m/second. Larger droplets of more than 2 mm travelling at velocity of 10 m/ second is seen in coughing bouts. Simple exhalation carries the droplet to 1mm horizontal distance at velocity of 1m/second.

VIABILITY OF DROPLETS:

Factors that impact the viability of the airborne microorganism are temperature , humidity , radiation, open air. SARSCoV2 are less than 100nm in size.

The tolerance of the virus laden phenotype depends on the viral phenotype, composition of aerosols, physical characterstics in surrounding environment. There are also association of the covid 19 infection with air pollution, as they could bind to the particulate matter and be airborne. In a closed environment, virus can be airborne by forces caused by the local ventilation pattern and travel further away through dispersion and diffusion.

CLINICAL MANIFESTATION OF COVID:

PULMONARY INVOLVEMENT:

The severity of the infection ranges from total absence of symptoms to mild pneumonia and in certain case severe disease, associated with pneumonia, shock, respiratory failure, death from multiorgan failure. This was noted as most common disease manifestation. Patient can have dry cough, fever, sputum production, fatigue, dyspnoea. Though covid 19 causes ARDS, atypical ARDS are getting common. There is acute interstitial pneumonia and diffuse alveolar hemorrhage and macrophage infiltration. There is formation of alveolar edema and hyaline membrane with thickening. The vascularity of the lung are involved in the form of hyaline thrombosis, vessel wall edema, intravascular neutrophil infiltration and immune cell infiltration.

This microthrombi with the lung causes pulmonary microinfarction, hemorrhage and pulmonary hypertension and right ventricular stress. This presentation has been aptly named as the pulmonary intravascular coagulopathy (PIC), which is further contributed by the hypoxemia and mechanical ventilation.

Hypoxia is the presenting feature of covid 19 in most instances, but it is mostly gradual and paradoxically well tolerated, gaining the name ‘HAPPY HYPOXIA’ or silent hypoxia, which is typically seen early and a atypical feature of ARDS. In typical ARDS, lung compliance is not preserved and the

hypercapnia will stimulate the sensation of dyspnoea. But in covid ARDS, the reverse happens as there is no stimulation of sensation of dyspnoea by hypercapnia. Various factors contribute to the presentation, the infection severity, patient co morbidities, physiological reserve and the time between disease onset and hospitalization , host immune response all can contribute.

One more factor that can contribute to the severity following ARDS is the excessive increase of proinflammatory cytokines like IFN, TNF-a. this results in cytokine storm and causes endothelial dysfunction ,damage vascular barrier and capillary leak, diffuse alveolar damage.

CARDIOVASCULAR MANIFESTATION:

Previous hypertension, diabetes and previous cardiovascular disease has been associated with very worst outcome and various complications are associated with poor survival. This also includes presence of obesity. Covid 19 can directly facilitate myocardial injury and lead to myocarditis due to ACE2 expression. The downregulation of ACE2 can also lead to compromise of myocardial function following direct damage. The hyperinflammatory syndrome caused by the excess of cytokines can lead to the activation of macrophages and leucocyte and adhesion to vulnerable atherosclerotic lesion and can cause plaque disruption and coronary occlusion predisposing to myocardial infarction and acute coronary syndrome(ACS).

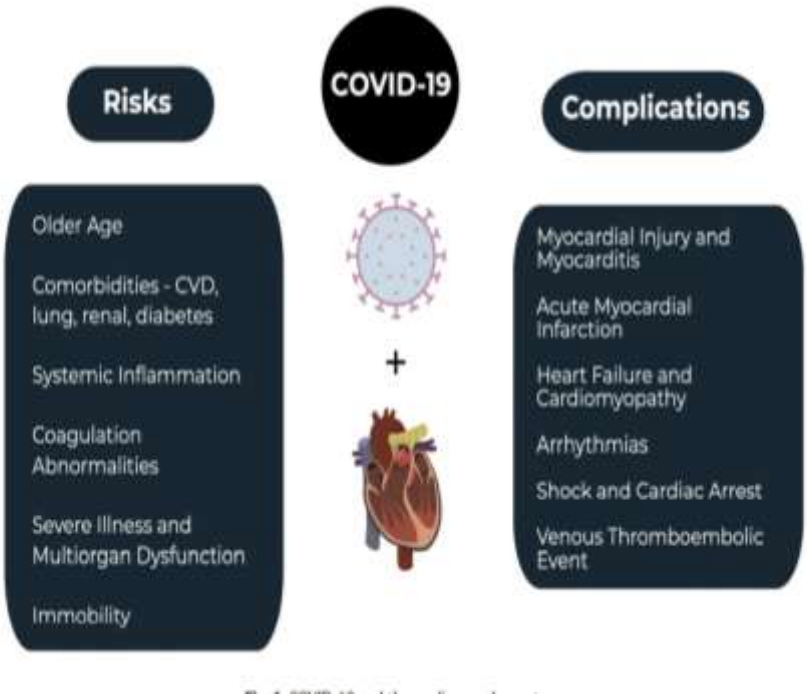
There is also activation of endothelial cells and dysfunction of microvasculature leading to myocardial ischemia and injury. The type II myocardial infarction which is supply demand mismatch is also possible with covid 19 infection, this may be due to hypoxia, sepsis induced hypotension and cytokine storm induced hypotension. The presence of systemic infection can increase the metabolic demand of peripheral tissues and end organ demand,that can create a burden on the already failing heart. Right heart failure can occur in these patients with ARDS and acute lung injury.

Certain patients infected with covid 19 can also have fulminant myocarditis that present with severe left ventricular systolic dysfunction and cardiogenic shock. Arrythmias are another life threatening manifestations of covid 19 in cardiovascular system. Ventricular tachycardia and ventricular fibrillation are most frequently and most common arrythmias. Atrial tachycardia is more common in patients on mechanical ventilation. Sustained ventricular arrythmias are more commonly a manifestation of acute covid myocarditis. Various medical treatment that are suggested and that are frequently used are also predisposing to arrythmia and these include azithromycin, chloroquine phosphate, hydroxychloroquine. These can predispose to torsades de pointes and mortality due to QTc prolonagation. Dysarrythmia in the setting of elevated troponin I should make one consider myocarditis, myocardial infarction and acute coronary syndrome. Kawasaki

like syndrome characterized by macrophage activation syndrome and circulatory dysfunction is also reported in certain case reports.

There is increased risk of venous thromboembolism in covid 19. The contributing factor include abnormal coagulation status, multiorgan dysfunction, systemic inflammation and critical illness. It is associated with significantly elevated D-DIMER. Very much elevated D-DIMER levels is associated with pulmonary embolism which are confirmed by CT Pulmonary angiogram and which is associated with death.

Dysautonomia could be a part of covid 19 infection, either as a acute manifestation of the infection or a long term sequelae of the disease called “LONG COVID”. It is the malfunction of the autonomic nervous system either due to dysfunction , failure and overactivity of the parasympathetic and sympathetic components. One of the feature of such dysautonomia is the POTS postural orthostatic tachycardia syndrome with features of lightheadedness, fatigue, dyspnoea, presyncope, Shortness of breath, chest pain , sleep disturbance , gastrointestinal symptoms. Various mechanisms that can lead to POTS are invasion of the medullary system, brain stem, and autoimmunity.



Medication that are approved for covid that are associated with cardiovascular comorbidity include fravipravir that interact with statins, antiplatelets, anti coagulants, anti arrythmics and can cause hemolytic anemia. Lopinavir /ritonavir can interact with antiplatelets, statins, anti coagulants and anti arrythmics. Ribavirin can interact with anticoagulants and can cause severe hemolytic anemia. Remedesvir can cause hypotension and arrythmias. Interferon cause direct myocardial toxicity and hypotension, ischemia, worsen cardiomyopathy, altering cardiac conduction.

Methylprednisolone can interact with anticoagulant and cause fluid retention and electrolyte change. Patients recovered are also noted to have edema and fibrosis of cardiac tissue evidenced by the imaging with cardia

MRI. But in most cases it is not clear that whether fibrosis was present before covid infection or has developed following infection. This fibrosis is hypothesized to be due to the replacement fibrosis of the necrotic myocardial cells, this appears to be the main mechanism of the fibrosis. Also the infiltration of the myocardium with immune cells and presence of cytokine storm can cause the transfer of the fibroblast to myofibroblast conversion and can cause subsequent remodeling of the matrix.

HEMATOLOGICAL MANIFESTATION :

There is a significant impact of the hematopoietic stem cells and hemostasis. The cardinal laboratory manifestation is lymphopenia that has also some impact in the prognosis. The cytokine storm occurs at around the 7-15 days from the onset of the initial symptoms, during this time significant lymphopenia can manifest. Platelet /lymphocyte ratio , neutrophil/lymphocyte ratio all have prognostic value in determining disease severity. Lymphocytes are directly infected by the SARS CoV 2, through ACE2 receptors and cytokine can increase lymphocyte apoptosis. There is also atrophy of the lymphoid organ like spleen mediated by cytokine elevation. Among the lymphocyte population it is the CD4 and CD8 T lymphocytes that are predominantly reduced neutrophils was also elevated in severe covid 19. Apoptotic and immature granulocyte forms are also observed in the peripheral smear, these may be due to the cytokine storm and hyperinflammatory

syndrome. Hemophagocytosis has been seen in bone marrow of covid 19 patients. Also there is an increase in pleomorphic megakaryocyte, plasma cell hemophagocyte and macrophages. The active platelets production are seen in bone marrow. Blood group O has been associated lower severity on infection, but other blood groups are associated with severe infection.

Thrombocytopenia was more significantly associated with severity of the disease, though variation occurs, people who fail to recover have very low platelet counts. Biomarkers such as LDH, CRP, IL6 also prove to be better prognosticators of dismal prognosis.

Coagulation parameters like prothrombin time, activated partial thromboplastin time, prolongation with increased fibrin degradation product and thrombocytopenia can point to disseminated intravascular coagulation (DIC). Endothelial cell activation and damage due to virus binding can be increasing the risk of venous thromboembolism. These coagulation abnormalities noted in the covid can point to characterstics of both prothrombotic coagulopathy and consumptive coagulopathy. These can manifest as feature of hypercoagulability together with severe hyper inflammatory state. There is diffuse bilateral pulmonary inflammation, that has lead to the emergence of the pulmonary specific vasculopathy. The complex mechanism and phenotype of the covid coagulopathy resembles the TMA (thrombotic microangiopathy), that is mediated by the complement,

rather than sepsis associated. Initially there is no consumptive coagulopathy as if in like sepsis or DIC, but as the disease, this can cause consumptive coagulopathy and the alveolar macrophages release urokinase type plasminogen activator, that can cause systemic fibrinolysis and elevated D-DIMER levels.

Red blood cells of patients infected with covid have increased oxidation of structural proteins and have altered lipid metabolism. This could reduce the hemoglobin levels to a milder extent and was suggested to prognostically impact management, but at present no recommendations are officially.

GASTROINTESTINAL AND LIVER INVOLVEMENT:

As previously discussed about the site of presence of ACE2 receptors in the intestine , there is inflammation and occurrence of diarrhea in infected patient and the binding of SARSCov2 is much higher than the strain and this accounts for higher incidence of infection and inflammation are more. The most common of the symptoms reported are the nausea, vomiting, and loss of appetite, then diarrhea and abdominal pain which are present in the minority of the people. The patients with gastrointestinal symptoms take a long time to admission from onset of symptoms than the patients without these symptoms. The occurrence of diarrhea is correlated with the illness severity as more critically ill people have diarrhea as their initial manifestation. There are reports of renal insufficiency increasing in incidence associated with the

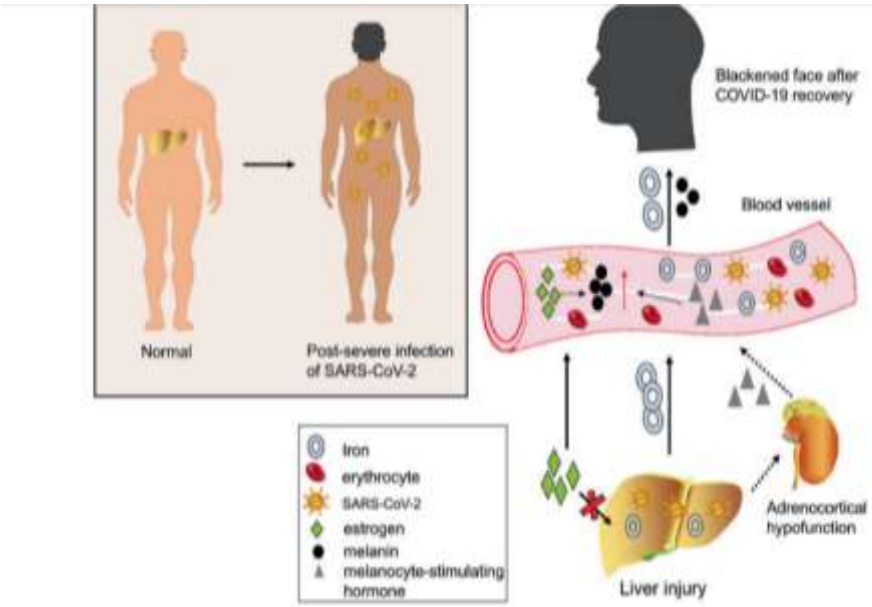
diarrheal symptoms. The inflammasome activation and impaired intestinal barrier is associated with increased risk of cardiac injury. This is concluded by correlating plasma levels of intestinal fatty acid binding protein, marker of intestinal leakage and marker of intestinal homing. The ventilator support was also needed more in patients with diarrhea than those who do not have.

