Pathophysiology study and sex differences response in COVID-19 patients

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Abstract
Coronavirus disease 2019 (COVID-19), an extremely infectious illness caused by a novel Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) that has spread over the worldwide, has become one of the most difficult public health problems of our time. Age and gender are two major characteristics that influence the risks and outcomes of numerous diseases. Our study will investigate and compare the difference in hematological, biochemical, and serological biomarkers between sexes in order to evaluate severity and pathogenicity. Clinical records were taken from 150 SARS-CoV-2 positive patients were included in this study; the infection was confirmed by real time reverse transcriptase polymerase chain reaction. Blood samples subjected to measure changes in hematological parameters and serum subjected to measure biochemical test including ferritin, creatinine, CRP, D-dimer, and liver function enzyme either for ELISA test to measure serological biomarkers including IgM, IgG, TNF-α, IFN-γ, IL-6, and IL-10. 90 (60%) of whom were male and 60 (40%) of whom were female. Our study found a significant increase in CRP, IgM, IL-6, IL-10, TNF-α, IFN-γ, AST, ALP, and TBIL levels in males compared to females, and the age group most susceptible to SARS-CoV-2 infection was 41-60 years. Based on these findings, we concluded that males and those of older age had a high prevalence of severity and progression than females.
Keywords: SARS-CoV-2, COVID-19, biochemical test, hematological biomarker, serological parameter

1. Introduction

Coronavirus disease 2019 is caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2, which has the possibility to become a worldwide pandemic in 2020 [1,2]. It has expanded to about 230 areas and countries throughout the world and over 630 million individuals had been infected and over 6.5 million had died as of October 26, 2022 [3]. SARS-CoV-2 is a β coronavirus belonging to the coronavirus family [4], which is a new distinct coronavirus associated to severe acute respiratory syndrome (SARS-CoV) and Middle East Respiratory Syndrome related coronavirus (MERS-CoV) and has a genetic similarity of 79% with SARS-CoV and 51.8% with MERS-CoV [5,6].
Coronavirus disease 2019 has posed significant clinical issues, leading to its very different clinical presentation, which can vary from asymptomatic, moderate, to severe cases [7,8]. Infected individuals may have any of the following symptoms: Fever, coughing, muscle aches, sputum secretion, headache, hemoptysis, and diarrhea [9], and breathlessness, while in severe instances, advanced symptoms such as acute respiratory distress syndrome (ARDS), sepsis, multi-organ failures, and death emerge [10]. The clinical course of the illness can be difficult to predict, with some patients having severe symptoms and potentially fatal problems, while others proceed slowly to critical disease [11,12]. In severely infected individuals, the SARV-CoV-2 virus that causes inflammation of the lung, resulting in a cytokine storm and an increase in inflammatory biological indicators including C-reactive protein (CRP), Interleukin-6 (IL-6), interferon gamma (IFN-λ), and tumor necrosis factor-alpha (TNF-α), haemostasias system profile including D-dimer, platelets, and serum biochemical analyses for liver and kidney activities [13,14].
Epidemiological evidence collected earlier that host factors such as gender and age might enhance COVID-19 severity [15]. However, some studies have found that the older age of male patients with COVID-19 are considered the high risk of requiring intensive care therapy, worse results, and mortality than their female counterparts [16]. This gender difference is not totally surprising that previous studies has shown that males of all ages are more susceptible than females to other respiratory tract infections including SARS and MERS [17]. This could be associated to genetics and sex hormones, which can alter both viral receptor expression and the differential control of immune responses [18]. In general, men's immune systems are less robust, making them more susceptible to a variety of infectious diseases. In contrast, females develop higher innate and adaptive immune response and are relatively resistance to viral infections [19]. on the other hands, female hormones may provide protection against COVID-19 illness [20]. In contrast, androgens may predisposition males to a more severe COVID-19 progress [21]. Moreover, testosterone suppresses immunological functioning [18]. In this study, we performed a detailed study of clinical features associated with SARS-CoV-2 infection to analyze the association biomarkers including complete blood counts, CRP, D-dimer, ferritin, creatinine, IgM, IgG, IL-

doi.org/10.31838/hiv23.02.148 Received: 25.10.22, Revised: 10.11.22, Accepted: 19.12.22.

