Assessment of concentrations of interleukin IL-1β, interleukin 2, interleukin 15 and level of Testosterone hormone in COVID-19 infertile patients

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Abstract The male reproductive system relies on the proper production of sperm. Spermatogenesis is a complex process that calls for the cooperation of many different elements. The presence of pro-inflammatory cytokines such as interleukin 1 beta (IL-1β), interleukin 2, and interleukin 15 in the male reproductive tract may be responsible for the manifestation of physiological processes in the testes, epididymis, and sperm of the male reproductive system. However, the present study aimed to determine the effect of Coronavirus disease (COVID-19) on the concentration levels of IL-1β, interleukin 2, and interleukin 15 in males. Moreover, to assess its impact on the hormone. A total of the n=95 clinical samples from the infertile patients confirmed COVID-19 patients involved the analysis of serum or plasma samples from 95 patients to measure levels of Testosterone hormone, IL15, IL-1B, and IL-2 by using an ultrasensitive enzyme-linked immunoabsorbent. The results showed a significant increase (p<0.05) of IL-1B, IL-15 and a decrease in IL-2 level in infertile; also, testosterone evaluated in the serum and plasma of SARS-CoV-2 patients showed a positive connection between the level of hormone and IL-1B, IL-15, While negatively related with IL-2 patients infected with coronavirus compared with non-infected infertile patients. Male individuals with COVID-19 who experience infertility exhibited a combination of various factors that are linked to reduced testosterone hormone levels. These factors include advancing age, obesity, and cytokine alteration; there is an observed increase in IL-1B and IL-15 levels and a decrease in IL-2 concentration in these patients.

Keywords: coronavirus disease, testosterone, cytokines, spermatogenesis, infection

1. Introduction

Since SARS-CoV-2 infection was first discovered in December 2019 in Wuhan City in China, it spread rapidly and a global pandemic of COVID-19 has occurred. According to several recent studies on SARS-CoV-2, the virus primarily infects the respiratory system but may cause damage to other systems. ACE-2, the main receptor for entry into the target cells by SARS-CoV-2, was reported to abundantly express in testes, including spermatogonia, Leydig and Sertoli cells. Nevertheless, there is no clinical evidence in the literature about whether SARS-CoV-2 infection has an impact on male reproductive health.

The ongoing global outbreak brought on by the SARS-CoV-2 virus persists in exerting significant impacts on both human health and healthcare systems across the globe. In the Chinese city of Wuhan, the first SARS-CoV-2 infections in humans were reported in December (Phelanet al., 2020). This phenomenon's origin is considered zoonotic in nature, although the specific animal reservoir is currently being investigated. Nevertheless, the recently identified SARS-CoV-2 exhibited the capacity for human-to-human transmission, leading to its swift global dissemination. The disease resulting from the SARS-CoV-2 virus was officially designated as COVID-19 by the World Health Organisation (WHO) on February 11th, 2020. On March 11th 2020, the World Health Organisation (WHO) officially determined COVID-19 to be a pandemic. From very mild upper respiratory illnesses to severe primary pneumonia that may result in respiratory failure and death, the clinical range of SARS-CoV-2 infection seems to be fairly wide (Zhou et al., 2020). Previous viral epidemics have recorded different effects on male reproductive processes, increasing the likelihood of viral infections in the male reproductive system. Furthermore, COVID-19 affects more men than women, unlike SARS (male-to-female ratio of 2.7:1 vs 1:1.25, respectively(Huang et al., 2020). Moreover, it has been observed that SARS-CoV-2 exhibits a prolonged persistence in the human body compared to SARS-CoV. According to Kessler et al., (2020) the potential for a resurgence of SARS-CoV-2 remains a possibility even following its eradication (Wong et al., 2020); because of this, it is a disease that has a significant economic and medical impact.
The clinical manifestations of COVID-19 exhibit notable similarities to those observed in SARS (Epelboin et al., 2017) and observable effects of COVID-19 on the digestive and cardiovascular systems (Liu et al., 2018). Various viruses, such as EBOLA, Human Immunodeficiency Virus (HIV), ZIKA, Hepatitis B virus (HBV), Hepatitis C Virus (HCV), MUMPS, Human papillomavirus, Herpes and numerous others, have been shown to have a variety of different impacts on the reproductive health of men (Wu et al., 2019). Several notable harmful effects of these viruses include disrupted spermatogenesis, impaired sperm motility, decreased sperm counts, and altered hormonal levels, among others (Li et al., 2020). Elevated body temperatures that persist, such as those experienced during viral infections, have the potential to disrupt the integrity of the blood-testis barrier (Fijak et al., 2018); the available experimental evidence also indicates that even slight scrotal heat stress has the potential to induce the blood-testis barrier (BTB) leakages, thereby facilitating the passage of macromolecular substances into the testis (Wang & Xu, 2020).