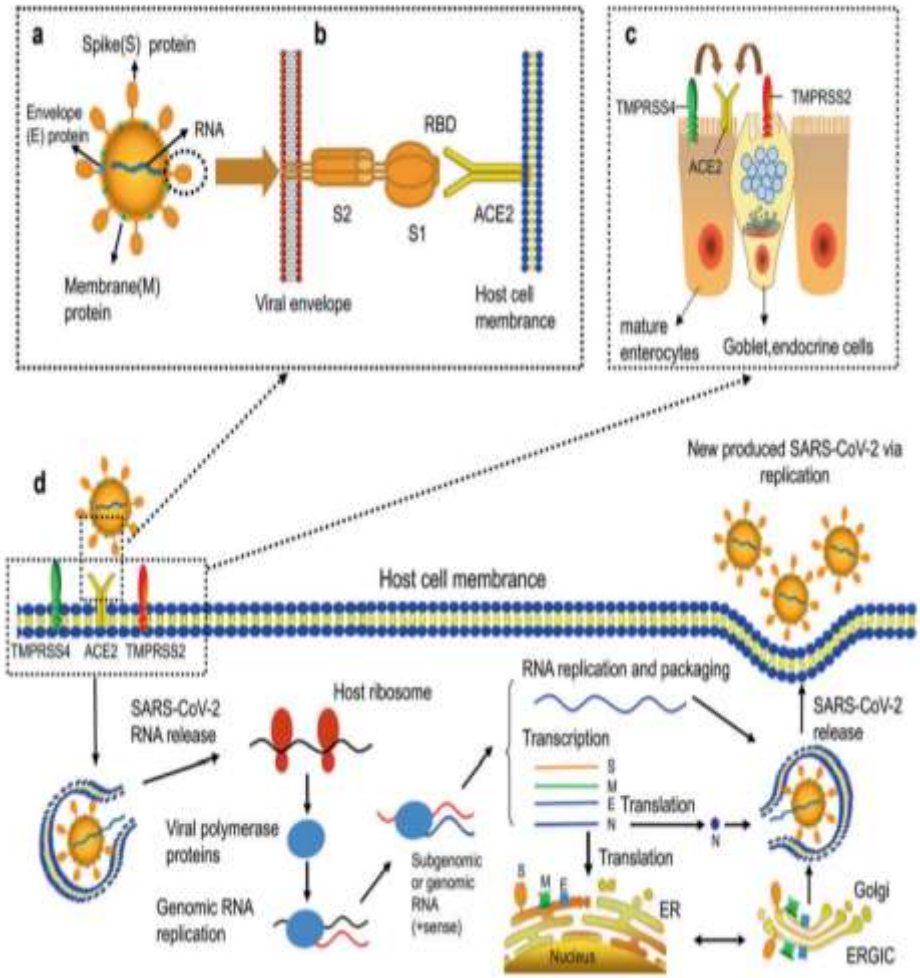
Use of proton pump inhibitors which are classically used to treat peptic ulcer disease and gastric acid reflux disease, decrease the stomach acid may be beneficial to the people with that condition , but can increase the vulnerability of the gut to get infected with corona virus. Since the binding of the SARSCoV2 to the ACE2 receptors occurs at a neutral Ph. Though the stomach does not contain receptors for SARS CoV2, the use of proton pump inhibitors can facilitate the infection by decreasing the acid secretion of the stomach making the pH from 1-3.5 increase and enable the virus to enter the small/large intestine that has pH of 7.5- 8.0 . This also correlation to the dose of PPI as people taking two to three doses PPI can get positivity more than who takes one dose. The use of Histamine H2 blockers is not associated with such a elevated risk. Patients who have taken PPI in past 30 days have increased risk of severe clinical outcome by 90%.

It is not surprising to notice the stool samples from covid 19 patients are tested positive, as it was positive in the forerunner MERSCoV. These provide sufficient evidence to suggest to say that feco-oral route of

transmission is a potential route of transmission. The stool positivity rate of covid 19 infection gradually decreases. The diarrhaeal patient have high viral load than those who do not have, the viral load were higher during the third and fourth week of disease onset.

Certain Chinese people who have suffered from covid had a sequel of darkened face and pigmentation during recovery. This is due to liver injury and multiple organ injury. The mechanism that are proposed are inactivation of the estrogen due to liver dysfunction increasing the conversion of tyrosine to melanin by tyrosinase which are normally inhibited by thiamine and estrogen, increased secretion of melanocyte stimulating hormone from the anterior pituitary, increased blood iron levels that can spread to the face and cause blackened face. All patients have abnormalities of AST and ALT, also there is an increase in bilirubin levels. The correlation states that the elevation of the AST/ALT twice as high is noted in the patients with severe pneumonia than in patients with mild pneumonia. In patients with NAFLD, there is increased production of proinflammatory cytokines from kuppfer cells. This change the state of macrophage polarization and the increase the proinflammatory ligands. The liver damage is mostly by the immune mediated mechanisms and direct viral mediated damage

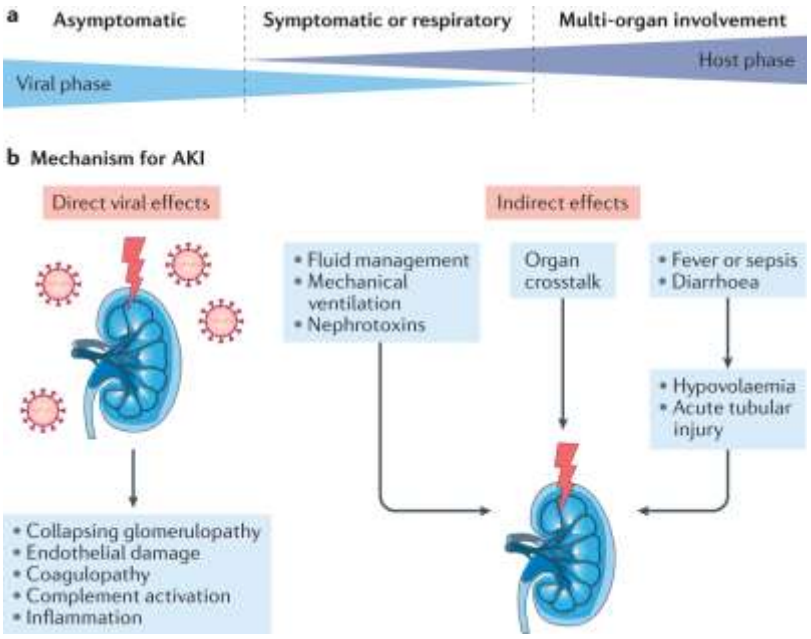

Normal
Post-severe infection of SARS-CoV-2
Blackened face after COVID-19 recovery
Blood vessel
Iron
erythrocyte
SARS-CoV-2
estrogen
melanin
melanocyte-stimulating hormone
Adrenocortical hypofunction
Liver injury


a
Spike(S) protein
Envelope (E) protein
RNA
Membrane(M) protein
b
RBD
S2
S1
ACE2
Viral envelope
Host cell membrance
c
TMPRSS4
TMPRSS2
ACE2
mature enterocytes
Goblet,endocrine cells
d
New produced SARS-CoV-2 via replication
Host cell membrance
TMPRSS4 ACE2 TMPRSS2
SARS-CoV-2 RNA release
Host ribosome
RNA replication and packaging
Transcription
Translation
SARS-CoV-2 release
Viral polymerase proteins
Genomic RNA replication
Subgenomic or genomic RNA (+sense)
Translation
ER
Golgi
ERGIC
Nucleus

RENAL INVOLVEMENT:

There is mild to moderate proteinuria along with the higher levels of TNF a, IFN gamma, IL8 in patients admitted to ICU. These are due to the cytokine release syndrome which causes cytokine storm, which is similar to sepsis associated AKI, in which there is kidney injury caused by uncontrolled inflammation. The mechanism of this injury may be due to monocyte, lymphocyte and virus induced proinflammation. There is alteration in the renal hemodynamics and patients with mild to moderate disease do not have acute kidney injury and they are mostly subclinical. There is an elevated urea and creatinine, that confer an elevated risk of injury and high risk of death. In certain studies, AKI was the most common extrapulmonary complication noted. ACE2 expression is more in the kidney than in the lungs and present in the apical membrane of the proximal tubule and also at the podocyte in the lower levels. Patients with diabetic kidney disease and Ckd are associated with higher incidence of the acute kidney injury, since they have a higher expression of ACE and down regulation of the ACE2, that can lead to the proinflammatory and profibrotic state. One another possibility of the kidney injury is the hypoxia caused by the pneumonia seen in severe disease. Microthrombi and hypercoagulable state found in covid 19 pneumonia tends the affect the microvasculature of the kidneys and cause acute kidney injury. Patient with AKI can have volume overload and electrolyte derangements that

can compound the damage caused by the ARDS in the covid 19 pneumonia. Since they have only few functioning nephrons, the remaining nephron compensate by hypertrophy. There is also glomerulosclerosis, tubular atrophy, interstitial fibrosis, that can be decompensated further by covid infection.



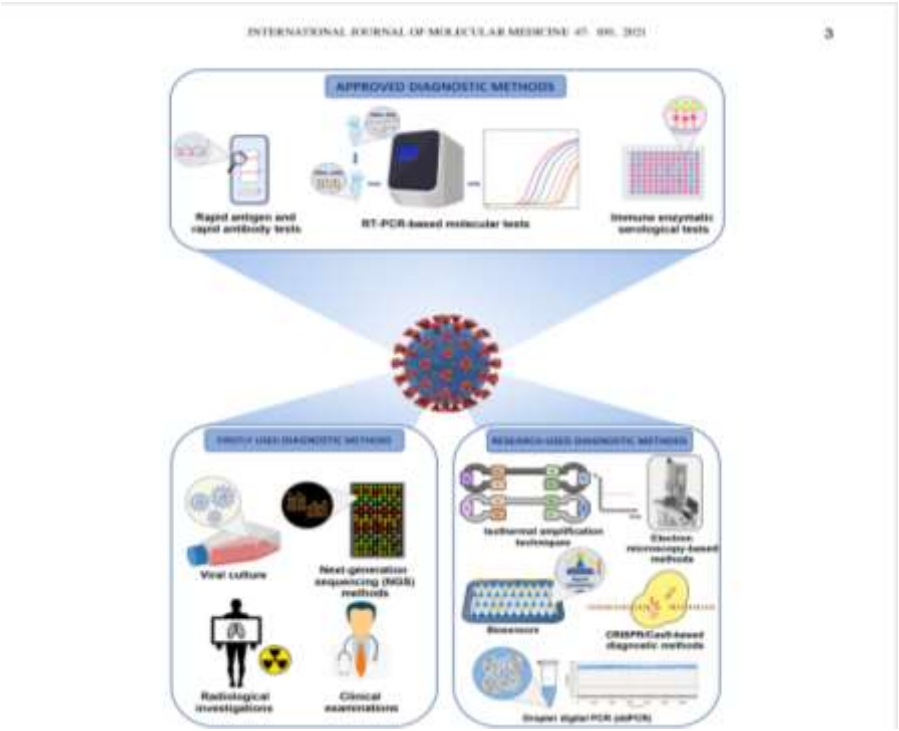
DIAGNOSSIS OF COVID 19 :

Diagnosis of Covid 19 pneumonia relies on the detection of the SARS Cov2 virus by the reverse transcriptase polymerase chain reaction(RT-PCR) of the sample taken from the upper airway, lower airway or Broncho alveolar lavage. The primarily preferred method is the upper respiratory tract sample, that is obtained either through the nasopharyngeal swab (NP), oropharyngeal swab. Though bronchoscopy can be used to obtain the sample from the lower respiratory tract, it is not routinely recommended or used since it poses substantial threat for other patients and health care worker as they are aerosol

generating procedures. Bronchoscopy can be used for patients who are intubated, when they have negative results from the upper respiratory tract samples. It can also be used when the diagnosis is not certain, when all the safety precautions have been made, tracheal aspiration could also be done in intubated patients, who cannot be subjected to the bronchoscopy procedures. The lower respiratory tract samples yield more positive results than the upper respiratory tract samples.

The studies indicate that SARS CoV 2 was isolated from the lower respiratory tract more than the upper respiratory tract, with the levels of viral RNA were higher from the lower respiratory tract than from the upper respiratory tract secretions, with a lower cyclical threshold values. The viable virus particle is also isolated from the blood, stool, urine specimens. The virus particles are isolated from these sites for weeks long but their viability is questionable. Among the upper respiratory tract smaples, the nasopharyngeal swab is more reliable than the oropharyngeal swab as indicated from certain studies, since the nasopharyngeal swab can reach the correct area to be sampled in the nasal cavity, it is tolerable to the patient and safer for the health care worker. But the patient presenting with specific clinical symptoms pertaining to the oropharynx like pharyngitis will be suitable for the oropharyngeal swab.

The specificity of the swab testing done for the covid is around 95-98%, but due to swab contamination false positive results are obtainable in asymptomatic patients. The sensitivity rate approachs to be around 66-80%, therefore the validity of the test in the people who are in close contact with the symptomatic positive people is questionable. The sensitivity could reach even around 50-60% in those without any evidence of symptoms or proven disease. But in a heavily exposed person, the presence of single negative test does not exclude the SARS CoV 2 infection and a repeat sample is to be done from the same site or deeper airway. Over time the positivity of the SARSCoV2 in these samples decrease and to confirm the presence of covid infection the fecal samples are used in those who have negative nasopharyngeal swab. A greater persistence of SARSCoV2 is seen in the gastrointestinal tract, even for more than a month following infection. The cause of the reduced sensitivity is the inappropriate timing for specimen collection, either too early or too late, inappropriate handling of the specimen, technical errors that can occur, quality of the specimen is poor.



METHOD OF OBTAINING THE NP SWAB:

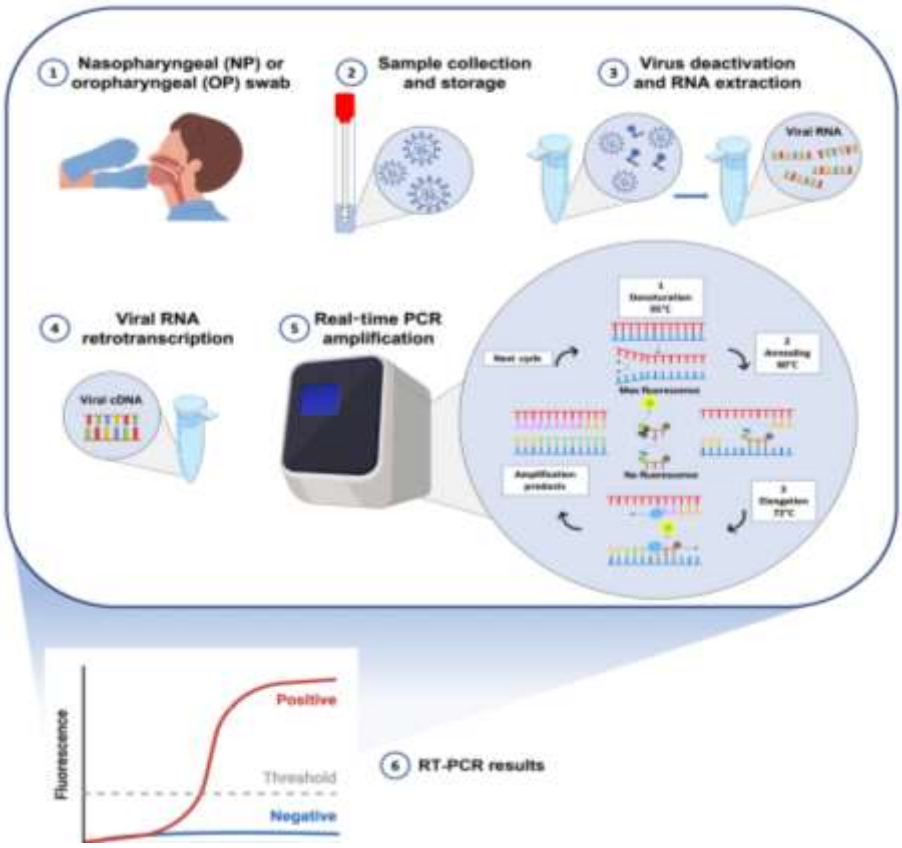
To properly obtain the NP swab , insert the swab deeply into the nasal cavity and as the patient is flinching it indicates that the swab is in the target area It should be left for the 10 seconds and then twisted 3 times. In order to be safe while collecting the swab, PPE equipment should be used. If the personal protective equipment is not available, other method of sample collection from the upper respiratory tract like salivary collection should be used.

