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Review Article

Considerations about Genomic and Proteomic of (SARS-CoV)-2 and Concern Variants

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Abstract

A novel coronavirus that related to previous SARS-CoV and Middle East respiratory syndrome coronaviruses, has been emerged in the end of 2019 in China and rapidly wide spread all over the world causing what is termed pandemicCOVID-19 disease. The disease presented firstly by flu-like symptoms but may be dramatically exaggerate in certain infected patients to develop acute respiratory distress syndrome that may be fatal. The causative agent was termed severe acute respiratory syndrome coronavirus (SARS-CoV)-2, and suggested to be of bat corona virus's origin. One of the criticalmain challenges is how to diagnose precisely the disease without confusion with another flu and respiratory distress causing viruses. Theinvestigated epidemiology, genomic structures and proteinsof the novel virus as well as different diagnostic methods were rathermentioned in this article.

Introduction

A short time ago at the end of 2019, one of the Chinese business and trade cities; Wuhan experienced a rapidly spread outbreak of an unusual respiratory distress. The disease threatened over seventy thousand and caused the death of more than eighteen hundred individuals within the first two months (Wu *et al*., 2020).In February 2020, WHO announced the disease as a global pandemic caused by an unprecedented coronavirus (Mahase, 2020, WHO, 2020).

The novel virus was termed as Wuhan coronavirus or 2019 novel coronavirus (2019-nCov) by the Chinese researchers and defined as a member of the β group of coronavirus. Later on, the International Committee on Taxonomy of Viruses (ICTV) named the virus as SARS-CoV-2 and the disease as Coronavirus disease 2019 (COVID-19) (Lai *et al*., 2020, Wang *et al*., 2020).The COVID-19 has implemented in more than five and half million confirmed cases, with over 350,000 deaths globally, as of May 27, 2020.More badly, it induced unusual disruptions; social distancing, working stoppage, cities lockdowns and travel restrictions resulted in remarkable weakness in world and consequently individual economy(WHO, 2020). There major present–day challenges face the health and research organizations are the hardness of containing the huge spread of COVID-19, the pattern of the disease that displays a varied range of clinical signs which may confused with other respiratory illnesses (Tan, 2020).Until a commercial vaccine becomes attainable, there isa great needing of acquiring efficient methods for precise identification of asymptomatic cases

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that result in spreading of the virus to close contacts. Hence, this identification facilitates theavoiding of unnecessary quarantines of negative persons and the spread of infection by positive ones ((Jones, 2020, Mizumoto*et al*., 2020).

Knowing the origin, evolution, primary hosts, how the virus transmits and infects aids in the establishment of limitationand control strategies. Moreover, analysis of the virus genome and structure promotes the development of accurate diagnosis (Adhikari*et al*., 2020).The diagnosis of COVID-19 may little relies on clinical signs, radiological picture or blood picture, so it is important to develop more complicated diagnostic techniques based on viral genomic sequencing as essential tool for determining the rate and degree of mutational variances associated with SARS-CoV-2 and for more efficient vaccine development (Linda *et al*., 2020).

This current essay provides an updated overview on the origin, transmission, structure, clinical and laboratory diagnosis that obtained from various published researches via searching among different databases.

1. Etiology

SARS-CoV-2 which is belongs to subgenus *Sarbecovirus*, the subfamily *Orthocoronavirinae* in the family of *Coronaviridae* of the order *Nidovirales* (Zhu N., 2020).

The family *Coronaviridae* is subdivided to four subgroups; alpha (α), beta (β), gamma (γ) and delta (δ). Corona viruses developed their name from presence of crown-like spikes on the outer surface of the virus. Coronaviruses are considered as minute sized viruses; (65–125 nm in diameter) and their genome comprise a positive single-stranded RNA as a nucleic material, size ranging from 26 to 32kbs in length (Fig. 1)(Cui *et al*., 2019).

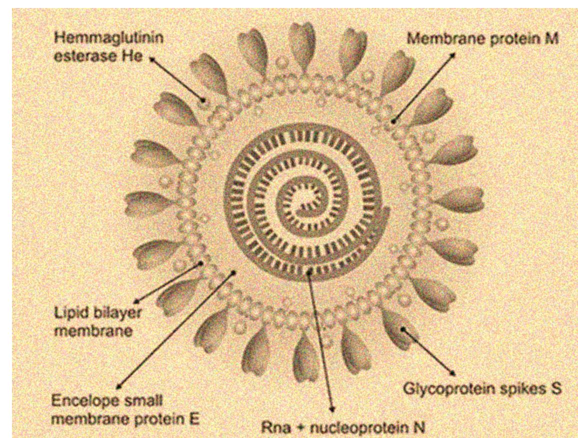

Fig. (1): Diagrammatic structure of Corona viruses

2. Epidemiology:

The pandemic shot up exponentially at the beginning of 2020, and might be drop in the sea due to delayed case notification and shortage in testing kits (Li *et al*., 2020). One of the important containment plans is determination of the source of origination to control the infection, as a member of coronaviruses, SARS-CoV-2 has been suggested to be of non-human origin, and may have been transmitted to humans through hosts.Initially, a group of researchers purposed snakes to be the potential host of the SARS-CoV-2 or even supposed it was a recombinant virus of bat and snake coronaviruses (Cui *et al*., 2019, Ji*et al*., 2020).

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Otherwise, the most studies discussed the historic background 0f other coronaviruses reservoirs. Going back to 2001, anti-bodies against SARS-coronavirus were detected among Hong Kong healthy persons by 2.5% frequency rate using molecular assessment. These findings have given a hypothesis that SARS-coronavirus may be circulating in humans before causing the outbreak in 2003 (Zheng*et al*., 2004).

The researchers suggested certain carnivores; raccoon dogs and palm civets as secondary hosts through examination of the samples isolated from the civets at the food market which displayed positive results for viral RNA detection (Kan*et al*., 2005), later on supposed *Rhinolophus* bat as a source of viral replication (Shi and Hu 2008).On the other side, the MERS- coronavirus having camels as a zoonotic source or primary host (Paden *et al*., 2018), and was also detected in *Pipistrellus* and *Perimyotis* bats (Annan *et al*., 2013) displaying that bats are the key host and transmitting medium of the virus (Lau *et al*., 2013).

Furthermore, the genomic similarity feedbacks of novel coronavirus with SARS-like bat viruses probed the declaration that not snakes as firstly thought but only bats could be the key reservoirs (Lu *et al*., 2020). Even so, to uproot, the virus, more investigationsare required to be conducted in the aspects of the identification of the intermediate zoonotic source that caused the transmission of the virus to humans; Figure (2), (Muhammad *et al*., 2020).

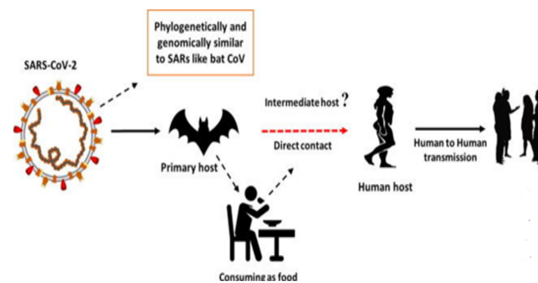

Fig. (2): Tracking of the origin and hosts of novel corona virus (Muhammad *et al*., 2020)

The onset of the first cluster cases reported an exposure history to the Huanan seafood (wild animal) wholesale market in Wuhan. However, phylo-epidemiologic analyses suggested that Huanan market was not the origin of (CoVID-19),the virus was imported from elsewhere and boosted in the crowded market, after which it spread rapidly with infected travelers to the whole of China and to other countries (Huang *et al*., 2020).

3. Genome and phylogeny

Based on whole genomes phylogenetic analysis, ten Chinese and five USA current SARS-CoV-2outbreak isolates were sequenced using the gamma distribution MEGA 7.0 version. The obtained data exposed that the isolates are nearly identical across theS-gene based phylogeny suggesting a monophyletic clade (Malika*et al*., 2020).The phylogenetic tree of family *Coronaviridae* falls into two clades. The Beta-coronavirus genus constitutes one clade, while the other clade comprises the Alpha, Gamma and Delta-coronaviruses (Chan *et al*., 2020).

The studies manifested that the 2019- nCoV is in the same Beta-coronavirus clade as SARS-CoV and MERS-CoV in the parallel manner to SARS-like bat CoVs indicating their close relation. Investigations also demonstrated that the genome of SARS-CoV-2has the highest identity (89% identical) with that of a SARS-like bat CoV, (isolated in China from horseshoe bats between 2015 and 2018), 82% identical to human SARS-CoV, but distant

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from and less related to the MERS-CoVs., (about 50%) Fig. (3). This proposed a different viral evolution from SARS and MERS, and pointed out to the suggestion of that bats are the potential wild reservoir (Benvenuto *et al*., 2020, Paraskevis *et al*., 2020, Wu *et al*., 2020).

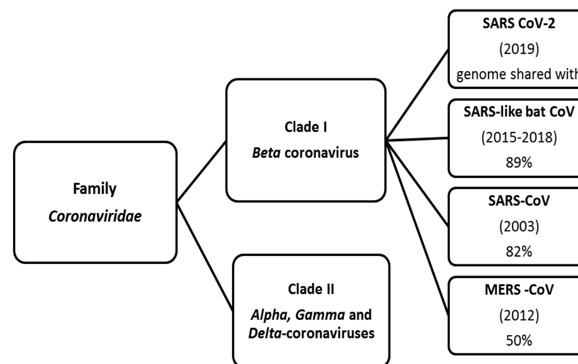

Fig. (3): Schematic diagram of phylo-genomic relations of novel corona virus

Primer novel virus genome analysis demonstrated a close evolutionary association with the SARS like bat coronaviruses; through determining the first three genomes, namely Wuhan/ IVDC-HB-01/2019 (HB01), Wuhan/IVDCHB-04/2019 (HB04), and Wuhan/IVDC-HB-05/2019 (HB05), (Zhou *et al*., 2020).

