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Analysis of semen parameters of recovered patients after COVID-19

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Abstract

Aim: The COVID-19 has spread rapidly around the world. Apart from primarily targeting the lungs and causing acute respiratory distress syndrome with severe respiratory symptoms and high risk of death, the disease can also cause damage to other organs such as the heart, kidney, and testis. Studies have shown that men are more likely to get infected by the disease than women and the death rate due to the disease is found to be higher in men. We aimed to show the effects of COVID-19 on sperm parameters.

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DOI: 10.5455/annalsmedres.2022.10.317 Materials and Methods: In the study, semen analyzes of COVID-19 patients and healthy control groups were compared according to the presence or absence of PCRconfirmed SARS-CoV-2 positivity in hospital records. Patients with COVID-19 (+) were the patients who had mild disease. A total of 510 participants, 53 COVID-19 patients and 457 healthy control groups. After positivity confirmation of recovered COVID-19 patients using PCR, semen analysis was performed after an average of 157 (24-355) days. Also, in order to evaluate the short-term and long-term effects of the disease, patients were grouped as those with less than 90 days and more than 90 days after receiving the positive PCR result.

Results: While the mean progressive motility of sperm was $41.45\pm(19.21)$ in the recovered COVID-19 patients, it was $38.35 \pm (21.74)$ in the control group. The mean total motility was $55.74 \pm (19.34)$ in recovered COVID-19 patients, where as it was $51.84 \pm (22.45)$ in control group. The percentage of normal sperm morphology was $11.45\pm(5.97)$ in recovered COVID-19 patients, however it was $11.19\pm(8.08)$ in control group. The sperm concentration was found to be $68.60\pm(56.22) \ge 10^6$ /ml in recovered COVID-19 patients, but it was $74.10\pm(61.72) \ge 10^6$ /ml in control group. Finally, the mean sperm immobility was $44.26 \pm (19.34)$ in recovered COVID-19 group, however it was $48.16 \pm (22.45)$ in control group.

Conclusion: In the study, when the semen analysis results of patients who recovered after COVID-19 and healthy control groups were compared in terms of semen parameters according to the recovery time period, no statistically significant difference was found. More studies are needed to fully understand the effects of COVID-19 on the reproductive system.

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Introduction

Coronavirus disease 2019 (COVID-19), which is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is known to have symptoms such as shortness of breath, cough, fever, gastrointestinal disturbances, fatigue and myalgia. In the course of the infection, about 80% of the cases are known to be mild, 5% are known to be critical, whereas 3% of the cases are known to be fatal. The main risk factors include advanced age, obesity, hypertension, diabetes and chronic respiratory diseases [1,2].

SARS-CoV-2 uses serine protease transmembrane protease serine 2 (TMPRSS2) for preparing the Spike (S) protein.

SARS-CoV-2 is an enveloped virus containing singlestranded (ss) and positive-sense ribonucleic acid (RNA). It is a member of β -coronavirus subgroup. It includes nucleocapsid (N) protein, envelope (E) proteins, membrane (M) proteins that aid viral assembly and surface spine (S) proteins. S glycoproteins are the largest structures that are essential for the virus to enter the host cell. They consist of two important subunits, S1 and S2, respectively. Using S1 and S2 subunits, the virus binds to the cell membrane of host cell and fuses to the target cell membrane, respectively [3,4].

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Angiotensin converting enzyme (ACE2) receptor is used by SARS-CoV-2 in order to enter the host cell. The ACE2 receptor plays a vital role in COVID-19 pathogenesis causing damage to host cell. Therefore, it can be stated that cells with high ACE2 expression are more susceptible to SARS-CoV-2. ACE2 receptors are found in the liver, kidney, testes, myocardium, gastrointestinal tract and especially in the lungs, which are the main targets of the disease [5,6].

Males have high levels of TMPRSS2 and ACE2 expression as compared to females. ACE2 is expressed in spermatogonia, Leydig and Sertoli cells, while TMPRSS2 is expressed in germ and somatic cells [5,7]. Furthermore; due to inflammatory responses, cytokine storm, high fever, increased oxidative stress or side effects of drugs used during treatment, DNA fragmentation and defective human sperm function may occur and spermatogenesis may be paired in patients infected with SARS-CoV-2, thus leading to a lower fertility rate in females or infertility in males [8-10].

The infection rate of SARS-CoV-2 varies according to age groups and gender. Males have a higher prevalence of the disease than females. Hospitalization and death rates in intensive care due to COVID-19 have been shown to be higher in males. Gender-related predisposition is attributed to genetic, immunological and hormonal differences [11,12]. Testicular expression of ACE2 varies with age. With increasing age, low expression levels have been reported. Young male patients are found to be more likely to experience testicular damage due to COVID-19 than older male patients [2,13].