RT-PCR (REVERSE TRANSCRIPTASE POLYMERASE CHAIN REACTION):

RT-PCR testing kit available targets two genes of the viral genome E and RdRP gene. The E gene is a specific gene for all SARSCoV related virus and it is the RdRP gene, that tests positive only in covid 19 infection.

RT-PCR techniques have a low level of detection of SARSCoV2 RNA. In the start of the pandemic many of the cases were labelled negative falsely, which was due to the sensitivity of the RNA primer used.

Therefore though the RT-PCR can be considered as the gold standard for diagnosing covid 19 infection , this can be subject to many limitations and criticism, which can lead to many false positive or false negative results. With lower viral loads, many asymptomatic or pauci-symptomatic people have lower sensitivity with RT-PCR results. The RT-PCR based tests depending on the protocol can be completed within the 4-8 hours and the semiautomatic tests can be completed within 24 hrs.



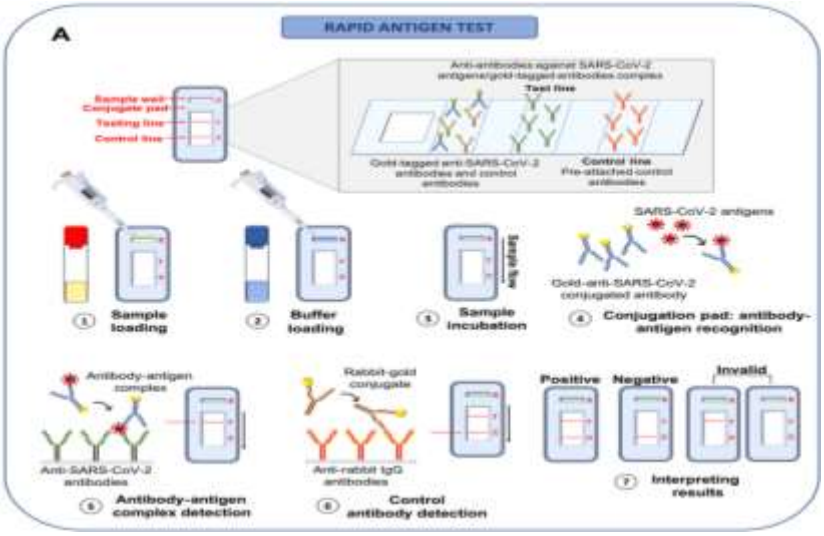
RAPID ANTIGEN TEST:

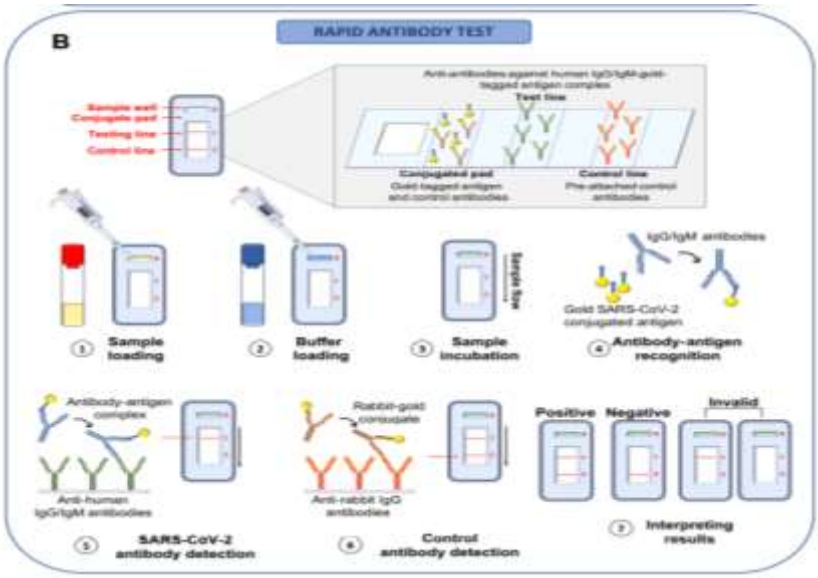
In order to overcome the delay poised by the RT-PCR based tests, the rapid antigen/antibody tests are developed. These are more rapid in execution with the time of 15-30 min and are affordable at lower cost and they are easily performed by anyone requiring no special training. These test are based on the lateral immunoflow assay and are used to detect SARSCoV2 viral antigen particularly the spike protein.

One another similar test that use the same principle is the rapid antibody test, but it detects human IgA, IgG, IgM against the SARSCoV2 antigen. In this test, the blood or saliva sample is tested. The test results are read by naked eye and are available in a short period of time and used as a point of care test. They yield only qualitative and quantitative results, that can establish whether the individual is positive or not and cannot determine the viral load. In case of the antibody test, these can detect only the presence or absence of antibody, but cannot differentiate whether the patient is actively infected or recovered. Important limitations of these tests are their lower sensitivity which is 56.2%, specificity of 99.5%, false negative rate of the rapid antigen test are 27.9% , and there are no false positive results.

The limitations are mainly due to the low viral load seen in some of the patients and low antibody response mounted by the infected patients. Though the viral antigen are found in the sample shortly after the infection their

biological stability is limited. The rapid antibody test detecting the IgG/IgM antibodies, do not detect them immediately after infection , but takes around 3-4 weeks for the antibodies to be formed and hence , such time to detect them by the tests. IgA antibodies can present in the serum at one week of infection, so they can be used to detect the earlier infection. Therefore the rapid tests are not for precise diagnosis, and they should always be confirmed by the RT-PCR analysis.

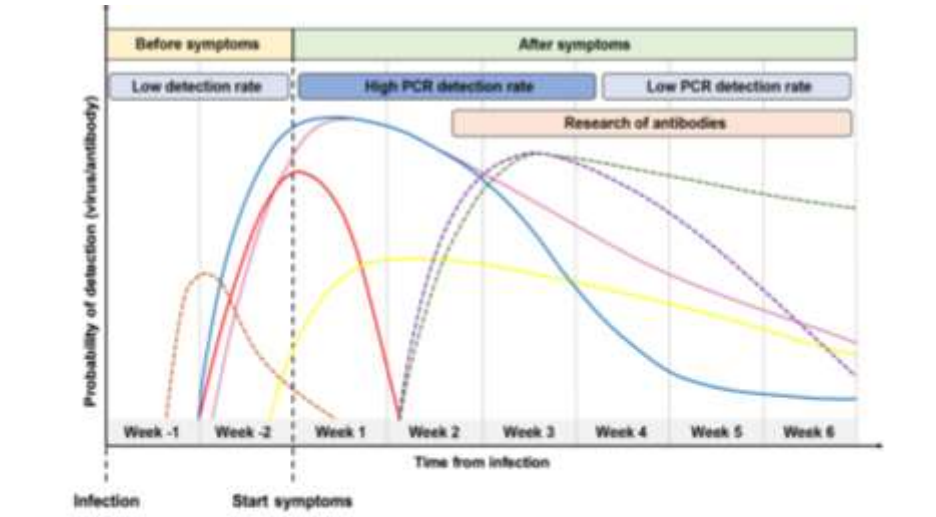




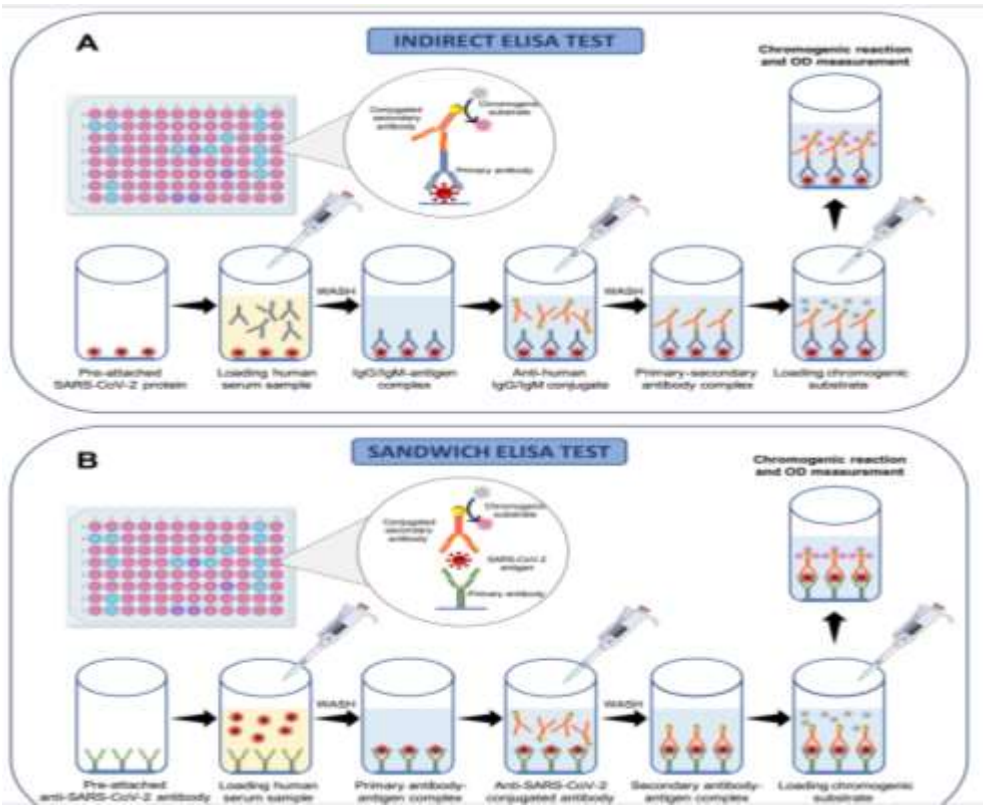
IMMUNOENZYMATIC TEST FOR COVID:

Most of the available test has the basic principle of the indirect ELISA. These are chemiluminesence colorimetric test. They contain both parts , either to detect the antibodies or antigen. Currently used to detect the antibodies to IgG/IgM which are specific for SARSCoV2 antigen especially the spike protein (S). Recent test kit to detect IgA antibodies are introduced as they are first antibody to be produced following exposure to virus. So the wide use of these test include the immunosurveillance of the covid 19 and to check for the seroprevalence as in this study., to confirm the seroconversion of the patients and to confirm immunocompetence is acquired against the covid 19 infection. Also by detecting the viral protein and IgA antibodies against the SARSCoV2, they are used for large scale screening statergies.

The sensitivity and the specificity of the test range from 75.6% to 100% and 85% to 100% respectively. These sensitivity and specificity depends upon the timing of the infection and timing of the screening. But these antibody test are better for large scale screening and large scale surveillance campaign, due to the rapidity of the method and can analyse multiple samples at a time, and precise quantification of the antibodies and antigen is possible.


Before symptoms
After symptoms
Low detection rate
High PCR detection rate
Low PCR detection rate
Research of antibodies
Probability of detection (virus/antibody)
Week -1
Week -2
Week 1
Week 2
Week 3
Week 4
Week 5
Week 6
Time from infection
Infection
Start symptoms


LEGEND
Nasopharyngeal swab
Fecal swab
BALF/Spit (PCR)
Virus isolation in the respiratory tract
IgM antibodies
IgA antibodies
IgG antibodies


A
INDIRECT ELISA TEST
Chromogenic reaction and OD measurement
Pre-attached SARS-CoV-2 protein
Loading human serum sample
IgG/IgM-antigen complex
Anti-human IgG/IgM conjugate
Primary-secondary antibody complex
Loading chromogenic substrate
B
SANDWICH ELISA TEST
Chromogenic reaction and OD measurament
Pre-attached anti-SARS-CoV-2 antibody
Loading human serum sample
Primary antibody-antigen complex
Anti-SARS-CoV-2 conjugated antibody
Secondary antibody-antigen complex
Loading chromogenic substrate

OTHER METHODS OF DIAGNOSIS:

NEXT GENERATION SEQUENCING:

These are the key techniques for identifying the SARSCoV2 and used for developing currently used molecular diagnostic methods. With the help of NGS, the entire genome of the SARSCoV2 is fully characterized and establishing it in the beta coronavirus genus. NGS is not suitable for diagnostic purpose commercially as it is prohibited by its cost, requiring high cost technology, training of personnel in bioinformative skills and molecular techniques. These NGS are aptly used for the epidemiological purposes and to detect the newly emerging molecular variants and detect the emerging mutants and for the development of the new vaccines. One of the most useful test is the Amplicon based metagenomic sequences, the amplicon sequencing allows the amplification of the SARSCov2 viral RNA and metagenomic sequencing to detect any abnormalities in the host microbiome or secondary pathogen that caused secondary worsening of the health status of the patient.

VIRAL CULTIRE AND ELECTRON MICROSCOPY :

These are pivotal at the initial phase of the epidemic, they observe the main characteristic of the virus. They are used to identify the typical structure of the coronavirus which is nucleocapsid enclosed in a crown like envelope which is composed of spike proteins, gaining the name coronavirus. Though they are useful in developing the diagnostic test that are used today, the viral

culture technique is time consuming and needs specific equipment with appropriate biosafety levels. So they are recommended only for the research studies, which should be carried out in the labs that are equipped with Level 3 biosafety cabinet. Electron microscopy cannot be afforded by everyone and it requires trained personnel and specific skills in preparing the sample and image analysis. They also have low diagnostic sensitivity and specificity.

CRISPR/CAS BASED COVID TESTING :

This is a bacterial nucleoprotein complex, conferring resistance to external plasmids. This is the noble prize winning invention for chemistry in the year 2020, which is technology of genome editing (molecular scissors). It uses the loop mediated thermal amplification (LAMP) for retrotransciption and the complementary DNA will be detected by the CRISPR/CAS detection system. This has been used by certain international agencies as apart of core testing because of their smart execution , which do not require specific lab setting, rapidity of the procedure and very low cost. The US FDA has given emergency use authorization for the Sherlock CRISPR /CAS 12 a detection system, which uses the Cas13 a nuclease activity. This has a sensitivity and specificity that approaches 100 % and a more rapid execution than RT-PCR.

