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Original Research

# Association of ABO and Rh blood groups, Demographics and Susceptibility to SARS-CoV-2 Pandemic and its Complication

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**Abstract:**

Coronaviruses (CoVs) are members of the family Coronaviridae, the enveloped viruses that possess extraordinarily large single-stranded RNA genomes ranging from 26 to 32 kilobases in length. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a strain of coronavirus that causes COVID-19 (coronavirus disease 2019), the respiratory illness responsible for the ongoing COVID-19 pandemic.The virus previously had a provisional name, 2019 novel coronavirus (2019-nCoV), SARS-CoV-2, the viruses emerged in December 2019 in Wuhan of China and a great effort is being undertaken to contain its spreading. To find the association of the SARS-CoV-2 with blood type and Rh 105 smples were collected from different places in Thiqar governorate. The samples were collected from patients at different ages and genders. The period of study was from October 2021 to May 2022. The frequency distribution of blood type among 105 SARS-CoV-2 infected individuals represented the observed a high frequency of of blood group O+ (n=44, 41.9%) followed by A+ (n= 26, 24.8%) , B+(n=23 , 21.9% ) ,AB+(n= 9, 8.6% ), O- (n=2 ,1.9 % ) ,B -(n=1, 1.0% ) respectively , and no patients were A-, No association between blood group B- and risk of SARS-CoV-2 infection was found. Result indicated that 10 (23.26%) of type A , 4(13.95%) of type B , 6(9.30%) of type AB and 23(53.49%) of type O on ICU . While there was 16 (25.81%) of type A , 20 (4.84%) of type B ,3 (32,26%) of type AB and 23 (37.10%) of type O that weren't need for ICU care . Also there were 42(97.67%) positive rhesus and 1(2.33%) negative rhesus needed for ICU care .while 60 (96.77%) positive rhesus and 2 (3.23%) negative rhesus weren't need for ICU care. although, SARS-CoV-2 was significantly more seen with the blood group O. Concluded that SARS-CoV-2 complication increase in obese patients and elder patients and may led to death. SARS-CoV-2 was significantly more seen with the blood group O. There is no clear association between SARS-CoV-2 and blood group, however we found that O+ and A+ most susceptible to the infection. Patient with blood type A are most frequent admission to the intensive care unit.

Keywords: SARS-CoV-2, COVID-19, Rh blood groups

**Introduction:**

Coronaviruses (CoVs) are a positive-sense single-stranded RNA virus that cause diseases in humans and animals. The human coronaviruses (HCoVs) were first identified as causes of acute upper respiratory infection (URI) in 1962. Over the past few years, HCoVs have more often been found to be associated with severe upper and lower respiratory tract infection (RTI). They have been identified as a main cause of pneumonia in older adults and immuno compromised patients . Over the last two decades, two highly pathogenic human coronaviruses were identified including coronaviruses associated with severe acute respiratory syndrome (SARS-CoV-2) and the Middle East respiratory syndrome (MERS-CoV) which emerged in different regions of the world . On 31st 56 December, 2019, a new strain of coronavirus was isolated and named as severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) by the International Committee on Taxonomy of Viruses (ICTV) from patients with pneumonia of unknown etiology in Wuhan city, China. On 11 59 th March, 2020, the World Health Organisation (WHO) announced that COVID-19 is a "public-health emergency of international concern" . This genome size increase enhances genomic plasticity, thus permitting alteration via mutations and recombination, resulting in higher genetic diversity and higher chances of cross-species transmission ***.(1)***

***Causes***

**Virology and pathogenesis:**

Coronaviruses are enveloped single-stranded RNA viruses that are zoonotic in nature and cause symptoms ranging from those similar to the common cold to more severe respiratory, enteric, hepatic, and neurological symptoms.Other than SARS-CoV-2, there are six known coronaviruses in humans: HCoV-229E, HCoV-OC43, SARS-CoV, HCoV-NL63, HCoV-HKU1, and MERS-CoV. Coronavirus has caused two large-scale pandemics in the last two decades: SARS and MERS.(3)

To detect the infection source of COVID-19, China CDC researchers collected 585 environmental samples from the Huanan Seafood Market in Wuhan, Hubei Province, China on 1 January and 12 January 2020. They detected 33 samples containing SARS-CoV-2 and indicated that it originated from wild animals sold in the market . Then, researchers used the lung fluid, blood, and throat swab samples of 15 patients to conduct laboratory tests.(4)

**Symptoms:**

CoVID-19 can occur in three possible states: mild, moderate, and severe. Patients under 60 years of age and without relevant comorbidities can present the disease in a mild or moderate state, while patients with significant comorbidities may present a moderate state. Moderate cases also present symptoms of pneumonia[60]. Adult patients with a severe COVID-19 state manifest tachypnea (³30 breaths•min-1), £ 93% oxygen saturation at rest, the PAFI index defined as the ratio of partial arterial pressure of oxygen (PaO2)/inspired oxygen fraction (FiO2)-1 £ 300 mm Hg (if the patient is found 1000 m above sea-level, the formula must be corrected).

The clinical manifestations of patients with COVID-19 include fever, fatigue, dry cough, shortness of breath, and acute respiratory distress syndrome. White blood cell count is usually normal or low. There may be lymphopenia; a lymphocyte count < 1000 has been associated with a more severe condition; the platelet count is usually normal or slightly low. C-reactive protein and erythrocyte sedimentation rate are generally elevated, but procalcitonin levels are usually normal. A high level of procalcitonin can indicate a bacterial coinfection. (5)

**Viral Transmission:**

At present, the exact mechanism of transmission of SARS-CoV-2 is still not completely understood. Human-to-human transmission via droplets is the main route of transmission within a susceptible population. Chinese health authorities reported an R0 of 1.4–2.5 on January 23, 2020, to the WHO International Health Regulations (2005)

Emergency Committee. The scientific literature showed that SARS-CoV and MERS-CoV are viable in environmental conditions that facilitate oral-fecal transmission. SARS-CoV has been detected in sewage water of two Chinese hospitals in which patients with SARS were treated, and MERS-CoV was found to be viable on different surfaces at low temperature and low humidity. SARS-CoV-2 was detected in stool of patients with COVID-19 pneumonia, as well as in respiratory samples. Thus, it is plausible that also SARS-CoV-2 can be transmitted via the oral-fecal route as well as via fomites***.(6)***