Recent reports have proposed that Angiotensin-Converting Enzyme 2 may serve as the cellular receptor for SARS-CoV-2, potentially facilitating its entry into the male reproductive organs. The expression of ACE2 is highly prevalent in the testes, encompassing various cell types such as spermatogonia, Leydig cells, and Sertoli cells (Salam & Horby, 2017). Moreover, a hypothesis suggests that the binding of SARS-CoV-2 to the ACE2 receptor could lead to an upregulation of ACE2 expression, subsequently triggering an inflammatory response that may disrupt the regular functioning of Sertoli and Leydig cells (Song et al., 2011). The possibility of SARS-CoV-2 infection in the male reproductive tract (MRT) should not be completely dismissed, especially considering past viral infections like ZIKA, EBOLA, HIV, and similar cases (Sharlip et al., 2002). This study’s main objective was to determine if COVID-19 cytokine involvement was common and what effect it had on infertile males with azoospermia, oligozoospermia, asthenoteratospermia, and leucocytospermia.

2. Materials and Methods

2.1. Patients group

A total of n=95 clinical samples (Serum and plasma samples) from the infertile patients included first n=45 from laboratory confirmed COVID-19 patients and n=50 from those who were not infected who attended the laboratory of the Fertility Center in AL-Sadder Medical City in the Province of Najaf, AL-Najaf Health Directorate / Ministry of Health /Iraq during the period from 1 April 2020 to 30 March 2021.

2.2. Questionnaire

The patient data included the following demographic and clinical variables: age, sex, and body mass index; also, all patients were questioned about COVID-19 infection, previous diseases, drug intake, Diabetes type II, Chronic Heart Diseases and scrotal Doppler ultrasound and hormonal evaluation.

2.3. Sampling and Analysis of Sperm

Semen samples are obtained from individuals following a period of sexual abstinence lasting 3-5 days. This is done in a clean, disposable, sterile and dry container using masturbation in a calm environment adjacent to the laboratory dedicated to analysing seminal fluid under microscopic examination. The evaluation of semen quality encompasses various parameters, including sperm concentration, total sperm count, motility, and morphology. These assessments adhere to the guidelines outlined by the World Health Organisation WHO2010.

2.4. Blood samples collection and processing

Blood samples were collected from participants by extracting 3 millilitres of blood using sterilised medical needles via the brachial vein. Subsequently, the extracted blood was transferred into gel tubes, with each tube containing 3 ml of blood. Subsequently, the tube is subjected to ambient temperature conditions for 30 minutes to induce blood coagulation. Following this, the samples are subjected to centrifugation at a rate of 3000 revolutions per minute for 10 minutes, thereby facilitating the separation of serum from other constituents present in the blood. The serum was extracted using a micropipette and subsequently transferred into Eppendorf tubes for biochemical analysis. The tubes were then stored at a temperature of -20°C.