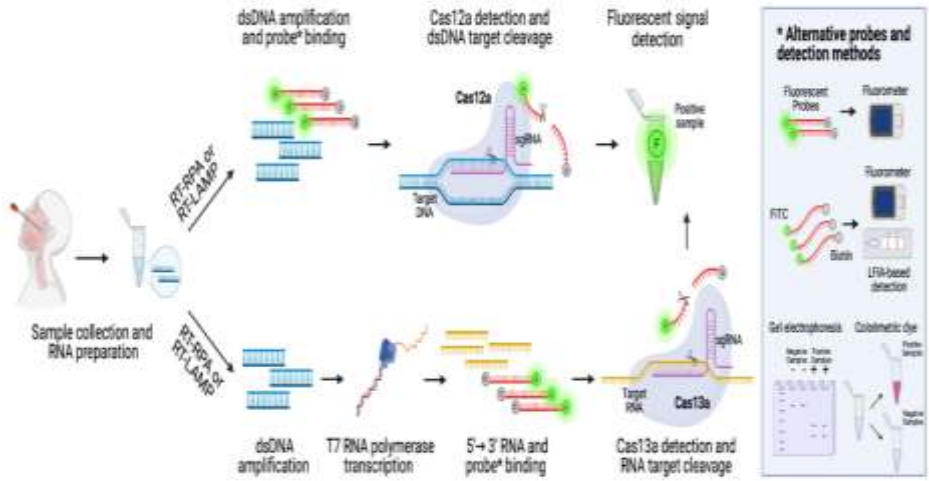


Figure 9. Schematic workflow of CRISPR/Cas-based methods for the diagnosis of COVID-19 infection. CRISPR/Cas12a systems effectively detect SARS-CoV-2 viral RNA after cDNA synthesis through the recognition of a specific SARS-CoV-2 sequence that is cleaved by Cas12a activity resulting in the collateral cleavage of fluorescent probes. In the same manner, CRISPR/Cas13a systems effectively detect SARS-CoV-2 RNA that is cleaved by Cas13a activity resulting in the collateral cleavage of fluorescent probes. Apart from fluorescence, alternative detection methods are based on the use of colorimetric dyes, electrophoresis gel and LFIA cartridge. CRISPR/Cas, clusters of regularly interspaced short palindromic repeats/Cas; LFIA, lateral flow immunoassay.

IMAGING IN COVID 19 :

The most preliminary radiological test is the chest x-ray and computed tomography (CT) imaging. Chest x -ray is used to detect lung abnormality either caused by the infection or neoplasm. In the first phase of pandemic, the Chest x-ray was proficiently used to detect the lung abnormalities like interstitial space and alveolar involvement in covid 19, but these are widely used for the patients with moderate to severe symptoms, these progress with the disease course and will progress more severely in the elderly. But the disadvantage of the chest x-ray is same as in many other pulmonary conditions, as they cannot be clearly defined as in a CT, even though X-Ray is a low cost investigation.

The CT scan has a better resolution power with a sensitivity approaching 95 -100% although they have a low specificity, since the same picture can also obtained with other infections rather than covid 19. Its usefulness is more in an asymptomatic patient in whom the covid RT-PCR testing will be negative in view of low viral load. Even the infection can be detected at early stages of infection, but it is not able to differentiate in chest x-ray the infection that present with atypical symptoms. The major limitation of the CT-SCAN is the cumulative dose of radiation, that are a problem when progress is followed up, but this can be overcome by using low dose CT scan, ultra low dose CT scan.

The CT finding that are considered hallmarks of covid 19 are ground glass opacities, which are prominent in the peripheral, lower lobe , sometimes multiple subsegmental areas of consolidation. With time they will progress and flow thicken along with disease progression. Certain atypical findings include pleural effusion detected only in 5%, cavitation, masses, lymphadenopathy but these should point to other diagnosis, rather than covid 19. A standardized system of reporting and assessment of the covid 19 lung infection was developed which was started in march 2020, provides improved communication with referring physicians and for gathering the scientific evidence.

CORADS, which is the covid 19 report and data system, provide a suspicion of the pulmonary involvement in covid 19 with an unenhanced CT chest. With this the level of suspicion increases from low level of CORADS I to very high level of CORADS 5 and the additional categories are CORADS 0 which is insufficient examination technically and RT-PCR confirmed SARSCoV2 infection, which is CORADS 6. To be clear, the CORADS provide suspicion and the final decision need the laboratory test results, clinical features, the duration of symptoms and type of symptoms. The interpretation of the covid 19 infection according to the CORADS are as follows:

CORADS 0 – Not Interpretable – Scan Technically Insufficient For Scoring

CORADS 1 – Very Low Suspicion – Normal /Non Infection

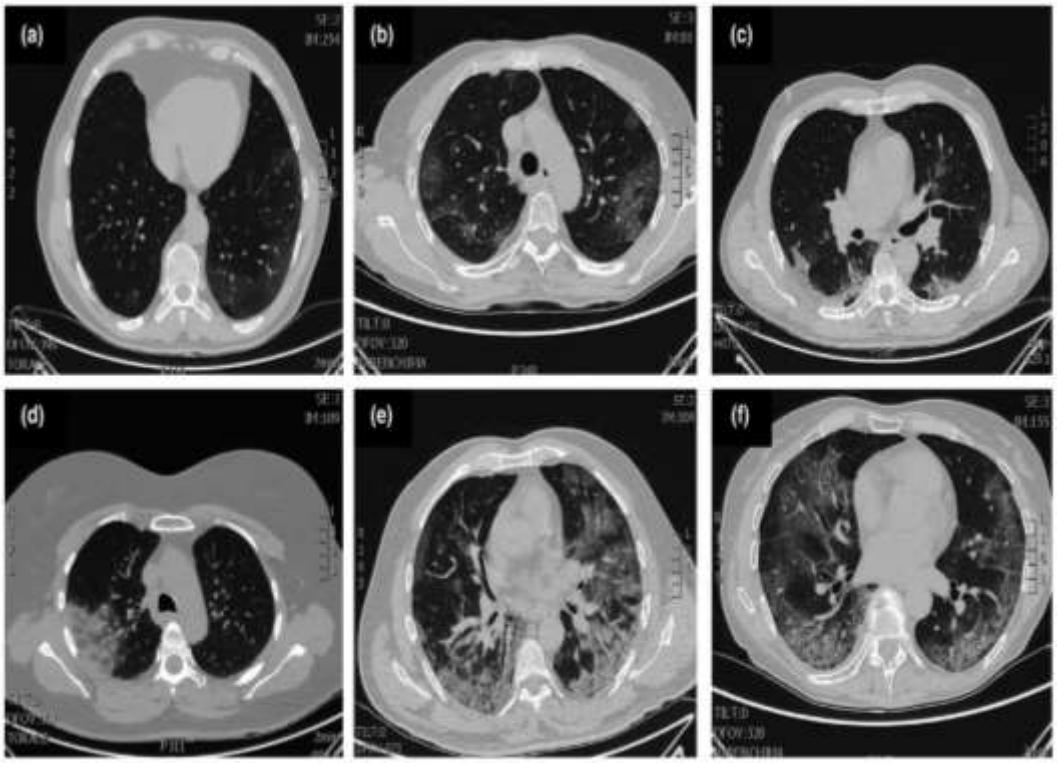
CORADS 2 – Low – Typical For Other Infection But Not Covid 19

CORADS 3 – Equivocal – Features Compatible With Other Diseases

CORADS 4 – High- Suspicion For Covid 19

CORADS 5 – Very High – Typical For Covid 19

CORADS 6 – Proven – RT-PCR Positive For Covid 19



Use of ultrasound in diagnosis of covid 19 in a limited number of cases, though that has a very low specificity and affected by factors such as disease severity ,patient weight and operator skill. It has a sensitivity of 75%, it can be used to monitor the disease progress by taking note of the kerley B lines, subpleural consolidation. The finding of CT and ultrasound are superimposed. The CT scan are better to detect the intraparenchymal lesion in the apex, and ultrasound are better to detect small subpleural lesion and small pleural effusion. The major finding are isolated, confluent B lines, irregular interrupted pleural thickening with air bronchogram. These lesions are mostly located in the lower, posterior area and with colour doppler mode it can be detected that these regions are having reduced blood supply.

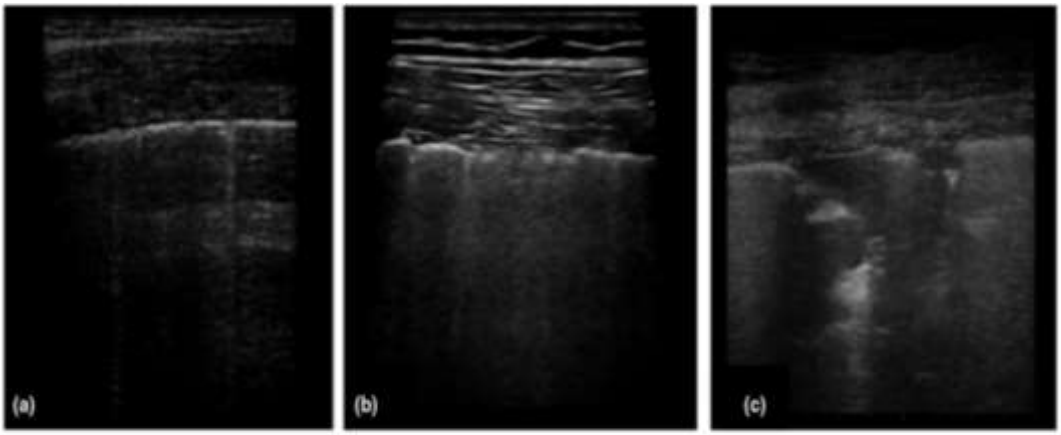


Fig. 2 *Patterns of COVID-19 at chest ultrasound. Early bilateral multifocal areas of interstitial syndrome. Interstitial pneumonia characterized by interstitial syndrome with B lines and preserved sliding sign. Advanced, organized pneumonia with interstitial syndrome associated with multiple subpleural consolidations and reduced sliding sign.*

SEVERITY OF COVID 19 :

It was proposed by the WHO(world health organization) that severity of covid 19 can be stated as mild , moderate , severe disease. The mild disease have fever, cough, and manifestation of upper respiratory tract symptoms and malaise, along with headache, loss of smell, taste. Moderate disease patients has lower respiratory tract symptoms with infiltrates on chest X-ray, but these patients can be maintained on room air saturation. Patients who developed complications are those who have severe symptoms which include hypoxia of saturation at <93% in atmosphere and the pao2/FiO2 being <300 mm hg with respiratory distress and rate being more than 30 beats/ min, involvement of the more that 50% of lung parenchyma. Various factors also contribute to the severity of covid 19 and these include age of the patient co morbidities, genetic factors, viral associated factors and socioeconomic background and sex. Considering the age people who are elderly tend to have more severe disease.

Older age people have more morbidity and mortality than their younger counterparts. Patient with multiple comorbidities have severe illness and they resulted in higher rates of hospitalization, ICU admission, intubation and mechanical ventilation. The risk factors that have the evidence of the proof are cancer, cerebrovascular accident, chronic kidney disease, chronic lung disease, diabetes, HIV, down syndrome , smoking, pregnancy BMI > 30 Kg/m2 , tuberculosis. The severe disease in the elderly discussed above is due to the association of such comorbidity and one another cause being the weaker immune system. There are social difference in the susceptibility to severe infection, severe infections are more common in blacks, Hispanics and south Asians, who have a higher mortality even when they are present in US,UK. Symptomatically patients who have fever and fatigue are reportedly having severe disease.

Certain laboratory markers help in predicting the disease severity in covid 19. Among them are the neutrophil /lymphocyte ratio (NLR) which will be more than 3 in severe disease. It is a marker of severe inflammatory stress. Presence of severe disease is a stressful condition and these patients have a higher NLR. Patient with severe disease have an elevated SGOT of more than 40 and have an increased risk of severe disease than patients who have normal levels. The varied mechanisms causing these are discussed previously, in the section of pathogenesis of liver injury.

Serum electrolytes also have a prognostic value, the deranged values particularly the low sodium and potassium are associated with severe disease than the normal value. This could affect the body's appropriate stress response and can lead to more severe disease, leading to varied complications and death. With the WBC count both leucopenia and leucocytosis are associated with severe covid 19 and death. Presence of decreased leucocyte count can increase the potential threat from serious infection and expression of already existing pathogens and elevated white cell count is associated with exuberant immune response mediated damage of organs and systems.

Other laboratory abnormalities associated with poor prognosis and severe illness are thrombocytopenia, increased lactate dehydrogenase, increased inflammatory markers like CRP, ferritin, IL 6, increased D-DIMER, increased prothrombin time, increased fibrinogen and increased creatinine phosphokinase. The deficiency of VITAMIN D and certain other micronutrients are linked to covid 19 severity but they always challenged by certain confounders.

Those with severe disease have higher loads of viral RNA in their respiratory specimen and these people also have viral RNA detected in their blood. Among the genetic factors, it has been stated the people with TYPE O blood group have associated with lower infection risk and disease severity and people with TYEP A group have associated higher risk.

VARIANTS OF COVID 19:

One of the important concern of the viral pandemic is the problem of mutation and the properties they confer to the virus to evade the host immunity, test of detection, diagnosis, and treatment directed. As the covid is spreading from person to person, the viral mutation occurs. Various researches indicate that initially that the changes in the SARSCoV2 will be much slower than the mre common occurring HIV infection. But the spike protein which is the essential component of the virus for penetrating into the cell is more important which respect to the mutation. It has been always a misnomer in epidemiology in using the terms mutation, strain, variants to confer one and single unified meaning. Mutations are the change in the sequence of the nucleotide, variants are the genomes that differ in their sequence, variant can be termed as a strain when it has different phenotypic properties like transmission, virulence.

Since the start of the September 2020, various variants have been defined out of which the following have special mention. Among the variants there are variant of concern and variant of interest.

ALPHA VARIANT:

Defined initially as a variant of concern by the WHO, this was first identified in September in England, UK. Though it was thought more transmissible initially at around 70 %, now it was found to be 30 – 40 %

transmissible than that of original virus. Concerning the vaccines the moderna mRNA vaccine is 100% effective against the alpha variant and with Pfizer BioNTech 93.7% efficacy.

BETA VARIANT :

A variant of concern first reported from south Africa on may 2020. There is 50% increase in transmission and a big concern is its ability to evade the existing vaccines. The efficacy of the Pfizer and Moderna vaccine are lower against the variant and it was around 72 – 75 %. Efficacy of Astrazeneca is around 82%.

GAMMA VARIANT :

It is a variant of concern first identified in Manaus, brazil at around end of November 2020. It is 1.7-12.4 times higher transmission than SARSCoV2. The gamma variant has a higher infection rate among those who have completed 2 dose of vaccine at around 60 % compared with the infection rate of 75 % in unvaccinated population.

DELTA VARIANT:

Variant of the concern first identified in the Europe then in US, there was a steady increase in the case all over Asia, South Korea, Thailand. In India it was First identified in October 2020. It is considered the most transmissible form of SARSCoV2 and reported to be 60% more transmissible than alpha variant. Considered as an alpha variant improved version, the mutation it has

acquired helps it to be more infective in the airway, such that infected person can expel more virus into the surrounding air. And certain study indicated that around 1260 times higher virus in the SARSCoV2 delta variant infection. As it has better infection properties the infection can occur even at a lower dose exposure. The oxford Astrazeneca vaccine has 67 % efficacy and Pfizer BioNTech has 88% efficacy against the delta variant. Delta plus variant is delta virus with a K417N mutation, this variant is primarily seen in young people. Data are showing that antibodies formed in the vaccinated people are effective against this variant.

