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Comparison of Haematological parameters among COVID-19 patients and a group of Healthy Control

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بِسْمِ اللهِ الرَّحْمنِ الرَّحِيمِ

﴿ وَأَيُّوبَ إِذْ نَادَى رَبَّهُ أَنِّي مَسَّنِيَ الضُّرُّ وَأَنْتَ أَرْحَمُ الرَّاحِمِينَ * فَاسْتَجَبْنَا لَهُ فَكَشَفْنَا مَا بِهِ مِنْ ضُرٍّ وَآتَيْنَاهُ أَهْلَهُ وَمِثْلَهُمْ مَعَهُمْ رَحْمَةً مِنْ عِنْدِنَا وَذِكْرَى لِلْعَابِدِينَ ﴾

صدق الله العظيم

سورة الأنبياء: الآية (83 – 84)

الاهداء

الى... الينبوع الذي لايمل العطاء

الى...مــن حاكت سعادتي بخيوط منسوجه مــن قلبها (والدتي العزيزة)

الى... مــن سعى وشقى لأنعم بالراحة والهناء الذي لم يبخل بشيء مــن اجل دفعي في طريق النجاح الذي علمني ان ارتقي سلم الحياة بحكمه وصبر (والدي العزيز)

الى..مــن حبهم يجري في عروقي ويلهج بذكرهم فُــؤآدي(اخواتي الغاليات)

الى... شريك حياتي ورفيق دربي (زوجي الغالي)

اهدي هذا العمل المتواضع راجيه مــن الله عز وجل اني يجد القبول والنجاح

صهاب & غدير

شكر وتقدير

الحمد لله الذي هدانا لهذا وما كنَّا لنهتدي لولا أن هدانا الله، والصلاة والسلام على سيدنا محمَّد سيد الأنام، وعلى آله وصحبه الكرام.

يطيب لنا ونحن نضع لمساتنا الأخيرة على كتابة هذا البحث أن نتقدَّم بخالص شكرنا إلى أستاذتنا الدكتورة أنوار علي الحسيني ، متمنين لها دوام الصحة والعافية، لاقتراحها موضوع البحث، ودعمها المتواصل، وتوجيهاتها في سبيل إنجاز هذا البحث، داعين من الله لها بالخير والصحة، ولها منا كل الحب والتقدير والاحترام، وجزاها الله عنِّا كل خير.

كما نتقدم بالشكر والعرفان إلى رئاسة جامعة بابل، وعمادة كلية العلوم، وقسم علوم الحياة، لإتاحتهم الفرصة لنا في إكمال مشوارنا العلمي.

ومن الله التوفيق

Abstract

A cross-sectional study was conducted on Seventy-six (56 male and 20 female) unvaccinated Iraqi patients infected with COVID-19 and control group of 51 (20 male and 31 female) healthy individuals. Significantly more males were represented among the COVID-19 patients than females. No significant differences were found between different age groups of the study subjects among cases and controls. Measurements of haematological parameters suggested elevated numbers in WBC among the patient group except lymphocytes that was insignificantly lower in patient group than in control. Results of the study were consistent with published reports on the predictive value of haematological measurements in the prognosis of COVID-19 infection.

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Chapter One

Introduction and Literature Review

1.Introduction and Literature Review

1.1 Introduction

Coronaviruses, belonging to the Coronaviridae family, cause respiratory infection in mammals, such as bats, camels and masked palm civets, and in avian species (De Wit *et al*.,2016; Gong and Bao,2018). Symptoms and tissue tropism of coronavirus infection can vary across different host species (Fehr,2015). In humans, coronavirus infections may be asymptomatic or accompanied by fever, cough, shortness of breath and gastrointestinal irritation (Chen *et al*., 2020). In certain cases, particularly in elderly and immunocompromised individuals, coronavirus infections may lead to severe pneumonia and subsequently, the death of the patient (Jartti *et al*., 2011).

The World Health Organization WHO, (2020) later renamed the disease caused by SARS-CoV-2 as Coronavirus Disease-2019 (COVID-19).