6, IL-10, TNF-α, IFN-λ, and liver function enzyme are associated to the severity and pathogenicity of both sexes at various ages.

2. Materials and Methods

Sampling

A total of 150 nasopharyngeal swabs and blood samples were obtained from patients at the Emarati and Rozhawa Emergency Hospital in Erbil, Kurdistan Region of Iraq, among August 2021 and January 2022 who were infected with COVID-19 and tested positive for SARS-CoV-2. All specimen was diagnosed by rRT-PCR. In addition, data about sex and age were collected from the individuals. 50 healthy individuals of similar ages and sexes comprised the control group, and they tested negative for SARS-CoV-2.

SARS-CoV-2 RT-PCR Screening Test

Nasopharyngeal and/or Throat swab were taken and placed into viral transport medium (VTM) and kept at - - 80 C until assayed for viral RNA detection using a real time reverse transcriptase polymerase chain reaction (rRT-PCR) test. Viral RNA was isolated using the AddPrep Viral Nucleic Acid Extraction Kit, a specialized laboratory kit (addbionic, Korea). The real-time reverse transcriptase PCR experiment was carried out using the SARS-CoV-2 Nucleic Acid Detection Kit (PCR-Fluorescent Probe Method) (Zybio, China) according to the manufacturer guidelines.

Hematological test

Blood samples from positive SARS-CoV-2 detecting tests were subjected to a fully automated 3-part distinct analyser (Medonic, - Alpha, Germaley) to measure changes in parameters associated to the severity and progression of COVID-19 in both males and females.

Biochemical test

blood specimens were obtained in order to obtain serum from COVID-19 patients. The ferritin, creatinine, and liver function enzyme tests were done using (COBAS INTEGRA 400 plus system - fully automated biochemical analyzer, Germaley), and the CRP and D-dimer levels were determined using the kit with an auto-analyzer Nano-Checker 710 Reader (nanoditech, USA).

Serological tests

blood specimen was obtained in order to collect serum from COVID-19 patients. Serum cytokine values in the specimen were measured using commercially available Enzyme Linked Immunosorbent Assay (ELISA) kits for IgM, IgG, TNF-α, IFN-γ, IL-6, and IL-10 (Sunredbio, China) at wavelength of 450 nm according to the manufacturer 's instruction.

3. Statistical Analysis

The data from this inquiry were given as the mean and standard deviation (SD). The findings were analyzed utilizing one way analysis of variance (ANOVA) to investigate differences among all groups, followed by Tukey's multiple comparisons test, and figures were generated with GraphPad Prism 9. The study was carried out using a 95% level of confidence and a statistical significance level of 5%. When a p-value less than or equal to 0.05 was declared statistically significant ($p \leq 0.05$). The subscripts asterisks characters *, **, *** indicated a significant difference among SARS-CoV-2 patients and the control groups; the subscripts hash characters #, ##, ### indicated a significant difference among the male and female groups. They are significant $p<0.05$, $p<0.01$, $p<0.001$, and $p<0.0001$, respectively.

4. Results

The study included 150 patients, all of whom were RT-PCR positive for COVID-19 infections. In comparison to 50 individuals with RT-PCR negative, 60% were males and 40% were females. Most of the patients 22% belong to 51-60 years and 21.33% belong to 41-50 years. According to the findings, patients between the ages of 41- 60 are at higher risk of getting infected with COVID-19.

Table 1: Baseline characteristics of the sample by sex and age.

Age group	Total 150	male		Female	
		No.	%	No.	%
21-30	7	3	42.8	4	57.2
31-40	21	7	33.4	14	66.6
41-50	32	12	37.5	20	62.5
51-60	33	15	45.5	18	54.5
61-70	24	9	37.5	15	62.5
71-80	23	10	43.5	13	56.5
81-90	10	4	40	6	60

Antibody test results revealed a significant increase in IgM and IgG levels in patients with positive RT-PCR when compared to the control group. Moreover, as compared to the female group, the males had a significant increase in IgM levels and a slight increase in IgG levels (figure 1).