During the COVID-19 pandemic. For patients having more than one spermogram before the COVID-19 pandemic, the newest was considered. During the pandemic period, patients were not asked to show proof of negative COVID-19 PCR (polymerase chain reaction) because none of our patients had COVID-19 symptoms and according to the rules in that period PCR was not indicated. Spermogram appointment was postponed if the patient had any of the COVID-19 symptoms. We didn’t include patients who received medication (antibiotics, antivirals, anti-inflammatory, antioxidants) between the two compared spermograms. We didn’t also include patients who had undergone urogenital surgery (for varicocele, inguinal hernia) during that period.
2.5. **Hormone and cytokine quantification**

The study involved the analysis of serum or plasma samples from a cohort of 95 patients to measure levels of Testosterone hormone, IL15, IL-1B, and IL-2. This analysis was conducted in a clinical laboratory using an ultrasensitive enzyme-linked immunoabsorbent assay (ELISA) technique. The ELISA was performed using a commercially available Cytoscreen immunoassay Kit (Biosource International, Camarillo, CA, USA). The limitation of this study is the lack of data regarding whether the patients who presented during the pandemic had a history of COVID-19 infection. However, the main objective of this study was not to evaluate the effect of COVID-19 infection specifically but to demonstrate the overall impact of the pandemic. Therefore, this study did not examine the history of COVID-19 infection in the patients who presented due to infertility during the pandemic. Moreover, the results of this study pertain to the first year of the pandemic. Therefore, this study did not examine the history of COVID-19 infection in the patients who presented due to infertility during the pandemic. Moreover, the results of this study pertain to the first year of the pandemic.

2.6. **Statistical Analysis**

The widely recognised statistical software GraphPad Prism version 5 was utilised to analyse the variance table using a one-way ANOVA with Tukey’s multiple comparisons tests. This approach was employed to compare the measured parameters among the subdivided groups. The outcomes are given as (Mean Standard Error). To determine the correlation between markers and parameters, correlation coefficients are generated. The descriptive statistics and correlation coefficients were conducted using mega stat (Version v 10.12) for Excel 2007(13).

3. **Result and Discussion**

3.1. **Clinical infertile men distribution**

Of a total 95 cases men semen and blood sample collected, the age for infertility patients is significant increase (p<0.05) (38.12 ±0.42years) for azoospermia, (36.42±1.32years) in Oligospermia astheno teratospermia, witha significant increase (p<0.05) of BMI in both Azoospermia (35.34±0.6 kg/m2) and Severe asthenoligiteratospermia, leucocytospermia (31.52±0.7 kg/m2).

3.2. **Effect IL-1B level in infertile men**

Numerous studies have been undertaken in the wake of the COVID-19 pandemic to gain insights into the progression of the disease, establish effective therapeutic methods, and devise strategies for mitigating its transmission. The quantification of potential adverse effects resulting from the COVID-19 illness on various human organ systems, including the male reproductive system, is a matter of great importance and concern for healthcare professionals. The decline in sperm quality observed worldwide is indicative of various existing factors that exert a detrimental impact on male fertility (Jørgensen et al., 2001).

The results showed a significant increase (p<0.05) of IL-1B level in infertile patients infected with corona virus compared with non-infected infertile patients (Figure 1).

![Figure 1 The distribution of IL1B in infertile patients.](https://www.malque.pub/ojs/index.php/msj)
Epididymitis and orchitis can occur simultaneously in cases of viral infections (Fijak et al., 2018). The proximity of these two elements can result in undesirable outcomes such as reduced spermatozoa function, altered secretion of sex hormones, and dysregulated release of inflammatory cytokines. These outcomes have the potential to occur because of the existence of these two factors (Ma et al., 2020). It was discovered that four out of six patients had IgG depositions in their seminiferous epitheliums, which points to the possibility of an autoimmune response. It is feasible to postulate that the observed precipitation of IgG in the seminiferous tubules of these COVID-19 patients may be attributable to a secondary autoimmune reaction caused by the viral infection. This secondary autoimmune response is comparable to the autoimmune orchitis found in prior instances of SARS-CoV infection.

