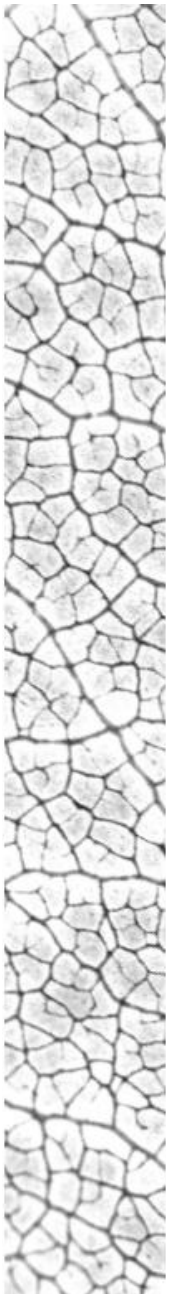

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Human sperm DNA fragmentation and its correlation with antioxidant markers and sperm characteristics in Iraqi males

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Abstract

Background: The determination of sperm DNA fragmentation, as a significant addition to semen routine examination, has been applied in some medical andrology laboratories. This study aimed to determine the association between sperm DNA fragmentation (DFI) and semen quality and antioxidant and fertility status.

Methods: Semen samples from 88 infertile males (36 asthenozoospermia, 22 oligoasthenozoospermia) and 30 normospermia were analyzed for sperm DNA fragmentation. Sperm parameters were assessed using the World Health Organization guidelines.

Results: After their sperm DFI was examined, it was found that the sperm DFI of asthenozoospermic and oligoasthenozoospermic patients were higher than the normozoospermic males. The activity of SOD in seminal plasma was significantly increased ($P < 0.05$) in oligoasthenozoospermic subjects as compared to normospermic groups. Significant differences were noted in spermatozoa SOD activity between normospermic and asthenozoospermic compared to oligoasthenozoospermic subjects. The results showed that sperm DFI was positively related to infertility duration.while negatively related to progressive motility and seminal plasma catalase. Correlations between seminal antioxidant status and semen parameters were also evaluated in this study. Spermatozoal SOD activity showed a significant correlation with sperm concentration.

Conclusion: Sperm DNA fragmentation is one of the main source of male infertility, which associated with poor fertility outcomes in couples. Our results have shown that sperm DNA fragmentation index was almost significantly linked to all values reflecting semen quality, indicating that sperm DFI might be an important cause leading to the decline of semen parameters.

Keywords: sperm DNA fragmentation, sperm parameters, antioxidants, SD1, SOD

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INTRODUCTION

Infertility is a disease of the reproductive system. It can be defined as the inability of an individual or a couple at reproductive age to achieve pregnancy after one year or more of regular unprotected sexual intercourse (Vander Borght, & Wyns, 2018). Over the world, infertility challenges approximately 15% of couples who are trying to conceive (Bisht, et al. 2017)., wherein the fertility problem is equally distributed between the male and female partner (Coutton, et al. 2016). However, the depiction that half of the infertility cases derive from the male's semen quality, maybe an underestimation. This is potentially related to the tendency to blame infertility problems on the female in places such as the Middle East or Northern Africa, where accurate record-keeping, and inadequate health care and diagnosis antagonizes reliability of the statistical interpretations. Furthermore, clinical investigations adopt a different definition of infertility (Agarwal, et al. 2015).

Oxidative stress is a condition wherein a lulled performance of the antioxidant system occurs in conjunction with a high level of reactive oxygen species (ROS). ROS is a collective term for a group of very reactive molecules generated from the metabolism of diatomic oxygen. Diatomic oxygen does not react with other molecules spontaneously, so cells activate O_2 by temporary conversion to a ROS. ROS is needed for several functions in the routine maintenance of live sperm cells. Specifically, to react O_2 molecules with important biochemical they are broken to form an equimolar number of monoradicals via an enzymatic system capable of overwhelming the required energy