**Complictions Of Coronavirus:**

1. Respiratory system involvement
2. Cardiovascular involvement
3. Kidney involvement
4. Hematologic involvement
5. Coagulopathy
6. Electrolyte imbalance
7. Liver involvement
8. Endocrine involvement

**The Aim of Research:**

The world is heavily suffering from the COVID-19 pandemic for more than a year, with over 191 million confirmed cases and more than 4.1 million deaths to date. Previous studies have explored several risk factors for coronavirus disease 2019 (COVID-19), but there is still a lack of association with ABO blood type. This study aimed to find out the relationship between the ABO blood group and COVID-19 outcomes in Iraq/ thiqar

**Literature review:**

**Covid-19 Outbreak :**

In late December 2019, a previous unidentified coronavirus, currently named as the 2019 novel coronavirus, emerged from Wuhan, China, and resulted in a formidable outbreak in many cities in China and expanded globally, including Thailand, Republic of Korea, Japan, United States, Philippines, Viet Nam, and our country (as of 2/6/2020 at least 25 countries). The disease is officially named as Coronavirus Disease-2019 (COVID-19, by WHO on February 11, 2020). It is also named as Severe Pneumonia with Novel Pathogens on January 15, 2019 by the Taiwan CDC.COVID-19 is a potential zoonotic disease with low to moderate (estimated 2%–5%) mortality rate. Person-to-person transmission may occur through droplet or contact transmission and if there is a lack of stringent infection control or if no proper personal protective equipment available, it may jeopardize the first-line healthcare workers**.(8)**

Bats are the zoonotic reservoir of CoV and several viruses. It has been noted that bats usually harbor viruses and demonstrates no clinical symptoms. More than millions of years, bats and viruses have been co-existing and co-evolving. **(9)**

The intermediate host is an animal that plays a significant role in the transmission of the virus from natural hosts to others. The intermediate hosts may be domestic animals and these animals themselves might suffer diseases caused by bat-borne or closely related CoV. Swine acute diarrhea syndrome coronavirus was transmitted from bats to pigs(10).

**SARS COV- 2 in Iraq:**

The Corona Virus of 2020 was spread in Iraq starting from February 24, 2020 in the city of Najaf, when a sample of an Iranian religious student was examined and the result was positive for its injury to the Corona virus associated with severe respiratory syndrome. Then other cases were revealed with Covid-19, and the total confirmed cases in Iraq were 2,131,500 cases, including 24,267 deaths, and the number of recovered people reached 2,071,838 until January 20, 2022. ***(12)***

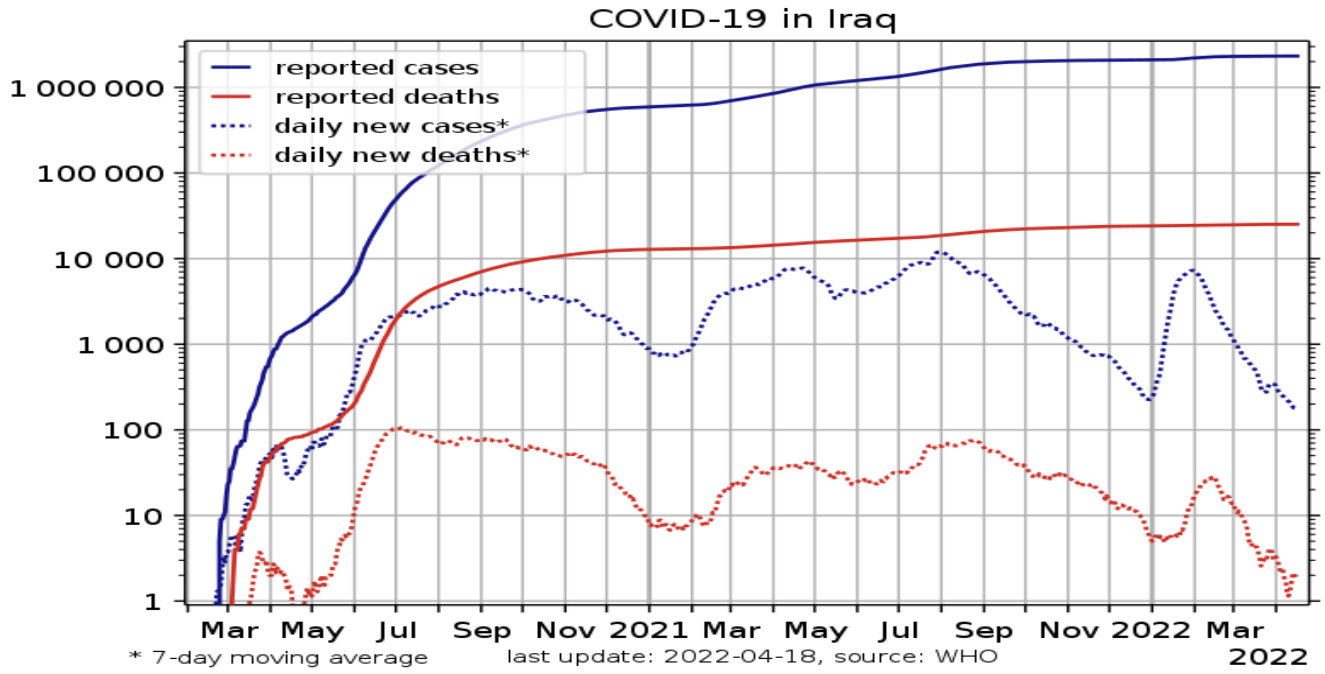


**Figure (2): Covid-19 virus statistics (12)**

## Classification of Covid-19

Coronaviruses (CoVs) are spherical and approximately 125 nm in diameter, with club-shape spikes projecting from the surface of the virus giving the appearance of a solar corona, prompting the name, coronaviruses Within the envelope is the helically symmetrical. nucleocapsids, which is actually uncommon among positive-sense RNA viruses. CoVs are classified under the order Nidovirales, family Coronaviridae, and subfamily Orthocoronavirinae (Fig.3). With genome sizes ranging from (26 to 32) kilobases (kb) in length, CoVs have the largest genome for RNA viruses. Based on genetic and antigenic criteria, CoVs have been organized into four groups: alphacoronavirus (α-CoV), .**(13)**