The disease was rapidly sweeping through the whole country and has spread to the other more than 150 countries and territories around the world. On March 13, 2020, the World Health Organization (WHO) declared the novel coronavirus outbreak to be a pandemic (WHO,2020), resulting in lockdown and life restrictions in worldwide in the attempt to prevent and slow the spread of the virus.

1.2 Evolution of the Coronavirus

Coronaviruses were named after the Latin word corona, meaning crown or halo, owing to their crown-like spikes on the surface as seen when viewed under an electron microscope (Pyrc *et al*., 2007). Coronaviruses are enveloped viruses containing a non-segmented, single stranded, positive-sense RNA genome of approximately 32 kilobases,

thus making it the largest known genome for an RNA virus (Zhang *et al*., 2020). Coronaviruses belong in the subfamily coronavirinae of the coronaviridae family, in the order of nidovirales. The Coronavirinae subfamily consists of four genera: alphacoronavirus, beta coronavirus, delta coronavirus and the gamma coronavirus, with the SARS-CoV-2 strain being classified under the beta coronavirus genus based on the genome sequence analysis (Zhu and Zhang,2019). The coronavirus genome is known to have a 50 cap and a 30 poly (A) tail; therefore, upon infecting the host cell, the genome acts as an mRNA for translation of the replicase polyproteins required for viral replication (Sawicki *et al*., 2007). Coronaviruses have been reported to predominantly reside in an animal reservoir, such as bats, mice, rats, chickens, dogs, cats, horses, and camels (Van der Hoek *et al*., 2004). Recently, the virus has developed the ability to initiate an epidemic by adapting to humans via zoonotic transmission, similar to the previous Zika virus outbreak in 2015 (Sharma and Lal, 2017). Bats have been reported to be the primary carrier and reservoir for a vast range of viruses, including the coronavirus, thus making the animal–human species barrier cross highly probable due to the large number of bats that congregate within the community and their ability to travel long distances (Kahn and McIntosh, 2005).

1.3 Classification and Origin

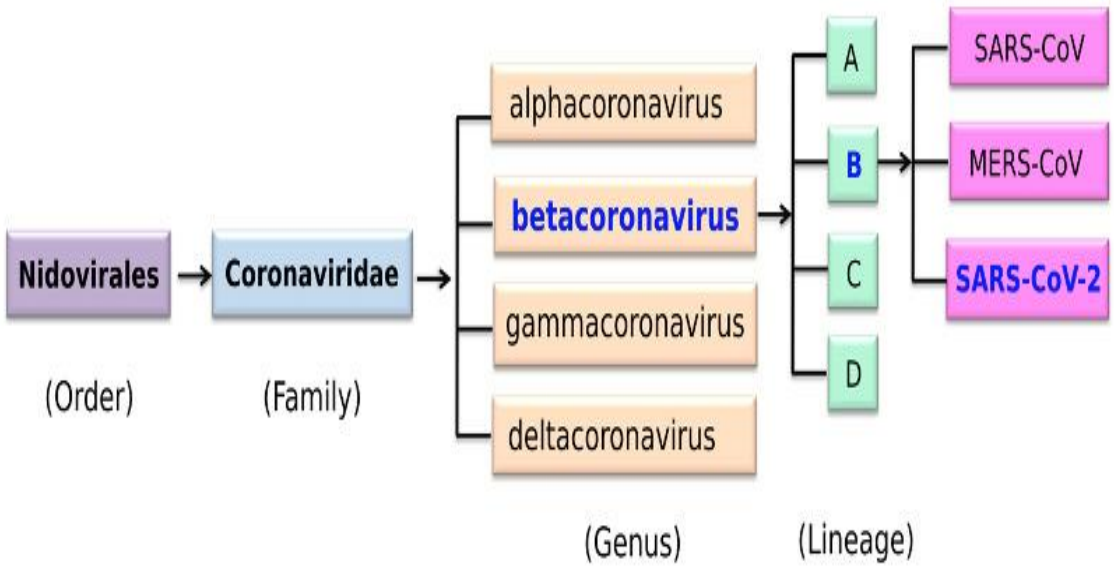
Coronaviruses belong to the Nidovirales order and Coronaviridae family. They are classified according to their predominant genetic characteristics located within ORF1ab (pp1ab) replicase polyprotein. The most distinctive coronavirus characteristics are:

1. The genome size is around 30 000 base pairs; coronaviruses are ribonucleic acid viruses with the largest genomes. This expansive

coding ability appears to provide and necessitate many gene expression strategies.