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barrier (Wernerman, 2012).However, with an underactive antioxidant system, ROS are not quenched as in normal healthy function, which can overwhelm the cell and fragment DNA. Generally, thirty to eighty per cent of infertile males have above-average levels of ROS, which points to the potential of ROS to have pathological effects. Measuring ROS indirectly can be achieved by estimating the antioxidant capacity of enzymatic and non-enzymatic defence molecules present in the seminal system (Ko, Sabanegh Jr, & Agarwal, 2014). In the Vitamin E family α-tocopherol is the most abundant in human cells and has antioxidant activity against oxygen radicals and other free radicals. Due to the hydrophobic character, α-tocopherol is localized in the cytoplasmic membrane where it acts as a breaker to the chain reaction the characterizes LOP. Estimating α-tocopherol content gives some indication about the status of the sperm membrane (Choudhury, Tan, & Truswell, 1995). The major ROS in a sperm cell is (•O2−), converts either spontaneously or by superoxide dismutase (SOD) to hydrogen peroxide. By catalase (CAT) enzymes the hydrogen peroxide is converted to the non-harmful products H2O and O2. Glutathione (GSH) acts as a substrate for the two CAT enzymes, glutathione peroxidase (GP) and glutathione reductase (GR). These two enzymes carry out the CAT process leading to the elimination of H2O2 (Choudhury, Tan, & Truswell, 1995, Lopes, 1998]. Thus, the amount of SOD, CAT, GSH, α-tocopherol in sperm cytoplasm and seminal plasma provides an indication of approximate ROS levels and the degree of SDF can be interpolated from that by calculating sperm fragmentation index (SFI). Finally, this index can be linked with patient infertility with OS and ROS.

According to the World Health Organization (WHO) guidelines, routine semen examination is the primary technique for evaluating men fertility. Routine semen examination can offer an incomplete estimation of male fertility potential and is not constantly competent to clarify the reason for male infertility. Indeed, several cases of men infertility are caused by sperm DNA damage, which routine semen quality examines fail to identify (Agarwal, & Allamaneni, 2005). Since sperm DNA fragmentation may be a key mark of male infertility and considering the principal defence delivered by seminal antioxidants against oxidative damages, this study aimed to (1) assess the levels of enzymatic antioxidants (CAT, SOD and Vitamin E) in the human spermatozoa and seminal plasma of Iraqi infertile men and to (2) evaluate their effects on sperm DNA fragmentation and associated sperm alteration.

MATERIALS AND METHODS

Subjects

This present study included 88 men as volunteers, who donated semen for analysis at the Al-Kafeel Specialized Hospital. The study was approved by the local Ethics committee of the Hospital. The study was conducted from March 2019 to December 2019. The patient parameters that were recorded and analysed included age, height, weight, body mass index (BMI), alcohol status, smoking status, fertility history, and occupation. A professional andrology doctor interviewed all contributing males. After the initial screening, data were collected from all participants. According to the 5th edition of the World Health Organization (WHO) guideline, the patients were divided into the following groups based on their semen parameters. Normozoospermic: thirty men (n=30). Their ages ranged from 21 to 30 years. They underwent seminal fluid analysis and passed the guideline limits of semen parameters, and were considered as the control group, asthenozoospermic: (n=36). Their ages ranged from 21 to 50 years old. Their fertility is distinctly less than normal levels for sperm motility, but with normal levels for sperm concentration and morphology; Oligoasthenozoospermic patients (n=22) their ages ranged between 20-50. They were diagnosed as oligoasthenozoospermic according to their semen parameters, which failed to pass the lower limits of number and motility.

Exclusion criteria

patients who have varicocele, diabetes, any acute infection, those using any medication or antioxidant supplementation, and patients suffering from leucospermia.

Collecting and Analyzing Samples

Semen analysis

All specimens were collected at a laboratory facility by masturbation after 2-7 days of ejaculatory abstinence. All specimens were collected into clean and sterile polypropylene containers that are non-toxic to spermatozoa. After labelling with patient code and time of collection, the specimens were covered tightly and transported to the incubator set at 37 ºC and checked continuously to see the liquefaction state. After complete liquefaction, basic semen parameters were assessed according to the WHO's 2010 guidelines. For each sample an aliquot of one to two ml (depending on volume availability) was added to a special conical tube and centrifuged at 1500xg for 10 minutes at 1ºC (MEGAFUGE 40R- Thermo Scientific) to separate plasma from spermatozoa. Then plasma and cells were stored at -35 until biochemical analysis.