Later on, in-depth genome notation of the novel virus was carried out with a comparison to related coronaviruses; 338 bat SARS-like CoV, 1,008 human SARS CoV, and 3,131 human MERS-CoV, whose genomes were published before January 12, 2020 (release date: September 12, 2019) from Virus Pathogen Database and Analysis Resource (ViPR) (http://www.viprbrc. org/) and NCBI. It was founded that the genome of SARS-CoV-2 is almost identical to those three coronaviruses, with only five nucleotide differences in the genome of ~29.8 kb nucleotides. The genome of coronaviruses whose size domains approximately 26- 32 kb, is the largest among all known RNA viruses, with G + C contents varying from 32% to 43% and contains an inconstant number (at least 6) of ORFs (Song *et al*., 2019).

To realize the greatest output from their courted genomes, viruses frequently avail what is called alternative open reading frames (ORF), in which translation is launched from a start codon within a presented gene and, getting out of frame, brings about a distinguished protein product (Meier *et al.,*2006).

The SARS-CoV-2genome was defined to possess 14 ORFs encoding 27 proteins. The first ORF "*orf1ab*" is the largest gene, looking over approximately 67% of the entire genome encodes pp1ab protein and other 15 non-structural proteins (nsps), while the *orf1a* gene encodes for pp1a protein with 10 nsps , both previous genes located at the 5''- terminus of the genome. However, the other ORFs resided at the 3'' -terminus of the genome encode structural as well as eight subsidiary proteins (3a, 3b, p6, 7a, 7b, 8b, 9b, and orf14),(Cui *et al*., 2019, Chen *et al*., 2020, Lu *et al*., 2020, Wu *et al*., 2020).

4. SARS-CoV-2 Proteoms

At the proteome scale analysis, the SARS-CoV-2 is extremely similar to the related Beta-coronaviruses, unless there are some notable variances. For instance, the 3a, 3c and 8b accessory proteins are both closest to the SARS CoVs but varied in the amino acids numbers with the absence of 8a. On the other hand, the encoded structural proteins of pp1ab, pp1a,

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envelope, matrix, nucleocapsid as well as accessory protein 7a, showed a close relation to SARS-like bat CoVs, but regarding the spike S glycoprotein, the SARS-CoV-2is closest to the bat CoVs.

Given the side of SARS-CoV-2 discrimination, the protein differences may represent in the amino acids chain length or amino acid substitutions, these variations may result in structural and functional segregation from other SARS-CoVs.

Apart from identity presents in nonstructural protein 7 (nsp7), nsp13, envelope, matrix, or accessory proteins p6 and 8b, in total, there were 380 amino acid replacements between sequences of SARS-CoV-2and the parallel assent sequences of SARS and SARS-like viruses. For instance; 102, 61 and 27amino acid substitutions are located in nsp3, nsp2 and spike protein respectively. Moreover, four replacements in the C-terminal of the receptor-binding subunit S1 domain (Figure 2) are located in two peptides previously reported to be antigens for SARS-CoV, (Guo*et al*., 2004, De-Ming *et al*., 2020, Renhong*et al*., 2020).

The researchers cannot give sensible captions about the reasons of presence or absence of those amino acid substitutions. The presence or absence of the substitutions could affect the host tropism and transmission property of the SARS-CoV-2compared to other parallel CoVs.

The four major structural proteins of coronaviruses are the spike surface glycoprotein (S), small envelope protein (E), matrix protein (M), and nucleocapsid protein (N), fig (4:a,b,c,d), (Chang, *et al*., 2014; Li, 2016; Bianchi *et al*., 2020;Max Perutz Labs, 2020, Zhu *et al*., 2020).

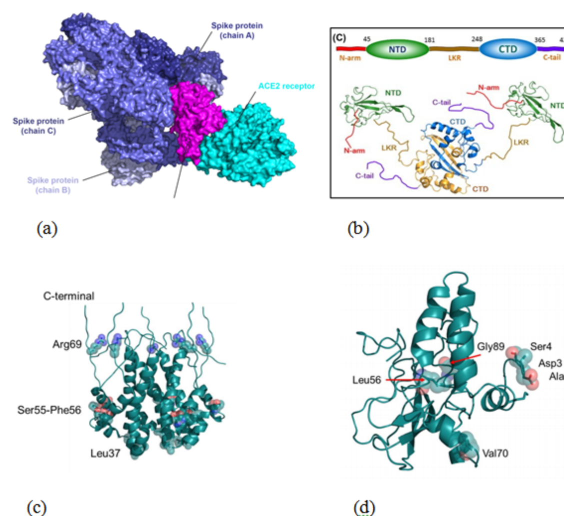

Fig. (4): The four structural proteins of SARS-CoV-2,

(a): spike S protein; (b): nucleoproteinN protein;(c): envelopeE protein; (d): matrixM protein.

4.1. Spike glycoprotein

Spike glycoprotein (S protein) is type 1 membrane trimeric protein inserted in envelop protein forming the spikes on the virus surface which give the characteristic crown-like appearance of corona viruses, (Fang , 2020).The receptor-binding domain (RBD) is a part of a protein sequence, life independent tertiary structure which binds to a specific atom or

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molecule. It is substantial because they help splicing, assembling, conformational changing and translating proteins.

Generally, the coronavirus S protein comprises two prime domains: the S1 subdomain at the N-terminus of the protein interposes binding to the target receptor of the host cell and the C-terminus S2 domain enhances fusion of the virus membrane with cellular membrane of the host cell, (Li, 2016).

It is reported that S protein of the novel coronavirus is modified via homologous recombination; a mixture of bat SARS-CoV and a not known Beta-CoV . The S1 subdomain of SARS-CoV2 includes 424–494 amino acids (AA), S1 C-terminal contain core structure of 5 antiparallel B-sheet (B1, B2, B3, B4, and B7) and short concave outer surface. It is declared that SARS-CoV-2 S protein contains 1273 AA; near SARS-CoV '1255 AA', but less than MERS-CoV '1353 AA'. Otherwise,MERS-CoV RBM is flat surface that is 4 anti-parallel B-sheet. The S2 subunit contains a fusion peptide, 2 heptad repeat domains HR1 and HR2, a transmembrane (TM) domain , and an endodomain (E) (Rota *et al*., 2003, Li, 2016, Benvenuto, *et al*., 2020, Li *et al*., 2020).

The spike surface glycoprotein plays an essential role in binding to receptors on the host cell and determines host tropism. The RBD comes into direct contact with the extracellular binding site on ACE2 known as the peptidase domain (PD) (Bosch *et al*., 2003). There are two cleavage sites in the S protein, arginines R667 and R797. The R667 site is at the division between S1 and S2 and cleavage at the R797 site results in the final S2 polypeptide (Millet and Whittaker, 2015). It is also reported that Spike glycoprotein of the novel coronavirus is modified via homologous recombination. The spike glycoprotein of SARS-CoV-2 is the mixture of bat SARS-CoV and a not known Beta-CoV.There are some variations in RBD amino acid contact with ACE2 ofSARS-COV2 in apart from SARS-CoV, fig (5:a,b); these differences make SARS-COV2 is stronger binding to ACE2 via spike glycoprotein (Li *et al*., 2020, X *et al*., 2020).

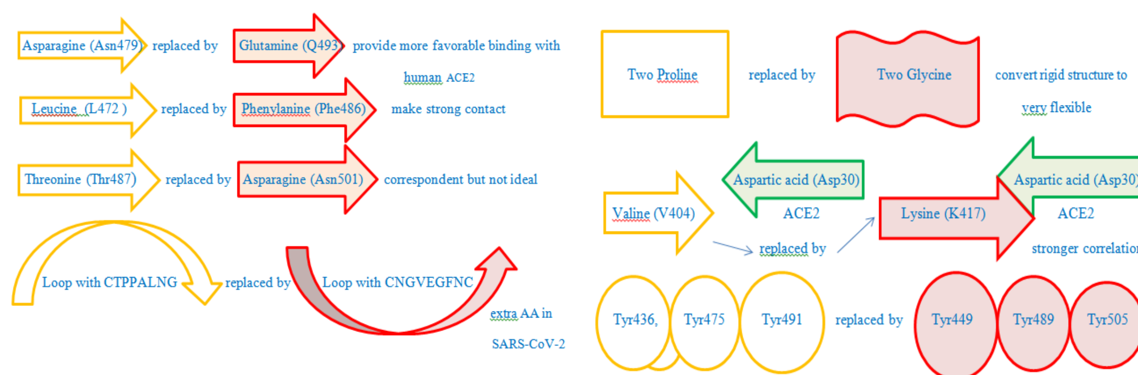

Fig. (5:a,b): Some variations in RBD (domains) amino acid contact with ACE2 betweenSARS-COV2 and SARS-CoV.

4.2.Nucleocapsid protein (N)

Nucleocapsid protein "N" protein is a basic structural protein that protein binds to the virus genome forming ribonucleoprotein known as nucleocapsid. N protein is more stable than spike protein so it becomes a target for antiviral therapy, SARS-CoV-2 N protein sequence contains 419 amino acid and shares 89.74 % SARS-CoV N protein but only 48.59 % of MERS-CoVone, (Kang, *et al*., 2020).

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The three structural domains have characteristics common to all coronavirus N proteins, N-terminal domain (NTD, 45–181 AA) of the SARS-CoV- 2 N protein acts as RNA-binding domain, thenSer/Arg (SR)-rich linker is responsible for phosphorylation. Finally, the C-terminal domain (CTD, 248–365 AA) acts as a dimerization domain for oligomerization (Dinesh, *et al*., 2020).

On the whole, the C-Terminal of N protein is identical in all coronavirus, so the variations are mainly focused on N-terminal domain.Unlike, the common folded N-terminal nitrogenous base binding channel tail, SARS-CoV-2 has extended outward tail. This unique pattern leads to change charge distribution of N protein nucleotide surface making easier accessibility. Moreover, the phosphate group binding site in SARS-CoV-2 N-NTD has larger side chain amino acid compared to other coronaviruses. Finally, the edge of nitrogenous base in SARS-CoV-2 N-NTD has Arg 89 amino acid compared with Tyr 102 causing increase polar properties. As for the C-terminal domain fragment contains a short multiple hydrophobic interaction dimerization core besides positive charged regions, the N protein is able to bind to single-stranded RNA (ssRNA), single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) with greater affinities., (Kang, *et al*., 2020).