ETA VARIANT:

Seen in 72 countries and first detected in December 2020, information available are scarce , it can reduce the neutralising activity of the monoclonal antibody and convalescent plasma and hence it has been lablelled as variant of interest .

IOTA/KAPPA VARIANTS:

Much information is not available on the IOTA variant but it has been declared as the variant of interest with reduced susceptibility to monoclonal antibody treatment. It was first discovered in the New York city. Kappa is also a variant of interest first identified in India. It is found in October 2020, been reported so far in 55 countries.

LAMBDA VARIANT:

Initially discovered in peru by December 2020, within 3 months it became the dominant variant accounting for the dominant variant causeing infection accounting for 80% of cases. It has faster mutational capabilities and this could affect its response to the vaccines and antibodies and have faster transmission. It is variant of interest.

OMICRON VARIANT :

The latest addition to the long line of variants is the B1.1.529 variant also known as the omicron variant. Reported from south Africa on 24, November 2021. This contains a large number of mutations and the evidence suggest increased rate of reinfection with this variant. This variant is feared to have certain growth advantage. It has been stated that 3 doses of the Pfizer vaccine can neutralize the omicron variant, but he results are too early to be taken into consideration. Though the reinfection rate is higher, the severity of the variant infection is much lower than the other variants, and milder than the delta infection.

VACCINES FOR COVID:

The first ever practice of vaccination started by around 15^{th} century against the deadly small pox and surprisingly it was the Chinese who practiced it. The method of grinding up smallpox scabs and blowing the matter into nostril is called variolation. It was Edwars jenner's innovation beginning with

his successful 1976 use of cowpox material to create immunity to small pox , quickly made the practice widespread. His method underwent medical and technological changes over the next 200 years and eventually resulted in the eradication of the small pox. Innovative techniques now drive the vaccine research, with recombinant DNA technology and new delivery techniques leading scientist in new direction.

Since the major protective effect is to be attributed to the antibodies against the Spike protein and in particular against its receptor binding domain. Most vaccines are designed to elicit the immune response against the trimeric SARSCoV2 spike (S) protein. A polyclonal antibody against the multiple epitopes of the S protein beyond the RBD(receptor binding domain) might for example inhibit the viral attachment, provide additional neutralizing activity and prevent post attachment fusion. Such a vaccine would mitigate the possibility of immune escape by mutation. Various vaccine platforms are available based on attenuated SARSCoV2 viruses, based on inactivated SARSCoV2 viruses, based on SARSCoV2 protein, naked DNA based vaccines, mRNA based vaccines, based on viral vectors

ATTENUATED VACCINES:

This involves decreasing the virulence of the vaccine by passing it in serial cultures, and these can be rapidly produced. Since it is a actual live virus ,a robust immune response with good immunological memory. The

disadvantage they have are the possibility of the attenuated to get reverted to wild type virus on inoculation, there is cross reactivity with naturally acquired corona infection, in immunocompromised person there is prolific multiplication of the attenuated strain. The following are the candidate vaccines in this category:COVI-VAC – intrnasal live attenuated vaccines by Codagenix , this vaccine is in produced in india by the serum institute of india and are in phase I trail .

INACTIVATED VACCINES :

Inactivated pathogen vaccines use a dead form of the virus, thus ensuring a better safety profile than live attenuated vaccines sometimes losing their immunogenicity rendering this strategy less efficacious than live attenuated pathogen immunization. The following vaccines involved in this category SINOVAC – 2 doses – from Chinese based company, on the phase 4 trials. BHARAT BIOTECH – COVAXIN – 2 doses, crossed the phase 3 trials by January.

SUBUNIT/PROTEIN BASED VACCINES :

Protein subunit candidates against SARSCoV2 using different immunogens principally different forms of the entire spike protein or its receptor binding domain(RBD). A general rule, perfusion stabilized viral glycoproteins are usually more immunogenic, thus being more attractive vaccine target. It eliminates severe adverse effects, but there is necessity to

increase booster doses and optimize the adjuvant added for stronger and more durable immunization. The following examples include the vaccine in this classes.

NOVOVAX – 2 doses, crossing the phase 3 trial

MEDICAGO – 2 doses, on its phase 3 trial

mRNA BASED VACCINES :

mRNA needs to reach the cytoplasmic and endoplasmic reticulum ribosomes in order to be translated in to protein. Administered as a encapsulated in the lipid nanoparticle(LNP) vectors that can encapsulate efficiently nucleic acid and potentially enable tissue penetration. The disadvantage is that the mRNA vaccines are unstable than DNA ,and these commonly require temperature between -70 c and -20 c for long term storage that complicate the distribution logistics of these kind of vaccines. Candidate vaccines are as follows:

Pfizer BioNTech VAACCINE - 2 doses , on the use in united states

MODERNA VACCINE - 2 doses , final results in the united states .

DNA VACCINES :

These vaccines are delivered intradermally through short electric pulse, uptaken by the cutaneous antigen presenting cells(APC) (macrophages, monocytes and dendritic cells , presented to the naïve T cells in the secondary

lymphoid organs , leading to cellular adaptive responses. Nebulization of these vaccines can cause mucosal immunity by way of pulmonary APC. Oral intake of these vaccines can provide GALT immunity by presenting the plasmids to the APC present in the peyers patches. The major advantage of these vaccines are DNA molecules are quite stable, permitting the storage of DNA vaccines at +4 *C, thereby simplifying the distribution of these types of vaccines.

VECTOR BASED VACCINES:

These are divided into the replication preserved and replication deficient. Various viral vectors are the human adenovirus, chimpanzee adenovirus. The main disadvantage of the vector based vaccine is pre-existing immunity against the vector that can impair the magnitude of the elicited immune response. Multiple vaccination regimes produce antibodies against the viral vector produced after the prime vaccination can decrease the immunogenicity of booster administrations. Following are the notable mentions

ASTRAZENECA – 2 DOSES. From oxford based using the chimpanzee adeno vector

JOHNSON & JOHNSSON – single dose – uses human adeno vector Ad26.

SPUTNIK V – 2 doses, made in Russia , uses two different adenoviral vectors for the two dose (Ad26 and Ad5).

Efficacy of the vaccine is a tricky part to be handled , since the actual efficacy of the vaccine is gauged only if there is substantial reduction in the transmission, reduce the chance of acquiring the infection , reduce the chance of getting a sever infection , reduce the mortality. There are certain surrogate endpoints in measuring the efficacy of the vaccine, which is the sero conversion of antibodies but whether it is sufficient to provide protection against infection or disease is unclear. Even if the antibodies are sufficiently providing protection against the infection the titre for protection is not known. Cellular immune response are described in response to infection, which is likely to be an important component of the protective adaptive immune response. This is because people who are seronegative had T cell response to the SARSCoV2 spike protein. But the particular cellular signature required for protection is unknown.

India, one of the greatest vaccine manufacturers in this world gives up to 60 % of the global vaccine supply. Vaccines with Emergency Use Authorisation in india are COVISHIELD, COVAXIN, SPUTNIK V and MODERNA.

COVAXIN is the first indigenous covid 19 inactivated vaccine developed and manufactured by Bharat Biotech in collaboration with Indian council of medical research (ICMR) and the National Institute of

Virology(NIV), which is dosed at 28 days apart , 2 doses, which is stored as multidose vials stored at temperature of 2-8*C.

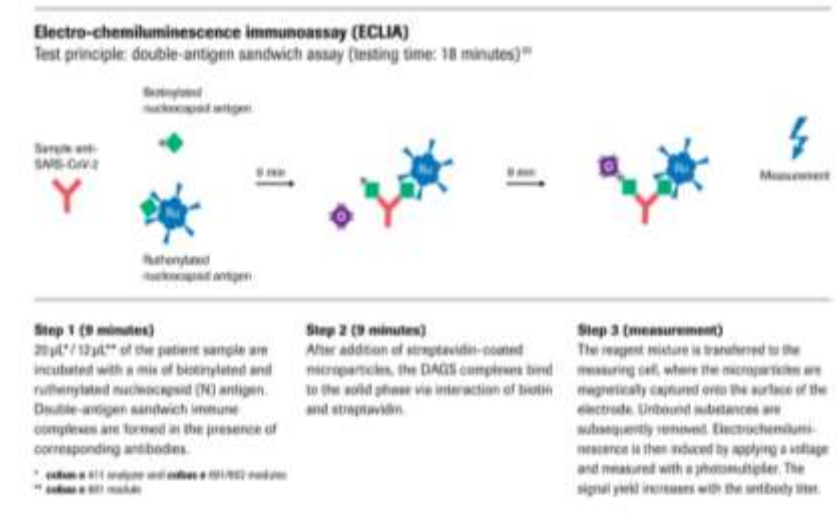
COVISHIELD is the Indian version of the replication deficient adenoviral vector vaccine developed by the Oxford university and Astrazeneca. It is manufactured by the serum institute of india. It is dosed at 4 -12 weeks interval and stored as multidose vials to be used within 6 hours, and stored at 2-8 *C.

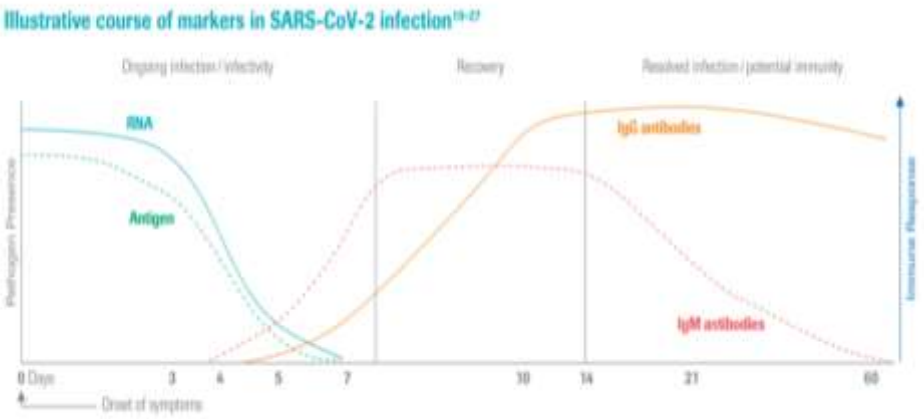
SPUTNIK V vaccine is an adenoviral vector vaccine made by the Russian manufacturer Gamelaya institute and DR.REDDY'S lab private limited. It is a heterologous vaccine which employs 2 different viral vectors. Dosed at an interval of 21 days apart.

ECLIA ASSAY TO QUALITATIVELY DETECT ANTIBODIES:

The ELECSYS anti SARSCoV2 immunoassay is ised for the invitro detection of the antibodies, qualitatively for the SARSCoV2 which will be present in the human serum and plasma. This assay uses the recombinant nucleocapsid protein for the detection of the antibodies. Based on the methods applied the median time during which the seroconversion is obtained is around 10-13 days from the symptom onset for IgM , which peaks at 2- 4 weeks, and vanishes around 6-7 weeks. For IgG the seroconversion happens at around 12-14 days from symptom onset, peaks at 3- 6 weeks and remains high at around 6-7 weeks. The neutralizing antibodies target the spike and

nucleocapsid right from 9th day onwards. The following picture explains the mechanism of the ECLIA test (electro-chemiluminescence immunoassay:





MATERIALS AND METHODS

STUDY POPULATION: This study will be conducted on 200 health care professionals in GRH, Madurai during the study period of 6 months

DATA COLLECTION: Samples will be collected from 200 health care professionals in Govt Rajaji Hospital Madurai during the study period of 6 months, after proper informed consent

DESIGN OF STUDY: Observational - CROSS SECTIONAL

FINANCIAL SUPPORT: DEPARTMENT OF GENERAL MEDICINE

MATERIALS AND METHODS:

Under aseptic precaution around 5 ml of blood sample was collected from each patients by venipuncture at the cubital fossa, by using 23G needle. Blood was dispensed into a sterile test tube without anticoagulant. Samples were transported immediately to the ENDOCRINOLOGY lab. The blood was centrifuged at 2500 rpm and the serum was separated Analysis will be done with The separated serum with ELECYS SarsCov2 qualitative antibody assay kit. The assay uses a recombinant protein representing the nucleocapsid (N) antigen in a double-antigen sandwich assay format, which favors detection of high affinity antibodies against SARS CoV-2.

STATISTICAL METHOD

Statistical Analysis Of The Data Are Conducted Using The Software Ibm Spss 17.0 Version Statistical Difference Between The Groups Was Determined Using Student T Test .Subgroup Analysis Will Be Done According To The Age ,Gender , And Department/Ward Posted By Annova.

INCLUSION CRITERIA:

Age ≥18 years, to be health care professional in GRH, Madurai

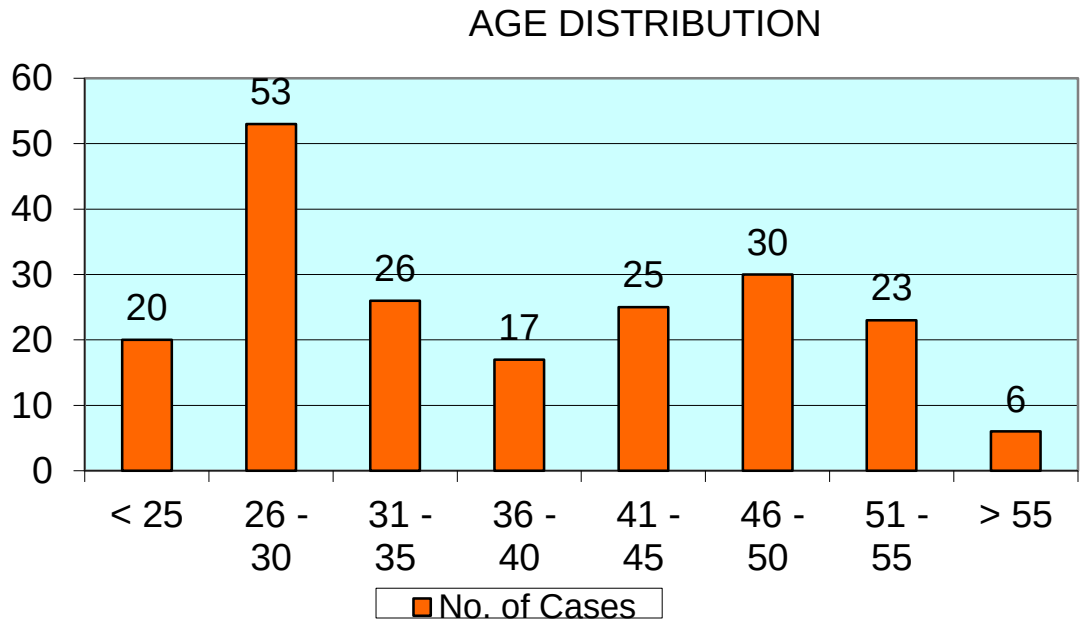
EXCLUSION CRITERIA:

Not to receive any other vaccine during the study period Suspected or confirmed immunosuppresive condition and infection H/O laboratory, imaging confirmed Covid 19, Clinically Symptomatic Individuals Recieved recent blood transfusions, immunoglobulins.

