Database screen: We had screened Google Scholar database with the keywords nCoV pathogenesis, molecular immune pathogenesis of COVID-19, virology of SARS-CoV-2, diagnosis of SARS-CoV-2 and advancement of nCoV diagnosis. Then further articles were screened for possible inclusion in the systematic review. Articles that have proper information about diagnosis, pathogenesis and virology were included for further review. The database files were cited using Mendeley application.

3. RESULT AND DISCUSSION

4.

A total of 130 articles were found after preliminary screening of the databases. After title and abstract screening, 15 articles were excluded. Full screening of the remaining 115 articles was done. Among these studies, after full-text screening, a total of 84 articles were included in the final review.

3.1 Virology of SARS-CoV-2

Origin, Family, and Genomic structure

In the end of 2019, nCoV appeared in local hospitals of Wuhan, Hubei, China. Notably, many kinds of live animals including seafood were available for sale in the market of China e.g., Huanan Seafood Market, before it was forced to close on 1st Jan, 2020 by China Center for Disease Control and Prevention. So, the CDC, China suggested the origin of the outbreak is Huanan Seafood Market as the SARS-CoV-2 samples were isolated from this area in the earlier phase. But this decision was disputed as there is no earlier reported case linked to the mention market.[1] In between two months, at least two different strains of nCoV have been discovered.[9] Till date, Chinese health authority conducted many epidemiological and etiological researches to find the origin of nCoV.[10]

From bronchoalveolar lavage of three patients from Wuhan Jinyiantan Hospital on 30th December, 2019, first nCoV was isolated.[11] After the analysis it was considered to be a member of beta-CoVs,[11][12] family *Coronaviridae* of Nidovirales order.[13] There are four subfamilies of CoVs according to serologically and genotypically: alpha, beta, gamma and delta CoVs. In between these groups the humans are affected (respiratory, hepatic, neurologic and enteric diseases) by alpha and beta CoVs.[14][15] According to the analysis, SARS-CoV-2 matches phylogenetically 79.5% with SARS-CoV and 50% with MERS-CoV[11][12][16] and the percent of sequence match is less than 90 between SARS-CoV-2 and other beta-corona viruses[11] which suggested the belonging of novel coronavirus is to lineage B (Sarbecovirus) of beta-coronavirus.[17]

SARS-CoV-2 contain positive single stranded RNA genome of size approx 29.8 kbp with a gene order of 5'-replicase open reading frame 1ab-S-E-M-N- 3'.[13] The nucleocapsid (N) is covered with bilayers of phospholipids and two types of spike proteins: spike glycoprotein trimmer (S) and hemagglutinin-esterase (HE). The envelope contains some structural protein along with S proteins which are membrane protein (M) and envelope protein (E).[17] The open reading frames (ORF), 1a and 1b are responsible for production of polyprotein 1a (pp1a) and polyprotein 1ab (pp1ab), which translates non-structural proteins (nsps).[13] The scientists have predicted the lengths of M, S, N, E and ORF3a genes of nCoV are 669, 3822, 1260, 228 and 828 nt respectively. The prediction also gives an idea of ORF8 gene length of 366 nt present in-between M and N ORF gene of SARS-CoV-2 which is similar to SARS-CoV.[17]

Another study observed that, nCoV protein sequences matches with Bat virus MG772934.1 (91.1%), Bat virus DQ022305.2 (79.7%) and SARS proteome (77.1%). Due to the mutation of ORF8, two variants of 2019-nCoV were observed: ORF8-5 and ORF8-L which changes the structural proteins.[18]

Properties of SARS-CoV-2

The physiochemical properties of nCoV from SARS-CoV or MERS-CoV as it resembles most of the same characters. nCoV is round or oval shaped with 60-100 nm diameter, inactivated by heating upto 56˚C for 30 minutes or ultraviolet rays. Substances like disinfectants e.g., diethyl ether, chlorine, 75%

ethanol, peracetic acid, chloroform is very active against nCoV.[19] According to various studies, on stainless steel, plastic the SARS-CoV-2 is more stable than cardboard and copper. The half-life of SARS-CoV-2 was higher in comparison with SARS-CoV.[20]

