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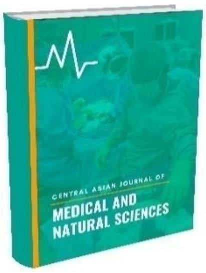

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Evaluation of Semen Characteristics and Interleukin 6 Levels (IL6) in Males Recovered from Covid-19

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Abstract: As of late, SARS-CoV-2 (coronavirus disease 2019, COVID-19) infections have spread around the world. Multiple organs can be affected by SARS-CoV-2, however the respiratory system is the primary target. Some people are worried COVID-19 may impair male processes for reproduction. In our investigation, semen samples from fifty males recovered from COVID-19 were collected for analysis of semen characteristics. All of the patient groups exhibited aberrant sperm quality. In addition, we compared the levels of interleukin 6 (IL6) between 50 COVID-19-recovered men of reproductive age and 50 age-matched controls. A higher serum Interleukin 6 (IL6) level were observed in patients group. The regression analysis indicated testosterone level was negatively associated with Interleukin 6 (IL6), while both luteinizing hormone (LH) and prolactin were showed positively correlation with Interleukin 6 (IL6).

Key words: COVID-19, analysis of sperm characteristics, Interleukin 6 (IL6), luteinizing hormone (LH), testosterone, and prolactin.

Introduction

A new coronavirus-associated infection (also known as coronavirus disease 2019, COVID-19) has swiftly spread and produced a global pandemic since its initial disclosure in December 2019. On June 15, 2020, more than 7,970,00 cases had been reported from over 200 countries or regions. Because of its extremely high sequence similarity (80%) with SARS-CoV, the beta-coronavirus responsible for COVID-19 has been classified as SARS-CoV-2 (1). However, indications of numerous organs or systems, such as cardiovascular, urinary, gastrointestinal, and liver diseases, have been recorded, in addition to the typical COVID-19 symptoms of fever, dry cough, exhaustion, and dyspnea (2).

SARS-CoV-2 acquires entry to host cells with the help of the cellular serine protease (TMPRSS2) and angiotensin-converting enzyme 2 (ACE2) (3). Single-cell RNA sequencing profiling of humanxtestes, that ACE20is mostly concentrated inxspermatogonia,xLeydig, and Sertolixcells (4). In a retrospective analysis of 1099 cases, it was determined that approximately 55% of COVID-19 patients were of reproductive age (15–49) and that nearly 60% of COVID-190 patients were male. Consequently, It was recently hypothesized that SARS-CoV-2 could impair male fertility (5).

It is debatable whether or not COVID-19 is present in human sperm. It should be emphasized that a viral febrile sickness may have numerous effects on male reproductive function. In addition to viral invasion and injury, Fever, inflammation, hypoxia, and medicines can all have an impact on the the hypothalamic-pituitary-gonadal (HPG) axis and the development of male sperm.

According to autopsies of six SARS-related fatalities, (6) found evidence of orchitis despite the absence of SARS virus in the testes. Therefore, to learn how COVID-19 affects fertility, it is necessary to assess testicular endocrine function and spermatogenesis.

In the current investigation, we evaluated the sperm parameters of 50 COVID-19-infected men of reproductive age with those of 50 uninfected men of the same age. Current research confirms that SARS-CoV-2 can impact male reproductive health. During and after recovering from COVID-19, patients exhibited anomalous sex hormones levels and a decline in sperm parameters. At the autopsy, a virus was also discovered in testicular tissue (7). To further assess This study provides essential clinical evidence concerning COVID-19's Impact on men's reproductive health.

Objective: Analyze the sperm parameters of a COVID-19-recovered male and evaluation of IL-6 and its effect on male reproductive system.