N protein is considered a multifunctional; which binds with RNA for genomic protection, ensuring replication and transmission. Also associates with M protein during assembly. Moreover, N protein regulates host pathogens interaction such as actin reorganization, host cell cycle progression, and apoptosis. Correspondingly, it is able to induce immune response, so it is considered highly antigenic. Also, it can escape from immune system via prohibition of type I interferon and cytokines after virion infected the host cells, (Lin, *et al*., 2020).

4.3.Envelope (E) protein

The SARS-CoV2 E protein is a small sized; 75 AA, coded by E gene and considered a critical component of purified virus particles; act as integral transmembrane for ion channel activity and responsible for virion envelope morphogenesis, (Navratil, *et al*., 2020). Also CoV E may have an anti-apoptotic function by suppressing the Unfolded Protein Response (UPR), probably as a survival mechanism essential for virus dissemination, (DeDiego *et al*., 2011). Generally, the E protein in all coronaviruses comprised primary and secondary structures containing three domains. N-terminal domain is a short (8 amino acids) hydrophilic then followed by an unusually long hydrophobic transmembrane domain (25–30 amino acids with 2–4 cysteine residues) that formed a helical hairpin. The ending hydrophilic carboxyl C-terminal domain is long (40 amino acid), ubiquitinated required for proper virus assembly, (Schoeman, and Fielding, 2019).

In contrary of the common observation among *Coronaviridea* members that the E protein corresponding sequence in between is few;(e.g. MERS-CoV is 69, 5%) there is a 94.74% identity shared between SARS-CoV2 'E protein' sequence and that of SARS-CoV.SARS-CoV2 E protein is a pentameric and one unit of E protein consist of seven α-helices and eight loops , so that the ion channel activity of ‘SARS-CoV2 E’ proteins is modulated via pentameric ion channel, (Gupta, *et al*., 2020). There are four differences between SARS-CoV and SARS-CoV-2 E proteins; two replacements in BH-3 like helix and two in N-terminal, fig.(6), (Navratil, *et al*., 2020).

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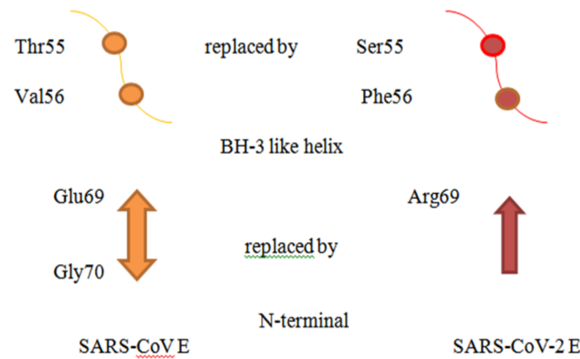

Fig. (6): The variations in amino acids ofE protein betweenSARS-COV2 and SARS-CoV.

4.4.Matrix M protein

It is essential in virus assembly, and plays an important role; turns cellular membranes into factories where virus and host factors join to make new virus particles. The M proteins from SARS-CoV-2 as well as SARS-CoV, and MERS-CoV are targeted to the vicinity of the Golgi apparatus. It is suggested that M protein promotes assembly by interacting with the viral ribonucleoprotein (N protein) and S glycoproteins at the budding site, besides creation of a network of M-M interactions which have the ability to exclude some host membrane proteins from the viral envelope, (Neuman*et al*., 2011, Hasöksüz*et al*., 2020).

4.5.Non- structural proteins of SARS-CoV-2 (Nsp):

The nonstructural proteins are coded by *ORF*1ab which encodes the *ORF*1ab polyprotein that contains from 1 – 7096 amino acid fig (7).

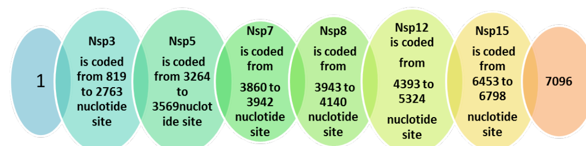

Fig. (7): *ORF*1ab sites of different non-structural proteins of SARS-CoV2.

4.5.1. Proteases (Nsp 3&Nsp 5)

Nsp 3 is a papin like protease (PLpro), whileNsp 5 constitutes3-chymotrypsin-like protease (3CL pro), and Mpro which are encoded by *orf*1ab gene.These enzymes are important for virus replication and the translation of the polypeptide from the genomic RNA to protein component, (Shankar, *et al*., 2020). The three-dimensional structure of SARS-COV-2Mpro is highly similar to SARS-CoVMpro, sharing about 96%. The Nsp5 is about 305 amino acid, and the differences between them are only 12 amino acid that are at positions 33, 44, 63, 84 86, 92, 132, 178, 200, 265, 283 and 284 , and on the same line, 3C- like protease sequence is 100% identical, (Zhang, *et al*., 2020).

Nsp5 is asymmetric unit contains only one but two of these polypeptides associate to form a dimer designated (protomer A and B). Each protomerconsist of 3 domain in which domains I and II (10-99 and 100-182 AA, respectively) are six-stranded antiparallel β-barrels

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that contain the substrate-binding site between them in the cleft. Domain III (198-303 AA) contains five α- helices that show globular cluster and shared in regulating dimerization of the Mpro. A Long loop (185–200 AA) links between domain II and domain III that participate in the formation of the substrate binding pocket. There are twelve cysteine residues across the protein molecule with six are buried in the core and the other six are exposed to the surface, one of them (C145) located in the catalytic center that lies in a cleft between domain I and domain II, (Jin, *et al*., 2020).3C like protease and papin like protease are important enzymes for Viral RNA translation to polyprotein process that they work on more than 11 cleavage sites on the large polyprotein 1ab (replicase 1ab),(Zhang, *et al*., 2020).

4.5.2. Nsp 9

Nsp9 in SARS-CoV2 is encoded from *orf*1ab polyprotein and share 97% sequence of Nsp9 in SARS-CoV.The apo-Nsp9 SARS-CoV2 structure is related closely to that belongs to SARS, and also like other Nsp9 homologues in which it exhibits an unusual fold that is not found outside of coronaviruses. The core of fold is a small 6-stranded enclosed β-barrel that showed series of extended loops projected outward. Two loops (β2-3- and β3-4) are projected from open face of barrel carry positive charge, rich glycine, and think to involve RNA binding, (Littler, *et al*., 2020).

4.5.3. Nsp12 (RNA-dependent RNA polymeras RdRp)

Nsp12 in SARS-COV-2 is encoded from orf1ab polyprotein and is closely related to that of picoronavirus (about 500 AA) apart to that of The SARS-CoV nsp12 (932 AA). SARS-CoV2 differs from SARS-CoV in 31AA mutations; 22 AA are located in N-terminal of nsp 12 , the remaining nine amino acids are located in C-terminal of nsp12 and one of them (S783A) is a non-conservative mutation, (Robert and Ward,2019).

SARS-COV-2 NSP 12 consists of C-terminal domain, and right hand polymerase domain, then Nidovirus unique N- terminal extension domain which contain nidovirusRdRp-associated nucleotidyltransferase (NiRAN)besides addition N-terminal β-hairpin. Finally, there is an interface domain connect between the right hand and NiRAN, (Peng, *et al*., 2020).

Polymerasedomain (right hand) in SARS-COV-2 structure is like structure of others in human associated coronaviruses that contains polymerase motifs A-G that form active site, template/primer entry, nucleoside triphosphate (NTP) entry, and nascent strand exit paths that is positively charged and solvent-accessible, (Gao, *et al*., 2020). On the other hand, NiRAN in which a portion of the N- terminal extensiondomain (residues 4 to 28 and 51 to 249) consists of two (other Nidoviruses have eight) helices with a five-stranded β-sheet at the N terminus , and is considered a genetic marker for arrangement of Nidovirales that no viral or cellular homologs is identified, (Shannon, *et al*., 2020).

Nsp12 alone is of little activity, so it requires co-factor such as Nsp 7 and Nsp 8 for stimulating its polymerase activity that catalyzes the synthesis of viral RNA and is necessary in replication and transcription,(Robert and Ward, 2019).

4.5.4. Nsp 7 and Nsp 8

Nsp7 and Nsp 8 in SARS-COV-2 are encoded from *orf*1ab polyprotein, and found to be bind to Nsp12 to form an active polymerase "apoRdRp complex". This complex includes one nsp12, one nsp7 and two nsp8; the polymerase domain of nsp12 binds to nsp7 and nsp8, leaving the other nsp8 molecule sited on the top of the finger subdomain and interacting with the interface domain that make structure more flexible and stabilize, (Peng*et al*., 2020).

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4.5.5. Nsp 15 endoribonuclease

Nsp15 in SARS-COV-2 is encoded from *orf*1ab polyprotein and shares 88% sequence identity and 95% similarity with SARS-CoV Nsp15.SARS-CoV2 Nsp15 is two monomers as asymmetric unit. The structure of SARS-CoV-2 Nsp15 monomer is very similar to other Nsp15s from coronaviruses, (Fu*et al*., 2020).

Nsp15 consists of three domain that is N-terminal domain, middle terminal and C-terminal catalytic NendoU domain with several loops. N-terminal domains consist of three β strands as a antiparallel β-sheet (strands β1, β2, and β3) wrapped, around two α-helices (α1 and α2). Middle domain consist of 12 βstrands (4 β to 13 β strands) and three short helices and finally the C-terminal catalytic NendoU domain consist of 6 βstrands as two antiparallel β-sheets with their edges is site for catalytic substance. The concave surface of the β-sheets is flanked by five α-helices.Previous structure is a subunit of monomer, but latterly this monomer arranges for forming hexamer that is important for enzymatic activity. C-terminal NendoU monomers assemble into a double-ring hexamer. The largest difference between SARS-CoV-2 and SARS-CoV seems to occur in the position of middle domains. The differences with H-CoV-229E are still more significant and show shifts in positions of α-helices, β-sheets and loops, (Kim*et al*., 2020).

5. Diagnosis of COVID-19

5.1. Clinical signs, course and prognosis

The incubation period for COVID-19 was firstly calculated to be about five days, which was based on 10 patients only (Li *et al*., 2020). An epidemiological analysis was conducted on 181 American cases, for which days of exposure and symptom onset could be appraised accurately. The study supposed a median incubation period of 5.1 days; that 97.5% became symptomatic within 11.5 days (CI 8.2 to 15.6 days) of being infected, and that extending the cohort to the 99th percentile results in nearly all cases developing signs in 14 days after exposure to SARS-CoV-2 92 (Lauer *et al*., 2020).The symptoms of COVID-19 vary amongst persons and populations even those of positive RT-PCR results; ranging from asymptomatic, mild flu-like symptoms and others showed dyspnea, severe interstitial pneumonia, ARDS and multi-organ dysfunction, (He *et al*., 2020).