Morphology and Sperm Deformity Index (SDI)

At least ten fields were randomly viewed. Normal morphology (normal form) and sperm deformity index was assessed by using Kruger/Tygerberg strict criteria that is outlined in the Fifth Edition of the WHO manual (WHO, 2010). By focusing only on the completely visible sperm, with head and tail, avoiding any overlap with other sperm or other types of cell or debris, 200 sperms

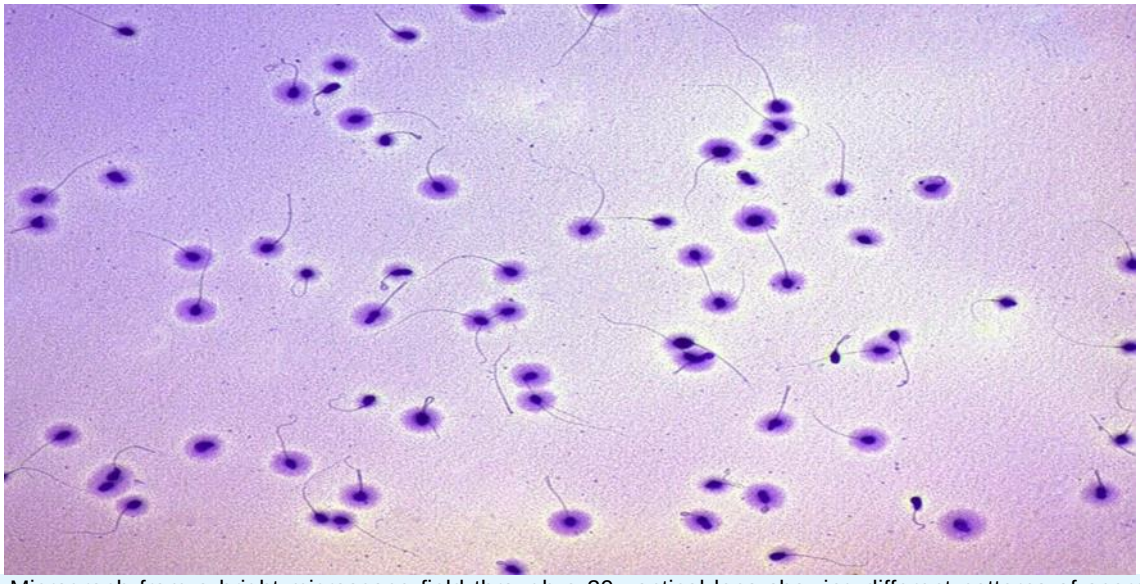

Fig. 1. Micrograph from a bright microscope field through a 20x optical lens showing different patterns of sperm DNA fragmentation

were counted using an 'eight key' mechanical counter and examined. Normal sperm morphology was expressed as a percentage by dividing the total number of normal sperm by the total number of examined sperms, multiplied by 100%. Normal sperm should have an intact head and tail. The head must be oval shaped and smooth, with a normal acrosome area occupying 40-70% of the head. The midpiece should have a slender shape and have the same length as its head and be aligned with the major axis. The residual cytoplasm should be more than one third of the length of the sperm head (MORTIMER, & MENKVELD, 2001)

Sperm DNA Fragmentation Assay

The technique that was used to estimate DNA fragmentation was the Sperm Chromatin Dispersion Test (SCD), which is a fast and reliable test to assess the amount of fragmented DNA in sperm cells. This test is commonly called the Halosperm test. A commercial kit (Avicenna Research Institute-Iran) was used in this study. The procedure that was used was in accordance with other studies (Fernández, 2003, Fernández,., 2005).

At least 300 sperm were counted and screening at random field, focusing on halo with sperm body head and tail (As shown in **Fig. 1**). Overlapping halos were excluded from the count. The size of the halo was measured by use of the measuring tool provided with the camera software. Sperm with a big halo (equal or larger than sperm head) and medium halo were considered as signs of intact sperm without fragmented DNA. No halo and a halo smaller or equal to one third of the size of the sperm head were considered as sperm with fragmented DNA. DNA fragmented was expressed as a percentage by dividing the fragmented sperms over the total number of sperms counted, multiplied by 100.