2. Expression of many non-structural genes by displacement of the ribosomal framework.
3. Various unique or unusual enzyme activities are encoded within the large polyprotein replicase-transcriptase.
4. Expression of downstream genes by synthesis of 3' nested sub genomic messenger ribonucleic acid, confirming close resemblance to host messenger ribonucleic acid.

Nidovirales order name is derived from these nested 3' messenger ribonucleic acid. The main differences within nidovirus families are in the number, type, and size of structural proteins. Because viruses mutate quite easily, it has been established that strains isolated from different sources must have more than 90 percent homology (at the level of amino acids), within conserved replicase domains for them to be considered the same species (de Groot *et al*., 2012). Based on these criteria, coronaviruses, according to the Coronavirus Study group of International Committee on Taxonomy of Viruses, are classified into four genera, divided by phylogenetic groupings, alphacoronavirus (a-coronavirus), beta coronavirus (β-coronavirus), gamma coronavirus (γ-coronavirus) and delta coronavirus (δ-coronavirus) (Woo *et al*., 2012). Within genus β-coronavirus there are four lineages: lineages A, B, C, and D (Figure 1).

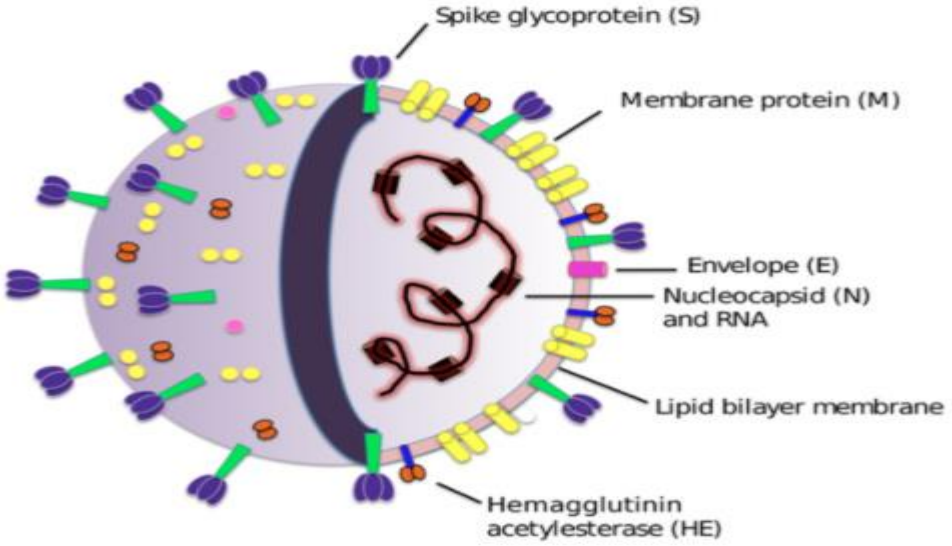


Source: elaborated by the authors.

Figure (1.1): Taxonomic classification of coronavirus, (Santos-Sánchez, N. F., Raúl Salas-Coronado,2020).

1.4 Coronavirus virions structure and SARS-CoV-2 action mechanism

Coronavirus virions contain four major structural proteins. These are spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins. All these are encoded within the viral genome 3'-end (Figure 2 and Table 1). Coronaviruses are approximately spherical and moderately pleomorphic. Virions have generally been reported to have average diameters of 80 to 120 nanometers. Protein S (~ 150 kilodalton) uses an N-terminal signal sequence to gain access to the host. This protein forms a distinctive spike structure on the virus surface (Godeke *et al*., 2000). S spikes project about 17 to 20 nanometers from the virion surface, with a thin base that swells to a width of about 10 nanometers at the distal end (Sugiyama and Amano,1981).