Furthermore, the infiltration of leukocytes may have adverse effects on the functionality of Leydig cells, leading to a reduction in testosterone production. This infiltration can also cause damage to the blood-testis barrier and destroy the seminiferous epithelium (Xu et al., 2006). The cumulative extent of damage, in conjunction with the presence of inflammatory cytokines, can trigger an autoimmune reaction characterized by the deposition of IgG within the tubules (Xu et al., 2006). Moreover, a previous study has documented the presence of IgG deposition in the seminiferous epithelium, interstitium, and vascular endothelium during experimental autoimmune orchitis (Itoh et al., 1991).

3.3. Effect IL-2 level in infertile men

The results showed a significant increase (p<0.05) of IL-2 level in infertile patients infected with coronavirus compared with non-infected infertile patients (Figure 2).

![Figure 2 The distribution of IL-2 in infertile patients.](image)

Interleukin-2 (IL-2) is primarily synthesized by T-cells upon activation. Elevated levels of interleukin-2 (IL-2) correlate with vascular leak syndrome (Krieg et al., 2010). Lung endothelial cells that express high-affinity IL-2, the receptor of IL-2, are believed to cause life-threatening pulmonary edema by inducing vascular permeability via IL-2 binding. The results of this study demonstrate a significant association between distinct cytokines and chemokines and the severity of the disease in both male and female individuals. In the male population, a positive correlation exists between the augmentation of a range of inflammatory cytokines and the severity of the disease. In this context, there appear to be alterations in the homeostasis of factors related to angiogenesis and endothelial function (Rafi-Janajreh et al., 1991).

3.4. Effect IL-15 level in infertile men

The results showed a significant increase (p<0.05) of IL-15 level in infertile patients infected with the coronavirus compared with non-infected infertile patients (Figure 3). Additionally, other comorbidities observed within this cohort are linked to modifications in sex hormones.

Individuals diagnosed with type II diabetes are more susceptible to experiencing lower levels of testosterone, although no significant impact on estradiol levels has been observed. Furthermore, advancing age may further compound this issue, as testosterone levels tend to decline as individuals age. This is noteworthy in the context of male patients diagnosed with COVID-19, as the median age of such patients is reported to be 62 years (Cheung et al., 2015). Furthermore, a notable percentage of male patients exhibited heightened levels of estradiol, which were found to be associated with obesity in certain cases. This observation aligns with prior findings indicating that individuals with obesity may exhibit altered ratios of...
testosterone to estradiol, which can be attributed to increased aromatase activity. Aromatase is an enzyme that is predominantly expressed in adipose tissues. (Jensen et al., 2004). It is noteworthy that there exists a correlation between heightened levels of estradiol in male patients and heightened levels of IL-type markers, which have been identified as a poor prognostic indicator in patients with COVID-19. (Tay et al., 2020).

![Image](https://www.malque.pub/ojs/index.php/msj)

**Figure 3** The distribution of IL-15 in infertile patients.

3.5. Correlation between testosterone and cytokine levels in male COVID-19 patients

The levels of testosterone that were evaluated in the sera and plasma of SARS-CoV-2 patients showed a positive connection between the level of hormone and IL-1B IL-15. While negatively related with IL-2, figure (4),(5),(6). The androgen receptor, often known as AR, is encoded on the X chromosome and exhibits broad expression across various cells and tissues. Androgen receptor (AR) exhibits a wide array of biological activities, playing crucial roles in the development and upkeep of various bodily systems, such as the hematopoietic, cardiovascular, reproductive, immune, musculoskeletal, and neural systems(Davey & Grossmann, 2016). Nuclear transcription factor AR binds to testosterone and dihydrotestosterone with a stronger affinity. Dihydrotestosterone is produced when the 5-alpha reductase converts testosterone, a prohormone. Once within the nucleus, the AR-testosterone/dihydrotestosterone complex binds to DNA’s androgen-response sites, activating multiple genes’ transcription. These genes include those required to establish immune responses against viruses and those involved in reproduction (Mills, 2014). The androgen receptor (AR) is expressed in male immune cells, and its functionality is primarily influenced by the levels of testosterone and dihydrotestosterone, which vary between men. Consequently, it appears improbable that individuals with insufficient levels of these crucial AR activating factors would be capable of mounting effective immune responses against viral infections. SARS-CoV-2 viral spike (S) protein attaches to the ACE2 receptors and engaging the cellular serine protease (TMPRSS2) for S protein priming, and also they both are existing in the testis (Hoffmann et al., 2020), raising the concern regarding infection of the testes and possible sexual transmission. ACE2 receptors are established at higher concentrations in the testes and confirmed the presence of ACE2, angiotensin (1-7), and its MAS receptors in the testicles, unambiguously in Leydig and Sertoli cells (Reis et al., 2010). These particular receptors are noted on developing sperm, production of testosterone, the male sex hormone. According to a recent study, it was observed that in 81 reproductive-aged men who have infected with COVID-19 the ratio of testosterone to luteinizing hormone was significantly reduced which was accompanied by C-reactive protein levels when compared to COVID-19 unaffected healthy individuals (Wu et al., 2019).

