The temporary impact of COVID-19 on semen deoxyribonucleic acid fragmentation

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Introduction Coronavirus disease 2019 (COVID-19) is characterized by impact on different systems of human body. Recently, several anti-COVID vaccines have been developed.

Material and methods In our study, we included two groups of males: GROUP1, anti-COVID vaccinated males, n = 46, and GROUP2, n = 43, non-vaccinated males, who all fell ill with the Coronavirus infection. A level of semen DNA fragmentation was characterized by Sperm DNA Fragmentation Index (SDFI) that was calculated before infection and compared with data at every month after laboratory recovery. The Mann–Whitney test was used to establish differences between parameters, with $p < 0.05$ considered significant.

Results Compared with the pre-COVID baseline we registered significant increasing of SDFI in each group of participants: 35.3 ±4.7% vs 18.6 ±5.8% in GROUP1, p = 0.0009, and 41.8 ±5.6% vs 19.2 ±6.1% in GROUP2, p = 0.0006. At the 2nd month after recovery SDFI in GROUP1 and GROUP2 continued to grow and reached its peak to 40.6 ±6.4% and 49.7 ±7.2% respectively. Thereafter SDF indexes in both Groups started to decrease, normalizing at the 7th month after COVID-19 recovery in GROUP1 and at the 9th month in GROUP2.

Conclusions COVID-19 causes a gradual increase in semen DNA fragmentation, which peaks at the 2nd month after recovery and is more pronounced in unvaccinated men. Normalization of SDFI occurs no earlier than at the 7th month in vaccinated and at the 9th month in non-vaccinated men.

Key Words: COVID-19 ‹› semen DNA fragmentation ‹› vaccination ‹› fertility ‹› spermatozoa

INTRODUCTION

In the beginning of 2020 AD a global pandemic of Coronavirus disease 2019 (COVID-19) has begun. Severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) was caused by single-stranded RNA virus that belongs to the Sarbecovirus subgenus of the Coronaviridae family, and is the 7th Coronavirus which able to infect humans [1].

The main COVID-19 symptoms were considered as: fever, cough, loss of taste and/or smell and tiredness. These symptoms may be combined with sore throat, headache/muscle aches, diarrhea, red or irritated eyes or rush on skin. The most serious, life-threating COVID-19 signs are: difficulty to breath, chest pain, confusion, problems of mobility and speech [2].

More COVID-19 patients presenting as much as 12 months later with strokes or heart attacks, and there is an increased risk of dementia or development of type 1 diabetes [3]. Different anti-COVID vaccines have been developed recently with proved clinical efficacy [4]. However, a part of individuals do not wish undergone vaccination because of reluctance or medical contraindications [5].

It has been previously suggested that persistent fever during viral infection may destroy the blood-testis barrier with invasion of virus into male reproductive system [6]. But, there is no evidence of presence

SARS-CoV-2 RNA in semen after COVID-19 infection [7, 8, 9].

Sperm Deoxyribonucleic acid (DNA) integrity is important for fertilization and development of healthy fetus. Sperm DNA may be damaged by different environmental/lifestyle influence like heat, toxins, tobacco/drug use, alcohol, mobile phones radiation etc. or endogenous factors like varicocele or different infections including COVID [10, 11]. A large number of reports show the direct correlation between sperm DNA damage and male infertility as well as pregnancy outcomes. The high levels of sperm DNA fragmentation (SDF) negatively impact the outcomes of both natural and assisted reproduction. Chen et al. analyzed the results of 10 articles and demonstrated that high SDF levels were associated with significantly lower pregnancy and delivery rates [12]. Recently it has been noted that SARS-CoV-2 infection reduces quality of sperm parameters including SDF [13].

Taking into account the proved principal role of sperm DNA on fertility and influence of coronavirus on semen quality, we aimed to detect the post-COVID dynamics of Deoxyribonucleic acid fragmentation in the semen of vaccinated and non-vaccinated males.

MATERIAL AND METHODS

Into our prospective two-cohort study we included two groups of males: GROUP 1, previously anti-COVID vaccinated males, n = 46, and GROUP 2, n = 43, non-vaccinated males, who all fell ill with the SARS-CoV-2 infection in 2021–2022 years when 'Delta' coronavirus strain persisted. Males were recruited among the healthy fertile partners of infertile woman who were the clients of reproductive clinics for in vitro fertilization.

Participants were healthy in general without serious comorbidities. Fifteen (16.9%) of them had a history of Gastroesophageal reflux disease and 7 (7.9%) – Chronic cholecystitis without exacerbation and need to cure. Males with varicocele, obesity, cancer, history of epididymitis/epididymo-orchitis, mumps, prostatitis, sexually transmitted diseases, tobacco smokers and alcohol/drug abusers were not included into the study because of proved impact of abovementioned conditions and lifestyle factors on semen quality [14, 15].