RNA: ribonucleic acid
Source: elaborated by the authors.

Figure (1.2): Coronavirus virion scheme with an essential set of structural proteins. (Santos-Sánchez, N. F., Raúl Salas-Coronado,2020).

Table (1.1): Coronavirus essential structures, (Santos-Sánchez, N. F., Raúl Salas-Coronado,2020).

Structure (Symbology)	Main function	Size (kDa)	Observations
Spike (S)	Determinant to viral infection by receptors recognition and for virus entry into the host cell cytoplasm.	150	Located in the membrane, it is a distinctive virus protein. The S1 domain (~700 amino acids) is required for receptor recognition and binding and contains two subdomains: N-terminal (NTD) and C-terminal (CTD). The S2 domain (~600 amino acids) docks fusion machinery to the host cell[13-15].
Membrane (M)	The structural protein is necessary for virus assembly and related to virus shape and size.	25 to 30	The most abundant protein in the viral envelope and contains three transmembrane domains[16,17].
Envelope (E)	Promotes virus assembly and release.	8 to 12	A transmembrane protein and the least abundant protein of viral envelope[18,19].
Protein (N)	Protein involved in viral genome replication, transcription and packaging, and also hinders the reproductive host cell cycle.	50	The most abundant protein in coronavirus and the only one present in nucleocapsid is abundantly expressed during infection. It contains two domains, an NTD and another CTD[20,21].

kDa: kilodalton.
NTD: N-terminal domain.
CTD: C-terminal domain.

1.5 Transmission of COVID-19

The virus that causes coronavirus disease 19 (COVID-19) is a highly transmittable and pathogenic viral infection and mainly transmitted through contact with respiratory droplets rather than through the air (Shereen and Khan ,2020). Primarily people can catch coronavirus disease 19 (COVID-19) from others who are infected. A single cough can circulate up to 3.000 droplets. These droplets can land on other people, and covering surfaces around them, however, several smaller particles will stay within the air. the virus is also shed for extended in fecal matter, thus anyone who not washing their hands thoroughly after visiting the toilet, bathroom could contaminate anything they touch like many respiratory viruses, including flu, Covid-19 can be spread by close contact with small droplets released from infected individuals' upper respiratory tract secretions, (Gray ,2020) e.g., sneezing, common cold or coughing from the nose and mouth. That is why to stay more than 1 meter (3 feet) away from a person who is sick. The virus can also be transmitted through surface contamination when these droplets land on objects and surfaces around the person and other individual touches these objects or surfaces and further touching their eyes, nose or mouth then these people catch COVID-19. Transmission of COVID-19, Figure 3.

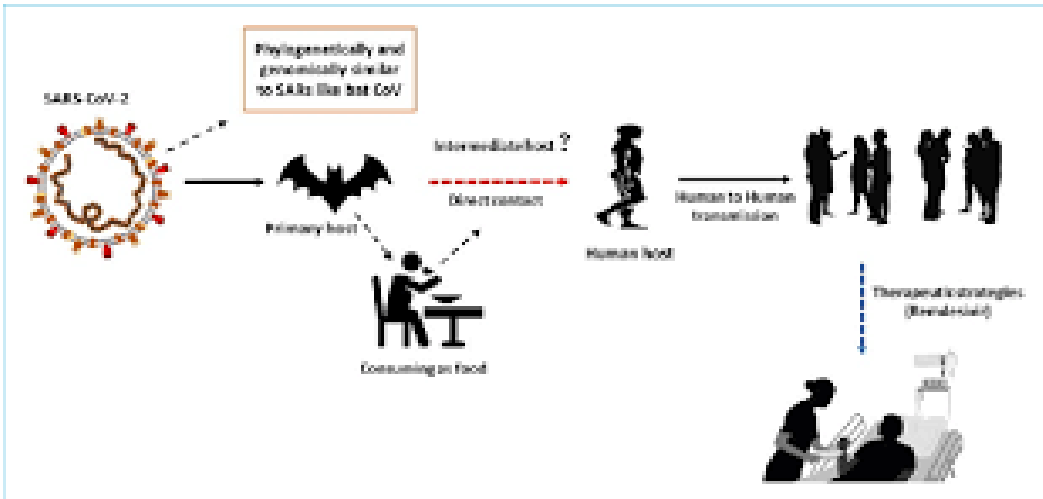


Figure (1.3): Transmission of COVID-19 to human host, (ScienceDirect.com).