RESULTS

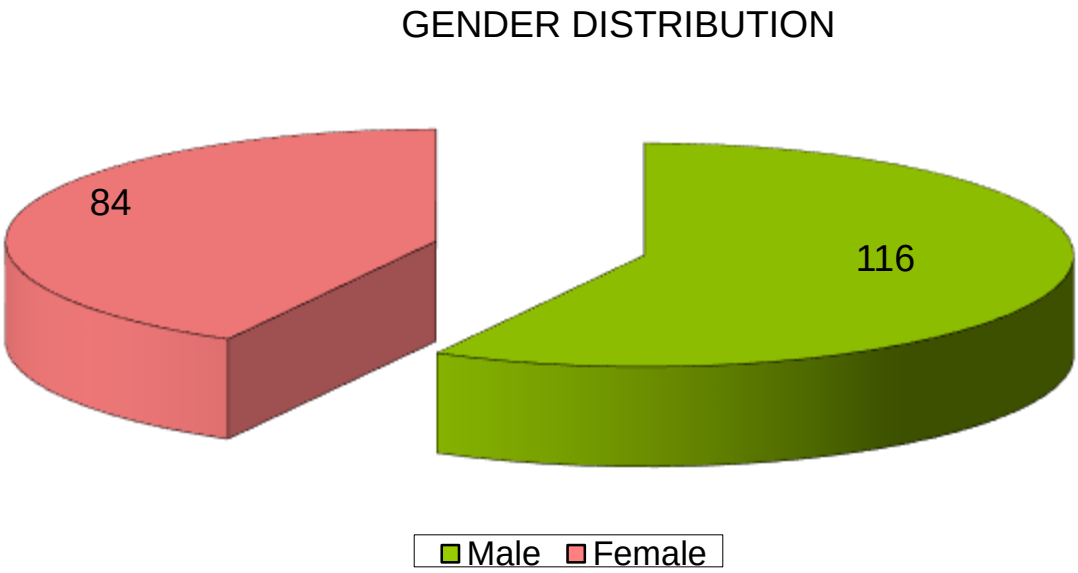
The study conducted was an observational study, that aims to enumerate the seroprevalence of antibodies IgG/IgM, to covid 19 in the health care population of government Rajaji hospital in Madurai. The results of the study are plotted in the charts and graph for the ease of the illustrator. The study population is characterized according to the age, sex, their occupation. Then the population is segregated according to their titres in correlation with their age, sex, and occupation. A titre value of more than (>1) is considered positive according to my study, as specified by the manufacturer of the kit.

The following tables and charts are illustrating and summarize my study



Age	No. of Cases
< 25	20
26 - 30	53
31 - 35	26
36 - 40	17
41 - 45	25
46 - 50	30
51 - 55	23
> 55	6
Total	200
Mean	37.75
SD	10.21

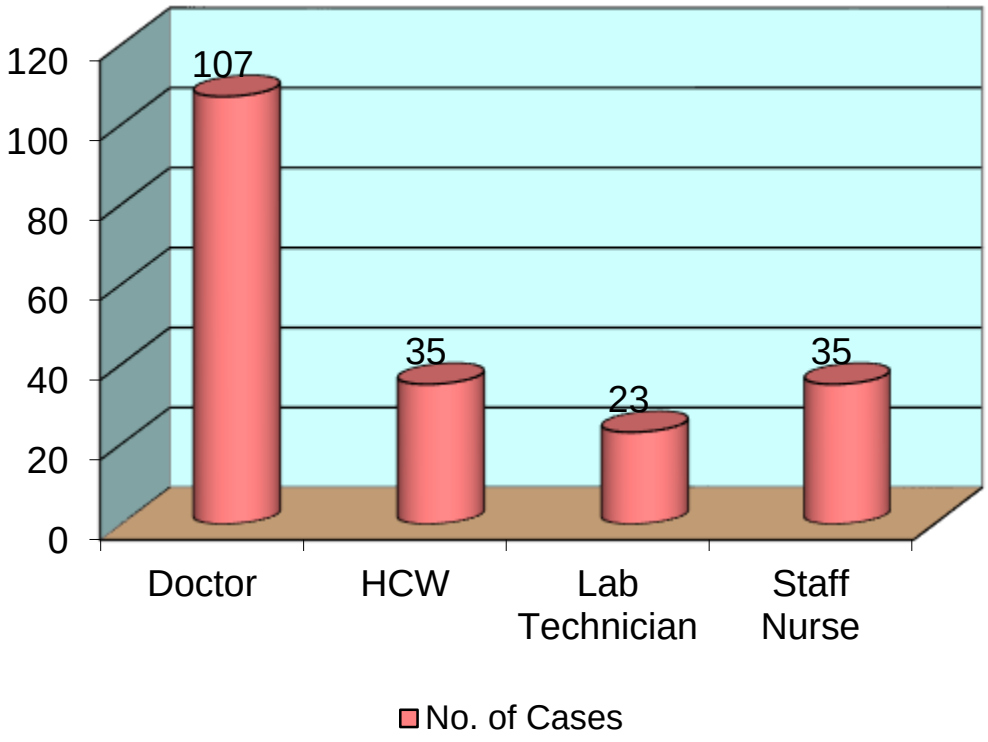
TABLE 1,CHART 1: illustrate the age wise distribution of the study group. Majority of the participants are in 26-30 years of age which is 53 out of 200. The 2nd major is the 46-50 years of age group.



Gender	No. of Cases
Male	116
Female	84
Total	200

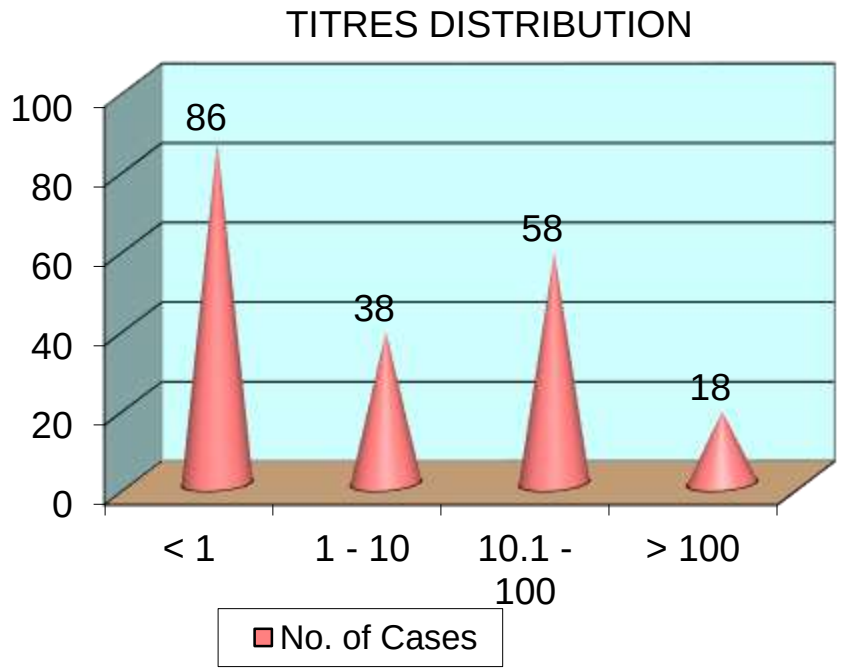
Table 2, Chart 2 : Illustrate the gender distribution with males being predominant .116 (58%) of the study population are males and 84 (42%) are females.

OCCUPATION DISTRIBUTION



Occupation	No. of Cases
Doctor	107
HCW	35
Lab Technician	23
Staff Nurse	35
Total	200

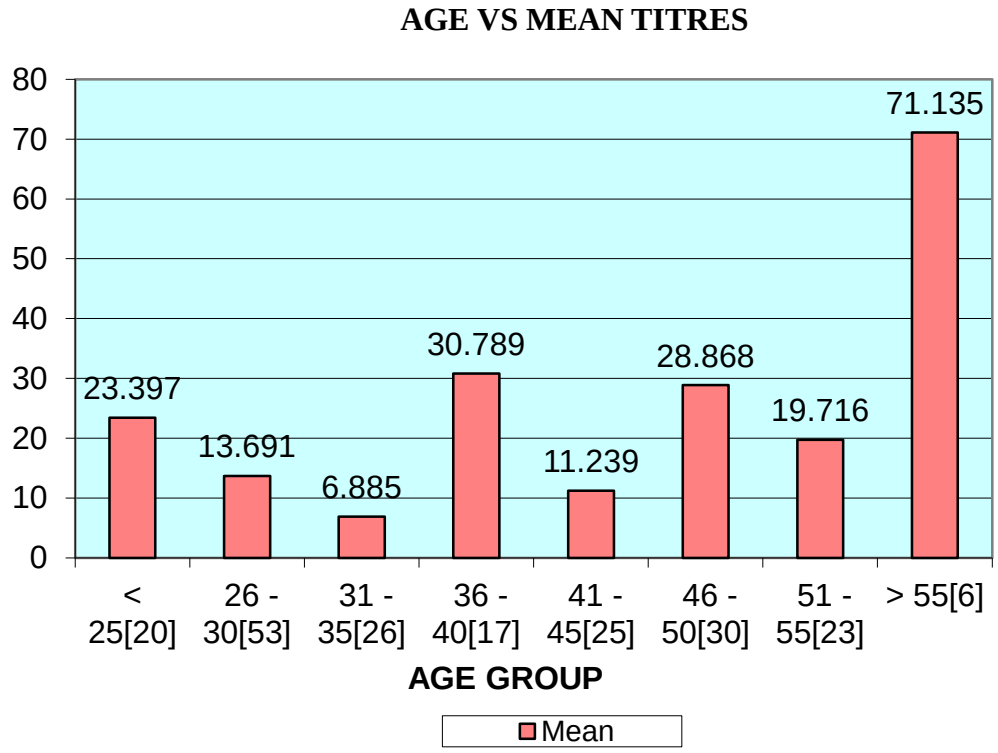
Table 3,Chart 3: Indicate the occupation wise distribution of the study population. Of the 200 107(53.3%) were doctors , 35 (17.5%) were hospital care workers, 23 (11.5%) were lab technicians , 35 (17.5%) were staff nurse.



Titres	No. of Cases
< 1	86
1 - 10	38
10.1 - 100	58
> 100	18
Total	200

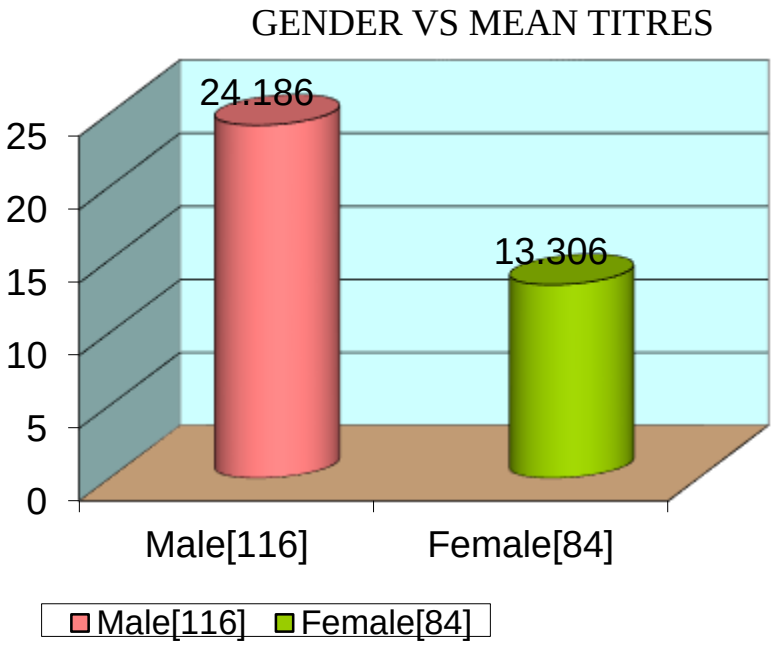
Table 4 , Chart 4 : indicates the distribution of the titre according to the values

43% had less than 1, 48% had titre 1-00 , 9 % had titre >100.



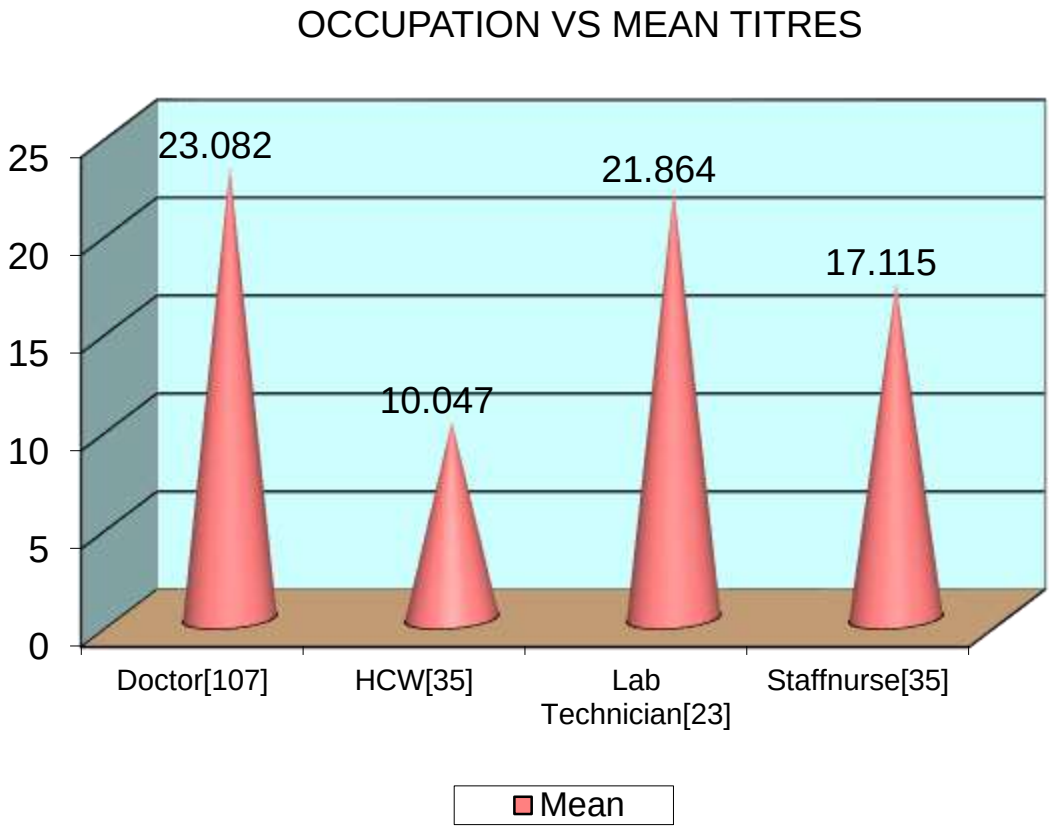
Age vs Titres	Mean	SD
< 25[20]	23.397	50.176
26 - 30[53]	13.691	29.993
31 - 35[26]	6.885	11.547
36 - 40[17]	30.789	52.013
41 - 45[25]	11.239	24.832
46 - 50[30]	28.868	44.964
51 - 55[23]	19.716	40.312
> 55[6]	71.135	74.139

Table 5 , Chart 5 : illustrate the distribution of mean titre across various age groups and it shows that in the age group of > 55 years , the mean value of the titre is at much highr range of 71.135.