Biochemical assay

The measurement of antioxidant enzymes (catalase, superoxide dismutase) and non-enzymatic antioxidant total vitamin E in sperm cells and seminal plasma was calculated accordingly. The separated centrifuged sperm cell, after melting in room temperature, were washed with NTPC medium three times. The sperm cells were mixed with 10 volumes of NTPC medium then the mixture was centrifuged at 1500xg for 10 min at 4°C. The pellets that were obtained were treated with 0.1% Triton X-100, then centrifuged at 3000xg with refrigerated centrifugation at 0ºC for 30 minutes. The supernatant was collected for measurement of the antioxidant in the sperm cell. The concentration of Triton X-100 (0.1%) does not affect enzyme activity and the plasma was previously separated and also melted in same condition (Hadwan, 2008)

- Catalase activity was measured by spectrophotometric assay (Hadwan, & kadhum Ali, 2018).
- SOD activity were measured as procedure of [Magnani, Gaydou, & Hubaud, 2000, Marklund, & Marklund, 1974].
- Total vitamin E level in seminal plasma and sperm were measured as procedure of (Quaife, Scrimshaw, & Lowry, 1949).

Statistical analysis

Statistical analysis was accomplished with Statistical Package for Social Sciences (SPSS) (version 23.0, SPSS Inc., Chicago, IL, USA). For comparison between two groups, one-way ANOVA was used. The independent correlation of sperm DNA fragmentation and antioxidants with semen parameters was tested by linear regression analysis with Pearson's correlation

Table 1. General characteristics of the participant's groups

Variables	Group 1 (n= 30)	Group 2 (n= 36)	Group 3(n= 22)	P-value
Male age (years)	28.733± 5.064	30.583 ±7.291	32.363± 6.373	NS
Height	1.733 ± 0.077	1.748 ± 0.060	1.72 ± 0.069	NS
Weight (kg)	81.7 ± 18.846	77.69 ± 13.396	80.772 ± 8.579	NS
BMI (kg \ m^2)	27.209 ± 6.587	25.355 ± 3.921	27.38 ± 3.299	NS

Values are presented as mean ± Standard Deviation
Group 1, normal semen parameters; group 2, asthenozoospermic males; group 3, oligoasthenozoospermic males. The mean difference is significant at the 0.05 level

Table 2. Descriptive statistics and comparison of semen parameters and sperm DFI in the study population (n = 88)

Variables	Group 1 (n= 30)	Group 2 (n= 36)	Group 3(n= 22)
Semen volume (ml)	[a] 3.113 ± 1.371	[a] 3.394 ± 1.076	[a] 3.054 ± 1.148
Sperm concentration (*10^6 \ ml)	[a] 81.0313 ± 57.8	[b]40.322 ± 21.559	[c]9.631 ± 3.140
Total sperm count (*10^6/ejaculate)	[a] 247.019 ± 95.107	[b]135.973 + 87.106	[c]28.540 ± 13.365
Sperm Motility (%)			
Progressive motility (%)	[a] 43.066 ± 8.106	[b]18.75 ± 8.573	[b]15.136 ± 8.356
Non-progressive motility (%)	[a] 34.833 ± 8.626	[ab] 30.888 ± 11.911	[b]27 ± 8.613
Immotile sperm (%)	[a] 21.832 ± 8.713	[b] 50.361 ± 15.022	[c] 57.318 ± 12.833
Normal sperm morphology (%)	[a] 45.933 ± 9.187	[b] 34.833 ± 11.149	[b] 33.772 ± 9.601
Vitality (%)	[a] 77.9 ± 9.102	[b] 51.416 ± 14.952	[c] 43.136 ± 12.687
SDI (%)	[a] 0.921 ± 0.283	[b]1.306 ± 0.344	[b]1.289 ± 0.393

Values are presented as mean ± Standard Deviation
Group 1, normal semen parameters; group 2, asthenozoospermic males; group 3, oligoasthenozoospermic males. Means with different letters indicate significant differences.