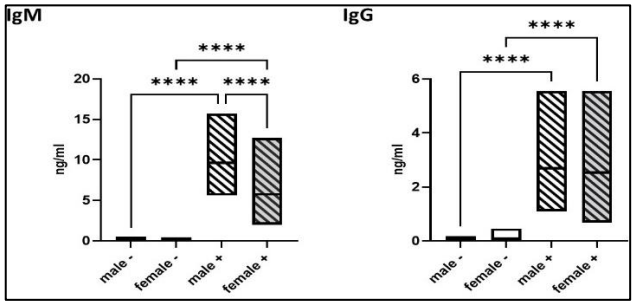

*Figure 1: The level of IgM and IgG in positive SARS-CoV-2 patients from both male and female compared with the Control group. Male - = control male, female - = control female, male + = positive male, female + = positive female. *<0.05, **=0.01, ***<0.001, ****<0.0001*

There are significant differences in the parameters of the hematological variables of SARS-CoV-2 infection when compared with the control group. Furthermore, no significant variations in the level of

hematological markers were identified when males and females were compared (Table 2).

Table 2: Comparison between male and female for COVID -19 patients in hematological parameters.

Hematological parameter	control		SARS-CoV-2 Patients	
	male	female	male	female
WBC	7.72 ± 0.42	7.55 ± .7	14.43 ± 7.84 ***	13.3 ± 1.69 ***
LYM%	32.77 ± 9.26	32.92 ± 8.9	12.34 ± 7.28 ***	8.15 ± 7.21 ***
MID%	6.89 ± 1.76	6.01 ± 2.61	11.17 ± 0.28	9.25 ± 19.94
GRA%	59.94 ± 11.31	60.57 ± 11.52	82.66 ± 24.39 ***	88.1 ± 2.89 ***
RBC	4.89 ± 0.51	4.63 ± 0.04	3.95 ± 0.91 ***	3.75 ± 0.21 ***
Hb	13.91 ± 1.2	13.13 ± 0.63	10.88 ± 3.6 ***	10.4 ± 0.56 ***
HCT	42.55 ± 1.55	39.71 ± 3.11	31.94 ± 4.66 ***	30.76 ± 0.84 ***
MCV	87.1 ± 6.36	85.62 ± 7.42	84.82 ± 1.97 ***	83.73 ± 3.11 ***
MCH	27.64 ± 14.77	28.18 ± 2.12	29.03 ± 2.19 ***	28.62 ± 0.21 ***
MCHC	32.71 ± 1.41	32.85 ± 1.41	34.86 ± 0.35 ***	34.95 ± 1.27 ***
PLT	237.76 ± 119.5	291.24 ± 61.51	285.1 ± 346.76 ***	300.73 ± 45.96 **
MPV	8.6 ± 1.41	8.67 ± 0.21	9 ± 0.28 ***	8.93 ± 0.63 ***

The results showed a substantial increase in the levels of CRP, D-dimer, and ferritin and no substantial differences were seen in the level of creatinine for COVID-19 patients when compared with the control group. On the other hands, the level of CRP was significantly higher in the male compared to the females, but no significant differences were seen in the level of D-dimer, ferritin, and creatinine in males compared to the females (figure 2).