Direct and indirect as well as short-term and long-term effects of COVID-19 on male fertility are inevitable. In this study, the aim was to evaluate effects of SARS-CoV-2 on semen parameters of the patients who applied to the andrology laboratory of our hospital during the COVID-19 pandemic, by examining the semen parameters of the control and patient groups retrospectively according to the COVID positivity confirmed by PCR.

Materials and Methods

For this study, approvals were obtained from the Scientific Research Platform of the Ministry of Health (Decision No: $2020-12-17T09_{16}_{00}$, Dated: 20/12/2020) and Malatya Clinical Research Ethics Committee (Decision No: 2021/01, Dated: 29/12/2020).

The study included 510 male patients admitted to Malatya Training and Research Hospital between January, 2021 and July, 2021 with a mean age of 32.1 (17-68), and were in stable clinical condition at the time of the study. After 3 days of sexual abstinence, semen samples were taken by masturbation and were kept in sterile containers, and semen analyzes were performed within one hour. All semen analyzes were performed in accordance with the sixth edition of the WHO Manual for the Laboratory Examination and Processing of Human Semen, 2021.

The patients, who were younger than 17 years of age, had a chronic disease and used drugs that could affect semen parameters, had undergone any urogenital operation, had azoospermia and whose sperm parameters could not be measured by the device and the patients, who had received COVID-19 treatment in the hospital, were excluded from the study. The study was conducted with 457 healthy control participants and 53 patients who were tested positive for real-time reverse transcriptase polymerase chain reaction (rRT-PCR) in pharyngeal swab samples and had mild disease without hospitalization. Semen analyzes were performed in all participants.

PCR and semen analysis performed after an average of 156 (24-355) days confirmed the positivity of recovered patients. After determining the PCR (+), the patients were grouped as those with less than 90 days (n=10) and those with more than 90 days (n=43) of recovery time, so that the short-term and long-term effects of the disease could be evaluated. The semen analysis included sperm concentration, sperm motility (progressive motility, total motility and immobility), semen volume and percentage of normal morphology.

Statistical analysis

In this study, the data were analyzed using the open-source statistical software Jamovi v.1.6.9 (https://www.jamovi.org.). Kolmogorov Smirnov test was used as normal distribution test. In the analyses, Kruskal Wallis and Mann Whitney U tests were also used. The descriptive statistics were number and percentage in categorical variables, and average, standard deviation, minimum, and maximum value in numerical variables. Non-parametric tests were used in the comparisons of the independent groups, since the numerical variables did not provide a normal distribution condition. The Mann-Whitney U-test was used in the comparison of two independent groups, while the Kruskal–Wallis test was used for comparisons of more than two independent groups. P value smaller than 0.05 (P<0.05) was considered to be significant.

Results

Mean age of 510 patients who underwent semen analysis in the andrology laboratory of our hospital was found to be $32.10\pm(7.51)$ (17-68), while the mean age of the healthy control group was found to be $32.06\pm(7.37)$. The mean age of recovered COVID-19 patients was determined as $32.42\pm(8.74)$ (Table 1).

Oligozoospermia refers to sperm concentrations below established reference limits (e.g. 16 million/ml, 95% confidence interval 15–18 million/ml; WHO 2021). Of all participants whose semen were analyzed, 65 (sperm concentration below 16 million/ml) had oligozoospermia. Of the participants with oligozoospermia, 59 (14.2%) were found to be in the healthy control group and 6 (11.32%)were in the recovered after COVID-19 group. The mean semen volume of all patients participating in the study was $2.85\pm(0.77)$ ml. The mean progressive motility (PR) and the mean total motility (PR+NP) were found to be $38.68 \pm (21.50)$ and $52.24 \pm (22.16)$, respectively. The percentage of normal morphology was $11.22 \pm (7.88)$, and the sperm concentration was 73.52 ± 61.14) x 10^6 /ml. Sperm immobility was found to have an average of 47.76(22.16%). The sperm concentration, sperm motility (total motility,

Table 1. Semen parameters of recovered COVID-19 patients and control group.

	Total	Control	Recovered COVID-19 patients	P value	
Number of patients for whom sperm analysis was performed	510	457	53		
	X±SD or n (colon %)				
The average age (17-68)	32.10 (7.51)	32.06 (7.37)	32.42 (8.74)	.992	
Semen volume (mL)	2.85 (0.77) (0.5-8)	2.85 (0.77) (0.5-8)	2.83 (0.76) (1-5)	.882	
Progressive motility (PR, %)	38.68 (21.50)	38.35 (21.74)	41.45 (19.21)	.161	
Total motility (PR+NP, %)	52.24 (22.16)	51.84 (22.45)	55.74 (19.34)	.132	
Normal morphology, %	11.22 (7.88)	11.19 (8.08)	11.45 (5.97)	.170	
Sperm concentration, x10 ⁶ /ml	73.52 (61.14)	74.10 (61.72)	68.60 (56.22)	.595	
Immotility (IM, %)	47.76 (22.16)	48.16 (22.45)	44.26 (19.34)	.132	

 Table 2. Comparison of semen results by time interval after recovery.