We excluded males who suffer from chronical diseases and take medecines able to impair human spermatogenesis like Methadone hydrochloride, Nitrofurantoin, Paroxetine mesylate/paroxetine hydrochloride, Nifedipine, Colchicine, Dexamethasone/dexamethasone sodium phosphate, Sulfasalazine etc. [16].

During the process of preparation for in vitro fertilization all of them were obligated to make spermograms where they have been demonstrated normozoospermia according to latest WHO criteria [17].

All of the participants from Group 1 completed a full vaccination course by the Pfizer-BioNTech COVID-19 vaccine ('Comirnaty'). The participants from Group 2 did not get anti-COVID vaccination because of religious restrictions and/or reluctance. All of the participants with previously registered normozoospermia had moderate SARS-CoV-2 infection confirmed by PCR testing (nasopharyngeal swab) and characterized by any of the various signs and symptoms of COVID-19 (e.g., fever, cough, sore throat, malaise, headache, muscle pain, nausea, vomiting, diarrhea, loss of taste and smell) with evidence of lower respiratory disease during clinical assessment or imaging and who have an oxygen saturation measured by pulse oximetry (SpO2) ≥94% on room air at sea level [18]. Participants were non-hospitalized, took paracetamol to relieve pain and fevers, kept hydrated and took cough medicines.

Clinical and demographic characteristics of participants are presented in the Table 1.

A level of DNA fragmentation in all semen samples was detected before SARS-CoV-2 infection and every month after laboratory recovery confirmed by PCR testing. Semen samples were collected after 3-5 days of sexual abstinence. We used the sperm chromatin dispersion test with original DNA breakage kit that can detect DNA damage in nuclei of spermatozoa. Diagnostic technique was made according to Fernández et al. Sperm suspensions either isolated from semen by gradient centrifugation or prepared from sperm were embedded in an agarose microgel on slides and treated with 0.08 N HCl and lysing solutions containing 1% sodium dodecyl sulfate, 0.8 M dithiothreitol and 2 M NaCl. Thereafter the slides were sequentially stained with 4',6-diamidino-2-phenylindole and/or the Diff-Quik reagent, and the percentages of sperm with nondispersed and dispersed chromatin loops were monitored by fluorescence and brightfield microscopy, respectively [19].

The nucleotides from spermatozoa with fragmented DNA either do not show a dispersion 'halo' or the 'halo' is expressed insignificantly (2) while the specific 'halo' in normal spermatozoa was pronounced (1). (Fig. 1).

The 'Halosperm®' in vitro diagnostic kit (Halotech Dna, SL) has been used that can be considered as a quick and easy test. DIFF– QUICK®/Panoptic stain has been used after the sample processing with 'Halosperm'. Spermatozoa with damaged DNA were without 'halo' (1) while those without DNA fragmentation were pointed by dispersion 'halo' (2). After viewing the each semen sample in the bright-field of microscope, we divided the spermatozoa as follows (Fig. 1).

The Sperm DNA Fragmentation Index (SDFI) was calculated as percentage of cells with fragmented DNA in 500 spermatozoa by formula:

$$\text{SDF index (\%)} = 100 \times \frac{\text{Number of spermatozoa with fragmented DNA}}{\text{Number of spermatozoa counted}}$$

Statistical data were presented as: 'Mean value ± Statistical Deviation'. The results were analyzed using the Mann–Whitney test to establish differences in the performance of both groups: the difference was considered as statistically significant at $p < 0.05$. For analyzing of SDFI changes we calculated relative increasing (RI) of parameter in percents comparing with pre-COVID SDFI baselines inside each Group [20].

The sample size was assessed for comparison using the Wilcoxon-Mann-Whitney test (comparison of two groups). With $\alpha = 0.05$, Power = 95%, and Effect Size d = 0.8 ('large effect'), the minimum required sample size is 44 patients for each group. The proper sample size calculations were conducted before the study began.

Study was approved by the local Ethics Committee and Institutional Review Board (№ 23.02.2021/18). All included participants declared their informed consent in writing.