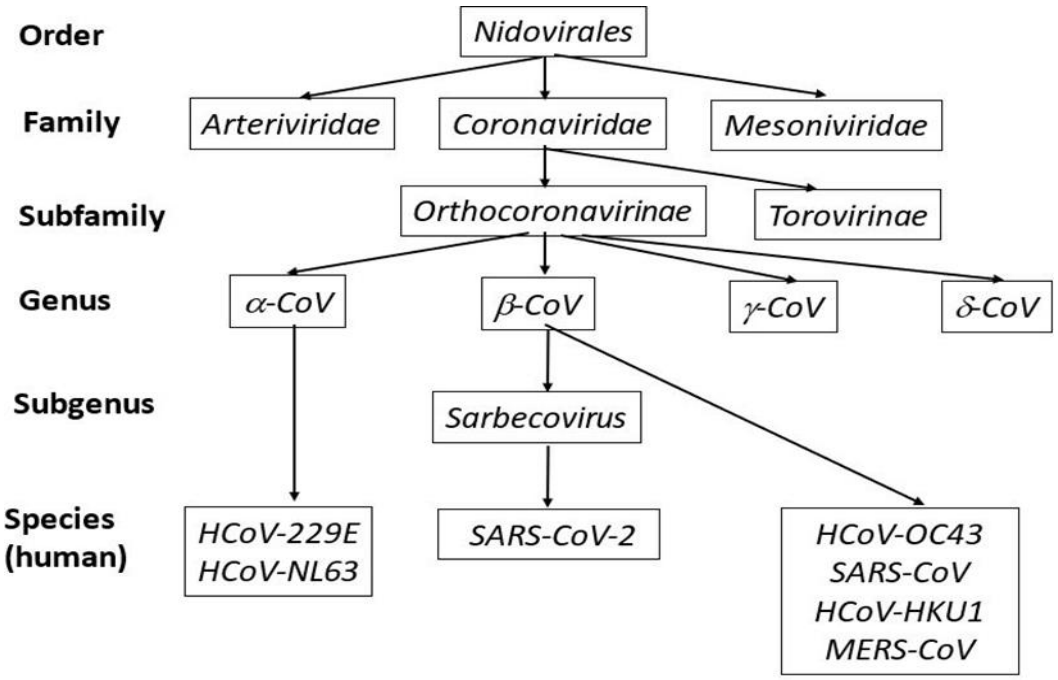


**Figure3: Classification of Human Coronaviruses *(13)***

All members of the Coronaviridae family share the following characteristics:

**1-Virions**: enveloped and decorated with large (15–20nm) surface projections

**2-Nucleocapsid**: helical, comprised of genome and multiple copies of a single basic phosphoprotein species (N)

**3-Envelope**: contains a variable number of viral membrane protein species, two of which seem to be conserved family-wide and are essential for virion morphogenesis and/or infectivity (at least in coronaviruses):

- a 200- to 250-aa triple-spanning integral membrane protein M

- an extensively N-glycosylated, 1100- to 1600-aa class I fusion protein S which forms peplomers.

**4- Genome:** positive sense RNA, linear, unimolecular, infectious, 26–32kb in length, capped, poly-adenylated and structurally polycistronic. ***(14)***

## 1.2.4 Genomic Structure of Covid-19

Coronaviruses are known to have a large genome, with sizes rang-ing from 26 to 32 kilobases***.(15)*** Corona, a Latin word for crown ,depicts the spike-like projections on its surface. They consist of the following structural proteins:

**(1) Trimeric spike (S) protein:**
The S1 protein recognizes ACE2receptors found in the lungs, heart, kidneys, intestines, esophagus, liver, and blood vessels, and is attached to the host cell membrane. The host cell proteases (serine 2, cathepsins, trypsin, and furin) cause cleavage of the spike protein, resulting in the fusion of the virus inside the host cell, and this is mediated by S2 protein of the virus***.(16)***

**(2) Envelope (E) protein:**
The envelope proteins are the smallest and they are mainly present in the ER and the Golgi Apparatus, where they are responsible for the assembly and release of virus from the host cell. Hence, they are well expressed during viral replication.

**(3) Membrane (M) protein:**
The membrane glycoproteins are surface proteins and they are the most abundant proteins of the virus. Their structure is comprised of the N-terminal domain on the outside of the virus, three trans membrane domains ,and the C-terminal domain found inside the viral membrane***.(17)*** It helps in the formation and gives shape to the virus envelope, and it also controls the assembly of various components of the virus***.(18)***

**(4) Nucleocapsid (N) protein:**
The nucleocapsid protein is bound to the ssRNA of the virus. Its function is to breakdown the defense mechanism and deregulate the cell cycle of the host cell and to assist in the assembly of the virus by interacting with other structural proteins. It packages the viral genome into capsids to protect it***.(19)***

**(5) Hemagglutinin-esterase (HE) protein:**
Hemagglutininesterase, a glycoprotein, helps in the attachment and destructionof sialic acid receptors to the host cell surface.

**Genomic structure of SARS-CoV-2 and its shape:**

The SARS-CoV-2 genome is similar to that of typical CoVs and contains at least ten open reading frames (ORFs). The 5'-terminal two-thirds of the genome ORF1a/b encodes two large polyproteins, which form the viral replicase transcriptase complex. The other ORFs of SARS-CoV-2 on the one-third of the genome encode the same four main structural proteins: spike (S), envelope (E), nucleocapsid (N) and membrane (M)proteins, as well as several (fig 4) accessory proteins with unknown functions which do not participate in viral replication***.(20)***

**Mechanism of Entry and Replication Inside the Human Cell:**

The virus enters the body through the nose, eyes, or mouth. The spike protein binds specifically to the ACE2 receptors present on the type 2 pneumocytes in the alveoli in the lungs, just like the SARS-CoV1. The type 2 pneumocytes produce surfactants that reduce the collapsing pressure and also decrease the surface

tension in alveoli. (***22)***

The binding of the ACE2 receptor allows the entry of the virus into the host cell due to host cell proteases that cleave the spike protein of the virus. The virus enters the host cell either by direct cell entry by membrane fusion or by endocytosis***.(23)***

Unlike a typical flu virus that travels to the nucleus once inside the host cell, the SARS-CoV-2 releases its positive-sense RNA into the host cell cytoplasm. This RNA is translated into polyproteins, pp1a and pp1ab. These help in the replication and transcription of the viral RNA. The replication of positive-sense RNA using RNA-dependent RNA polymerase enzyme gives a negative-sense RNA. The negative-sense RNA is either replicated to give positive-sense RNAs (incorporated in the viral genome) or transcribed. The transcribed mRNAs can be translated to produce viral proteins, like the spike, membrane, envelope, and nucleocapsid proteins. ***(24)***

**Covid-19 pathogenesis:**

COVID-19, caused by the SARS-CoV2 virus, is a potentially fatal disease that represents a major global public health concern. The SARS- CoV2 virus infects the lower respiratory tract and causes pneumonia in humans, with symptoms that appear milder than SARS or MERS infection, but ultimately becomes a lethal disease of hyper inflammation and respiratory dysfunction ***(26)***

bySARS-CoV2 infection and disease can be approximately divided into three phases:

I.an asymptomatic phase with or without detectable virus.