Host Cell Entry

To enter into the cell, SARS-CoV-2 takeover the angiotensin converting enzyme-2 (ACE2) as a functional receptor.[21][22] Angiotensin converting enzyme-2, a type I membrane protein, presented in, heart, lung, intestine and kidney which majorly connected with heart diseases. N terminal peptide domain and C terminal collectrin like domain are present on ACE-2. ACE-2 gives a direct bonding location for S proteins of coronaviruses, to break angiotensin-1, for producing other angiotensins (1 to 9).[23] The process of viral membrane structural arrangement to fuse with cell membrane of host is initiated by S1 subunit. The viral membrane binds with host receptor cell through hinge like movements of receptor binding domain (RBD).[15][24] Many research evidences suggest that the binding affinity of SARS-CoV-2 with human ACE-2 is 10-20-fold higher than SARS-CoV. To observe the potential infection effect of SARS-CoV-2, S protein RBD which is in connection with ACE-2 was analysed.[25] According to another research ACE-2-B0AT1 complex may be bind simultaneously to two S proteins.[26]

Ecology of SARS-CoV-2

All coronaviruses which have effects on human are zoonotic in origin and the natural hosts for any coronavirus it is most likely the bat.[27] Chinese horseshoe bats, Rhinolophdae family in Yunnan, China found to be very close regarding SARS-CoV.[28][29] Some bat CoVs like BatCoV RaTG13 shows similar sequence up to 96% nt with SARS-CoV-2.[29]
Naturally BatCoVs cannot affects humans unless until it undergoes through mutation and recombination in any host animal.[30] Different studies shows that SARS-CoV-2 may be originated from pangolin as the sequence of nCoV and pangolin CoVs matches 99%.[31] Till now, different researches are ongoing to track the SARS-CoV-2 animal host.

Variation of Genome

The earlier genomics were obtained from nine patients of COVID-19 which was 99.98% identical match.[16] Other scientists, on analysing 103 genomes have found two type of major evolution of SARS-CoV-2: *S* and *L*. The *L* type is more aggressive and spreads rapidly as it has severe selective pressure but *S* type has weaker selective pressure so it may persist slowly.[32] These extracted RNA are very unstable, so strong surveillance is required to control SARS-CoV-2.[10]

3.2. Pathogenesis of SARS-CoV-2

The knowledge about COVID-19 pathogenesis is poorly understood as it is a new strain but from the previous studies of MERS-CoV and SARS-CoV gives an idea of nCoV mechanism as it resembles almost similar symptoms and gene sequence.[33]

Entry and replication of Coronavirus

Protein S of CoVs is main responsible for entering into the virus cells.[34] The spike glycoprotein present on the envelope of SARS-CoV-2 makes a bond with ACE-2 of the host cell.[22] This host receptor may be different for SARS-CoV and MERS-CoV i.e. CD209L[35] and DPP4[36] respectively. Through fusion of the membranes between plasma membrane and virus, which occurred at the S2′ position of S protein leads to various proteolytic cleavage, as a result the invasion is completed.[37] After entering the host cell, structural proteins and polyproteins formation starts when the viral RNA comes into cytoplasm and replication of genome started.[38] The glycoprotein forming by the viral genome enters into endoplasmic reticulum membrane and Golgi membrane. The combination of viral RNA and nucleocapsid protein will generate a nucleocapsid. Then, the endoplasmic reticulum Golgi

intermediate compartment (ERGIC) will be the germination hub for viral particles and when the released virus particles containing vesicles will bind with the plasma membrane it releases the virus in the host body.[34] A group of scientists have found a molecule, N-(2-aminoethyl)-1 azirdine-ethanamine as an inhibitor of angiotensin converting enzyme-2 which block the fusion of SARS-CoV RBD with the host cell.[39]

Antigenic action in coronavirus infection

Anti-viral immunity of the body will represent the antigen presenting cells to the viral antigen and the major histocompatibility complex or human leukocyte antigen will present the antigenic peptides which will be recognized by the virus specific cytotoxic T-cells. So, we can understand the pathogenesis of SARS-CoV-2 through the antigen presenting cells but there is not much reports of COVID-19. We only get the research papers from MERS-CoV and SARS-CoV.[40] According to researches the MHC-1 is the main for SARS-CoV presentation.[41] Various researches shows that a number of polymorphisms of human leukocyte antigen e.g., HLA-B*0703, HLA-DRB1*1202, HLA-B*4601,[42] HLA-Cw*0801,[43] can access the SARS-CoV susceptibility. But another research shows that polymorphism like HLA-A*0201, HLA-Cw1502 and HLA-DR0301 may protect from infection like SARS.[44] Apart from polymorphisms, mannose binding lectin is also an antigen presenting cell related to infection of SARS-CoV.[45]