Table 3. Percentage of DNA fragmentation and antioxidant profiles in the study population (n = 88)

Variables	Group 1 (n= 30)	Group 2 (n= 36)	Group 3(n= 22)
Sperm DFI %	[a] 28.163 ± 5.278	[b] 36.130 ± 5.564	[b] 35.222 ± 6.219
Seminal Plasma Catalase activity	[a]0.185 ± 0.088	[a]0.184 ± 0.136	[a]0.180 ± 0.099
Spermatozoa Catalase activity	[a]0.414 ± 0.016	[a]0.408 ± 0.057	[a]0.407 ± 0.045
Seminal Plasma SOD activity	[a] 0.217 ± 0.099	[ab]0.338 ± 0.320	[b] 0.505 ± 0.596
Spermatozoa SOD activity	[a] 0.643 ± 0.700	[a]0.514 ± 0.436	[b]1.232 ± 0.843
Seminal Plasma vitamin E	[a]5.341 ± 2.946	[a]5.731 ± 3.194	[a]5.594 ± 1.180
Spermatozoa vitamin E	[a]28.068 ± 5.615	[a]27.822 ± 6.349	[a]30.226 ± 3.869

Values are presented as mean ± Standard Deviation
Group 1, normal semen parameters; group 2, asthenozoospermic males; group 3, oligoasthenozoospermic males. Means with different letters indicate significant differences.

test. The significance level was set as two-tailed, and p < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The study design included three groups based on the semen parameters. Group 1 involved males with normal semen analysis (normozoospermia); group 2 involved males with asthenozoospermia and group 3 consisted of males with oligoasthenozoospermia. Statistic description of the general characteristics of infertile groups and controls was given in **Table 1**. As shown, there were no statistically significant differences (p>0.05) in the distributions between normozoospermic and infertile groups.

Table 2 shows the results of the comparison of semen parameters in subgroups of infertile men (asthenozoospermic and oligoasthenozoospermic) with normospermic group. Semen volume was not statistically different between normozoospermic and infertile groups. Otherwise, sperm concentration was significantly higher (P< 0.05) in controls than the two infertile groups.

Total sperm motility was also higher in normozoospermic sperm as compared to that from the asthenozoospermic and oligoasthenozoospermic groups (P< 0.05). Yet, normal morphology was significantly lower in asthenozoospermic and oligoasthenozoospermic groups compared to controls (P<0.001). Besides, SDI was significantly higher in asthenozoospermic and oligoasthenozoospermic groups when compared to controls (P<0.001).

In this study, semen samples were obtained from men undertaking routine infertility assessment. After their sperm DFI was examined, it was found that the DFI of sperm from asthenozoospermic and oligoasthenozoospermic patients was higher than that from normozoospermic males (p<0.001). Results are shown in **Table 3**.

According to WHO guidelines, routine semen examination is the primary technique for evaluating male fertility. Routine semen examination can offer an incomplete estimation of male fertility and is not consistently competent as a method to clarify the reason for male infertility. Indeed, several cases of male infertility are demonstrated to be a consequence of sperm DNA damage, which routine semen quality examines fail to identify (Agarwal, & Allamaneni, 2005).

Our results demonstrate that highly significant increases in the sperm DFI, evident in the asthenozoospermic and oligoasthenozoospermic groups compared to controls, are in agreement with the observations of previous studies [Zribi,et al. 2011- Atig, et al. 2017 Shamsi, et al. 2009).] who found that sperm

DNA fragmentation was significantly higher in asthenozoospermic and oligoasthenozoospermic patients than normozoospermic.

The high prevalence of sperm DNA damage in infertile semen samples as compared to normospermic was stated previously (Smit, et al. 2010).The threshold of DFI at >30% is associated with a noticeable and significant decrease in pregnancy and delivery rates (Bungum, et al. 2007).. Similarly, another study established that males with sperm DFI of ≥ 26.1% have 2.84 times higher risk for infertility than males with sperm DFI of < 26.1%(Wiweko, & Utami, 2017).confirming that the probability of pregnancy for the group with DFI >30% is very low than that for the group with a DFI value <30% (Evenson, & Wixon, 2008).