1.6 Detection methods of COVID-19

The whole-genome sequencing of SARS-CoV-2 is one of the most comprehensive approaches for viral nucleic acid identification and quantification with high accuracy and reliability, making it possible to identify SARS-Cov-2 unequivocally. The first complete SARS-CoV-2 genome sequence available from GenBank has an accession number of MN908947. This sequencing allowed the identification of five typical open reading frames in the SARS-CoV-2 genome, ORF1ab polyprotein (7096 amino acids), protein S (1,273 amino acids), protein E (75 amino acids), protein M (222 amino acids), and protein N (419 amino acids). Reverse transcription polymerase chain reaction is the most popular test method for SARS-CoV-2 detection that commonly uses samples from the back of a patient's nose or mouth (Khailany *et al*., 2020). From these samples, ribonucleic acid is extracted and converted into its complementary deoxyribonucleic acid via a transcription reaction in reverse transcriptase enzyme presence. Later, specific gene fragments within complementary deoxyribonucleic acid are amplified using primers

for specific targets. Primers are generally obtained from three regions of viral genome with conserved sequences:

1. RdRP gene (ribonucleic acid -dependent gene that expresses ribonucleic acid polymerase) in open reading frame of ORF1ab region.
2. Gene that expresses protein E.
3. Gene that expresses protein N.

Table 4 shows the most representative COVID-19 diagnostic methods and some of their characteristics, (Santos-Sánchez, N. F., Raúl Salas-Coronado,2020).

Table 1.2:General characteristics of SARS-CoV-2 detection methods.

Diagnostic method	Detection Mechanism	Sample	Test result	Target gene	Generalities
RT-PCR	Polymerase chain reaction, fluorescence. Molecular assay.	Nasal, throat, saliva, bronchoalveolar lavage fluid.	Quantitative (4 h, including RNA extraction).	For ORF1ab region expression of proteins E and N (N1, N2 and N3).	Viral RNA is reverse transcribed to cDNA, and specific cDNA gene fragments are amplified. Test is specific, fast, sensitive, need for bulky instrumentation. LoD: 3.6 to 8.3 copies/reaction^{-1}.
Isothermal nucleic acid amplification	TMA, 3SR, NASBA, SMART, SDA, RCA, LAMP, IMDA, HDA [72], SPIA, cHDA, iLACO [74], CRISPR (DETECTR), molecular assay.	Nasal, throat, saliva.	Qualitative (45 to 70 min with manual RNA extraction).	For ORF1ab region expression of proteins E and N.	Use and amplification of viral RNA and/or viral RNA is transcribed into cDNA[76], fast, sensible and low cost. LoD: 70 to 300 copies/μL^{-1}.
EIA, ELISA	Antibody detection, colorimetric interpretation. Immunoassay.	Blood, serum, blood plasma, bronchoalveolar lavage fluid.	Qualitative and quantitative.	IgA, IgG and IgM immunoglobulins (the last two based on protein N).	Indirect detection of SARS-CoV-2. Fast and specific.