Semen characteristics				
	Recovery< 90 days n=10	Recovery>90 days n=43	Control n=457	P value
Age, years	33.90 (9.94)	32.07 (8.53)	32.06 (7.37)	.944
Volume, mL	2.95 (1.01)	2.80 (0.70)	2.85 (0.77)	.956
Sperm concentration, x10 ⁶ /ml	63.05 (51.80)	69.89 (57.70)	74.10 (61.72)	.840
Progressive motility (PR, %)	38.30 (18.33)	42.19 (19.55)	38.35 (21.74)	.320
Total motility (PR+NP, %)	56.60 (19.53)	55.53 (19.52)	51.84 (22.45)	.320
Immotility (IM, %)	43.40 (19.53)	44.47 (19.52)	48.16 (22.45)	.320
Normal morphology, %	9.80 (5.05)	11.84 (6.15)	11.19 (8.08)	.299

progressive motility and immobility), semen volume and percentage of normal morphology values for the control group and who recovered after COVID-19 are shown in Table 1.

When the semen parameters of the recovered COVID-19 patients and the participants in the control group were compared, no statistically significant difference was found. Although not statistically significant, it was observed that there was a slight increase in normal morphology percentage, total motility and progressive motility parameters of the patients who recovered from COVID-19. However, as shown in Table 1, a slight decrease in sperm concentration and immobility levels was observed.

After the PCR (+) results, 53 patients who recovered from COVID-19 were divided as those with less than 90 days of recovery time (n=10) and those with more than 90 days of recovery time (n=43). Age, sperm concentration, progressive motility, semen volume, immobility, total motility, and sperm percentages of control group and the patient group were compared according to the recovery period. There was no statistically significant difference between the groups (Table 2).

Our results, although not statistically significant, showed an increased trend in the percentage of sperm concentration, progressive motility, immobility and normal sperm morphology in patients with more than 90 days (43) of recovery time when compared to patients with less than 90 days of (10) recovery time. Furthermore, semen volume and total motility also decreased relatively (Table 2).

Discussion

For more than two years, COVID-19 has threatened people all over the world, regardless of class from the very first day. Pandemic that caused more than 6 million deaths worldwide continues to affect people socially, economically, physiologically and psychologically. Within the scope of combating the virus, countries have implemented different strategies such as isolation, social distancing, mask usage, monitoring of infected cases, rapid tests and vaccine development studies.

Apart from causing respiratory diseases and affecting the lungs, the virus, which has become a major pandemic threat worldwide, causes histopathological or functional changes in the male genital system [5]. Many viruses such as mumps virus, HIV, HSV and Zika virus are known to have harmful effects on male fertility. In addition, previous studies have shown that SARS having a clinical picture similar to COVID-19, may cause orchitis and focal testicular atrophy [14,15].

Infertility, affecting approximately 10% of the world's population, can be defined as the inability to achieve clinical pregnancy despite having regular unprotected sexual intercourse for 12 months or more. Semen analysis of the individual is extremely important in the evaluation of male fertility potential [16]. As COVID-19 infection causing devastating health problems all over the world is more serious and fatal in males, it is extremely important to fully understand its possible effects on the male reproductive system [17].

ACE2 and TMPRSS2 play important roles in the entry of SARS-Co-V-2 into the host cell. After entering host cell,

the virus down-regulates ACE2 expression, causing excessive angiotensin production, thus increasing reactive oxygen species and causing apoptosis. Excessive production of infection-induced cytokines may also affect spermatogenesis negatively by increasing the leukocyte infiltration and autoimmune response in the testis. COVID-19 infection may pose a potential risk to male fertility, including semen parameters, spermatogenesis, testicular endocrine function and erectile function [2,18,19]. Antiviral drugs, which are frequently used in the treatment of COVID-19, are also shown to decrease testosterone, impair spermatogenesis, decrease sperm count and cause abnormalities in semen parameters [2, 9]. High fever caused by COVID-19 may impair spermatogenesis. Therefore, semen parameters may tend to decline between 72 and 90 days after infection [10].

In a study conducted on males at their reproductive ages in Iran, Maleki and Tartibian compared the data of patients infected with COVID-19 and healthy control group. After COVID-19 positivity, markers of oxidative stress and inflammation, apoptotic variables, seminal ACE2 activity and semen quality parameters were measured throughout the study at 10-day intervals. As a result of previously conducted studies, it was observed that semen quality parameters in patients with COVID-19 deteriorated at an early period after infection and remained significantly low during subsequent measurements. According to this study, the disturbances observed in semen quality parameters were thought to be caused by the deterioration in seminal inflammatory and redox responses caused by COVID-19 infection [20].