Material and Techniques

This research was evaluated and approved by the Medical Ethical Committee of the AL-Najaf Health Directorate/Ministry of Health/Iraq in Najaf Province. One hundred male patients were selected, all of age at conception (median age 33, range 25-50). admitted to the Fertility Center's laboratory in AL-Sader Medical city AL-Najaf Health Directorate between September 1, 2021 and January 31, 2022 for semen characteristics analysis. Patients were recruited on a continuous basis as long as they met the inclusion criteria by using a strategy of convenient sampling. The blood draws were part of a standard medical procedure. We measured angiotensin-converting enzyme 2 (ACE2) in the leftover blood from our lab tests. As a result of the fact that the leftover specimens of serum were thrown away as biohazardous waste and the method did not impose any additional burdens or cause any damage to the patients, there was no additional burden or risk associated with the procedure. Patients and controls were comprised of males who underwent sex hormone testing and sperm analysis prior to marriage as part of a fertility assessment or when their spouses intended to get pregnant. The control group was comprised of males who underwent sex hormone testing and sperm analysis as component of a test to determine fertility prior to marriage or when their individuals intended to conceive. 100 age-matched males ((median age 33 years, range 25-50)) were chosen at random and their sperm characteristics were recorded. Between September 1 and January 31, 2022. The patient group was cured of covid-19 and had children prior to infection, whereas the control group. was. uninfected. and. fruitful.. Each. participant provided verbal consentxafterxbeing. informed.

After 2 to 7 days of abstinence, participants provided a sperm sample that was analyzed within one hour of ejaculating. Certified andrologists assessed the semenxvolume, spermxconcentration, spermxmotility, and totalxmotile sperm count in accordance with World Health Organization (6th Edition) criteria. Progressive sperm (PR) at 32% and non-progressive sperm (NR) at 40% denote low motility. Calculating IL-6 concentrations in the blood was evaluated with an enzyme-linked immunosorbent test (ELISA), according the manufacturer's instructions (BT Lab Inc., Shanghai, China). According to the company's test manual and reagent description, the normal range was 20–320 ng/L. The socioeconomic status, multiple medical conditions, symptoms, diagnostic tests, and adverse events of each patient have been documented.

Exclusion criteria:

Excluded from this study are a patient's historyxof varicocele, cryptorchidismx, xcongenitalxdisorders, ximmunologicalxandxinflammatory diseases, hormonexdisruptions, diabetes, alcoholxabuse, and smokingxif he is (<20 __>55 years old.

Collection of blood

This study's blood samples were obtained from males recovered from COVID-19 by drawing 5 ml of blood and placing it in a clot activator tube (8). Blood samples were centrifuged (5000 rpm) for 5 minutes to separate serum after being placed in a clot activator tube and left at room temperature for 30 minutes to coagulate the blood. The serum was withdrawn using a micropipette, deposited in six Eppendorf containers, and frozen at -20 degrees (9).

Result and Discussion

Semen paramters of COVID-19-recovered patients

In the morning, 50 recovered patients' sperm samples were collected. Table 1 displays the sperm characteristics of each patient and control. The parameters of sperm were evaluated between COVID-19 patients and healthy controls (Table 1) (Figure 2). In contrast to the control group, at least one of the 50 patients had an aberrant value for at a minimum one semen parameter as defined by WHO. Patients had a substantially reduced overall average sperm count per ejaculate and average sperm concentration than controls (P= < 0.05). In contrast to control group, dramatically reduced average of motile and progressive mobility sperm were observed in case group (P <0.001), normal morphology (P= <0.001), and a higher ratio of oligospermia (P= <0.05). Patients had considerably higher ratios of oligospermia than controls (P <0.05), normal morphology comparison to healthy group (P = <0.001), and drastically reduced rates of motile and progressing motile spermatozoa than controls (P = <0.001). While all of the males in the control group had normal average of progressively motile or motile sperm, all of the patients had percentages below the WHO standard levels. Significant, statistically meaningful differences existed between patients and controls in the percentage of men with oligospermia and the percentage of men with aberrant sperm morphology.