The sperm DNA fragmentation index is considered as a more accurate biologic indicator of sperm quality because undamaged DNA is essential for the accurate transmission of genetic material to the egg cell (Zribi, et al. 2011). Spermatozoa DNA damage, characterized by DNA single or double-stranded breaks, happens throughout late spermatogenesis as a result of endogenous processes occurring in the testis/epididymis, or due to exogenous substances encountered by sperm after ejaculation. Exogenous DNA damage in spermatozoa is prompted by exposing spermatozoa to a harsh environment such as exposure to extraordinary levels of reactive oxygen species (Aitken, Baker, & Sawyer, 2003).

Our study showed that asthenozoospermic and oligoasthenozoospermic men compared with normozoospermic do not have deficient spermatozoa or seminal plasma vitamin E levels. Our results contrast with an earlier study suggesting that the seminal plasma vitamin E levels were lower in the infertile male's ejaculate when compared to the fertile males (Bassey, et al. 2019). However, variations in the criteria used in this study may explain this discrepancy.

Our data were consistent with previous studies that found no significant change in catalase levels between asthenozoospermic, oligozoospermic and normozoospermic groups (Tomar, et al. 2017. Ben Abdallah, 2009). Still, several other investigations have found elevated rates of catalase activity in normozoospermic compared to the abnormal groups [Shamsi,2009, Vatannejad, 2017]. Yet, a recent study found that the activity of catalase was decreased in seminal plasma of ejaculate from males with normal sperm motility compared with semen from asthenozoospermic males (Marzec-Wróblewska,., 2019). Which in parallel with a previous study showing that the levels of catalase and total antioxidant capacity were significantly reduced in asthenozoospermic, asthenoteratozoospermic, and oligoasthenoteratozoospermic males when compared with the healthy males with normozoospermia [Khosrowbeygi, & Zarghami, 2007]. These results lead to the suggestion that a deficiency of antioxidant defence may play a main part in male infertility.

However, the activity of SOD in seminal plasma was higher in oligoasthenozoospermic subjects as compared to normospermic groups. The variations observed were significant ($P < 0.05$). Significant differences were noted in spermatozoa SOD activity between normospermic and asthenozoospermic compared to oligoasthenozoospermic subjects and the obtained results are shown in **Table 3**. This result confirms previous observations reported by other authors Rehman, et al. 2020] who described a significant increase in SOD activity in the infertile group compared to the fertile group. Yet, several other studies didn't agree with our findings and reported elevated rates of SOD activity in males with normal sperm criteria than in those with sperm dysfunctions Dorostghoal, et al. 2017)].

Different studies have examined the association between the seminal plasma anti-oxidative defence and sperm parameters, however, the outcomes are controversial (Otasevic, et al. 2019) and, so far, such lack of agreement keeps the argument open. These variances may be due to differences among inclusion and exclusion criteria for sample selection. In addition, the differences in sample size or the procedures used might account for this inconsistency.

Next, we analyzed the correlations between sperm DFI, semen parameters and antioxidants biomarkers in asthenozoospermic and oligoasthenozoospermic samples. The results showed that sperm DFI was positively related to infertility duration ($r= 0.289$, $p< 0.028$) augmented in parallel with SDI ($r= 0.289$, $p< 0.028$), while negatively related to progressive motility ($r= -0.378$, $p< 0.003$) and seminal plasma catalase ($r= -0.269$, $p< 0.041$). Although significant correlations between DFI and semen analysis findings were noted in the overall study population when the data of infertile males (asthenozoospermic and oligoasthenozoospermic) were analyzed separately, the correlation between sperm concentration and sperm DFI was not significant. Data are shown in **Table 4**.

Sperm motility is a key factor of male fertility as active flagellar movement is vital for successful fertilization. Factors such as genital infections, maturational abnormalities in the epididymis, defects in flagellar axonemes or defects in metabolism cause impairment of sperm motility leading to infertility. A recent study showed significantly high percentages of DNA damage in males with abnormal spermatozoa morphology (Ammar, Mehdi, & Muratori, 2020).