II. a non-severe symptomatic phase with upper airway involvement.

III. a severe, potentially lethal disease with hypoxia, 'ground glass' infiltrates in the lung, and progression to acute respiratory distress syndrome (ARDS) with high viral load***(27)***

The coronavirus genome encodes four major proteins: spike (S), nucleocapsid (N), membrane (M), and envelope (E). The S protein is responsible for viral entry into target ACEII expressing cells of the body. Approximately 75 percent of the SARS-CoV2 genome is identical to the SARS-CoV genome, and the amino acid residues required for receptor binding are the same between these two viruses; both viruses use the angiotensin converting enzyme 2 (ACE-2) receptor to infect airway epithelial cells and endothelial cells. ***[28].***

ARDS is the main cause of death in COVID-19 disease, and appears to cause similar immune pathogenic features in SARS-CoV and MERS-CoV infections .One of the main features of ARDS is the cytokine storm - an uncontrolled systemic inflammatory response resulting from the release of pro-inflammatory cytokines and chemokine's by immune effector cells High blood levels of cytokines and chemokines have been detected in patients with COVID-19 infection, including: IL1-β, IL1RA, IL7, IL8, IL9, IL10, basic FGF2, GCSF, GMCSF, IFNγ, IP10, MCP1, MIP1α, MIP1β, PDGFB, TNFα, and VEGFA [6]. The ensuing cytokine storm triggers a violent inflammatory immune response that contributes to ARDS, multiple organ failure, and finally death in severe cases of SARS-CoV-2 infection, similar to SARS-CoV and MERS-CoV infections .Patients infected with COVID-19 showed higher leukocyte numbers, abnormal respiratory findings, and increased levels of plasma pro-inflammatory cytokines (Fig.7) The direct cause of death from acute COVID-19 involves cytokine storm damage to lungs and multiple organs of the body: heart, kidney and liver, leading to multiple organexhaustion ***(29)***

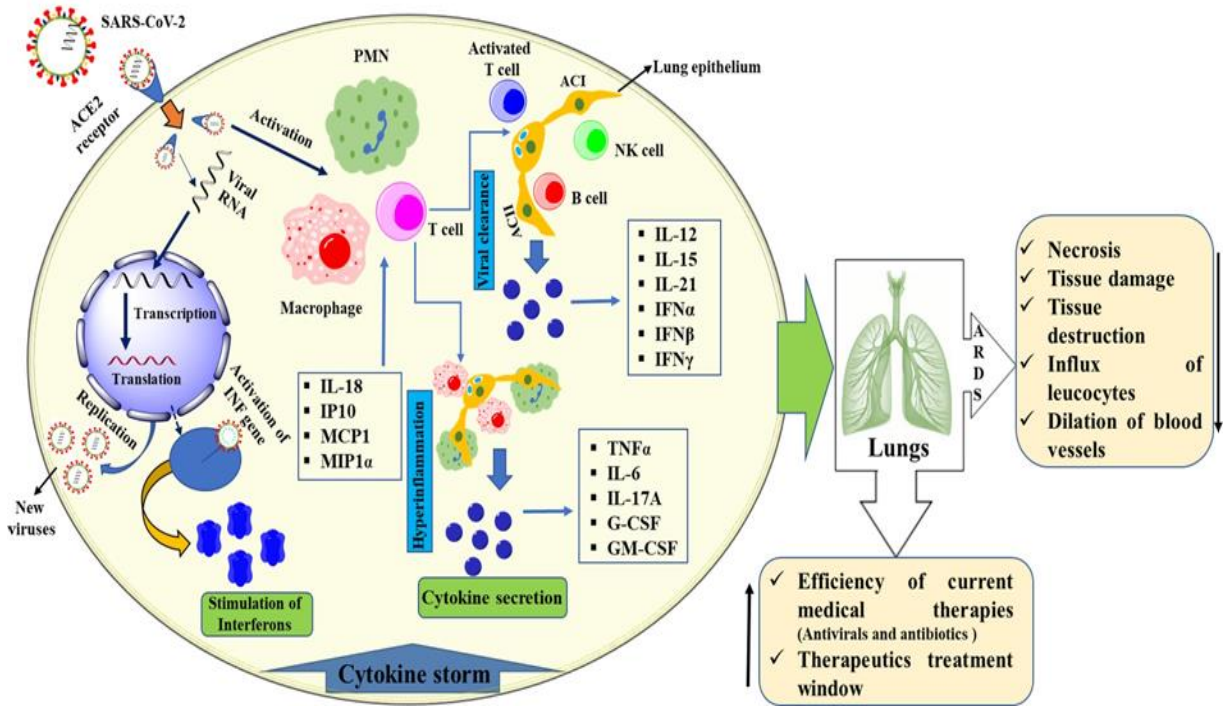


**Figure.5. Schematic representation of COVID-19 pathogenesis and cytokine storm with possible effects. SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; ACE2: angiotensin-converting enzyme 2; PMN: polymorphonuclear granulocyte; AC: alveolar cell; NK: natural killer). *(31)***

***Covid-19 Relationship with - Rh-blood group***

The ABO blood group system mainly involves the A and B antigens and their corresponding antibodies. The antigen-encoding gene is located on chromosome 9q34.1–34.2. It consists of the A, B, and O alleles, and there are a total of 4 genetic phenotypes (A, B, O, and AB blood types) ***[32].*** Differences in blood group antigen expression can increase or decrease host susceptibility to many infections. Blood group antigens can play a direct role in infection by serving as receptors and/ or co receptors for microorganisms, parasites, and viruses. In addition, many blood group antigens facilitate intracellular uptake, signal transduction, or cell adhesion through the organization of membrane micro domains. Blood group antigens can modify the innate immune response to infection ***[33].***