In our study, the semen parameters of our patients were measured 24-355 days after the patient received the PCR (+) result. We had data of 10 patients with less than 90 days of recovery time. All the patients in our study were mild COVID-19 patients. After receiving the PCR (+)result, patients with more than 90 days of recovery time showed an increased trend in progressive motility, sperm concentration and normal sperm morphology percentage as compared to patients with less than 90 days of recovery time. Although the difference was not statistically significant in our study, it was noticed that the sperm parameters improved over time. In this study, the ability to clearly see COVID-19 effect on sperm parameters may have been limited due to the fact that all the recovered patients had mild disease and the sample size was small.

In a study conducted by Ma et al., semen samples were taken from 12 patients for analysis of semen parameters and SARS-CoV-2 virus detection, and it was observed that 8 of the patients had normal sperm characteristics, whereas semen quality decreased in 4 patients. According to the study, the total motile sperm count of two of the three patients whose sperm parameters were compared before and after COVID-19 was slightly decreased when compared with previous records [21].

In a study performed by Ruan et al, data of 74 patients aged between 20-50 years who recovered from SARS-CoV-2 and had the disease at different intensities were compared with that of a healthy control group. In the comparison, a decrease in total sperm count, sperm concentration and total motility was observed. Patients having recovery time longer than 90 days showed worse sperm quality than those with shorter than 90 days [22].

In a study performed on sperm analysis of 43 patients who recovered after COVID-19 in Italy, Gacci et al. showed that as the severity of the disease increased (patients hospitalized, not hospitalized, treated in intensive care), the total sperm count decreased. In addition, according to the study, young and sexually active males recovering from COVID-19 were found to be at serious risk in terms of oligo- cryptozoospermia development [23].

There were 5 patients with azoospermia in total. Of the patients, 4 (5.97%) recovered after COVID-19, whereas one (0.19%) was in the healthy control group. However, in order to conduct our study in the best possible way, patients with azoospermia were not included in the study. Considering the patients with azoospermia, the rate was found to be very high in patients who recovered after COVID-19. Our patients had not received COVID-19 treatment at the hospital and had a mild illness. Therefore, pre- and post-COVID-19 data of our patients were not available.

In another study by Temiz et al. including 30 patients and a control group before and after COVID-19 treatment, sperm morphology was found to be the lowest in COVID-19 patients among the semen parameters they compared. According to them, this difference might be related to acute patient stress. COVID-19 along with its treatment did not have a specific devastating effect on male sexual health in a short time [24].

In a study comparing sperm parameters of mild and moderate COVID-19 patients and control group, Holtmann et al. reported that the patients with moderate COVID-19 infection had a statistically significant decrease in sperm quality when compared with control group and patients who recovered from mild infection [25]. In this study, only patients with mild disease were included and no statistically significant results could be obtained.

Pazir et al. compared both pre-semen and post-semen parameters of 24 participants who had recovered from mild COVID-19 and showed that total motile sperm count and total motility were significantly decreased in patients having mild history of COVID-19 [18]. In our study; although not statistically significant, it was observed that the sperm parameters of the patients with more than 90 days (43) of recovery time after receiving PCR (+) result had a slight improvement in overall sperm parameters as compared to the patients with less than 90 days of recovery time. As time elapsed after COVID-19 positivity, sperm parameters tended to improve.

In their study on 69 patients, Erbay et al. compared pre- and post-COVID-19 spermiogram samples, and they showed that sperm motility and viability in the group with mild disease and all semen parameters in the group with moderate disease were statistically significantly decreased [26].

The researchers conducted so far have many shortcomings such as small sample size, lack of a control group, lack of COVID-19 values of patients, heterogeneity of the population, uncertainty regarding the smoking habits of the patients, the absence of patient groups with different intensities of the disease, patients with chronic diseases and not excluding the patients with drug use affecting sperm parameters. When the studies in the literature are evaluated, it is observed that COVID-19 affects male fertility negatively by decreasing sperm parameters.

Lack of pre-COVID-19 values for the same patient and the small sample size are the limitations of this study.

Fewer studies have been performed regarding COVID-19 effect on sperm. Many variables need to be considered in the evaluation of male fertility. More studies are required to fully understand long-term effects of infection on the male reproductive system in individuals infected by COVID-19.

Ethics approval

Approvals were obtained from the Scientific Research Platform of the Ministry of Health (Decision No: 2020-12-17T09_16_00, Dated: 20/12/2020) and Malatya Clinical Research Ethics Committee (Decision No: 2021/01, Dated: 29/12/2020) for this study.

Conflict of interest

The authors have no conflicts of interest to declare.

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