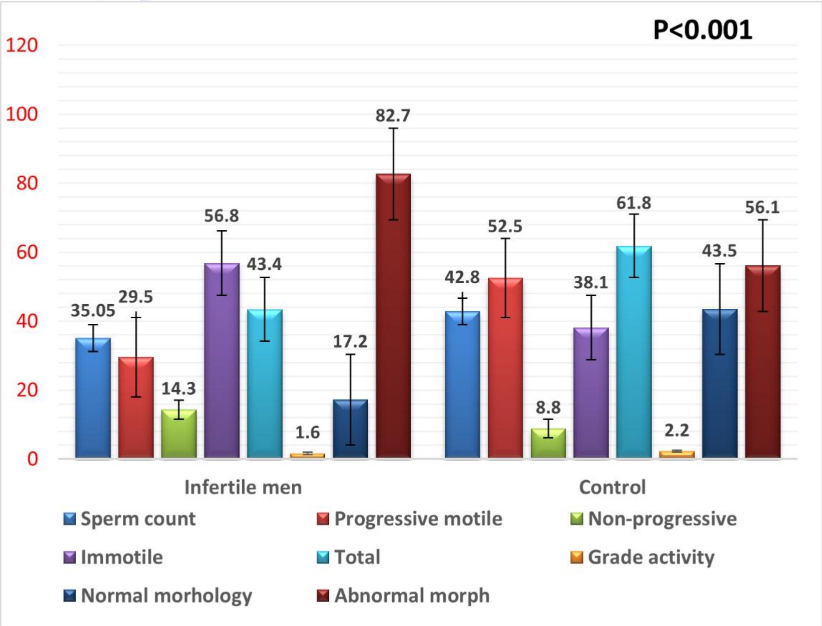

Figure (1): semen parameters comparison among infertile and control group.

Table (1): semen parameters comparison among infertile and control groups

***: a**: significant at <0.05**; b**: highly significant**.**

Semen parameters		Infertile males Mean ± SD* (N.50)	Control Mean ± SD (N.50)	P-value
Sperm count (million/ml)		± 12.6 35.05 [a]	± 20.8 42.8	P: <0.05
Motility (%)	Progressive	± 6.3 29.5 [b]	± 15.4 52.5	P: <0.001
	Non-progressive	± 6.1 14.3 [b]	± 4.5 8.8	P: <0.001
	Immotile	± 22.4 56.8 [b]	± 15.7 38.1	P: <0.001
	Total	± 13.1 43.4 [b]	± 16.4 61.8	P: <0.001
Grade activity		± 0.6 1.6 [b]	± 0.5 2.2	P: <0.001
Morphology	Normal	± 4.4 17.2 [b]	± 7.9 43.5	P: <0.001
	Abnormal	± 14.4 82.7 [b]	± 20.2 56.1	P: <0.001

IL-6 levels in patients recovered from COVID-19.

This study divided individuals into patient and control groups based on the typical range of blood IL-6 values (20–320 ng/mL). The median blood IL-6 concentration in the patient group was 79.1 ng/L, compared to 55.9 ng/L for the healthy group. Patients with increased serum IL-6 levels were more obese when compared to patients with normal serum IL-6 levels, when compared to the healthy group, the IL-6 group showed considerably greater levels (P <0.001).

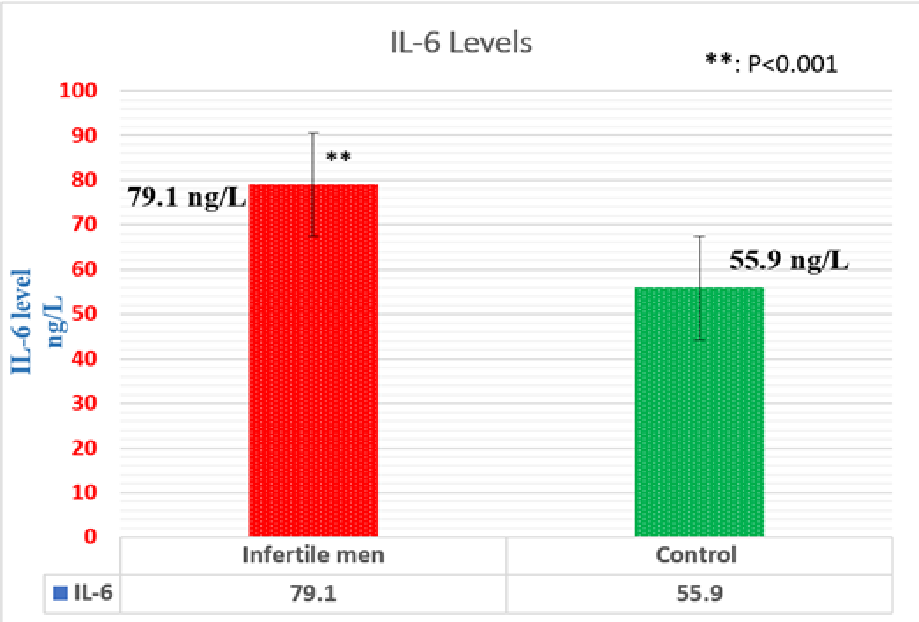

Figure (2): Level of IL6 among infertile and control group.