The outbreak of COVID-19 is quickly spreading worldwide. As of June 2020, there were over 8 million confirmed cases of COVID-19 worldwide, and the total number of deaths from this disease had exceeded 400,000. There are a rising concern by several studies on impact of COVID-19 upon male reproductive health. Based on the little evidence to date, it can be proposed that the possible pathogenicity and attack of COVID-19 on testicular tissues might affect testicular spermatozoa quality leading to poor male fertility. Special attention should be focused to evaluate an appropriate intervention in the young couples fertility throughout and after this outbreak, specifically for those infected with SARS-CoV-2 virus.

As is common of this family of viruses, binding of SARS-CoV-2 to its receptors ACE-2 and TMPRSS 2 that the virus fuse with the membrane and enter the cells, followed by translation and replication of the proteins. The replication complex, which makes more RNA of the SARS-CoV-2 are packaged in Golgi. SARS-CoV-2 release outside to spread to other cells. It is a matter of concern, evidence exists that correlated coronaviruses with severe orchitis. Whereas sperm counts can be diminished by high temperature alone, the question of other possible long-standing effects on male gametes is critical. Specially, whether the shedding of virus in some individuals, which may impact the safety and storage of gametes.
In conclusion, there is an excessive possibility of testicular damage and later infertility following COVID-19 infection. The probability of testicular damage might be caused by either direct viral invasion via binding of SARS-CoV-2 virus to ACE-2 receptors or secondary to immunological and inflammatory response. Therefore, follow-up studies of reproductive function of recovered male patients are necessary to investigate this possibility.

The levels of sex hormones in individuals infected with COVID-19 have also been investigated due to their direct effect on semen parameters. Male sex hormones vary dramatically with acute illness or physiological stress, so it is important to remember that these early results may be disputable.

Figure 4 The correlation between IL-1B and Testosterone of infertile men patients.

Figure 5 The correlation between IL-2 level and testosterone of infertile men patients.
4. Conclusions

This study demonstrates elevated concentrations of the cytokines IL1B and IL15 in males infertile with infected Covid 19 and lower concentrations of the cytokines IL-2 concentration in males infertile with infected Covid 19. Also, the results showed a positive correlation between IL-15 and IL-1B and Testosterone hormone and a negative correlation between IL-2 and Testosterone hormone. It is conceivable that altering the tolerance to sperm cells in both the male and female reproductive tracts and lowering the conditions that are favorable for fecundation and implantation could be accomplished by increasing pro-inflammatory cytokines, such as IL-1B, and decreasing immunosuppressive cytokines, such as IL-2. More study that considers both pro- and anti-inflammatory substances is necessary to determine how important the immunological balance of semen is to human fertility.

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Ethical considerations

The Ethical Committee of the Department of Laboratory Investigation, Science Faculty, University of Kufa, approved this research and cooperation with Al-zahra Hospital in Najaf -Iraq. Informed consent was obtained from all the participants.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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