RESULTS

At the first month after laboratory recovery from SARS-CoV-2 infection that was confirmed by PCR testing, the SDFI of semen in vaccinated participants from Group 1 was 35.3 ±4.7% vs 41.8 ±5.6% in Group 2 of non-vaccinated males, $p = 0.017$. Comparing with start baseline, pre-COVID data we registered significant increasing of SDFI in each group of participants: 35.3 ±4.7% vs 18.6 ±5.8% in Group 1, $p = 0.0009$, and 41.8 ±5.6% vs 19.2 ±6.1% in Group 2, $p = 0.0006$, with relative increase of SDFI +89.8% in Group 1 and +117.7% in Group 2, $p = 0.028$ (Table 2). We found that at the second month after recovery SDFIs in Group 1 and Group 2 continued to grow and reached their peaks to 40.6 ±6.4% (+118.3% comparing with baseline) and 49.7 ±7.2% (+158.9% comparing with baseline) respectively, $p = 0.008$. At the third month after COVID recovery we noted

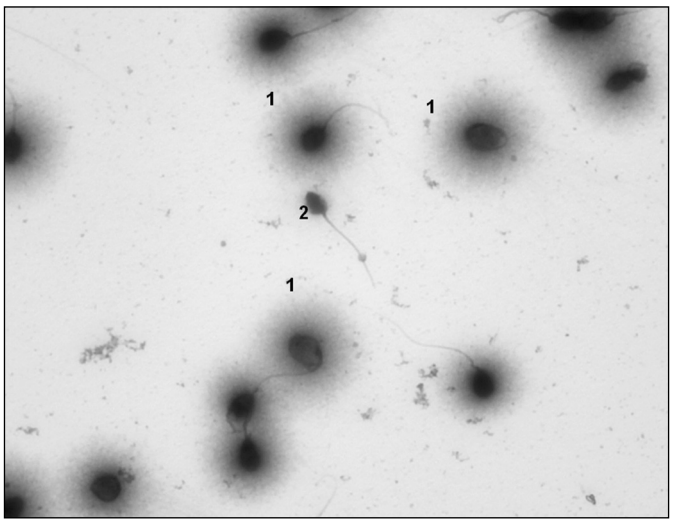

Figure 1. *Normal spermatozoa without DNA fragmentation marked by specific 'halo' (1) and spermatozoa with fragmented DNA without 'halo' (2).*

Table 1. *Clinical and demographic characteristics of participants*

VARIABLES		GROUP 1, n = 46	GROUP 2, n = 43	p
Age		32.6 ±4.8	33.7 ±5.1	0.867
BMI		26.6 ±2.7	27.8 ±3.2	0.752
Marriage time		4.2 ±1.9	3.8 ±1.4	0.631
Previous IVF Cycles, n	0	29	31	-
	1	10	7	-
	2	14	10	-
Pre-COVID SDFI baseline		18.6 ±5.8	19.2 ±6.1	0.743
Maximal COVID body temperature		38.2 ±1.6	39.7 ±0.5	0.018#
Duration of fever, days		5.3 ±1.6	7.6 ±2.3	0.007#
Duration of Covid-positive status, days		13.3 ±3.8	17.5 ±4.2	0.0006#
COVID severity		Moderate	Moderate	-
Oxygen saturation, %		96.4 ±2.4	97.1 ±2.6	0.431

SDFI – semen DNA fragmentation index; BMI – body mass index; n – number of patients
– statistically significant difference

Table 2. *Post-COVID dynamics of semen DNA fragmentation indexes in both Groups.*

		Group 1, n = 46	Group 2, n = 43	p
		SDFI (RI), %(+%)	SDFI (RI), %(+%)	
Baseline		18.6 ±5.8	19.2 ±6.1	0.631
Post-COVID months	1	35.3 ±4.7 (+89.8)	41.8 ±5.6 (+117.7)	0.017#
	2	40.6 ±6.4 (+118.3)	49.7 ±7.2 (+158.9)	0.008#
	3	37.4 ±4.3 (+101.1)	44.4 ±6.1 (+131.3)	0.013#
	4	32.7 ±6.2 (+75.8)	39.6 ±7.9 (+106.3)	0.015#
	5	26.5 ±4.9 (+42.5)	33.8 ±5.3 (+76.0)	0.018#
	6	24.8 ±5.1 (+33.3)	29.7 ±6.7 (+54.7)	0.021#
	7	20.1 ±4.6 (+8.1)	26.3 ±5.8 (+37.0)	0.014#
	8	19.7 ±5.7 (+5.9)	24.1 ±6.2 (+25.5)	0.028#
	9	18.8 ±4.8 (+1.1)	20.3 ±5.3 (+5.7)	0.539
	10	19.1 ±4.7 (+2.7)	20.6 ±5.2 (+7.3)	0.783

SDFI – semen DNA fragmentation index; RI – relative increasing of SDFI comparing with pre-COVID SDFI baseline in the Group; n – number of patients
– statistically significant difference