In our study, the patient group had significantly increased IL-6 levels compared to the fertile group. This result was consistent with previous findings that COVID-19 patients had elevated IL-6 levels (61.9 ng/L) (10). Similarly, (11) discovered that the patient group had 100 ng/L more IL-6 than the control group. Laboratory results revealed that the levels of IL-6 (10 ng/ml) between the two groups were significantly different (12). (13) Compared to a control group, Elevated levels of IL-6 were found in the blood samples of 102 COVID-19 patients in the hospital with moderate, severe, and critical illnesses.xPatients with severexCOVID-190infection hadxplasma levelsoof cytokinesxsuch IL-20, IL-60, IL-70, IL-100, Tumor necrosis factor, and monocyte chemoattractant protein-1(MCP-1) that had been much higher than those of healthy controls (11,14). (15) suggest that serum IL-6 levels may serve as a crucial indicator of the progression and severity of COVID-19. According to the above, IL-6 measurement could be used as a swift method for patient identification. with a greater likelihood of disease progression.

Discussion

In the research, the mean sperm count of the infertile group was 35.05 percent. compatible with other research., including (16,17), in which the mean sperm count in patients was 30.63 %, and (18), in which the mean sperm count was 39.2 %. together with Current research confirms that COVID-19 can impact male reproductive health. During and after recuperation from COVID-19, Patients exhibited a decline in the quality of their sperm and aberrant levels of sexual hormones. Consequently, the testis may be an infection site for COVID-19, which could have a negative impact on fertility in men. Furthermore, once the virus is found in sperm, SARS-CoV-2 can infect sperm that express ACE2 (7).

Both SARS-CoV and SARS-CoV-2 use the ACE2 receptor and the viral spike protein, which share 80% of their genetic sequences, to infect cells. Protein S, transmembrane serine protease 2 (TMPRSS2) is responsible for activating the spike protein. Cells of the male reproductive system express both the ACE 2 receptor and the TMPRSS2 protease, these cells include spermatogonia, Sertoli, and Leydig cells, as well as prostatic epithelial cells (19).

Patients under the age of 30 may have a higher risk of spermatogenesis, which is essential for male reproductive health, as evidenced by the higher concentration of ACE2 expression found in young male gonads (20), this highlights the importance of the current investigation. As shown in table (4.2) that sperm motility is divided into three categories: progressive, non-progressive, and immotile. The percentage for progressive (PR) sperms is 29.5% with (P value < 0.001), the average for non-progressive (NPR) sperms is 14.3% with (P value < 0.001), the percentage for immotile sperms is 56.8% with (P < value 0.001), and the percentage for total motile sperms is 73.3% with (P value < 0.001). It is consistent with previous research., such as (16), in which the average percentage for progressive (PR) rate was 22.38% and the mean total motility rate was 33.49 % in patients, and (18), in which the average percentage for progressive (PR) rate was 37.1% and the mean total motility rate was 44.4% in patients. Ruan et al. (2021) discovered that COVID-19 patients in recovery had considerably decreased total sperm count and total motility in semen analysis compared to healthy controls, considering the reality that most of the patient's semen parameters were still within the WHO-recommended range. There are statistically significant differences between normal morphology of infertile patients (17.2 ± 4.4) and controls (43.5 ± 7.9), with a (P value < 0.001) figure (4.2), and between abnormal morphology of infertile patients (82.7 ± 14.4) and controls (56.1 ± 20.2), with a (P value < 0.001) figure (4.2). It is consistent with other studies, such as (18) study, in which the mean number of recovered patients with anomalous morphology was 94.0. On the basis of the patients' sperm analysis, in our study, the percentages of sperm count, normal morphology, and total motility decreased significantly. There was a statistically remarkable difference among the patient and the controls in terms of count of sperm, overall motility, progressive motility, and morphology. Additionally, there were statistically significant rises in the percentages of spermatozoa with abnormal morphology and immotility, indicating that sperm quality may require additional time to recover or that these individuals had low sperm mobility and high abnormal morphology before contracting. Human spermatogenic stages are thought to be around 74 days in duration (21), these findings suggest that COVID-19's aftereffects may require additional time to recover.