Numerous studies have been published to date on the relationship between blood groups and disease- These studies include Hepatitis B, Hepatitis C, HIV, West Nile Virus, SARS CoV and SARS CoV-2 viruses***. (34)***

In all these studies raised an issue that some blood groups may be susceptible to viral infections and some groups may be protective. Although many models of this predisposition or protectionism have been established, the mechanism has not been fully elucidated and has been suggested as possible causes. Natural antibodies of the ABO system to block the interaction of SARS CoV spike protein and angiotensin converting enzyme 2 may be considered as one of the reasons suggested***(35).***

Some studies show that the ABO blood group antigen improves the overall inflammatory response. Single nucleotide polymorphisms at the ABO locus have been found to increase levels of two important serum inflammation markers, TNF- and soluble intercellular adhesion molecule-1 (ICAM-1), and the increase of TNF- causes inflammation***. (36)***

***Symptom of Covid-19 Infection:***

The complete clinical manifestation is not clear yet, as the reported symptoms range from

mild to severe, with some cases resulting in death. ***[37]***

have shown that the most common symptoms are fever (in 88.7% of patients), cough (67.8%),

fatigue (38.1%), sputum production (33.7%), shortness of breath (18.7%), myalgia or arthralgia (14.9%), or sore throat (13.9%). In addition, symptoms from the nervous and digestive systems should also be considered. ***[38]***

also indicate headache (53.3%) as an important symptom of the disease, as well as nausea, vomiting, or diarrhea (30%). Figure (8) shows both typical and less frequent symptoms of COVID-19. In many, the infection remains asymptomatic. ***[39]***

implied that patients with mild symptoms were reported to recover after 1 week while severe cases experienced progressive respiratory failure due to alveolar damage from the immunological response to the virus, which may lead to death. Severely affected patients are referred to intensive care units because of acute pneumonia, sepsis, septic shock,

or acute respiratory distress syndrome. The Incubation period for COVID-19 lasts( 2–14 )

days, but current studies indicate that it can be as long as (21 )days ***[40]*** .

**Techniques that detect covid-19:**

COVID-19 clinical diagnosis is based primarily on epidemiological history, clinical symptoms, and confirmation of various laboratory tests, including CT screening, NAAT, and serology. The coronavirus disease spreads rapidly by droplet particles generated by an infected person's coughing and sneezing. Asymptomatic carriers complicated the diagnosis based on COVID-19 manifestations. As a result, appropriate diagnostic methods are needed for people to mitigate and counteract rapid viral transmission. Since SARS CoV-2 is an RNA- based virus, it can be identified using any current RNA detection format. The viral genome must be reverse transcribed into a DNA complement by reverse transcriptase. After the SARS COV-2 genome was sequenced entirely, the primary diagnostic technique, RT-PCR, was developed.

**1-Chest Computed Tomography (CT) Scanning:**

A chest computed tomography (CT) scan is an excellent tool for identifying viral pneumonia, and it has previously been used to identify lung abnormalities in SARS and MERS situations. Chest computed tomography (CT) is initially used as a key investigative instrument for COVID-19 detection in mainland China. The chest CT results of COVID-19-infected patients indicate patchy penetration that proceeds to ground-glass opacities .CT of the chest has been shown to be more vulnerable than X-rays ***(44).***

**2- Nucleic acid amplification tests (NAAT):**

NAAT is the most sensitive and frequently used test for detecting early viral infections. Several NAAT assays, including reverse transcriptase real-time PCR (RT-PCR), reverse transcription loop-mediated isothermal amplification (RT-LAMP), microarray, and high throughput sequencing, have been established for the fast and precise diagnosis of COVID-19 ***(44).***

**3- Reverse transcriptase real-time polymerase chain reaction (RT-PCR):**

Probe-based RT PCR assays, the gold standard tool for detecting SARS COV-2, have been used to target various genes in the SARS COV-2 genome, including S, E, N, RdRp, ORF1b, and ORF8. The E gene is targeted for SARS COV-2 screening, as recommended by WHO, accompanied by a confirmatory test targeting the rdrp gene. In comparison, the nucleocapsid protein genes N1 and N2 are endorsed by the CDC .

**4- Reverse transcription loop-mediated isothermal amplification (RT-LAMP):**

Under isothermal conditions, loop-mediated isothermal amplification (LAMP), a PCR- based nucleic acid amplification process, will efficiently and quickly amplify a target series. The technique employs four to six distinct primers that recognize four to six regions on the target genome and DNA polymerase, which elongates the chain at a constant temperature through strand displacement. This method can be reinforced in a regular water bath, and the enhanced product can be colored fluorescently for visualization***. (46)***

**5-Antigen detection system:**

An antigen is a compound that stimulates the immune system and induces antibody formation to destroy pathogens. Unlike PCR-based techniques, antigen studies explicitly recognize viral components without the need for thermal amplification . However, antigen tests, including PCR-based methods, show the status of virus infection, not the state of recovery. As opposed to nucleic acid checking, the assay had a sensitivity of 68% and an accuracy of 100%. ***(47)***

**6-Antibody detection system:**

An antibody is a protein that is generated by the immune system in reaction to an antigen. Antibodies bind to just one kind of antigen in order to kill it from the body. Antibodies are classified based on their C-terminus areas. The specificity of an antibody is defined by Complementary Determining Regions (CDRs) on its N-terminus. The C-terminus of antibodies identifies five types: IgM, IgD, IgG, IgA, and IgE .IgM is the primary antibody generated during SARS-COV-2 infection, while IgG is the usual prevalent and rich antibody in serum. An antibody examination will detect the involvement and frequency of IgG and IgM in blood/serum/plasma samples to determine if the body is fighting the SARS-COV-2 pathogen. Antibody research is usually conducted using lateral flow assays (LFA) and enzyme-linked immunosorbent assays (ELISA) ***(48)***