A current hypothesis is that if sperm DNA damage in some patient is exclusively from the failure to repair DNA damage happening during spermatogenesis, one could reasonably suppose that it would also correlate well with other categories of spermatogenic failure, like teratozoospermia. Otherwise, if DNA breakage in

Table 4. Correlations of sperm DNA fragmentation with semen parameters, and antioxidant biomarkers in 58 asthenozoospermic and oligoasthenozoospermic samples

Variables	coefficient correlation (r)	P-values
Infertility duration	**0.289***	**0.028**
Semen quality parameters		
Semen volume (ml)	0.192	0.149
Sperm concentration (*106 \ ml)	-0.017	0.898
Total sperm count (106/ejaculate)	0.049	0.717
Progressive motility (%)	**-0.378****	**0.003**
Non-progressive motility (%)	0.110	0.409
Immotile sperm (%)	0.155	0.246
Normal sperm morphology (%)	-0.160	0.230
Vitality (%)	-0.121	0.368
SDI (%)	**0.382****	**0.003**
Seminal Plasma Catalase activity	**-0.269***	**0.041**
Spermatozoa Catalase activity	0.186	**0.162**
Seminal Plasma SOD activity	-0.028	**0.839**
Spermatozoa SOD activity	-0.168	0.207
Seminal Plasma vitamin E	0.043	0.748
Spermatozoa vitamin E	**-0.123**	**0.324**

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Table 5. Correlations between antioxidant status and conventional semen criteria in 58 asthenozoospermic and oligoasthenozoospermic samples

Variables	Sperm concentration	Total sperm count (106/ejaculate)	Progressive motility (%)	Non-progressive motility (%)	Immotile sperm (%)	Normal sperm morphology (%)	Vitality (%)	SDI (%)
Seminal Plasma Catalase activity	NS	NS	NS	NS	NS	NS	NS	NS
Spermatozoa Catalase activity	NS	NS	NS	NS	NS	NS	NS	NS
Seminal Plasma SOD activity	NS	NS	NS	NS	NS	NS	NS	NS
Spermatozoa SOD activity	***r* =- 0.266 *** ***P* < 0.043**	NS	NS	NS	NS	NS	NS	NS
Seminal Plasma vitamin E	NS	NS	NS	NS	***r* = 0.301 *** ***P* < 0.022**	NS	NS	NS
Spermatozoa vitamin E	***r* = -0.340 **** ***P* < 0.009**	***r* = -0.346 **** ***P* < 0.008**	NS	NS	NS	NS	NS	NS

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

spermatids are generally as a result of the adverse effects of ROS, then an association with sperm motility may be reasonably expected (Erenpreiss, et al 2006).

The study of correlations displays that the concentration of catalase in the seminal plasma of the infertile men is negatively related to sperm DFI. This result does not support a previous study (Bousnane, et al. 2017in which no correlation between the concentration of catalase in the seminal plasma and sperm DFI has been reported.

Correlations between seminal antioxidant status and semen parameters were also evaluated in the current study and are presented in **Table 5**. Spermatozoal SOD activity showed a significant correlation (r= -0.266, P < 0.043) with sperm concentration. Additionally, a positive correlation was found between immotile spermatozoa and seminal vitamin E (r = 0.301, p< 0.022). However, spermatozoal vitamin E was negatively correlated with sperm concentration and total sperm count (r= -0.340, p< 0.009 and r= -0.346, p< 0.008 respectively) (as shown in **Table 5**).

An earlier study showed that the seminal plasma vitamin E was positively associated with sperm concentration, sperm motility and normal sperm morphology (Shete, & Hamid, 2016). A comparative examination is again hindered by variances in sample selection, the difference in semen analysis criteria and the method of assessing the tested parameters.

CONCLUSION

Our results demonstrated that sperm DNA fragmentation index was almost significantly linked to all values reflecting semen quality, indicating that sperm DFI might be an important cause leading to the decline of semen parameters. We predict that further studies with stricter controls will elucidate this pattern with high statistical significance.

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