All seven types of ACEs are present in spermatocytes, in addition, it has recently been shown that sperm express receptors for types 1 and 2 of angiotensin II, indicating that male reproductive cells may be an infectious COVID-19 host (22,23). In this case, it is predicted that SARS-CoV-2 infection will affect ACE2 activity, Recent data shows that COVID-19 infection directly impacts sperm of males, since sperm have been shown to contain the type 1 (AT1R) and type 2 (AT2R) angiotensin II receptors, these receptors are present in sperm (24). This may cause sperm senescence and acrosomal exocytosis (25). Moreover, by activating AT1R and AT2R, angiotensin II may have an impact on sperm fertility and motility (26). RNA from SARS-CoV-2 has not been identified in sperm specimens from cured or new cases of infection, patients with a moderate infection were found to have substantially reduced quality of sperm (measured by sperm count and sperm motility), men who had survived from a moderate illness and those in the healthy group (27).

Similarly, (28) Four individuals (33.3%) were found to have poor sperm quality, exhibiting lower sperm concentrationxand motilityxas well as an elevated DNAxfragmentation index. Examining sperm parameters, we discovered the fact that progressive sperm motility decreased subsequent to COVID-19 treatment compared to prior to the COVID-19 infection, whereas immotility increased. Our findings indicate that COVID-19 impairs sperm motility (17).

In our study, Males with infertility had a significantly higher BMI than fertile males. Obesity has multiple effects on male fertility, including a fat-related a lack of testosterone, decreased spermatogenesis, and erectile dysfunction (29). In fact, COVID-19 erious cases there is a rise in IL-6 levels is correlated with an increase in viral dose (11). The levels of interleukin (IL)-6 are significantly higher in individuals with severe COVID-19 relative to the comparison group ($P < 0.0001$) (30). IL-6 concentrations in seminal fluid are correlated with Sertoli cell secretory activity (31). In other studies (32,33), Blood-testis barrier (BTB) disruption by IL-6 was implicated in the pathogenesis of autoimmunity-related orchitis.

According to (34), if a human body is harmed or becomes infected, the cytokine IL-60plays axrole in stability, alleviates acute tension. Excessivexproduction of IL-60is a significant contributor to the onset and progression of acute and chronic inflammatory disorders, once stress is removed from the body. Multiple research studies show Somatic testicular cells (Sertoli, Leydig, and peritubular cells) secrete high amounts of cytokines like interleukin-10(IL-1) and interleukin-60(IL-6) under typical Conditions; these cytokines are essential for spermatogenesis and sperm development (35). Li and colleagues also observed 248 samples of semen from COVID-19 inpatients, which demonstrated a decrease in sperm concentration in patients with elevated levels of interleukin-6 compared to healthy controls (36). According to (37), cytokines can affect the functions of Sertoli cells, and their altered levels can compromise spermatogenesis.

Severe intimal proliferation is associated with Human vascular endothelial cells release interleukin-6 in response to rejection-induced inflammation (38). Leydig and Sertoli cells are the principal generators of cytokines, while integral testicular somatic cells also produce some (IL-1, IL-6) (39), They can influence fertility control and reproductive physiology in general. Sperm include immune cells, which include those responsible for the production of tumor necrosis factor (TNF-), interleukins, interferon (IFN-), and some of its soluble receptors, TNF- is also secreted by mesenchymal cells, Sertoli cells, and spermatogonia. Consequently, it's possible that human sperm include cytokines like IL-6, IL-8, and TNF in a typical amount (40). Increased in dyspermia cases and in inverse association with spermiogram parameters, (39), IL-6 regulates spermatogenesis. By activating TLR2 receptors on LC, inflammatory mediators diminish testosterone production (41). In general, Increasedxlevels of IL-60,xTNF-, and IL-12, as well as decreasedxlevels of serum testosterone, are hallmarks of aging (42).

Conclusions:

The inflammatory cytokine IL6 in men who recovered from Covid-19 causes a decline in spermatogenesis in Sertoli and Leydig cells. These results suggest that COVID-19 infection may downregulate spermatogenesis in males via direct damage of testicular function. Further studies are warranted in order to fully elicit the molecular mechanisms and interactions of these molecules on male spermatogenesis.

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