**7-Lateral flow type assays (LFA):**

The molecules of interest are found in the patient's blood serum through a port in lateral flow style assays (LFA). The material is collected on the sample pad, and then capillary action is used to transfer it across the strip. When they come into contact with the first hand, antibodies labeled with gold nanoparticles adhere to the target molecule in the sample. Depending on if the test is for IgG- or IgM-class antibodies or both, the view window may reveal one, two, or three stripes. If the test is negative with just one kind of antibody, the control line will only display one stripe. ***(49)***

**8-Enzyme-Linked Immunosorbent Assay (ELISA):**

Goal molecules for an Enzyme-Linked Immunosorbent Assay (ELISA), recombinant viral antigen, are covered onto the exteriors of plastic wells. After being processed, the sample, which is the patient's blood, is added to the wells. When antibodies such as (IgG or IgM) against the target antigen are detected in a sample, this is referred to as a binding event. Washing removes the excess, and an enzyme-dependent color change reaction (usually horseradish peroxidase) reinforces target antibody binding. ***(50).***

**Chapter two:**

**Materials & Methods:**

**Material**

| **Material** | **Company** | **Origin** |
|---|---|---|
| 1-PCR | BIO-RAD | American (USA) |
| 2-Extraction device | BIOCOMMA | Spain |
| 3-Microcenterfuge | BECKMAN COLTER | Germany |
| 4-Mix | BIOBASE | China |
| 5-Safety cabinet Hood | BIOBASE | China |
| 6-Autoclave | RITTER | Germany |
| 7-Pipette | DRAGON LAB | China |
| 8-Micropipette | SUPERTEK | China |
| 9-Multipipette | SUPERTEK | China |
| 10-Disposable pipette Tips | PakGent | China |
| 11- Blue Tips | PakGent | China |
| 12-Kit PCR | MACCURA | China |
| 13-Kit PCR | Lifotronic | Austria |
| 14- Kit Extraction | Lifotronic | Austria |

**Reagents and lsits:**

**maccura kit:**

| Component | Ingredient | 32tests | 64tests | 96tests |
|---|---|---|---|---|
| qRT-PCR reaction mix | Primer,probe,dntp, mg+2,buffer | 544μLx1 | 1800 μLx1 | 1632 μLx1 |
| qRT-PCR enzyme mix | Taq polymerase,uracil-dna glycosylase | 96 μLx1 | 192 μLx1 | 288 μLx1 |
| Negative control | Depc-treated water | 450 μLx1 | 900 μLx1 | 1350 μLx1 |
| Positive control | Armored RNA containing target gene fragment | 450 μLx1 | 900 μLx1 | 1350 μLx1 |
| Lnternle control | Armored RNA containing internal control gene fragment | 64 μLx1 | 128 μLx1 | 192 μLx1 |

**Lifotronic kit:**

| Components | 48 Rxns/kit | 96 Rxns/kit |
|---|---|---|
| SARS-CoV-2 Reaction solution | 880 μLx1tube | 880 μLx1tube |
| SARS-CoV-2 Enzyme mixture | 85 μLx1tube | 170 μLx1tube |
| SARS-CoV-2 Positive control | 100 μLx1tube | 100 μLx1tube |
| SARS-CoV-2 Negative control | 100 μLx1tube | 100 μLx1tube |

**Blood group kit:**

**Method to Test Blood Group:**

**Aim:**

The basic purpose of conducting this experiment is to understand the basic concept of the different ABO blood group system and with the help of this procedure get to know our blood group and type.

**Materials Required:**

- Toothpicks
- Blood sample
- Alcohol Swabs
- Lancet
- Clean glass slide
- Sterile cotton balls
- Biohazard disposal container
- Monoclonal Antibodies (Anti-A, B, and D)

All these equipment will be readily available in a blood test tool kit.

**Procedure:**

- First, take a glass side and mark three circles on it after cleaning the slide.
- Unpack the Monoclonal Antibodies (MAB) kit. Now with the help of a dropper, add the Anti-A, Anti-B and Anti-D in the first, second and third circle respectively in a sequential order.
- Keep the slide aside safely without disturbing.
- Now you need to wipe the ring finger with the alcohol swabs and rub gently near the fingertip, where the blood sample will be collected.
- You need to prick the ring fingertip with the lancet and wipe off the first drop of the blood.

• As blood starts flowing out, allow it to fall on the three circles of the glass slide by gently pressing the fingertip.

• We must apply pressure on the pricked part in order to stop the blood flow.Use the cotton ball if required.

• Mix the blood sample gently with the help of a toothpick and wait for a minute to observe the result.

**Maccura ki**

**Principle**

This product is designed as a multiplex real –time reverse –transcription pcr system, containing specific

Primers and fluorescent probes targeting ORF1ab,E and N gene of SARS-cov-2. Virus nucluc acid load is

Detected by monitoring fluorescence intensity of ORF1ab,E and N gene in real-time. In addition , the

Internal control is added to monitor the presence of pcr inhibitor within specimen , thus effectively

prevent fulse negative results .

Reagent preparation (in reagent preparation area)

- Take out and equilibrate all reagent components of this kit and a nucleic acid extraction or purification kit ambient temperature, vortex briefly
- Prepare qrt-pcr mix according to the following the table:

| Name | Reagent | Volume/tests | Number of tests |
|---|---|---|---|
| Qrt-pcr Mix | Qrt-pcr reaction mix | 17μL | N=n+2 |
| | Qrt-pcr enzyme mix | 3μL | |

Note The number of tests in N=n+2, where n is the number of samples to be tested, and 2 indicare negative control and positive control. Based on laboratory conditions, due consideration shall be given to the losses in the muture dispensing process

- Transfer the prepared reagents to the specimen preparation area.
- Sample preparation (in the sample preparation area)
- Nucleic acid extraction:

Add 2 u Lof internal Control per tast into Negative Control, Positive Control and specimen for nucleic acid extraction. Please refer to operation manual of supporting nucleic acid extraction or purification reagerits for detailed procedure.

Recommended volume of extraction and elution:

| Extraction reagent | vaume of sample | Volume of elution buffer |
|---|---|---|
| Maccura Mag-bind DeNA Extraction Kit | 200μL | 35 μL |
| QIAamp viral rna mini kit | 140 μL | 80 μL |

- Add specimens:

Add 20ML RNA template (nucleic acid extracted from Negative Control,Positive Control or specimen respectively) to the PCR reaction tube/plate containing qRT-PCR Mix, Final volume should be 40 u L/test. Close the lid or seal the plate immediately to avoid contamination, Spin down briefly and get ready to perform PCR reaction

- PCR amplification (in the thermal-cycling area)
- For setting, please refer to the operation manual of different instrument. An example setting of ABI Prism7500 is shown as below.
- Plate set-up: Place the PCR reaction tube/plate into the instrument, set Negative Control, Positive Control and test specimen in sequence.