The wide majority of individuals of more progress clinical patterns had one or more coexisting medical situations, such as diabetes, hypertension, and cardiovascular disorders, with elevated case fatalities amongst elderly and feeble patients, (Li *et al*., 2020, Yang *et al*., 2020).In the beginning of the disease (3-7 days of onset), it is difficult to differentiate COVID-19 from other respiratory diseases; as common signs are fever, cough (dry), fatigue, slight dyspnea, sore throat, headache and conjunctivitis. Occasionally, gastrointestinal involvement was reported with diarrhea, nausea and vomiting, (Chen *et al*., 2020, Yang *et al*., 2020).Then, with the advanced stage of the disease dyspnea develop within 5 – 13 days, continuous severity on 8 – 14 day the case develops acute respiratory distress syndrome (ARDS) in 9 day, chest pain, muscle pain, acute respiratory failure, septic shock, refractory metabolic acidosis, and formation of multi thrombi, (Huang *et al*., 2020, Zhang *et al*., 2020).

Infected children with COVID-19 are either asymptomatic or mild to moderate symptoms that appear 3 to 7 days of the onset; include fever, dry cough and fatigue, diarrhea, headache, few upper respiratory symptoms including nasal congestion and runny nose, some children showed mild pneumonia. Most of infected children recovered within 1-2 weeks of

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onset illness but few could progress badly towards lowering respiratory infections, rarely died, (Qiu *et al*., 2020, Shen *et al*., 2020).

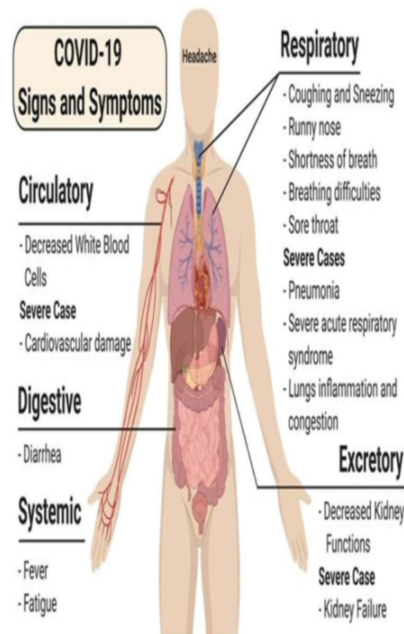

Fig. (8): Frequent symptoms of COVID-19

Moreover, there was an interesting observations; hyposmia, dysgeusia as well as loss of taste and smell senses, lead to suggest that SARS-CoV-2 could have neuro-invasive potential, but this hypothesis needs further investigations, (Desforges*et al*., 2020 Li *et al*., 2020, Sun and Guan 2020).

Interestingly, the course of the disease is asymptomatic or mild or in about 80–90% of cases, but becomes serious only in around 10% of cases, with dyspnea, hypoxemia and extensive (>50%) radiological involvement of the lung parenchyma. The bad consequence develops in around 5% of cases; a critical condition, pneumonia with respiratory failure, if shock with multi-organ failure occur, often lead to death in 2- 5%, (He *et al*., 2020, Wu and McGoogan, 2020,Xu*et al*., 2020).Also, the emergence of respiratory failure without subjective notice of dyspnea (silent hypoxemia) has also been reported and accompanied with hypocapnia induced by compensatory hyperventilation, (Xie *et al*., 2020).

The mortality rate is variable, probably due to various patient features and health conditions. It is also probable that quick saturation of intensive care facilities may have affected mortality rates, especially in high epidemic regions, (Wu and McGoogan, 2020). Until now, the mortality due to COVID-19 is appeared to be around 3%, lower than homologue other human respiratory coronaviruses; SARS-CoV (10%) and MERS-CoV (35%). The important concern is focused on the rapid and wide spread of the novel virus, so the actual mortality rate is still not determined, (Guan *et al*., 2020).Present guide points out that the prime risk factors for poor prognosis include elderly, diabetes mellitus, ischemic heart disease, hypertension, and chronic lung disease, (Zhou *et al*., 2020).

Giving the spot on pregnant women who undergo changes in immune function and physiological behavior; (such as diaphragm elevation, decreased oxygen intake and respiratory tract mucosal edema) that may be intolerant to hypoxia during pregnancy. So,

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pregnant women are under risk to respiratory microorganism and threatened pneumonia.So COVID-19 infection may be related to complications on pregnant women as, premature rupture of membranes (PROM) and preterm labor but in general the signs were mild to moderate and prognosis of the diseasehas almost gone well (Chen *et al*., 2020; Liu *et al*., 2020, and Zhu *et al*., 2020).

On the other hand, recent studies revealed that there are no proof for vertical transmission from mother to her fetus due to lack of maternal viraemia in case of SARS-COV2. The novel virus is poorly isolated from blood and plasma and not present in placenta tissue, cord blood, breast milk and amniotic fluid (Egloff*et al*., 2020 and Lang and Zhao, 2020). Whereas, pharyngeal swab obtained from neonates gave negative result via SARS-COV2 RT-PCR in most studies, but there are IgG and IgM either only or both in serum of some neonates with or without positive PCR.In case of few neonate of positive PCR,the antibodies were detected after 16 - 24hrs from onset, in some cases the neonate undergo fetal distress(Alzamora*et al*., 2020 and Zeng*et al*., 2020).

5.2.Radiological findings

Indelibly, Computed Tomography (CT) of the chest is applied that uses a special x-ray equipment to check abnormalities existed in other imaging tests. It helps in the diagnosis of unexplained cough, shortness of breath, chest pain, fever and other chest symptoms etiology. CT scanning is rapid, painless, noninvasive and precise, https://www.nibib.nih.gov/science-education/science-topics/computed-tomography-ct.

Regarding first week of COVID-19 onset patient's; the typical CT feedbacks were unilateral or multifocal ground glass opacities, especially on the peripheral and lower lung lobes, in addition to bilateral multiple lobular and sub-segmental areas of consolidation, particularly in ICU patients. More severity of the disease more number of lung segments involved and as the disease progress, the opacities tended to flow together and thicken, (Adhikari *et al*., 2020 and Cheng *et al*., 2020). The Nontypical CT findings comprise pleural effusion (only about 5%), masses, cavitation and lymphadenopathies; therefore, these would suggest alternative diagnoses, (Kanne*et al*., 2020).The CT sensitivity is variable; ranged from (86–97%) in patients with positive RT-PCR (Zhuang*et al*., 2020), and to(about 50%) in patients that show only constitutional and non-respiratory symptoms, (Kanne*et al*., 2020). It is found in conducted study that 56% of patients who displayed symptoms within 2 days had normal CT images (Bernheim*et al*., 2020).

Concerningother methods; conventional chest X-ray has low sensitivity (around 59%). On the other hand, ultrasound has a reasonable sensitivity (75%) but of a very low specificity and has been used as a diagnostic tool in a very limited number of cases. Ultrasound, and, despite being affected by factors such as disease severity, patient weight and operator skill, is estimated to be around (Bernheim*et al*., 2020,Yi *et al*., 2020).

Therewith, ultrasound may play a role in observing the progression of the disease through the detection of interstitial lung disease features, such as B lines and subpleural consolidations (Pascarella*et al*., 2020).

5.3.Laboratory findings

The most prevalent clinic- laboratory disorders recorded amongst 1099 hospitalized COVID-19 patients with pneumonia comprised lymphocytopenia (83%), thrombocytopenia (36%) and 34% had leucopenia (Guan *et al*., 2020), hypertransaminasemia and elevation in

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lactate dehydrogenase level have also been stated (Huang *et al*., 2020).The other significant observations were increased inflammation indicators; including ESR and C-reactive protein (CRP) level which may reaches 6 times in clinical sever cases and linked to increased mortality risk (Cheng *et al*., 2020, Young *et al*., 2020).Moreover, decreased calcitonin, and remarkable elevation in D-dimer, ferritin levels prothrombin time, and lactate dehydrogenasewas noticed in hospitalized patients (Ruan*et al*., 2020). In severe cases, laboratory finding show what is termed "cytokine storm" ; high elevation cytokine and chemokines that include IL2, IL7, IL10, GCSF, IP10, MCP1, MIP1A, and TNFα (Huang *et al*., 2020, Wang *et al*., 2020, Zhang *et al*., 2020). Interestingly, increased troponin was also recorded in 7% of patients who thereafter died because of fulminant myocarditis so the high troponin level is considered a very bad prognosis (Driggin*et al*., 2020).A Chinese study revealed that the laboratory finding criteria in infected children displayed an increase in Procalcitonin, c-reactive protein, d-dimer, creatine kinase, creatine kinase MB, alanine aminotransferase, aspartate transferase with decrease in lymphocyte and leuckopenia (Qiu *et al*., 2020).

5.4.Molecular diagnosis

5.4.1. Real-Time Reverse-Transcription PCR assays (RT-PCR)

It is established that RT-PCR is the reliable diagnostic test that uses various clinical samples which proved to contain the virus nucleic acid (RNA) particles; nasal swab (72%),oropharyngeal swab about (32 %) tracheal aspirate,fibro-bronchoscope brush biopsy (46%) or bronchoalveolar lavage (BAL) specimens (93%). Primarily, the collection of upper respiratory samples via nasopharyngeal and oropharyngeal swabs for bronchoscopy were preferred, but its drawback may constitutes the hazard of aerosol infection for both patients and healthcare staff. Consequently, bronchoscopy can be taken in consideration only for intubated cases when upper respiratory samples are negative and other diagnostic tools would remarkably change the clinical management. So, bronchoscopy may be denoted when clinical and safety criteria are met and in the case of uncertain diagnosis (Cheng *et al*., 2020; Iwen *et al*., 2020; Shen *et al*., 2020 and Wang *et al*., 2020).