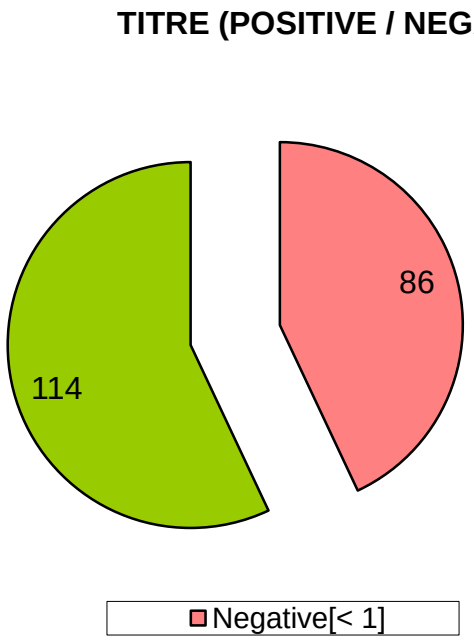
Gender vs Titres	Mean	SD
Male[116]	24.186	45.747
Female[84]	13.306	26.928

Table 6, chart 6 : Shows that the males have higher mean titres , than the females with 24.186 vs 13.304



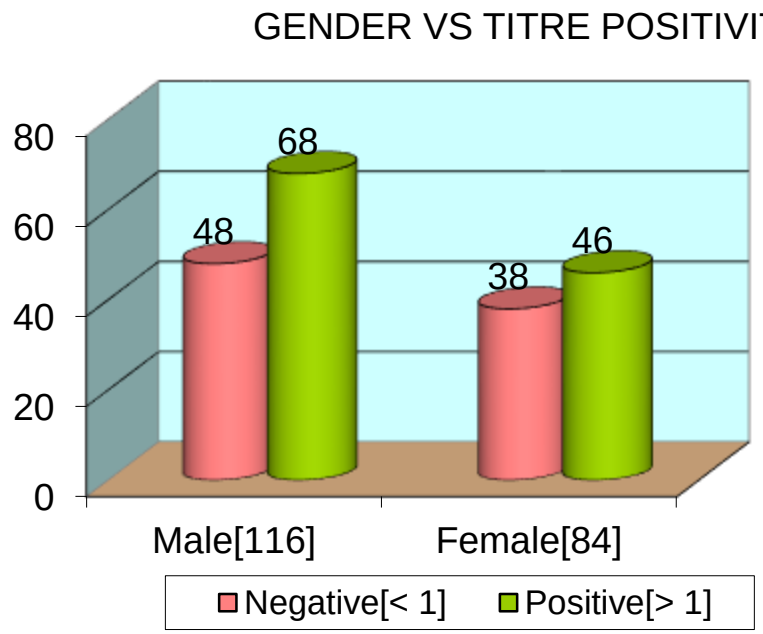
Occupation vs Titres	Mean	SD
Doctor[107]	23.082	45.455
HCW[35]	10.047	21.743
Lab Technician[23]	21.864	36.5
Staffnurse[35]	17.115	33.167

Table 7, chart 7 : illustrates the distribution according to the occupation wise that doctors have mean titres of 23.082 , following that lab technicans have titre of 21.864 , staff nurse have 17.115.



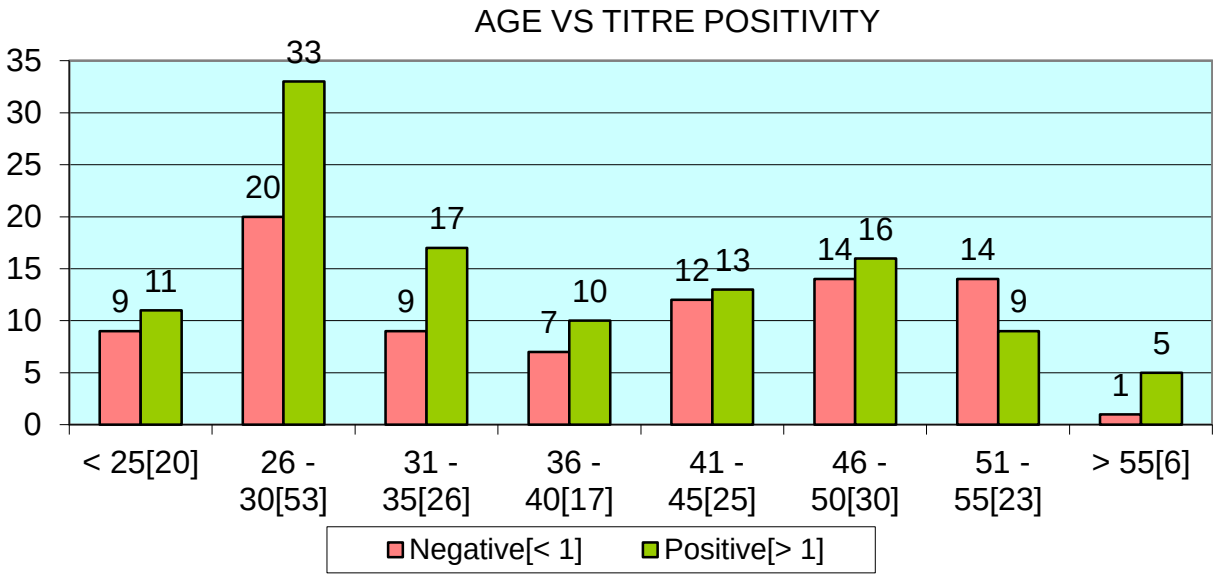
Titres	No. of Cases
Negative[< 1]	86
Positive[> 1]	114
Total	200

Table 8, chart 8 : shows the total positivity of the titres which is 114 in the study population of 200 which amounts to 57 %



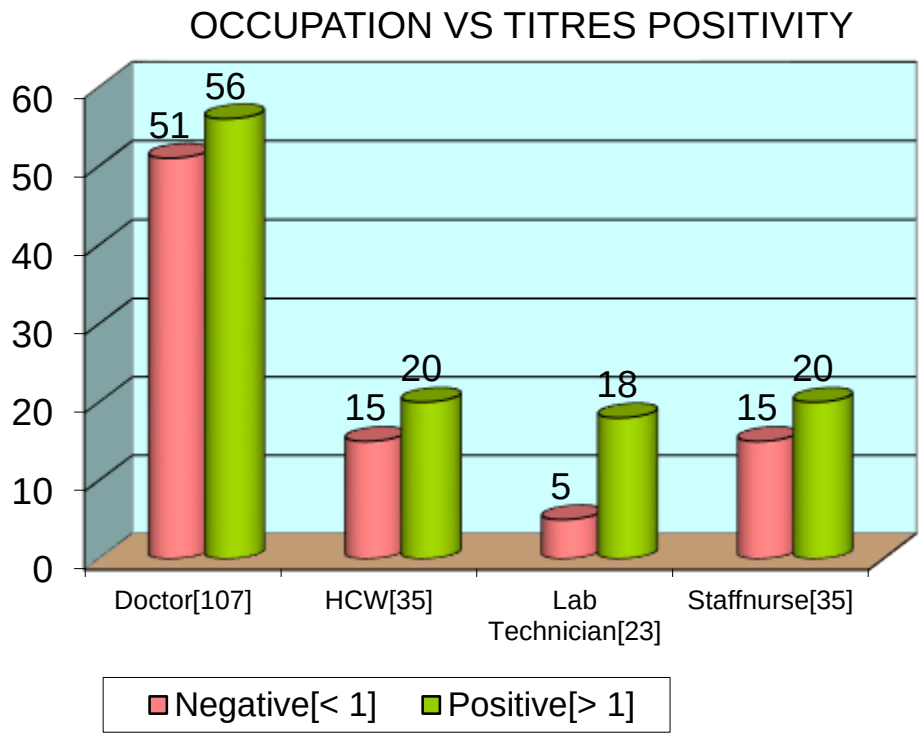
Titres vs Gender			
Titres vs Gender	**Negative[< 1]**	**Positive[> 1]**	**Total**
Male[116]	48	68	116
Female[84]	38	46	84
Total	86	114	200

Table 9, chart 9 : Illustrates the gender wise positive titre distribution. In which of the 116 male, 68 are positive (58.6%) and of the 84 females 46 are positive (54.7%).



Titres vs Age			
Titres vs Age	**Negative[< 1]**	**Positive[> 1]**	**Total**
< 25[20]	9	11	20
26 - 30[53]	20	33	53
31 - 35[26]	9	17	26
36 - 40[17]	7	10	17
41 - 45[25]	12	13	25
46 - 50[30]	14	16	30
51 - 55[23]	14	9	23
> 55[6]	1	5	6
Total	86	114	200

Table 10, Chart 10 : Illustrate the age wise positivity of the titres, which is high among 26-30 age group of which , in the 53 people 33 are seropositive which is 62.2% percent of this age group and lowest in the 51-55 year age group of which in the 23 participants 9 are positive (39.31%) and 14 are negative(60.85%) .



Titres vs Occupation			
Titres vs Occupation	**Negative[< 1]**	**Positive[> 1]**	**Total**
Doctor[107]	51	56	107
HCW[35]	15	20	35
Lab Technician[23]	5	18	23
Staffnurse[35]	15	20	35
Total	86	114	200

Table 11, Chart 11 : illustrate the occupation wise titre positivity , in which of the 107 doctors 56 are positive (52.33%), 20 of the 35 of the health care workers are positive (57.1%), 18 of the 23 lab technicians are positive(78.26%), 20 of the 35 staff (57%) are positive.

DISCUSSION

Covid 19 a disease of the viral pandemic has gotten into every aspects of this medical world. Since its discovery and molecular sequencing various antibody kits have been introduced for the commercial use and testing. The seroprevalence studies are done to estimate the disease burden in the society which is done by detecting the antibodies to the virus. These antibodies may or may not be protective which is not the aim of this study , but to detect their presence, though some claims have been made that the presence of these antibodies are protective against further infection. The antibodies measured are total antibodies and no differentiation is made here according to the study. People taken in this study as study population are those who are swab negative for RT-PCR and they don't have any past history of infection. This is essential as if the patient with a past history of infection are taken into account, the real burden of the disease could not be estimated.

The percentage of the people who are positive for the antibodies enumerate the asymptomatic form of infection and the potential for him to be silent spreaders and ensuring the chain of covid continues. Those people who are negative for the antibodies are most probably at increased risk of the infection from the subsequent exposure and these are the people who will be benefited from early vaccination though the vaccination is recommended for everyone even people who are previously infected and those with adequate

antibodies, the priority should be given for those people who are more vulnerable and seronegative.

The seroprevalence according to the gender and occupation wise as given in this study are segregated so that which strata and segment of the working community , that is much affected in noted and they can be targeted first for their protection. According to the study done here, it was the laboratory technicians who are having higher seroprevalence percentage with the population number chosen, that too in the 26- 30 year age group. These people may be having high exposure since they are involved in collecting and processing the samples from the patient and therefore are at more exposure rate, population group at second highest percentage are the staff nurse and workers as they are involved in shifting the patient who is a potential source of infection and who may have a high viral load like those admitted to intensive care units. The doctors who have a seroprevalence of 52.1% are having a low value because of their higher sample size comparing to the other occupational samples.

Though the people are seropositive , there is not evidence to be sure that they are protected from the covid 19 infection and even if they are protected, there is no protective titre ,that is present to say that they are adequately protected., and these people cannot be deffered of their vaccines., since they are active preventable strategies. The seroprevalence study done here are also excludes the people who are vaccinated since to avoid any vaccine induced

false positives. The efficacy of the vaccine is not determined by the seroprevalence studies or any other serological study other than that used while manufacturing and testing the vaccines. No commercial kit available in the market can confidently say the vaccine effectiveness. But as discussed before the results can be used to estimate the vulnerable population and can identify and target them for vaccination to break the chain.

Even when all the population are not seroprevalant and are not vaccinated , a considerable amount of the about 75-80% of the perevalance will be necessary to reduce the transmission by breaking the chain of transmission.

On treatment part , this serological study also is useful to choose patient for the antibody therapy, either monoclonal antibodies or convalescent plasma therapies. These people who are seronegative and who have heavy exposure can be subjected to monoclonal antibody treatment and for reducing the risk of infection after infection after exposure , reducing the severity of manifestations following infection and reducing the viral load present in these patients.

There is also a additional elements of the false positive reaction in these seropositive people. The antibodies could be showing positive even with infection to other virus like most commonly with CMV and EBV, dengue and other autoimmune condition like rheumatoid arthritis , SLE. But the test kit used in our case have 100 % specificity as specified by the manufacturer.

LIMITATIONS OF THE STUDY

1. The sample population chosen for this study is not equally distributed according to the age , occupation, sex. Hence comparing among them is not technically possible.
2. Small sample size is chosen and all are from the same tertiary care centre.
3. Since it is cross sectional observational study, the dynamics of the antibodies are not followed up.
4. This does not represent the general population of the Madurai city and logarithmic regression analysis could not be carried out.
5. Since the selection of the participants in the study are based on the exclusion criteria, which is based on the participants own perception of the symptoms in past, there is high possibility of the recall bias.

CONCLUSION

The present study shows that the seroprevalence of the covid antibodies in the health care workers of Government Rajaji Hospital , Madurai is around 57 % and the mean titres are higher among the males at 24.186 and among the occupational distribution , doctors have mean titres of 23.082, according to the age group the distribution is high among the 46- 50 yrs age group, which is 28.825.