- Fluorescence channel setting:

Delection channel FAM (Reporter: FAM, Quencher: None); ROX (Reporter: ROX, Quencher: None); Cy5 (Reporter Cy5, Quencher: None : Internal control channel: HEX or VIC (Reporter: HEX/VIC, Quenchor: None).

- Reaction volume: 40μL
- Set the cycling protocol:

| Step | | temperature | time | cycles |
|---|---|---|---|---|
| 1 | Revers transcription | 55 | 15 | 1 |
| 2 | Taq polymerase activation,pre-denaturing | 95 | 2 | 1 |
| 3 | Denaturation | 95 | 15 | 45 |
| | Annealing,extension,fluorescence acquisition | 58 | 35 | |
| 4 | Instrument cooling | 40 | 10 | 1 |

**Lifotronic kit:**

Precaution: All the reagents should be thawed completely before use, then vertex and spin down at 6,000rpm.

1.Sample treatment (Sample treatment zone) Extract RNA with viral RNA extraction kit. RNA extracted can be stored at -70°C or lower for 6 months. Positive control and negative control can be used without extraction.

2.Preparation of reaction solution (Mix preparation zone) Take N (N=negative control number + RNA sample number + positive control number) PCR tubes, add 18.3 μL reaction solution and 1.7 μL enzyme mixture to each tube.

| Components | Volume per test |
|---|---|
| SARS-CoV-2 reaction solution | 18.3 μL |
| SARS-CoV-2 enzyme mixture | 1.7 μL |

Centrifuge the PCR tubes at 6,000rpm for 30s and transport to sample treatment area.

3.Sample loading (Sample treatment area)

Add 20 μL RNA sample, negative control and positive control to the above PCR tubes respectively. Cap the tubes tightly and centrifuge at 6,000rpm for 10s and then transport to PCR amplification area.

4.PCR amplification (PCR amplification zone).

4.1.Put the caped PCR tubes into real-time PCR machine for amplification.

4.2.Thermal cycling setting:

| Steps | Temperature | Duration | Cycle |
|---|---|---|---|
| 1 | 50C | 10min | 1 |
| 2 | 95C | 3min | 1 |
| 3 | 95C | 10s | 40 |
| | 60C | 1min | |

Collect fluorescent signals at step 3: 60°C, 1min.. Fluorescent Channel: FAM for ORF 1ab gene, VIC for N gene, CY5 for internal control gene. The total volume: 40μL. NOTE: for ABI7500, VIIATM 7, Quant Studio 7 flex, choose 'none' as both passive reference and quencher.

4.3.Disposal after detection

Dispose the PCR tubes in a sealed bag after reaction and treat the used tubes as medical wastes.

5.Settings for result analysis Set the baseline at a region before the exponential amplification where

the fluorescent signals of all the samples are relatively stable (no significant fluctuations in all the samples); set the starting point (cycle number) away from the signal fluctuations at the starting phase of fluorescence collection; set the end point (cycle number) 1-3 cycles before the Ct of the first sample 4-15 cycles to enter exponential amplification. recommended. Set the threshold right above the highest point of the negative control amplification curve (irregular noise curve). are

6.Quality control Prior to evaluating the specimen results, the Positive control and Negative control should be interpreted using the Positive control and interpretation table below, and the Negative control curve must be performed correctly, otherwise the sample result is invalid.

| Channels Controls | Cycle threshold (Ct) value | | |
|---|---|---|---|
| | FAM | VIC | CY5 |
| Negative control | Ct = 40 or UNDET | Ct = 40 or UNDET | Ct = 40 or UNDET |
| Positive control | Ct ≤ 32 | Ct ≤ 32 | Ct ≤ 32 |

**Results and discussion:**

The clinical characteristics and outcomes of 105 patients included in the study according to their blood groups are presented in Table 1. The mean age of patients was (34.02) and that result agree with similar studies (51,52)

However the result of Greene et al.(53) disagree with the result of current study .

The BMI mean was (26.34 ) [54] .

**Table 1: Demographic characteristic of patients with covid19**

| | N | Mean | Std. Deviation | Std. Error Mean |
|---|---|---|---|---|
| Age | 105 | 34.02 | 12.609 | 1.230 |
| BMI | 105 | 26.34 | 5.39 | 0.526 |
| FAM | 105 | 23.35 | 4.459 | 0.435 |
| VIC | 104 | 23.37 | 4.479 | 0.439 |

As shown in (table 2 )a total of 105 COVID-19 infected individuals represented the observed a high frequency of of blood group O+ (n=44, 41.9%) followed by A+ (n= 26, 24.8%) , B+(n=23 , 21.9% ) ,AB+(n= 9, 8.6% ), O- (n=2 ,1.9 % ) ,B - (n=1, 1.0% ) respectively , and no patients were A - . also other studies [55,56] shows that O blood group is the highest frequency , while others [57,58] show Blood type O had the lowest frequency of disease positivity .

Disagree with other studies [59-61] the blood group A was more frequent in patients with severe COVID-19 infections compared to this study.

Logistic regression analysis showed that blood group B- was associated with a decreased risk of infection. [62]

while blood group O+ was associated with an increased risk [55,56].

No association between blood group B- and risk of COVID-19 infection was found .