It is found that SARS-CoV-2 RNA has been extracted from the upper respiratory tract specimens, and not only has been highly isolated in a cell culture of upper respiratory tract secretions and BAL specimens during the first 3 days after symptom onset"reasonably detectable for weeks" but also from an asymptomatic patient (Zou*et al*., 2020). Furthermore, numerous studies have demonstrated that SARS-CoV-2 RNA can also be detected in stool specimens (29 %) and blood (1 %)whileurine samples do not contain any amount of virus nucleic acid (Yong Zhang *et al*., 2020 and Zhang *et al*., 2020).

There are many RT-PCR techniques targeting different genes in 2019n-COV; (N, E, *orf*1aband RdRp genes)(Shen *et al*., 2020). The RT-PCR N gene assay was observed to be more sensitive, and take one hour and 15 min for each PCR run,(Chu, D. K., 2020). Ina performed dynamic study it is found that the first positive SARS-CoV-2 RT-PCR assay was 8 days after onset of symptoms, follow-up, the last positive result was after16 days and finally, the negative SARS-CoV-2 RT-PCR test result was 20 days from onset of symptoms (Xiao *et al*., 2020).

The RdRp gene is considered with a limit of detection (LOD) 3.6 copies ; the assay contains 2 probes , one of which reacts with SARS-CoV and SARS-CoV-2and the other one (RdRp-P2) reacts with 2019n-COV only. Usage of both or only one probe gives the same limit of detection for each virus (Corman *et al*., 2020). Moreover, there is another RT-PCR

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method that targets the RNA-dependent RNA polymerase (RdRp)/helicase (Hel) and be more sensitive and lower limit detection than the RdRp-P2 assay. In effect, the COVID-19-RdRp / Hel and COVID-19-N tests do not cross-react to SARS-CoV, other human-pathogenic coronaviruses, and respiratory viruses, in contrast to the RdRp-P2 assay (Chan *et al*., 2020). CDC recommended using RT-PCR assay contains PCR primer-probe sets for 2 regions of the viral nucleocapsid gene (N1 and N2) in the United States, this assay differs from the primary-probe sets of the World Health Organization targeting SARS – CoV-2 RNA-dependent RNA polymerase (RdRP) and envelope (E) genes, but both assays have a high analytical sensitivity and SARS – CoV-2 specificity (Cheng *et al*., 2020).

Finally, there is a RT-PCR assay intended to target SARSCoV2 nsp2; it is highly specific and sensitive compared to the COVID-19-RdRp / Hel assay, also it takes a shorter time of PCR response within an hour compared to COVID-19-RdRp / Hel assay so that the rapid results can facilitate the identification of suspected cases of COVID-19 and guide infection control and patient management (Cyril *et al*., 2020).

Regarding, the specificity and sensitivity of the RT-PCR test; the first concern seems to be very high, in spite of presence of some false-positive results due to swab contamination, especially in asymptomatic patients. The other concern; "sensitivity "rate is not clear, but is assessed to be around 66–80% (Ai *et al*., 2019).

On the other hand, RT-PCR test validity in asymptomatic persons who have been in close contact with symptomatic individuals is even less obvious; the rate of positivity could reach 50% without any indication of symptoms or assured infection (Zhuang*et al*., 2020).Eventually, a single negative test does not estranged SARS-CoV-2 infection, particularly in highly exposed individuals. Therefore, if the test is conducted using a nasopharyngeal swab specimen and at the onset of the infection, it may be recommended to repeat the test or collect a deeper respiratory tract sample, such as BAL (Pascarella*et al*., 2020).There are many established commercial kits that based on detection of previous genes; “Accula SARS-CoV-2 test” Mesa Biotech Inc. (N gene), “ID NOW COVID-19” Abbott Diagnostics kits (RdRP gene), “BioFire COVID-19 test” BioFire Defense, LLC (ORF1ab and ORF8), and others designed to detect one gene or more (Linda *et al*., 2020).

5.4.2. Real – Time Loop-Mediated Isothermal Amplification assays (RT-LAMP)

RT-LAMP method is a rapid and sensitivity method which used 4 – 6 different target sequence for recognizing 6 – 8 sequence of target gene within 1 hour. Moreover, the primers in this method consist of an outer forward primer (F3), an outer backward primer (B3), a forward inner primer (FIP) and a backward inner primer (BIP). A loop forward primer (LF) and/or a loop backward primer (LB) that were designed to accelerate the reaction that and finally result is seen by naked eye (Yan *et al*., 2020). The RT-LAMP is more preferred than RT-PCR; it takes a shorter time, does not require highly skill personal or high instrumentation plus it can be easily conducted in any site (not need certified laboratories). On other hand, its drawbacks are the implication of an internal PCR inhibition control and requiring of a complex primer model (Renfei*et al*., 2020).

5.4.3. Amplicon-Based Metagenomic Sequencing

Touching its name, this SARS-CoV-2 diagnostic technique based on a dual approach; the use of amplicon-based sequencing in accompanied to meta-genomics sequencing. Metagenomics sequencing is used mainly to address the background microbiome of infected persons, permitting the ability to rapidly identify not only SARS-CoV-2 virus but also other

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pathogens implemented in secondary infections that exaggerate the severity of COVID-19 symptoms. On the other hand, amplicon-based sequencing of SARS-CoV-2 let to perform contact tracing, molecular epidemiology, and studies of viral evolution (Uyaguari-Diaz *et al*., 2016). An Amplicon and metagenomicsMinION based sequencing were applied to rapidly (within 8 h) sequence the genome of both SARS-CoV-2 and the other microbiome present in nasopharyngeal swabs obtained from COVID-19 patients by the ISARIC 4C consortium (Moore *et al*., 2020).

5.4.4. CRISPR-Based Assays

A group of bacterial enzymes can recognize certain nucleic acid sequences found in prokaryotic organisms called Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) so called CRISPR-associated enzymes at which can be programmed to target and cut viral RNA sequences (Malina*et al*., 2013).Commercially, two companies, Mammoth Biosciences and Sherlock Biosciences, established independently gene-editing CRISPR methodology for detection of SARS-CoV-2 (Broughton *et al*., 2020 and Zhang *et al*., 2020).

Fortunately, these CRISPR-based methods do not require complex instrumentation and both low-cost and rapid (1 h), have great potential for point-of-care diagnosis (Tan, 2020). There are other molecular genomic based techniques that established for detection of previous human coronaviruses but haven't set for SARS-CoV-2 yet; Rolling circle amplification and nucleic acid hybridization using microarray (Wang *et al*., 2005, and Guo*et al*., 2014).

5.5.Serological and Immunological Assays

Despite, RT-PCR-based viral RNA detection has been broadly used in diagnosis of COVID-19, it cannot be used to check the progress of the disease phases and cannot be applied to vast identification of past infection and immunity (Linda *et al*., 2020).Serological testing is known as an assessment of body fluids for presence of immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies, wherefore shares an essential role in epidemiology and vaccine development. The body fluids specimens are primarily, the blood serum or plasma, also may comprise other biological fluids as saliva, sputum, etc... That analysis may be applied on either short-term (days to weeks) or long-term (years or permanence) tracks of antibody response, as well as antibody redundancy and diversity. IgM can point to early stage infection as it could be detectable in serum after a few days and lasts a couple of weeks upon infection, after that the immune response switch to IgG which can be an indicator of current or prior infection as well as sign for the presence of post-infection immunity (Udugama*et al*., 2020).Lately, the argument about the feasibility of the immunological assays has increased not only for the detection of antibodies themselves but also for the detection of pathogen-derived antigens via monoclonal antibodies (Maxim *et al*., 2014).

Otherwise, the immunological assays have an enormous prospect for the epidemiology of COVID-19, but tests outputs can be affected by at least three conditions: (1) a seronegative group with a positive PCR assays for SARS-CoV-2 infection; this may be attributed to the lag in antibody production following infection, (2) in contrary, a group may be seropositive yet negative for molecular assay results reflecting clearance of an earlier, milder infection, and (3) shortage in sensitivity and specificity of the assays (Hinton, 2020).

The low specificity issue is a very significant concern that the false positive results (cross reaction) may lead to deceptive antibody prevalence, consequently result in undesirable effect

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on the socioeconomic decisions and overall public trust in the results (FDA Fact Sheet 2020).The statement of SARS-CoV-2 exposure depends primarily on the detection of either IgM or IgG antibodies that are specific for different viral antigens involving, but not limited to, the spike glycoprotein (S1 and S2 subunits, receptor-binding domain) and nucleocapsid protein through approved antigen antibody reaction techniques (Hinton, 2020).

5.5.1. Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA is a microwell, plate-based assay technique designed for detecting and quantifying proteinaceous molecules. The assay can be qualitative or quantitative, and takes 1–5 h. ELISA is speedy, able to test multiple samples, but can be variable in sensitivity and is suitable for point-of-care determinations (Che*et al*., 2004).In the case of SARS-CoV-2; the plate wells are coated with a viral protein which binds to specific antibodies if present in the patient samples; this complex can be with an additional tracer antibody to produce a color or fluorescent-based readout (Linda *et al*., 2020).

5.5.2. Lateral Flow Immunoassay

This test is small, portable chromatographic fast assay; obtained in 10–30 min so it can be used at the point-of-care. It is cheap and not requires trained personnel, but provides only qualitative (positive or negative) results. The presence of the captured specific antibody–antigen complex is visualized as a colored test band (Linda *et al*., 2020).

5.5.3. Neutralization Assay

Neutralization assays depend on the ability of specific antibody to inhibit virus infection of cultured cells, so no cytopathic effects of viral replication were observed. Patient samples of whole blood, serum, or plasma are diluted and added at decreasing concentrations to the cell cultures inoculated by virus. The levels specific neutralizing antibodies (if exist), can be measured by determining the threshold at which they are able to inhibit viral replication in the infected cell cultures. Ordinarily, the assay take a long time to give its results; 3–5 days, but recent advances have minimized this to hours (Postnikova*et al*., 2019).There are limitations in performing this assay; requirement of cell culture facilities, and in the case of SARS coronavirus, availability of the Biosafety Level 3 (BSL3) laboratories. Despite these limitations, determination of neutralizing antibodies is essential in both the short term for the therapeutic implementation of recovery plasma and, in the long term, for vaccine development (Whiteman *et al*., 2020).