Host cell immunity

The virus-specific T and B cells stimulate the cellular and humoral immunity by the antigen presenting cells which activates the production of IgG and IgM. IgG antibody, specific for S and N can protect the body for long time where IgM only last for 12 weeks.[34][46] Recent researches Shows that the activation of CD8+ and CD4+ is higher but the count in peripheral blood is significantly low for SARS-CoV-2 patients.[47] Different researches on SARS-CoV patients shows that memory T-cells can recognise S-peptide up to four and six years after recovery.[48][49] These data may help in nCoV vaccine designing.

Cytokine storm

Recently published papers shows that SARS-CoV-2 induces the shedding of angiotensin converting enzyme-2 which results in a high activation of inflammatory factors like interferons, interleukins and chemokines.[50] In the earlier stage the viral replication triggers the chemokines and cytokines by causing damage to endothelial, epithelial cell and vascular leakage.[51] As a result of low ACE-2 levels, renin angiotensin system will be effected which will triggers more inflammation causing vascular permeability, ultimately leads to organ failure, acute respiratory distress syndrome etc.[10] Another study suggested that, viral cellular uptake can be improve by antibody dependent enhancement (ADE) through interaction of virus antibody complex and Fc receptor or different receptors, resulting enhancement of target cells.[52] The action between Fc receptor and the virus anti-S protein neutralizing antibodies complex may give an improvement in inflammation and replication of the virus in the lungs.[50]

Immune evasion

SARS-CoV and MERS-CoV have different strategies to dodge immune reactions in host cell. Pattern recognition receptors can identify the pathogen associated molecular pattern. But producing a double membrane vesicles with low PRRs, MERS-CoV and SARS-CoV can avoid host detection.[53] Against MERS-CoV and SARS-CoV infection, IFN alpha and beta can be helpful.[54] But the induction of IFN gets blocked by 4a-protein in MERS-CoV.[55] Alongside there are many proteins (ORF4b, ORF4a, ORF5 etc) which block the IFN regulatory factor-3 and activation of IFN beta promoter in MERS-CoV.[56] If we can closely monitor these processes and reverse the mechanism we may find a treatment strategy.

3.3. Diagnosis of 2019-nCoV

Several examinations are involved for confirming a COVID-19 case, firstly patient's travel history, clinical appearances and then lab investigation and radiological imaging (CT). Lab investigation involves blood culture, nucleic acid detection (NAAT, RT-PCR, LAMP), serological investigation, immune identification techniques (POCT, IIFT, ELISA).[57] The lab techniques commonly used for coronaviruses detection are listed in Table 2.[58]

Table 2: Laboratory techniques for detection of coronaviruses:[58]

Method	Characteristics	Test time	Reference
Antigen EIA	Rapid, poor sensitivity, some are CLIA-waived	<30 min	[59][60]
Antigen IFA	Good sensitivity and specificity, subjective interpretation	1–4 h	[61][62]
Cell culture	Gold standard, pure culture for further research and development, time consuming	1–7 days	[63][64]
Serology	Retrospective, cross-reaction	2–8 h	[63][22]
NAAT, monoplex, pan-HCoV	High sensitivity with universal coverage of all species of HCoV	1–8 h	[65][66]
NAAT, monoplex, specific-HCoV	High sensitivity and specificity for special species, potential quantification	1–8 h	[67][68]
NAAT, multiplex	High sensitivity and specificity, covering other pathogens, FilmArray RP EZ is CLIA-waived	1–8 h	[69][70]
NAAT, POCT	Rapid and safe, good sensitivity and specificity, some are CLIA-waived	15– 30 min	[71][72]

EIA- enzyme immunoassay; IFA- immunofluorescent assay; NAAT- nucleic acid amplification test; CLIA- Clinical Laboratory Improvement Act.