**Table 2: frequency distribution of blood group**

| | | Frequency | Percent | Cumulative Percent |
|---|---|---|---|---|
| Valid | A+ | 26 | 24.8 | 24.8 |
| | AB+ | 9 | 8.6 | 33.3 |
| | B- | 1 | 1.0 | 34.3 |
| | B+ | 23 | 21.9 | 56.2 |
| | O- | 2 | 1.9 | 58.1 |
| | O+ | 44 | 41.9 | 100.0 |
| | Total | 105 | 100.0 | |

**Figure 1: Frequence distribution of blood types among SARS-CoV-2**

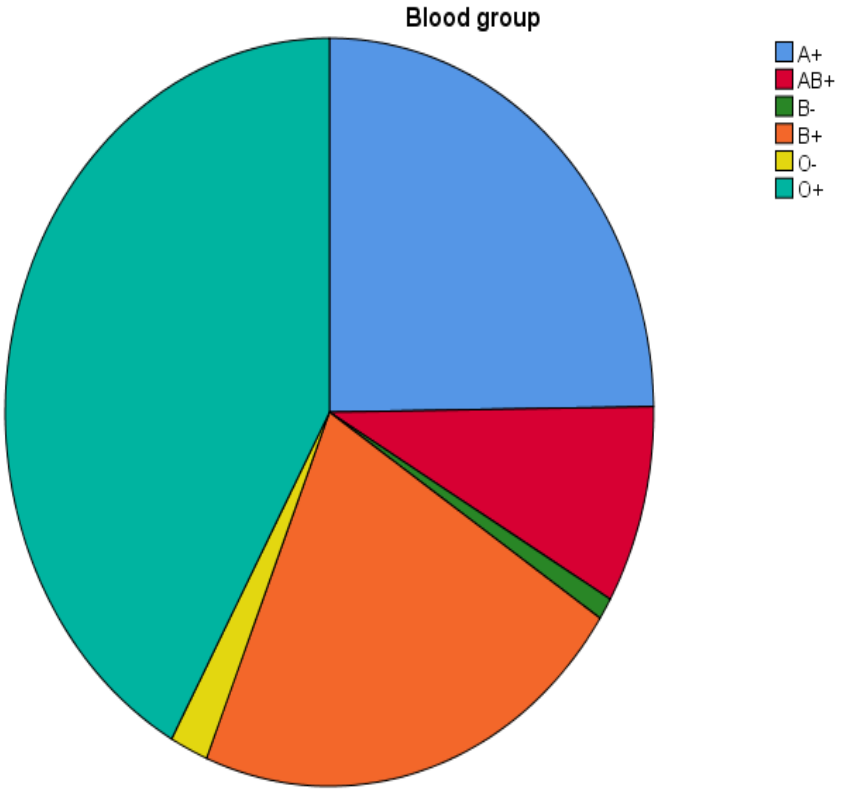


Table 4 show that 10 (23.26%) of type A , 4 (13.95%) of type B , 6 (9.30%) of type AB and 23 (53.49%) of type O on ICU . Kabrah et.al(60) found that O blood group was the highest blood type need for ICU care too .

While there was 16 (25.81%) of type A , 20 (4.84%) of type B ,3 (32,26%) of type AB and 23 (37.10%) of type O that weren't need for ICU care .

Also shown in (table 4 ), 42 (97.67%) positive rhesus and 1 (2.33%) negative rhesus needed for ICU care .while 60 (96.77%) positive rhesus and 2 (3.23%) negative rhesus weren't need for ICU care , there was no statistical significance [58,59] .

Though, COVID-19 was significantly more seen with the blood group O [55,56] . On the other hand Rh factor did not make any significant difference [63,58] .

**Table 4 : blood group and rhesus distribution among ICU care and without ICU care patients.**

| Variables | ICU care | Without ICU | X2 | P value |
|---|---|---|---|---|
| A | 10<br>23.26% | 16<br>25.81% | 9.939 | 0.0191 |
| B | 4<br>13.95% | 20<br>4.84% | | |
| AB | 6<br>9.30% | 3<br>32.26% | | |
| O | 23<br>53.49% | 23<br>37.10% | | |
| Positive | 42<br>97.67% | 60<br>96.77% | 0.074 | 0.2723 |
| Negative | 1<br>2.33% | 2<br>3.23% | | |

The purpose of this study is to evaluate the association between blood group ABO and CoVID-19 among the new cases , in (table 5 ) The data shows that the mean for Age, BMI, FAM and VIC of ICU care is 39.21 ± 15.24, 28.63 ± 5.926, 19.23 ± 2.671 and 19.44 ± 3.195 respectively and without ICU care it is 30.68 ± 8.691, 24.75 ± 4.383 , 26.21 ± 2.954 and 26.21 ± 2.954 respectively .

Results showed that there was a significant difference of Age (p=0.0004), BMI (p=0.0002), FAM (p<0.0001), VIC (p<0.0001) between patients with ICU care and without ICU care. The interaction tests showed that age, BMI, FAM and VIC have significant interaction with ABO when compared.

We demonstrate a big association between high BMI and raised risk of the composite outcome death in patients with COVID-19 . people with a high BMI had a doubled risk of death or prolonged intensive care. A high BMI was additionally related to death during intensive care.

Previous studies, each experimental and studies have systematically found higher status and severity of the COVID-19 pandemic course in people with obesity. A United Kingdom study as well as nearly seven million individuals all over multiplied risk of hospitalization and death because of COVID-19 in individuals with obesity [64] . Another study that enclosed over seventeen million adults ascertained increased risk of COVID-19 connected death with increasing obesity [65 ].

we also found that the severity of the disease increased with advanced ages [64].

Table 5 : the differences of anthropometric parameters and SARS-CoV-2 markers between ICU care patients and patients without ICU care.

| Variables | ICU care (mean±SD) | Without ICU care (mean±SD) | P-VALUE |
|---|---|---|---|
| Age | 39.21 ± 15.24 | 30.68 ± 8.691 | 0.0004 |
| BMI | 28.63 ± 5.926 | 24.75 ± 4.383 | 0.0002 |
| FAM | 19.23 ± 2.671 | 26.21 ± 2.954 | <0.0001 |
| VIC | 19.44 ± 3.195 | 26.21 ± 2.954 | <0.0001 |

**Conclusion and Recommendation:**

**Conclusion:**

1- SARS- CoV-2 complication increase in obese patients and elder patients and may led to death.

2- SARS-CoV-2 was significantly more seen with the blood group O

3- There is no clear association between SARS-CoV-2 and blood group, however we found that O+ and A+ most susceptible to the infection.

4- Patient with blood type A are most frequent admission to the intensive care unit.

**Recommendation:**

1- Total commitment to the destruction of WHO to prevent the distribution of virus and get vaccination.

2- Conduct studies on the incidence of SARS-Cov2 and its relation to the type of blood and genes that susceptible for virus infection.

3- Increase the size of population of study.

4- Find modern ways to detect, prevent virus infection and it's complication.

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