5.5.4. Luminescent Immunoassay

Luminescent immunoassays constitute techniques that lower the limits of detection for antibody-based reagents. Generally they comprise chemiluminescence and fluorescence. A peptide-based magnetic chemiluminescence enzyme immunoassay for diagnosis of COVID-19 has been developed (Cai*et al*., 2020).

Moreover, Diazyme Laboratories, Inc. (San Diego, California) advertised the availability of two new wholly automated serological tests for SARS-CoV-2 that are run on the fully automated Diazyme DZ-lite 3000 Plus chemiluminescence analyzer (Diazyme Laboratories, Inc. 2020).

5.5.5. Biosensor assay

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Biosensor tests are based on converting the specific interaction of biomolecules into a countable or observable readout via optical, electrical, enzymatic, and other ways. PathSensors Inc. declared a CANARY biosensor to detect the novel SARS coronavirus. This program utilizes a cell-based immunosensor that couples catch of the virus with signal amplification to give a result in 3–5 min. The biosensor is set to be available for research purposes since May 2020 (PathSensors, Inc., 2020). Complementary to molecular assays are the rapid antigen tests that permit detection of viral antigens. These tests depend on the ability of specific monoclonal antibodies to catch the viral antigens from an analytical sample, and not delimited in a particular format. They may involve a colorimetric enzyme immunoassay, an enhanced chemiluminescent immunoassay and more recently for the novel virus a fluorescence lateral flow assay for the detection of SARS-CoV-2 nucleocapsid protein (Che*et al*., 2004 and Diao*et al*., 2020).

Finally, however the serological and immunological tests have a great prospect for tracing the SARS-CoV-2 virus; most of these tests are still in the development stage (Linda *et al*., 2020).

Conclusion

A new respiratory infection has been emerged at the end 0f 2019, the disease started in China then spread rapidly and widely all over the world result in until now over 5 million patient and over 300000 deaths. A novel virus belonged to *Coronaviridae*has been confirmed as the causative agent and termed SARS-CoV-2. The primary zoonotic source of the novel virus still not defined, may bat was suggested as the main reservoir.The phylogenic studies revealed that the SARS-CoV-2 is setIn beta corona viruses clade, and is closer to SARS-like bat CoVs. The genomic structure and constitutional proteinsdisplayed some mutations and variances in comparison to other related human corona viruses.Because of the COVID-19 patient is either asymptomatic or shows flu-like signs, so become as potential source of infection. Eventhe radiological picture may not give the actual situation, so the early and precise diagnosis is needed to prevent the spread of infection.Combination of PCR and ELISA method make the detection of SARS-COV2 more sensitive and may overcome the cross-reactivity of the novel corona virus with SARS-CoV but these methods need more development in specificity and sensitivity.

Egypt vs. COVID-19

Until now, by the end days of May 2020, the situation in Egypt is still under control and not bad as happen in other European countries and USA. According to theofficial announcement of theEgyptian Ministry of Health mentioned that the number of confirmed infected individuals are 20000with cured 5100 (27%) and 800 (4%) deaths.

There are many Egyptian clinical trials, researches and projects to survey and diagnose the novel corona virus cases among the Egyptian population under supervision of Egyptian authorities and universities;

https://clinicaltrials.gov/ct2/show/NCT04336657

https://clinicaltrials.gov/ct2/show/NCT04354792

https://clinicaltrials.gov/ct2/show/NCT04374513

https://clinicaltrials.gov/show/NCT04319315

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https://clinicaltrials.gov/show/NCT04346043

https://clinicaltrials.gov/show/NCT04346056

https://clinicaltrials.gov/ct2/show/NCT04336657
https://clinicaltrials.gov/ct2/show/NCT04342637

References

Adhikari SP, Meng S, Wu YJ et al., (2020): Epidemiology, causes, clinical manifestation and diagnosis, prevention and control of coronavirus disease (COVID-19) during the early outbreak period: a scoping review. *Infect Dis Poverty,***9**: 29.

Ai T, Yang Z, Hou H et al.,(2020): Correlation of chest CT and RT-PCR testing in coronavirus disease 2019 (COVID-19) in China: a report of 1014 cases. *Radiology* 2019; 2020: 200642.

Alzamora M C, Paredes T, Caceres D, *et al*., (2020): Severe COVID-19 during Pregnancy and Possible Vertical Transmission. *American Journal of Perinatology*,s-0040-1710050. https://doi.org/10.1055/s-0040-1710050.

Annan A, Baldwin HJ, Corman VM, *et al*., (2013): Human beta-coronavirus 2c EMC/2012–related viruses in bats, Ghana and Europe. *Emerg Infect Dis*, **19** (3):456.

Benvenuto D, Giovanetti A, Ciccozzi S, *et al*., (2020): The 2019-new coronavirus epidemic: evidence for virus evolution. *J Med Virol*,

Bernheim A, Mei X, Huang M, *et al*., (2020): Chest CT findings in coronavirus disease-19 (COVID- 19): relationship to duration of infection. *Radiology*, 200463.

Bianchi M, Domenico B, Marta S, Pascarella A, *et al*., (2020): Sars-CoV-2 Envelope and Membrane Proteins: Differences from Closely Related Proteins Linked to Cross-species Transmission?Preprinted, April 2020.

Broughton J P, *et al*., (2020): CRISPR−Cas12-based detection of 779 SARS-CoV-2. *Nat. Biotechnol*. 2020.

Cai X, Chen J, Hu J, *et al*., (2020): A Peptide-based Magnetic Chemiluminescence Enzyme Immunoassay for Serological Diagnosis of Corona Virus Disease 2019 (COVID-19) medRxiv 2020.

Chan JF, Kok Z, Zhu H, *et al*., (2020): Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. *Emerg Microbes Infect*, **9** (2020): 221-236.

Chan JFW, Yip CCY, To KKW, *et al*., (2020): Improved molecular diagnosis of COVID-19 by the novel, highly sensitive and specific COVID-19-RdRp/Hel real-time reverse transcription-PCR assay validated in vitro and with clinical specimens. *Journal of Clinical Microbiology*, **58**(5).

Chang Ch, Ming-Hon H, Chi-FonCh, *et al*., (2014): The SARS coronavirus nucleocapsid protein – Forms and functions. *Antiviral Research*, **103**(3): 39-50.

This is an open access article under the terms of the Creative Commons Attribution-Non Commercial-Non Derivs License.

Che XY, Qiu LW, Pan YX, *et al*., (2004): Sensitive and specific monoclonal antibody-based capture enzyme immunoassay for detection of nucleocapsid antigen in sera from patients with severe acute respiratory syndrome. *J. Clin. Microbiol*.,**42** (6): 2629–2635.

Chen H, Guo J, Wang C, *et al*., (2020): Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: A retrospective review of medical records. *The Lancet*, **395**(10226): 809–815.

Chen N, Zhou M, Dong X, *et al*., (2020): Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *The Lancet***395**: 507– 13.

Chen Y, Liu Q, Guo D, *et al*., (2020): Emerging coronaviruses: genome structure, replication, and pathogenesis. *J Med Virol*.

Cheng MP, Papenburg J, Desjardins M, *et al*., (2020): Diagnostic testing for severe acute respiratory syndrome–related coronavirus-2: A narrative review. *Annals of internal medicine*.

Chu DK, Pan Y, Cheng SM, *et al*., (2020): Molecular diagnosis of a novel coronavirus (2019-nCoV) causing an outbreak of pneumonia. *Clinical chemistry*, **66**(4): 549-555.

Corman VM, Landt O, Kaiser M, *et al*., (2020): Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Eurosurveillance*, **25**(3): 2000045.

Cui J, Li F, and Shi ZL, (2019): Origin and evolution of pathogenic coronaviruses. *Nat. Rev. Microbiol*. 17: 181–192.

Cyril ChY, Chi-Chun H, Jasper FCh, *et al*., (2020): Development of a Novel, Genome Subtraction-Derived, SARS-CoV-2-Specific COVID-19-nsp2 Real-Time RT-PCR Assay and Its Evaluation Using Clinical Specimens. *Int J Mol Sci*. **21**(7): 2574.

De-Ming Y, Tai-Jay Ch, Mong-Lien W, *et al*., (2020): Hunting coronavirus severe acute respiratory syndrome coronavirus 2 (2019 novel coronavirus): From laboratory testing back to basic research. *J Chin Med Assoc*. **5**: 10.

DeDiego ML, *et al*., (2011): Severe acute respiratory syndrome coronavirus envelope protein regulates cell stress response and apoptosis. *PLoS pathogens,* **7**: e1002315.

Desforges M, Le Coupanec A, Dubeau P, *et al*., (2020): Human coronaviruses and other respiratory viruses: underestimated opportunistic pathogens of the central nervous system? *Viruses***12**: 14.

Diao B, Kun W, Jian C, *et al*., (2020): Diagnosis of acute respiratory syndrome coronavirus 2 infection by detection of nucleocapsid protein. *medRxiv* 2020, 2020. doi: https://doi.org/10.1101/2020.03.07.20032524

Diao B, Wen K, Chen J, *et al*., (2020): Diagnosis of Acute Respiratory Syndrome Coronavirus 2 Infection by Detection of Nucleocapsid Protein, medRxiv 2020,

Diazyme Laboratories, Inc. Announces Availability of COVID-19 Antibody Tests, News & Media. Diazyme Laboratories, Inc. March 23, 2020. www.diazyme.com/diazyme-laboratories-inc-announces-availability-of-covid-19-antibody-tests

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Dinesh DC, Chalupska D, Silhan J, *et al*., (2020): Structural basis of RNA recognition by the SARS-CoV-2 nucleocapsidphosphoprotein.

Driggin E, Madhavan MV, Bikdeli B. *et al*., (2020): Cardiovascular considerations for patients, health care workers, and health systems during the coronavirus disease 2019 (COVID-19) pandemic. *J Am CollCardiol*, **75**: 2352–71.

Egloff C, Vauloup-Fellous C, Picone O, *et al*., (2020): Evidence and possible mechanisms of rare maternal-fetal transmission of SARS-CoV-2. *Journal of Clinical Virology*, **128**: 104447. https://doi.org/10.1016/j.jcv.2020.104447

Fang Li, (2020): Structure, Function, and Evolution of Coronavirus Spike Proteins. *Annual Review of Virology,***3**:237-261.