Nucleic acid detection method

There are two methods for nCoV detection: high-throughput sequencing and real-time quantitative polymerase chain reaction – RT-qPCR.[12] The high-throughput sequencing is not preferred due to its high cost and equipment dependency. Whereas RT-qPCR is an effective and direct technique for identify viruses from blood and secretions from respiratory track.[73] Different studies trying to isolate live viral genome of nCoV from stool [74] and tear fluid.[75]

Scientists are conducting different type of RT-PCR and RT-LAMP for better and rapid identification like using iCycler thermocycler- IQSYBR Green SuperMix.[76] RT-LAMP isothermal amplification is also a rapid and very sensitive method in detection of cDNA or RNA of SARS-CoV-2 and the detection can be seen under natural light by the presence turbidity in the kit.[77][78] There are some guidelines for handling Nucleic Acid Amplification Test in different countries which are considered as standard operating procedure by WHO and the mechanism is according to their guidelines given in Table 3.[79]

Table 3: Current following protocol for Nucleic Acid Amplification Testing:[79]

Institute/Country	Gene targets
China CDC, China	ORF 1ab and N
Charitè, Germany	RdRp, E, N

HKU, Hong Kong SAR	ORF 1b-nsp14, N
National Institute of Infectious Diseases, Department of Virology, Japan	Pancorona and multiple targets, spike protein
National Institute of Health, Thailand	N
US CDC, USA	Three targets in N gene
Pasteur Institute, Paris, France	Two targets in RdRp gene
As listed in WHO	

The primers and probes used for RT-PCR for COVID-19 is listed in Table 4.[73]

Table 4: Primers and probes for real-time RT-PCR for COVID-19:[73]

Assay/use	Oligonucleotide	Sequence (a)
RdRP gene	RdRp_SARSr-F	GTGARATGGTCATGTGTGGCGG
	RdRp_SARSr-P2	FAM-CAGGTGGAACCTCATCAGGAGATGC-BBQ
	RdRP_SARSr-P1	FAM-CCAGGTGGWACRTCATCMGGTGATGC-BBQ
	RdRp_SARSr-R	CARATGTTAAASACACTATTAGCATA
E gene	E_Sarbeco_F	ACAGGTACGTTAATAGTTAATAGCGT
	E_Sarbeco_P1	FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ
	E_Sarbeco_R	ATATTGCAGCAGTACGCACACA
N gene	N_Sarbeco_F	CACATTGGCACCCGCAATC
	N_Sarbeco_P	FAM-ACTTCCTCAAGGAACAACATTGCCA-BBQ
	N_Sarbeco_R	GAGGAACGAGAAGAGGCTTG

(a)- W is A/T; R is G/A; M is A/C; S is G/C. FAM - 6-carboxyfluorescein; BBQ - blackberry quencher.

The comparison of real-time reverse transcription polymerase chain reaction between World Health Organisation and Centre for Disease Control and Prevention for diagnosis purpose of SARS-CoV-2 are given in Table 5.[80] The comparison of PCR and LAMP is given in Table 6.

Table 5: Comparison of rRT-PCR of WHO and CDC for diagnosis of SARS-CoV-2:[80]

Test	Molecular targets	Scope	Limit of blank	Specimens	Storage conditions
WHO	E gene	First-line screening	3.9 copies × reaction	Nasopharyngeal AND oropharyngeal swab or wash in ambulatory patients, lower respiratory specimens (sputum and/or endotracheal aspirate or bronchoalveolar lavage)	≤5 days: 2–8 °C
	RdRp gene	Confirmatory testing	3.6 copies × reaction		>5 days: ≤70 °C
	N gene	Additional confirmatory testing	N/A		(Dry-ice)
CDC	N1/2/3 gene	Combined assay	1.0–3.2 copies/μL	Nasopharyngeal AND oropharyngeal swabs, sputum, lower respiratory tract aspirates, bronchoalveolar lavage and nasopharyngeal wash/aspirate or nasal aspirate	≤4 days: 4 °C
	RNase P gene	Control assay	N/A		> 4 days: ≤70 °C

E gene - envelop gene; N gene- nucleocapside gene; RdRp gene- RNA-dependent RNA polymerase gene; RNase P gene- human RNase P gene.