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ABBREVIATION

SARSCoV	-	severe acute respiratory syndrome corona virus
IL – 6	-	INTERLEUKIN 6
IFN- A	-	INTERFERRON ALPHA
IFN – G	-	INTERFERRON GAMMA
IgG-	-	IMMUNOGLOBULIN G
IgA	-	IMMUNOGLOBULIN A
IgM	-	IMMUNOGLOBULIN M
CRS	-	CYTOKINE RELEASE SYNDROME
MIS-C	-	MULTISYSTEM INFLAMMATORY SYNDROME-CHILDREN
ARDS	-	ACUTE RESPIRATORY DISTRESS SYNDROME
GBS	-	GUILLIAN BARRE SYNDROME
CHADOX	-	CHIMPANZEE ADENO VECTOR OXFORD
ACE	-	ANGIOTENSIN CONVERTING ENZYME
ARB	-	ANGIOTENSIN RECEPTOR BLOCKER
MERS	-	MIDDLE EAST RESPIRATORY SYNDROME

RT PCR	-	REVERSE TRANSCRIPTASE POLYMERASE CHAIN REACTION
ELISA	-	ENZYME LINKED IMMUNO SORBENT ASSAY
AKI	–	ACUTE KIDNEY INJURY
CAD	-	CORONARY ARTERY DISEASE
CNS	-	CENTRAL NERVOUS SYSTEM

MASTER CHART

S.NO.	PATIENT NAME	AGE	SEX	OCCUPATION	TITRES
1	dr.sangumani	55	male	DOCTOR	0.183
2	dr.natarajan	55	male	DOCTOR	0.106
3	dr.dharmaraj	55	male	DOCTOR	0.089
4	dr.premkumar 56/m	56	male	DOCTOR	0.095
5	dr.tamilvanan	50	male	DOCTOR	0.647
6	dr.sridharan	40	male	DOCTOR	141.5
7	dr.murugan	54	male	DOCTOR	0.142
8	dr.manivannan	56	male	DOCTOR	13.2
9	dr.brilapavalam	51	male	DOCTOR	0.09
10	dr.subramaniyam	51	male	DOCTOR	0.207
11	dr.cheliyan	50	male	DOCTOR	0.944
12	dr.muthukumar	51	male	DOCTOR	124
13	dr.justin	52	male	DOCTOR	0.097
14	dr.amalraj	50	male	DOCTOR	0.862
15	dr.senthurajapandian	47	male	DOCTOR	181.1
16	dr.vasanthakalyani	40	FEMALE	DOCTOR	0.122
17	dr.saisathish	25	male	DOCTOR	26.79
18	dr.subbaih	49	male	DOCTOR	43.19

19	dr.senthil	55	male	DOCTOR	0.14
20	dr.pappaih	57	male	DOCTOR	163.3
21	dr.arulsundresh	49	male	DOCTOR	2.4
22	dr.malarvanan	41	male	DOCTOR	8.13
23	dr.kumardevan	42	male	DOCTOR	19.31
24	dr.s.krishnasamy	46	male	DOCTOR	0.669
25	dr.rajakokila	40	FEMALE	DOCTOR	0.277
26	dr.john bill clinton	25	male	DOCTOR	47.91
27	dr.nareshkumar	25	male	DOCTOR	93.81
28	dr.vishnu	24	male	DOCTOR	0.203
29	dr.vengadesan	28	male	DOCTOR	0.066
30	dr.vinay	25	male	DOCTOR	212.2
31	dr.palraj	29	male	DOCTOR	0.137
32	dr.prabhu	48	male	DOCTOR	11.22
33	dr.rr saravanan	50	male	DOCTOR	0.092
34	dr.deepansuresh	29	male	DOCTOR	128.9
35	dr.petho	28	male	DOCTOR	0.082
36	dr.aravindh kumar	25	male	DOCTOR	0.98
37	dr.sundaravel	24	male	DOCTOR	0.052
38	dr.fathhur rabbani	26	male	DOCTOR	121.2

39	dr.pavithran	28	male	DOCTOR	2.12
40	dr.antonsherly	27	FEMALE	DOCTOR	0.127
41	dr.noorulameen	25	FEMALE	DOCTOR	0.201
42	dr. priyadarshan	24	male	DOCTOR	8.49
43	dr.vinitha	24	FEMALE	DOCTOR	0.116
44	dr.mimijanet	24	FEMALE	DOCTOR	0.025
45	dr.gopinath	27	male	DOCTOR	0.056
46	dr.mukeshwar	24	male	DOCTOR	11.25
47	dr.reenajose	28	FEMALE	DOCTOR	0.265
48	dr.sumathy	26	FEMALE	DOCTOR	0.11
49	dr.muthukumar	49	male	DOCTOR	14
50	dr.sivakumar	40	male	DOCTOR	0.225
51	dr.sangeethA	38	FEMALE	DOCTOR	0.114
52	dr.ravindran	45	male	DOCTOR	0.962
53	dr.subha	40	FEMALE	DOCTOR	0.081
54	dr.manikandan	41	male	DOCTOR	0.056
55	dr.pandiselvam	49	male	DOCTOR	0.206
56	dr.mallika	42	female	DOCTOR	0.805
57	dr.senthilkumar	48	male	DOCTOR	69.26
58	dr.kannan	52	male	DOCTOR	0.205

59	dr.venkatesh	26	male	DOCTOR	12.85
60	dr.najima rani	45	female	DOCTOR	20.25
61	dr.sujatha sangumani	50	female	DOCTOR	0.124
62	dr.vinitha	27	female	DOCTOR	0.166
63	dr.nagarajn	62	male	DOCTOR	125
64	mrs.nagarajan	58	female	DOCTOR	124
65	dr.veeramani	51	male	DOCTOR	101.21
66	dr.sivakumar	48	male	DOCTOR	52.6
67	gayathri	26	female	labtechnician	0.873
68	dr.srisaravanan	35	male	DOCTOR	0.693
69	mupuram	44	male	HCW	0.997
70	dr.sheela	54	FEMALE	DOCTOR	48.54
71	dr.sairaman	27	male	DOCTOR	10.25
72	dr.sivayogini	27	FEMALE	DOCTOR	1.24
73	dr.nagarthinam	27	male	DOCTOR	0.243
74	dr.chakravarthy	28	male	DOCTOR	11.23
75	dr.anis preethi	27	male	DOCTOR	12.21
76	dr.akila	29	FEMALE	doctor	1.21
77	dr.nagma	27	male	DOCTOR	1.21
78	dr.vairamuthu	30	male	DOCTOR	22.12

79	dr.hasheem	27	male	DOCTOR	1.21
80	dr.ashwath	29	male	DOCTOR	0.21
81	dr.manojkumar	29	male	DOCTOR	1.254
82	dr.meenakshisundaram	29	male	DOCTOR	11.75
83	dr.manaoj kumar rl	30	male	DOCTOR	102.3
84	dr.naveen	34	male	DOCTOR	10.21
85	dr.karthick	32	male	DOCTOR	11.24
86	dr.rajeshkumar	30	male	DOCTOR	10.24
87	dr.arunprakash	29	male	DOCTOR	0.234
88	dr.vignesh	32	male	DOCTOR	1.241
89	dr.karthickeyan	31	male	DOCTOR	0.214
90	dr.kishore	30	male	DOCTOR	11.235
91	dr.aarthi	29	FEMALE	DOCTOR	0.236
92	dr.sethupathi	30	male	DOCTOR	1.213
93	dr.muthukumar	35	male	DOCTOR	2.35
94	dr.chandrganesh	35	male	DOCTOR	34.21
95	dr.santhanam	30	male	DOCTOR	0.285
96	dr.ansar fathima	31	FEMALE	DOCTOR	0.952
97	dr.kalaiselvan	36	male	DOCTOR	1.325
98	dr.jaiganesh	30	male	DOCTOR	13.54

99	dr.roopasree	32	FEMALE	DOCTOR	10.25
100	dr.vinothkumar	35	male	DOCTOR	1.235
101	savithri	35	FEMALE	staffnurse	2.355
102	kasthuri	23	FEMALE	labtechnician	2.369
103	devi	26	FEMALE	labtechnician	2.879
104	divya	32	female	staffnurse	26.25
105	keerthana	29	female	labtechnician	26.36
106	kayal	36	female	staffnurse	1.032
107	gangadevi	41	female	staffnurse	1.125
108	laksmipriya	42	female	staffnurse	1.258
109	annai	43	female	staffnurse	0.213
110	karthiga	24	female	labtechnician	1.326
111	malliga	23	female	labtechnician	0.213
112	kumari	45	female	staffnurse	11.32
113	umadevi	39	female	staffnurse	0.232
114	yogalakshmi	26	female	labtechnician	45.23
115	kiruthiga	23	female	labtechnician	3.123
116	thirumalaipriya	32	female	staffnurse	0.321
117	thangam	25	female	labtechnician	26.23
118	thirupathi	39	male	hcw	2.32

119	ibrahim	46	male	hcw	16.66
120	jannat	42	male	hcw	18.55
121	kumutha	49	FEMALE	hcw	0.325
122	chellam	32	FEMALE	hcw	0.987
123	jayanthi	54	FEMALE	hcw	0.214
124	gangadevi	51	FEMALE	staffnurse	12.36
125	shanti	37	FEMALE	staffnurse	25.36
126	tamil	47	FEMALE	hcw	27.58
127	kiruba	23	FEMALE	staffnurse	32.21
128	fiona	47	FEMALE	hcw	21
129	kirubakaran	27	male	staffnurse	12.87
130	nirmal	30	male	hcw	12.54
131	raja	27	male	labtechnician	98.21
132	manikandan	29	male	hcw	0.213
133	kamala	29	FEMALE	hcw	1.23
134	janani	29	FEMALE	labtechnician	2.213
135	murugesan	30	male	hcw	2.354
136	vigneshan	34	male	labtechnician	1.231
137	kokila	32	FEMALE	labtechnician	1.021
138	easwari	42	male	labtechnician	14.21

139	rajeshwari	35	FEMALE	labtechnician	0.213
140	lingeshwari	31	FEMALE	hcw	0.145
141	rajesh	51	FEMALE	staffnurse	1.021
142	nishanthi	43	FEMALE	labtechnician	14.21
143	poorna	47	male	labtechnician	0.213
144	kadhar	26	male	staffnurse	0.145
145	abirami	35	FEMALE	labtechnician	1.241
146	aadhi	22	male	hcw	0.214
147	radha	41	FEMALE	hcw	11.235
148	gopilakshmi	47	FEMALE	hcw	0.236
149	karthick	57	male	labtechnician	1.213
150	selvam	54	male	hcw	2.35
151	maheshwari	52	FEMALE	labtechnician	34.21
152	gayathri	49	FEMALE	staffnurse	0.285
153	govidaraj	43	male	hcw	0.952
154	anand	47	male	labtechnician	124
155	pratheepa	46	FEMALE	labtechnician	101.21
156	meera	37	FEMALE	staffnurse	52.6
157	preethy	28	FEMALE	labtechnician	0.873
158	ramasamy	24	male	hcw	0.236

159	mary	33	FEMALE	hcw	1.213
160	praveen	30	male	DOCTOR	2.35
161	vasantha	39	FEMALE	staffnurse	20.25
162	senthil	45	male	DOCTOR	0.124
163	logeshwari	42	FEMALE	DOCTOR	0.166
164	gokila	41	FEMALE	hcw	125
165	dharmaraj	40	male	staffnurse	124
166	tamilselvan	47	male	staffnurse	101.21
167	kamalraj	46	male	DOCTOR	52.6
168	radhi	42	FEMALE	staffnurse	0.873
169	ambika	54	FEMALE	staffnurse	0.106
170	gopalsamy	43	male	hcw	0.089
171	jeyaram	54	male	DOCTOR	0.095
172	keerthika	51	FEMALE	staffnurse	0.647
173	ilaiyaraja	36	male	DOCTOR	141.5
174	dhanalakshmi	39	FEMALE	staffnurse	0.142
175	ramkumar	34	male	staffnurse	13.2
176	jaya	47	FEMALE	hcw	0.09
177	viji	49	FEMALE	staffnurse	0.207
178	palanikumar	41	male	DOCTOR	0.944

179	santhidevi	51	FEMALE	staffnurse	124
180	sumathi	36	FEMALE	hcw	12.33
181	gomathi	34	FEMALE	hcw	11.25
182	kabilan	46	male	staffnurse	0.231
183	bose	30	male	staffnurse	0.258
184	jannath	27	FEMALE	hcw	0.365
185	kumutha	29	FEMALE	hcw	11.54
186	jayakumar	29	male	DOCTOR	1.547
187	lalitha	29	FEMALE	hcw	21.54
188	krishnaveni	30	FEMALE	staffnurse	0.213
189	gopikumar	34	male	hcw	1.326
190	ravikumar	32	male	staffnurse	0.213
191	deepan	42	male	staffnurse	11.32
192	ramaraj	35	male	hcw	0.232
193	sushila	31	FEMALE	DOCTOR	45.23
194	inbaselvi	51	FEMALE	hcw	3.123
195	jeyaraj	43	male	staffnurse	0.321
196	thirumurugan	47	male	hcw	26.23
197	sangaiya	26	male	staffnurse	2.32
198	radha	47	FEMALE	hcw	16.66

199	kanimozhi	42	FEMALE	staffnurse	18.55
200	lalitha	54	FEMALE	hcw	0.325

INSTITUTIONAL ETHICS COMMITTEE
MADURAI MEDICAL COLLEGE & GOVT. RAJAJI HOSPITAL, MADURAI
CDSCO:Reg.No.ECR/1365/Inst/TN/2020 &
DHR Reg.No.EC/NEW/INST/2020/484

Study Title	:	Seroprevalance of Covid antibodies in health care population of Government Rajaji Hospital, Madurai.
Principal Investigator	:	**Dr.Fathhur Rabbani. S**
Designation	:	PG in MD., General Medicine
Guide	:	Dr.M.Natarajan,MD., (Gen.Med.) Professor and Head of General Medicine
Department	:	Department of General Medicine Government Rajaji Hospital & Madurai Medical College, Madurai

The request for an approval from the Institutional Ethics Committee (IEC) was considered on the IEC meeting held on **20.07.2021** at GRH Auditorium, Govt. Rajaji Hospital, Madurai at 10.00 A.M

The Members of the committee, the Secretary and the Chairman are pleased to inform you that your proposed project mentioned above is **Approved.**

You should inform the IEC in case of any changes in study procedure, methodology, sample size investigation, Investigator or guide or any other changes.

1. You should not deviate from the area of work for which you had applied for ethical clearance.

2. You should inform the IEC immediately, in case of any adverse events or serious adverse reactions. If encountered during from study

3. You should abide to the rules and regulations of the institution(s)

4. You should complete the work within the specific period and if any extension is required, you should apply for the permission again for extension period.

5. You should submit the summary of the work to the ethical committee on completion of the study.

MEMBER SECRETARY,
IEC, Madurai Medical College,
Madurai.
Dr.K.RAADHIKA, M.D(Pharm)
Associate Professor
Member Secretary
IEC - Madurai Medical College
Madurai.

- 9 AUG 2021

CHAIRMAN,
IEC, Madurai Medical College,
Madurai
Prof. Dr. V. Nagaraajan
MD.,MNAMS.,DM.,DSC(Neuro).,DSC(Hon)
CHAIRMAN
IEC Madurai Medical College
Madurai

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CERTIFICATE

This is to certify that this dissertation titled **"SEROPREVALANCE OF COVID ANTIBODIES IN HEALTH CARE POPULATION OF GOVERNMENT RAJAJI HOSPITAL, MADURAI "** of the candidate **Dr FATHHUR RABBANI S** with registration number 201911108 for the award of M.D degree in the branch of GENERAL MEDICINE. I personally verified the urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file containing from introduction to conclusion pages and result shows 0 percentage of plagiarism in the dissertation.

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