TMA: transcription-mediated amplification.
3SR: self-sustained sequence replication.
NASBA: nucleic acid sequence-based amplification.
SMART: signal-mediated amplification of RNA technology.
SDA: strand displacement amplification.
RCA: rolling circle amplification.
LAMP: loop-mediated isothermal
DNA amplification.
IMDA: Isothermal Multiple Displacement Amplification.
HDA: helicase-dependent amplification.
SPIA: single initiator isothermal amplification.
cHDA: circular helicase-dependent amplification.
iLACO (iso-thermal LAMP based method for COVID-19).
CRISPR: Clustered regularly interspaced short palindromic repeats.
LoD: Limit of detection.
IgA: Immunoglobulin A.
IgG: Immunoglobulin G.
IgM: Immunoglobulin M.
RT-PCR: reverse transcription polymerase chain reaction.
ELISA: enzyme-linked immunosorbent assays.
EIA: enzyme immunoassays.
DETECTR: SARS-CoV-2 deoxyribonucleic acid (DNA) endonuclease-targeted clustered regularly interspaced short palindromic repeats trans reporter.

1.7 Laboratory tests

General findings

In the early stages of the disease, peripheral WBC count is normal or decreased and the lymphocyte count is decreased. Some patients have elevated liver enzymes, lactate dehydrogenase (LDH), muscle enzymes and myoglobin. Elevated troponin is seen in some critically ill patients. Most patients have elevated C-reactive protein and erythrocyte sedimentation rate and normal procalcitonin. In severe cases, D-dimer increases and peripheral blood lymphocytes progressively decrease. Severe and critically ill patients often have elevated inflammatory factors (Lippi,2020).

Pathogenic and serological findings

(1) Pathogenic findings: Novel coronavirus nucleic acid can be detected in nasopharyngeal swabs, sputum, lower respiratory tract secretions, blood, feces and other specimens using RT-PCR and/or NGS methods. It is more accurate if specimens are obtained from lower respiratory tract (sputum or air tract extraction). The specimens should be submitted for testing as soon as possible after collection (Trafton and Chu,2020).

(2) Serological findings: COVID-19 virus specific IgM becomes detectable around 3-5 days after onset; IgG reaches a titration of at least 4-fold increase during convalescence compared with the acute phase (Gilbert *et al*., 2022)

Chest imaging

In the early stage, imaging shows multiple small patchy shadows and interstitial changes, more apparent in the peripheral zone of lungs. As the disease progresses, imaging shows multiple ground glass opacities and infiltration in both lungs. In severe cases, pulmonary consolidation may occur. However, pleural effusion is rare.

Aim of this study:

Comparison of haematological parameters among a group of unvaccinated COVID-19 infected patients and a group of healthy controls to assess the value of hematological parameters in prediction of the prognosis of the COVID-19 infection.

Chapter Two

Materials and Methods

2.Materials and Methods

2.1 Subjects and Sampling

The study included 76 COVID-19 infected unvaccinated patients confirmed by CT-SCAN and RT PCR tests. This group was compared to 51 healthy individuals. Number of females in the patient group was 20 and males were 56 while 31 females and 20 males were among the healthy group. Data were collected from Merjan Hospital, Babylon from September 2020 to March 2021. Some of the blood samples were tested at AL Kindy laboratory, Babylon. These data were provided to the students who handled the biostatistical tests due to the lack of continuous regular presence of the students to the university based on the current regulations for combating the spread of COVID-19 in populations.

2.2 Statistical Analysis

Data were analyzed using SPSS (version 20, SPSS Inc. Chicago, Illinois, USA). The experimental results were analyzed using Chi square and expressed as percentage (%). Statistical significance of the results was assessed at $p \leq 0.05$.

Chapter Three

Results and Discussion

3. Results and Discussion

3.1 The Demographic Study

Seventy-six (56 male and 20 female) unvaccinated Iraqi patients infected with COVID-19 were enrolled in this study. The patients were clinically diagnosed by the physician based on a clinical examination, Chest computed tomography (CT) scan, and RT-PCR. The ages of the patients and control ranged between (19-80) years. whereas, the control group including 51 (20 male and 31 female) healthy individuals, table (3-1).

Table (3-1): Gender distribution among controls and COVID-19 patients.