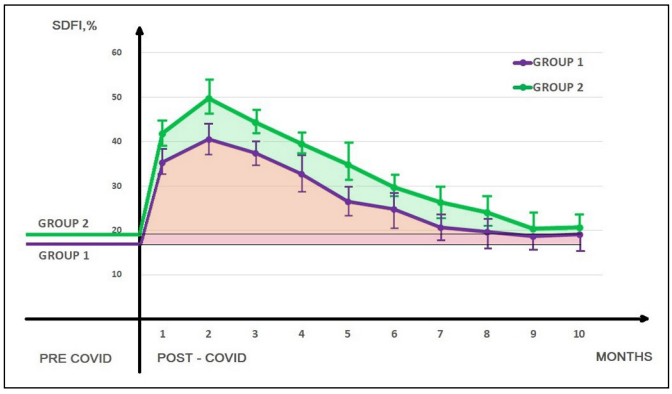

Figure 2. *Dynamics of post-COVID semen DNA fragmentation indexes (SDFI) in vaccinated (Group 1) and non-vaccinated (Group 2) males.*

decreasing of DNA fragmentation levels in both groups with a further downward trend. In Group 1 of COVID-vaccinated males SDFI returned to the pre-COVID level at the 7th month after recovery while in Group 2 SDFI has been normalized only at the 9th post-COVID month (Figure 1).

DISCUSSION

Pandemic of SARS-CoV-2 infection has dramatically changed human wellbeing everywhere in the world and significantly decreased the quality of life. Epidemic restrictions impacted usual lifestyle while resolved infection often left complications in various organs and systems of reconvalescents. A few recent Articles have described post-COVID changes of human semen, some of them are reporting about the growth of DNA fragmentation in semen samples after COVID [8, 13, 21]. Moryousef et al. presented case report of a male who demonstrated post-COVID increase of SDFI at 76%. Authors noted that at the 4th month after COVID his SDFI was 22%, which was similar to pre-COVID-19 baseline [22].

Comparing semen samples from COVID-positive and COVID-negative males, Shi et al. concluded about statistically lower SDFI in the COVID-negative group. Authors resumed that COVID-19 may adversely impacts male fertility, and their result can provide advisory guidance for doctors [23].

Hu et al. decided that post-COVID semen parameters showed a significant decline after a recovery time of 90 days and an improving trend after a recovery time of about 150 days [24].

In our prospective study which included 89 patients we aimed to detect the post-COVID dynamics of DNA fragmentation in the semen of vaccinated and non-vaccinated males. We confirmed that COVID-19 infection leads to increasing the percentage of spermatozoa with fragmented DNA in post-COVID period. Interestingly, that in post-COVID interval the constant increasing of SDIF remains with a peak at the 2nd month after laboratory recovering. We strongly agree with Depuydt et al, that such delayed effect may be explained by spermatozoa development cycle [13].

We also find that despite the same peak-time of SDFI elevation (2 months), in vaccinated and non-vaccinated males, percentage of fragmented DNA in semen of males without vaccination was significantly higher and time to normalization of their SDFI was longer. As presented in Table 1, maximal COVID body temperature in non-vaccinated males was higher than in their vaccinated coevals while fever lasts longer as well as period to laboratory recovery was more. That is only our assumption, but oxidative stress due to stronger intoxication and longer duration of viral persistence may play a role in the more DNA damage of semen in non-vaccinated males. This hypothesis needs future investigations with large number of involved patients.

The relatively small sample size and the absence of males with mild/severe cases might be considered as the main study's limitations. The small cohort size may not adequately represent the broader fertile male population impacted by COVID-19. Another limitation is the study of only one 'Delta' strain effects. It would be interesting to study the consequences of 'Omicron' strain or the other newest COVID-19 strains on semen DNA fragmentation. One else limitation is the fact that we have studied

the only effects of vaccination by the Pfizer-BioNTech COVID-19 vaccine. We consider it appropriate to know about influence of vaccination by other sertified anti-COVID vaccines on semen DNA fragmentation in the post-COVID period.

This area of research is relevant because new strains of coronavirus infection are emerging. Both urologists and specialists in reproductive medicine should orient their patients to the timing of low-risk repeated fertilization attempts. According to our data, COVID-vaccinated men who have contracted a COVID infection should expect normalization of the SDFI no earlier than 7 months after laboratory recovery, while unvaccinated men should expect normalization no earlier than 9 months.

Elevated semen DNA fragmentation has an impact on both miscarriage rates and birth weight in assisted reproductive technology [25]. Such negative consequences cannot be ruled out in the cases of natural fertilization.

Thereby, the potential long-term implication of our findings may be an additional belief in the need and necessity of ati-COVID vaccination for men at all and those from infertile couples especially. Rapid normalization of sperm DNA levels can be considered a strong argument for timely vaccination in males if they have no medical contraindications to the procedure.

CONCLUSIONS

COVID-19 causes a gradual increase in the level of DNA fragmentation in spermatozoa, which peaks at the 2nd month after recovery and is more pronounced in unvaccinated men. Recovery of SDFI indicators occurs no earlier than 7th month in vaccinated men and 9th month in non-vaccinated men. These time frames should be taken into account when guiding couples who are planning to conceive or males from infertile couples who are scheduled for IVF cycles.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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