FDA Fact Sheet(2020): Serological testing for antibodies to SARS-CoV-2 infection. U.S. Food & Drug Administration, April 17, 2020: 1–2.

Fu X, Li D, and Sun Y, (2020): orf1ab polyprotein [Severe acute respiratory syndrome coronavirus 2] GenBank: QIA20042.1

Gao Y, Yan L, Huang Y, *et al*., (2020): Structure of the RNA-dependent RNA polymerase from COVID-19 virus. *Science*

Guan W-j, Ni Z-y, Hu Y, *et al*., (2020): Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med,* 2020; **382**: 1708–20.

Guo JP, Petric M, Campbell W, *et al*., (2004): SARS corona virus peptides recognized by antibodies in the sera of convalescent cases. *Virology***324**: 251–256.

Guo X, Geng P, Wang Q, *et al*., (2014): Development of a single nucleotide polymorphism DNA microarray for the detection and genotyping of the SARS coronavirus. *J. Microbiol. Biotechnol*. **24** (10): 1445–1454.

Gupta MK, Vemula S, Donde R, *et al*., (2020): In-silico approaches to detect inhibitors of the human severe acute respiratory syndrome coronavirus envelope protein ion channel. *Journal of Biomolecular Structure and Dynamics*, (just-accepted), 1-17.

Hasöksüz M, Kiliç S, Saraç F, *et al*., (2020): Coronaviruses and SARS-COV-2.*Turk J Med Sci*. **50** (SI-1):549-556.

He F, Deng Y, Li W, (2020): Coronavirus disease 2019 (COVID-19): What we know? *J Med Virol,* 2020.

Hinton DM, (2020): Emergency Use Authorization for qSARS-CoV-2 IgG/IgM Rapid Test (Cellex Inc.). U.S. Food & Drug Administration, April 1, 2020: 1–8

Huang C, Wang Y, Li X,*et al*., (2020): Clinical features of patients infected with 2019 novel coronavirus in Wuhan. China. *The Lance* , **395**: 497–50.

Huang C, Yeming W, Xingwang L, *et al*., (2020): Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *The Lancet Journal*, **395** (10223): 497-506.

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Iwen PC, Stiles KL, Pentella MA, (2020): Safety considerations in the laboratory testing of specimens suspected or known to contain the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). *American Journal of Clinical Pathology*, **153**(5): 567-570.

Ji W, Wang X, Zhao J, *et al*., (2020): Cross-species transmission of the newly identified coronavirus 2019- nCoV. *J Med Virol*, **92** : 433-440,

Jin Z, Du X, Xu Y, *et al*., (2020): Structure of Mpro from COVID-19 virus and discovery of its inhibitors. *Nature* doi: 10.1038/s41586-020-2223-y.

Jones H, (2020): The Importance of diagnostic testing for COVID-19. Infectious Diseases Hub; April 2, 2020. www.id-hub.com/2020/04/02/the-importance-of-diagnostic-testing-for-covid-19/.

Kan B, Wang M, Jing H, *et al*., (2005): Molecular evolution analysis and geographic investigation of severe acute respiratory syndrome coronavirus-like virus in palm civets at an animal market and on farms. *J Virol*, **79**(18):11892–900.

Kang S, Yang M, Hong Z, *et al*., (2020): Crystal structure of SARS-CoV-2 nucleocapsid protein RNA binding domain reveals potential unique drug targeting sites. *ActaPharmaceuticaSinica B*.doi: 10.1016/j.apsb.2020.04.009.

Kanne JP, Little BP, Chung JH, Elicker BM, Ketai LH.*et al*., (2020): Essentials for radiologists on COVID-19: an update radiology scientific expert panel. Radiology 2020; 200527.

Kim Y, Jedrzejczak R, Maltseva NI, *et al*., (2020): Crystal structure of Nsp15 endoribonucleaseNendoU from SARS-CoV-2. *Protein Science*.

Lai CC, Shih TP, Ko WC, *et al*., (2020): Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and corona virus disease-2019 (COVID19): the epidemic and the challenges. *Int J Antimicrob Agents* 2020:105924.

Lang G, Zhao H, *et al*., (2020): Can SARS-CoV-2-infected women breastfeed after viral clearance? *Journal of Zhejiang University-SCIENCE B*, **21**(5): 405–407.

Lau SK, Li KS, Tsang AK, *et al*., (2013): Genetic characterization of Betacoronavirus lineage C viruses in bats reveals marked sequence divergence in the spike protein of pipistrellus bat coronavirus HKU5 in Japanese pipistrelle: implications for the origin of the novel Middle East respiratory syndrome coronavirus. *J Virol*, **87**(15):8638–50.

Lauer SA, Grantz KH, Bi Q, *et al*., (2020): The Incubation Period of Coronavirus Disease 2019 (COVID-19) From Publicly Reported Confirmed Cases: Estimation and Application. *Annals of internal medicine*. doi:10.7326/M20-0504.

Li B, Si H-R, Zhu Y, *et al*., (2020): Discovery of Bat Coronaviruses through Surveillance and Probe Capture-Based Next Generation Sequencing. *mSphere*, doi:10.1128/mSphere.00807-19

Li B, Yang J, Zhao F, *et al*., (2020): Prevalence and impact of cardiovascular metabolic diseases on COVID-19 in China. *Clin Res Cardiol*, **109**: 531– 38.

Li F, (2016): Structure, Function, and Evolution of Coronavirus Spike Proteins. *Annu. Rev. Virol*. **3**: 237–261.

This is an open access article under the terms of the Creative Commons Attribution-Non Commercial-Non Derivs License.

Li Q, Guan P, Wu X, *et al.*, (2020): Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. *N Engl J Med* (2020), doi:10.1056/NEJMoa2001316

Li YC, Bai WZ, Hashikawa T,(2020): The neuroinvasive potential of SARS-CoV2 may play a role in the respiratory failure of COVID-19 patients. *J Med Virol.*,**92**: 552– 5.

Lin Z, Gao Q, Qian F, *et al.*, (2020): The nucleocapsid protein of SARS-CoV-2 abolished pluripotency in human induced pluripotent stem cells.*bioRxiv*doi.org/10.1101/2020.03.26.010694

Linda JC, Linda VG, Jeffrey WS, *et al.*, (2020): Cynthia Liu Assay Techniques and Test Development for COVID-19 Diagnosis. *ACS Cent Sci*, 27; **6**(5): 591–605.

Littler D, Gully B, Colson RN, *et al.*, (2020): Crystal structure of the SARS-CoV-2 non-structural protein 9, Nsp9. *bioRxiv*.

Liu Y, Chen H, Tang K, *et al.*, (2020): Clinical manifestations and outcome of SARS-CoV-2 infection during pregnancy. *Journal of Infection*, S0163445320301092. https://doi.org/10.1016/j.jinf.2020.02.028

Lu R, Zhao X, Li J, *et al.*, (2020): Genomic characterization and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet*, **395**(10224):565–574.

Mahase E, (2020): Covid-19: WHO declares pandemic because of “alarming levels” of spread, severity, and inaction. *BMJ* 2020; **368**: m1036.

Malika YS , Shubhankar S , Sudipta B, *et al.*, (2020): Emerging novel coronavirus (2019-nCoV)—current scenario, evolutionary perspective based on genome analysis and recent developments. *Veterinary Quarterly***40**: (1), 68–76.

Malina A, John RM, Regina C, *et al.*, (2013): Repurposing CRISPR/Cas9 for in situ functional assays. *Genes Dev*. **27**(23): 2602–2614.

Max Perutz Labs, (2020): COVID-19 Q&A's - Max Perutz Labs.

Maxim DL, Niebo R, Utell MJ,*et al.*,(2014): Screening tests: a review with examples. *Inhalation Toxicol.* **26** (13), 811–828.

Meier C, Aricescu AR, Assenberg R, *et al.*, (2006): The crystal structure of ORF-9b, a lipid binding protein from the SARS coronavirus. *Structure*, **14**(7):1157-65.

Millet JK and Whittaker GR, (2015): Host cell proteases: Critical determinants of coronavirus tropism and pathogenesis. *Virus Res*.**16** (202): 120–134.

Mizumoto K, Kagaya K, Zarebski A, *et al.*, (2020): Estimating the asymptomatic proportion of coronavirus disease 2019 (COVID-19) cases on board the Diamond Princess cruise ship, Yokohama, Japan, 2020. *Euro Surveill* 2020: **25** (10).

Moore SC, Penrice-Randal R, Alruwaili M, *et al.*, (2020): Amplicon based MinION sequencing of 820 SARS-CoV-2 and metagenomicscharacterisation of nasopharyngeal 821 swabs from patients with COVID-19. *medRxiv* 2020.

This is an open access article under the terms of the Creative Commons Attribution-Non Commercial-Non Derivs License.

Muhammad AS, Suliman K, Abeer K, *et al*., (2020): COVID-19 infection: Origin, transmission, and characteristics of human coronaviruses. *J Adv Res*.**24**: 91–98.

National Center for Biotechnology Information, https://www.ncbi.nlm.nih.gov/

National Institute of Biomedical Imaging and Bioengineering (NIBIB) https://www.nibib.nih.gov/science-education/science-topics/computed-tomography-ct

National Institute of Biomedical Imaging and Bioengineering, https://www.nibib.nih.gov/science-education/science-topics/computed-tomography-ct

Navratil V, Lionnard L, Longhi S, *et al*., (2020): The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) envelope (E) protein harbors a conserved BH3-like sequence. *bioRxiv*.

Neuman BW, Kiss G, Kunding AH, *et al*., (2011): Structural analysis of M protein in coronavirus assembly and morphology. Journal of Structural Biology. **174**:11–22.

Paden C, Yusof M, Al Hammadi Z, *et al*., (2018): Zoonotic origin and transmission of Middle East respiratory syndrome coronavirus in the UAE. *Zoonoses Public Health*, **65**(3):322–33.

Paraskevis D, Kostaki EG, Magiorkinis G, *et al*., (2020): Full-genome evolutionary analysis of the novel corona virus (2019-nCoV) rejects the hypothesis of emergence as a result of a recent recombination event. *Infect Genet Evol***79**:104212.