Groups / Gender	Control	Patient	p-value
	No. (%)		
Female	31(60.78%)	20 (26.32%)	**0.035***
Male	20 (39.22%)	56 (73.68%)	
Total	**51 (100%)**	**76 (100%)**	

Results listed in table (3-1) indicated significant differences in representation of males and females among control and patients' participants as significantly more males were represented among patients' group than females. These results are consistent with data reported by several investigators who reported that severe and fatal outcomes are more commonly seen among male patients (Qian *et al*., 2020). Differences in women's and men's bodies due to their sex (biology) is thought to play a role in people's risk of illness and death due to COVID-19. Globally, there's no clear trend in terms of who is most likely to become infected by COVID-

19. However, across the vast majority of countries, a clear pattern in mortality has emerged; men appear more likely than women to die from COVID-19 once infected. This pattern varies by country and may be changing over time (Global Health,2020).

The age groups ranged from (19-80) years were divided into four groups, (14-24), (25-35), (36-55) and (≥56) years. No differences in representation among different age groups were found among patients and control. (Table 3-2). Although there were no significant differences in age range from (36-55) years (0.329) at ($P \leq 0.05$), the results suggest higher frequency of COVID-19 infection were reported among individuals within this group of age range., table (3-2).

A study by Farghaly and Makbou (2021) reported that age group C (40–49 year) was more commonly affected by COVID-19, while the least affected group was group F (≥ 70 years).

Several previous studies reported by Dong *et al*., (2020) and Zhao *et al*., (2020) indicated that sever cases of COVID-19 were diagnosed more commonly among older individuals suggesting that this group is at a higher risk for the disease.

Susceptibility to infection by SARS-CoV-2 varies by ages, where children are less susceptible than adults to becoming infected on contact with an infectious person, may explain the lower number of cases among children. Decreased susceptibility could result from immune cross-protection from other coronaviruses (Nickbakhsh *et al*., 2020), or from non-specific protection resulting from recent infection by other respiratory viruses (Cowling *et al*., 2012), which children experience more frequently than adults (Tsagarakis *et al*., 2018).

Table (3-2): Age distribution among controls and COVID-19 patients

Groups / Age (years)	Control NO. 51 No (%)	Patient NO. 76 No (%)	p-value
14-24	16 (44.44%)	20 (55.66%)	**0.329**
25-35	19 (36.54%)	17(51.52 %)	
36-55	16 (48.48 %)	33 (63.46%)	
≥56	0 (0%)	6 (100 %)	

Table (3-3) demonstrates the distribution of study groups according to total and differential count of WBC cells. The results showed there were significant differences between patients and control. There was lower number of lymphocytes in samples from patients' group than samples from the control group. However, the difference was not significant.

Table (3-3): White blood cell (WBC) distribution among controls and COVID-19 patients

Groups / Parameters	Control	Patient	p-value
	Mean ±S. E		
Neutrophils (cell×10^3/µl)	61.91±2.27	69.81±1.46	**0.003****
Lymphocytes (cell×10^3/µl)	33.31±2.5	28.32±1.8	**0.150**
Eosinophils (cell×10^3/µl)	2.22±0.3	0.98±0.1	**0.001****
Basophils (cell×10^3/µl)	0.24±0.02	0.85±0.14	**0.001****
WBC (cell×10^3/µl)	9.35±0.3	27.20±3.2	**0.002****

Besides, researchers had found that the level of lymphocytes is abnormally decreased in patients confirmed with SARS and COVID-19, whereas such a level is generally increased when facing common infectious diseases (He *et al*., 2005). Compared with young adults, the level of lymphocytes in older patients was significantly lower suggesting a vital indicator of the severity of COVID-19 in older patients (Guan *et al*., 2020). Besides these immune differences among older and younger people, the pre-existing conditions, such as obesity, drinking, smoking, and other unhealthful lifestyles, also contribute to the increased mortality of COVID-19, as these unhealthy habits are most commonly seen in the older group (Lauc and Sinclair,2020).

CONCLUSION

The study was consistent with published reports on the predictive value of haematological measurements in the prognosis of CONVID-19 infection.

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مقارنة المعايير الدموية ضمن المرضى المصابين بالكوفيد-19 مع مجموعة الاصحاء

من قبل

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