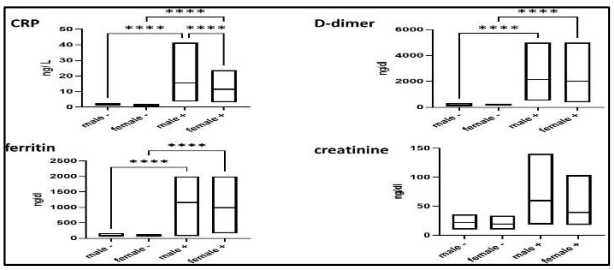

*Figure 2: The level of CRP, D-dimer, ferritin, and creatinine in positive SARS-CoV-2 patients from both male and female compared with the Control group. Male - = control male, female - = control female, male + = positive male, female + = positive female. *<0.05, **=0.01, ***<0.001, ****<0.0001*

There are significant differences in the parameters of the liver function enzyme for SARS-CoV-2 patients when compared with the control group. Besides, the level of AST, ALP, and TBIL was significantly elevated in the males when compared with females. While there are no significant differences were seen in the level of ALT between males and females (figure 3).

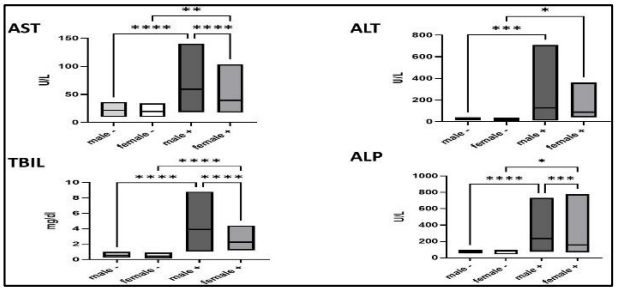

*Figure 3: The level of liver function enzyme in positive SARS-CoV-2 patients from both male and female compared with the Control group. Male - = control male, female - = control female, male + = positive male, female + = positive female. *<0.05, **=0.01, ***<0.001, ****<0.0001*

In this study, most COVID-19 patients had significantly higher blood levels of pro-inflammatory cytokines, such as IL-6, IL-10, TNF-α, and IFN-γ compared to the control group. When compared between male and female group we see that the level of the IL-6, IL-10, TNF-α, and IFN-γ were significantly higher in male group than in female group (figure 4).

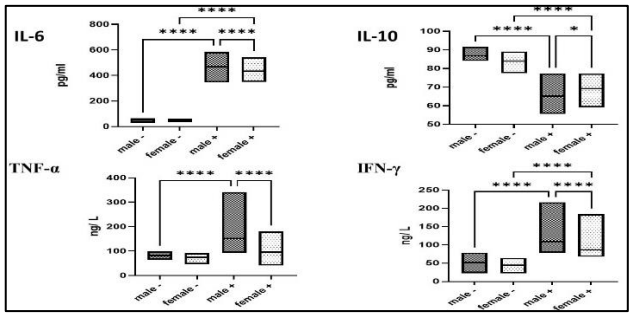

*Figure 4: The level of cytokine profile in positive SARS-CoV-2 patients from both male and female compared with the Control group. Male - = control male, female - = control female, male + = positive male, female + = positive female. *<0.05, **=0.01, ***<0.001, ****<0.0001*

5. Discussion

Coronavirus is a large virus family that causes diseases varying from the common cold to serious pneumonia and remains a global health problem [22], As well as age and sex are two critical characteristics related with risks and outcomes of COVID-19 illness. In this study, we have analyzed possible factors that may have contributed to a gender difference in 150 patients with COVID-19 clinical outcomes, especially in the rate of severity and mortality by using several parameters including hematological, biochemical, and serological parameters.

We found that males had a higher case COVID-19 severity rate among hospitalized patients. COVID-19 Associated mortality increases with age and was higher in males of all ages [23]. Several studies found a relationship between sex and illness outcome; males were more affected than females, and the ratio was higher in most countries [24,25]. Both genetics

and sex hormones may impact viral receptor expression and the differential regulation of immune responses. Moreover, they appear to be capable of modulating immunological and inflammatory responses, as well as the production of the Angiotensin-converting enzyme (ACE)-2 gene, which binds the SARS-CoV-2 viral spike protein [26,27]. Female hormones may give protection against COVID-19 illness [28]. Another factors which play critical role in increasing the prevalence of infection is lifestyle of males, such as smoking and alcohol drinking as compared to females [29]. Many research revealed that based on the clinical categorization of severity, males tended to acquire more severe cases than females [30]. A recent study conducted by Liu et al. on 4,880 patients that have respiratory symptoms and close relations with COVID-19 patients in the hospital in Wuhan found that males and the elderly people (>70 years) had a considerably higher risk of SARS-CoV-2 infection [31]. Furthermore, a study conducted by Jin et al. revealed that males had much higher mortality and severe symptoms than females [32].