Pascarella G, Strumia A, Piliego C, *et al*., (2020): COVID-19 diagnosis and management: a comprehensive review.*J Intern Med*. 2020 Apr 29. doi.org/10.1111/joim.13091

PathSensors, Inc. Announced the Development of a SARS-CoV-2 Biosensor. PathSensorsInc, PathSensors News and Press, March 24, 2020. www.pathsensors.com/psi-sars-cov-2-biosensor/

Peng Q, Peng R, Yuan B, *et al*., (2020): Structural and biochemical characterization of nsp12-nsp7-nsp8 core polymerase complex from COVID-19 virus. *bioRxiv.*

Postnikova EN, Pettitt J, Van Ryn CJ, *et al*., (2019): Scalable, semi-automated fluorescence reduction neutralization assay for qualitative assessment of Ebola virus-neutralizing antibodies in human clinical samples. *PLoS One* **14** (8):e0221407.

Qiu H, Wu J, Hong L, *et al*., (2020): Clinical and epidemiological features of 36 children with coronavirus disease 2019 (COVID-19) in Zhejiang, China: an observational cohort study. *The Lancet Infectious Diseases*.doi:10.1016/S1473-3099(20)30198-5.

Renfei L, Xiuming W, Zhenzhou W, *et al*., (2020): Development of a Novel Reverse Transcription Loop-Mediated Isothermal Amplification Method for Rapid Detection of SARS-CoV-2. *Virol Sin*. 2020 Apr: **1**: 1–4.

Renhong Y, Yuanyuan Z, Yaning L, *et al*., (2020): Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. *Science*. 27; **367**(6485): 1444–1448.

Robert NK and Ward AB, (2019): Structure of the SARS-CoV nsp12 polymerase bound to nsp7 and nsp8 co-factors .*Nat Commun***10**: 2342.

This is an open access article under the terms of the Creative Commons Attribution-Non Commercial-Non Derivs License.

Rota PA, Oberste MS, Monroe SS, *et al*., (2003): Characterization of a novel coronavirus associated with severe acute respiratory syndrome. *Science*.**300** (5624):1394-9.

Ruan Q, Yang K, Wang W, *et al*., (2020): Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. *Intensive Care Med* **46**(5):846-848.

Saahir K, Rie N, Aarti J, *et al*., (2020):Analysis of serologic cross-reactivity between common human coronaviruses and SARS-CoV-2 using coronavirus antigen microarray. *bioRxiv* 2020,

Schoeman D, and Fielding BC, (2019): Coronavirus envelope protein: current knowledge. *Virology journal*, **16**(1): 69.

Shankar AK, Bhanu D, Alluri A, *et al*., (2020): Whole Genome Sequence Analysis and Homology Modelling of Main Protease and Non-Structural Protein 3 of the SARS-CoV-2 reveals an Aza-Peptide and a Lead Inhibitor with Possible Antiviral Properties. *New Journal of Chemistry*.doi.org/10.1039/D0NJ00974A.

Shannon A, Le NTT, Selisko B, *et al*., (2020): Remdesivir and SARS-CoV-2: Structural requirements at both nsp12 RdRp and nsp14 Exonuclease active-sites. *Antiviral Research*, 104793.

Shen K, Yang Y, Wang T, *et al*., (2020): Diagnosis, treatment, and prevention of 2019 novel coronavirus infection in children: experts' consensus statement. *World journal of pediatrics*, 1-9.

Shi Z, and Hu Z,(2008): A review of studies on animal reservoirs of the SARS coronavirus. *Virus Res*, **133** (1):74–87.

Song Z, Xu Y, Bao L, *et al*., (2019): From SARS to MERS, Thrusting Coronaviruses into the Spotlight. *Viruses*, **11**: E59.

SunTand Guan J,(2020):Novel coronavirus and central nervous system. *Eur J Neurol*. org/10.1111/ene.14227.

Tan R,(2020): COVID-19 Diagnostics Explained.Asian Scientist; April 8, 2020.

Udugama B, Kadhiresan P, Kozlowski HN, *et al*., (2020): Diagnosing COVID-19: The Disease and Tools for Detection. *ACS Nano*, **14**: 3822.

Uyaguari-Diaz MI, Michael Ch, Bonnie L, *et al*., (2016): A comprehensive method for ampliconbased and metagenomic characterization of viruses, bacteria, and eukaryotes in freshwater samples. *Microbiome***4**:20.

Virus Pathogen Database and Analysis Resource, http://www.viprbrc.org/

Wang C, Horby PW, Hayden FG, *et al*., (2020): A novel coronavirus outbreak of global health concern. *The Lancet***395** (10223), DOI: 10.1016/S0140-6736(20)30185-9

Wang D, Hu B, Hu C, *et al*., (2020): Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected pneumonia in Wuhan, China. *JAMA*, **323**(11): 1061-1069.

This is an open access article under the terms of the Creative Commons Attribution-Non Commercial-Non Derivs License.

Wang W, Xu Y, Gao R, *et al*., (2020): Detection of SARS-CoV-2 in different types of clinical specimens. *JAMA*.**323**(18):1843-1844.

Wang WK, Fang CT, Chen HL, *et al*., (2005): Detection of severe acute respiratory syndrome coronavirus RNA in plasma during the course of infection. *J. Clin. Microbiol*. 43 (2), 962–965.

Whiteman MC, Antonello JM, Bogardus LA, *et al*., (2020): A virus neutralization assay utilizing imaging cytometry. WO 2020/036811, World Intellectual Property Organization, 20 February 2020.

World Health Organization, (2020): Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases: interim guidance, 2 March 2020, 2020.

World Health Organization,(2020):Novel coronavirus situation dashboard. Geneva, Switzerland: WHO, 2020.

Wu A, Peng Y, Huang B, *et al*., (2020): Genome composition and divergence of the novel coronavirus (2019-nCoV) originating in China. *Cell Host Microbe* 2020.

Wu D, Wu T, Liu Q, *et al*., (2020): The SARS-CoV-2 outbreak: what we know. *Int J Infect Dis*, **94**: 44–8.

Wu Z, and McGoogan JM. (2020): Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese center for disease control and prevention. *JAMA***323**: 1239– 42.

X Xu, P Chen, J Wang, *et al*., (2020): Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. *Sci China Life Sci*, **63**(3):457-460.

Xiao AT, Tong YX, Gao C, *et al*., (2020): Dynamic profile of RT-PCR findings from 301 COVID-19 patients in Wuhan, China: a descriptive study. *Journal of Clinical Virology*, 104346.

Xie J, Tong Z, Guan X, *et al*., (2020): Critical care crisis and some recommendations during the COVID-19 epidemic in China. *Intens Care Med*, **46**: 579– 82.

Xu Z, Shi L, Wang Y *et al*., (2020): Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancet Resp Med*, **8**: 420– 2.

Yan C, Cui J, Huang L, *et al*., (2020): Rapid and visual detection of 2019 novel coronavirus (SARS-CoV-2) by a reverse transcription loop-mediated isothermal amplification assay. *Clinical Microbiology and Infection*.**26**: 773-779.

Yang J, Zheng Y, Gou X, *et al*., (2020): Prevalence of comorbidities in the novel Wuhan coronavirus (COVID-19) infection: a systematic review and meta-analysis. *International journal of infectious diseases*.IJID **94**: 91– 5.

Yang X, Yu Y, Xu J, *et al*., (2020): Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. *Lancet Resp Med,***8**: 475– 81.

This is an open access article under the terms of the Creative Commons Attribution-Non Commercial-Non Derivs License.

Yi HS, Liu Y, Zhang Y, *et al*., (2020): A preliminary study on the ultrasonic manifestations of peripulmonary lesions of non-critical novel coronavirus pneumonia (COVID-19). *Research square*.

Yong Zhang CC, Zhu S, Shu C, *et al*., (2020): Isolation of 2019-nCoV from a stool specimen of a laboratory-confirmed case of the coronavirus disease 2019 (COVID-19). China CDC Weekly, **2**: 123–4.

Young BE, Ong SWX, Kalimuddin S, *et al*., (2020): Epidemiologic features and clinical course of patients infected with SARS-CoV-2.In Singapore. JAMA 323: 1488.

Zeng H, Xu C, Fan J, *et al*., (2020): Antibodies in Infants Born to Mothers With COVID-19 Pneumonia. *JAMA*. doi.org/10.1001/jama.2020.4861

Zhang F, Abudayyeh OO, Gootenberg JS, *et al*., (2020): A protocol for 775 detection of COVID-19 using CRISPR diagnostics (v.20200321). 776 Broad Institute of MIT and Harvard. www.broadinstitute.org/files/ 777 publications/special/COVID-19

Zhang JJ, Dong X, Cao YY, *et al*., (2020): Clinical characteristics of 140 patients infected with SARS-CoV-2 in Wuhan, China. *Allergy*.doi: 10.1111/all.14238.

Zhang L, Lin D, Sun X, *et al*., (2020): Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α-ketoamide inhibitors. *Science*, **368** (6489): 409-412.

Zhang W, Du R-H, Li B, *et al*., (2020): Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes. *EmergMicrob Infect* **9**: 386–9.

Zheng BJ, Guan Y, Wong KH, *et al*., (2004): SARS-related virus predating SARS outbreak, Hong Kong. *Emerg Infect Dis***10** (2):176.

Zhou F, Yu T, Du R, *et al*., (2020): Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *The Lancet* . **395**: 1054– 62.

Zhou P, Yang XL, Wang XG, *et al*., (2020): Discovery of a novel coronavirus associated with the recent pneumonia outbreak in humans and its potential bat origin. *bioRxiv*.

Zhu H, Wang L, Fang C, *et al*., (2020): Clinical analysis of 10 neonates born to mothers with 2019-nCoV pneumonia. *Translational Pediatrics*, **9**(1): 51–60. https://doi.org/10.21037/tp.2020.02.06

Zhu N, Zhang D, Wang X, *et al*., (2020): A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med*, **382** (2020): 727-733.

Zhuang GH, Shen MW, Zeng LX,*et al*., (2020): Potential false-positive rate among the 'asymptomatic infected individuals' in close contacts of COVID-19 patients. *Zhonghua Liu Xing Bing XueZaZhi* **41**: 485–8.

Zou L, Ruan F, Huang M, *et al*., (2020): SARS-CoV-2 viral load in upper respiratory specimens of infected patients. *N Engl J Med* **382**: 1177–9.

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