Table 6: Comparison between PCR and LAMP:[83][84]

LAMP	PCR
Isothermal and continuous amplification (Smaller, simpler, portable).	Thermal cycling (Multiple heating and cooling cycle; bulky and cumbersome).
Always requires sample concentration and Preparation (Time-consuming).	For virus detection, for example, influenza or human norovirus, LAMP assay offers one-step detection. Sample preparation steps are simplified.
Single protocol (Faster).	Multiple protocols (Complicated and requires a skilled technician).
Tolerate inhibitors and more stable.	Inhibitors hinder the reaction.
Diagnostic sensitivity > 95%.	Diagnostic sensitivity (95%) is currently reported lower than LAMP
Still exploring.	Established technique.

CRISP-FDS is a sensitive assay for SARS-CoV-2 detection with very less equipment and low cost. There are researches going on placing a microfluidic chip of RT-PRA based CRISPR-FDS which can be usable by any smart phone.[81] RT-RAA kit is also a promising tool for nCoV detection.[82]

Radio imaging and other diagnostic techniques

Although the RT-PCR is a specific test for detection of SARS-CoV-2 but still most physician suggested CT-imaging as they believe to be more sensitive. As in many cases it has been seen that the RT-PCR report is negative but according the CT scans the patient is probably affected by nCoV as the image clearly shows the bilateral and multilobar GGO which can be distributed peripherally or posteriorly. Some other findings may include septal thickening, pleural thickening, bronchiectasis etc. Some uncommon but considerable findings are pleural effusion, lymph adenopathy, pneumothorax, cavitation etc. Follow-up case findings may be high number of GGO, septal thickening etc. The changes in lungs can occurs within 10 days of symptomatic actions. There are five stages of CT finding: (a) ultra-early, (b) early, (c) rapid progression, (d) consolidation and (e) dissipation stage.[85] But CT findings are still limited as the changes of the lungs can be due to other viral infection like adenovirus, MERS-CoV and SARS-CoV.[86]

In addition to nucleic acid detection and CT-imaging many researchers are developing kit for immunological detection and the detection rate of such serological kit (POCT of IgG/IgM, ELISA) is higher than nucleic acid detection.[87] Infact, enzyme-linked immunosorbent assay using chemiluminescence (CLEIA) has also emerged as a newer technique to detect the viral nucleocapsid (N) antigen in nasopharyngeal aspirate (NPA). Similar studies have found, rS- and rN- based ELISAs may give us a specific confirmatory reaction for COVID-19.[88] Salivary detection of secretory immunoglobulin A specific for COVID-19 can be a beneficial research as animal model of this was successful for SARS-CoV.[89] Some researchers said that, IL-6 and D-dimer levels can give an idea about the severity of SARS-CoV-2 infection.[90]

4. CONCLUSION

The issue under consideration in COVID 19 pandemic is the asymptomatic infected cases or the very mild cases who are considerably a large number in the population. Testing them for viral RNA is a real impractical situation; therefore, development of a specific IgG kit is the need of an hour. With this development large scale sero-diagnosis will be possible and true rate of infection in the population will also be known making us to understand the disease better and human to human transmission will also be easily traced. Hence, a rapid-performing serologic assay is keenly required for the fute and current viral needed for the current and future irruption. Again, as a preventive measure, strict vigilance of viral changes in different hosts is very important.

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Conflicts of Interest

There are no conflicts of interest.

Summary of work done by the contributors

VS, KJ and NP planned the concept and design. VS, KJ, PPK and AS carried out the Literature search and drafting the manuscript. PPK, AS and NP contributed in Manuscript editing and interpretation of the results. All the authors listed in manuscript have contributed substantially to the writing and revising of the manuscript.

Abbreviations

SARS-CoV: Severe Acute Respiratory Syndrome Coronavirus; MERS-CoV: Middle East respiratory Syndrome Coronavirus; SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus-2; nCoV: novel Corona-virus; COVID-19: 2019 Coronavirus; RT-PCR: Real-Time Polymeric Chain Reaction; RT-LAMP: Reverse Transcription Loop-mediated Isothermal Amplification; HRCT: High Resolution Computerised Tomography; ORF: Open Reading Frame; IIFT: Indirect Immunofluorescence Test; POCT: Point-of-care Testing; ACE-2: Angiotensin Converting Enzyme-2; HLA: Human Leukocyte Antigen; NAAT: Nucleic Acid Amplification Testing; ELISA: Enzyme Linked Immunosorbent Assay RT-RAA: Reverse-Transcription Recombinase Aided Amplification

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