Serum levels of immunoglobulin SARS-CoV-2 IgM and IgG antibodies are utilized to assess immunological responses [33]. During virus infection, IgM antibodies give early-stage defense before the emergence of the class IgG response for long-term immunity and immunological memories. During the SARS-CoV-2 pandemic, multiple studies indicated that identifying viral-specific IgM and IgG is useful for diagnosis [34]. Furthermore, a study conducted by Liu et al. discovered that IgM antibody levels are connected with severity, whereas IgG antibody levels are not substantially associated with severity in the early stages of infection [31]. In our study, the outcome of immunoglobulin SARS-CoV-2 showed that the level of IgM was significantly higher in males than females and suggested that males may be positively related to the COVID-19 severity.

Inflammatory cytokines have the potential to dramatically change the activities of hematopoietic cells, particularly neutrophils, lymphocytes, and monocytes [5]. When SARS-CoV-2 infected humans, it caused lymphocytosis since lymphocytes are virus-fighting effector cells [35,14] and the process might be induced by either direct coronavirus infection on lymphocytes or immune-mediated lymphocyte death [36]. In our study, there was no significant differences between male and female in hematological data were seen between males and females, this finding agreed with those done by Alkhouli et al. and Statsenko et al. [37,38]. A study done by Morgan revealed that noticing CRP, D-dimer, ferritin, and liver function values in COVID-19 patients has a possible function of predict the case progression [39]. Another study conducted by Mahmood et al. revealed that CRP is an indirect diagnostic test for inflammation, with both pro-inflammatory and anti-inflammatory features [10]. Our results were agreed with previous study that showed a significant difference in the level of CRP in males compared with females. In certain instances, it might overwhelm tissue injury by activating the complement system and hence inflammatory cytokines, as well as the most common laboratory abnormalities to be seen were elevated levels of AST, ALT, ALP, and creatinine [40]. This suggested that COVID-19 associated to liver and kidney damage has been announced as enhanced serum liver enzyme levels. This appear crucial as abnormal liver function and kidney test are related with higher severity and mortality rate [38]. Our results showed a significant increase in the level of AST, ALP, and TBIL in males compared with females and our results was agreed with another study done by Zhao et al. [41]. SARS-CoV-2 highlights variations within this wide characterization. In male patients, several cytokines and chemokines, and antibody titers, are elevated [42]. A study conducted by Copaescu et al. and Karki et al. revealed that an increase in serum IL-6, TNF-α and IFN-γ was associated with the severity of COVID-19 [43]. IL-6 is important for regulating immunological and inflammatory responses [44] suggesting that IL-6, TNF-α and IFN-γ might be a possible therapeutic target for severe COVID-19 pneumonia infections [45,46]. According to a study conducted by Conti and Younes et al. males had higher respiratory levels of proinflammatory cytokines than females [47]. Our study showed the significant increase in the levels of IL-6, IL-10, TNF-α, and IFN-γ in the males compared with females and agreed with the outcome of previous study, this finding suggests that males have a more severe COVID-19 disease.

6. Conclusion

Coronavirus disease 2019 remains a major health problem globally. We investigated potential factors that might have led to a sex difference in COVID-19 patient outcomes, particularly the rate of severity. COVID-19 severity appears to be impacted by patients age and gender. In our study, there was a statistically significant differences in CRP, IgM, IL-6, IL-10, TNF-α, IFN-γ, AST, ALP, and TBIL levels in males in comparison with females, and the age group most susceptible to SARS-CoV-2 infection was 41-60 years. We discovered that the most susceptible and severe cases were seen in males and older age.

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