

Analysis on the association between sperm DNA fragmentation index and conventional semen parameters, blood microelements and seminal plasma ROS in male patients with infertility

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Abstract. We conducted this study to analyze the sperm DNA fragmentation index, conventional semen parameters, blood microelements and seminal plasma reactive oxygen species (ROS) in patients with male infertility to determine the association between each of the above male physiological parameters and DNA fragmentation index and infertility. Eighty cases of infertile males and 20 cases of normal males with children were divided into the infertility and control groups, respectively. Sperm DNA fragmentation index, conventional semen parameters, serum microelement content and seminal plasma ROS levels were detected, and the existing correlation between sperm DNA fragmentation index and the various physiological parameters were studied. The sperm DNA fragmentation index had no correlation with conventional sperm parameters. Our results demonstrated that zinc, lead and magnesium ions in the serum microelements were correlated with sperm DNA fragmentation ($p < 0.05$). Upon an increase in zinc and lead serum concentration, there was a subsequent increase in sperm DNA fragmentation ($p = 0.008$). Furthermore, when magnesium ion increased, it also caused an increase in sperm DNA fragmentation ($p < 0.05$). The seminal plasma ROS of infertile males was higher than that of males with children ($p < 0.05$). Our results suggest that sperm DNA fragmentation index is closely associated with the infertility

rate and microelements of serum and seminal plasma ROS can impact the formation of sperm DNA fragmentation. Therefore, the sperm DNA fragmentation index can serve as an important parameter to assess male infertility.

Introduction

Over the last decade, there has been continuous development of social productive forces as well as deterioration of the environment. At the same time, living standards have shown improvement, resulting in increased attention being given to infertility, with diagnostic rates showing a notable increase (1). Correlational studies have shown that the reasons of infertility are complex. In an infertile couple, approximately 40% of the time both the male and female have an infertility issue, approximately 25% of the time the issue is due to either the male or the female, or approximately 35% of the time, the issue is unknown (2). Currently, there is a lack of methods to diagnose male infertility in clinic. One of the most direct and most certain methods to diagnose males with infertility is to analyze azoospermia in clinic. In clinic, certain defects and shortages exist in the basic assessment of male fertility by analyzing sperm density and motility in routine sperm examination, as the assessment on male fertility is not only limited to the above indices (3,4).

Currently, scientists have been studying the correlation between sperm DNA damage and male infertility. Correlational studies have reported that there is a great difference in sperm DNA fragmentation indexes among the different infertile patients, but total results indicate that sperm motility of a mass of infertile patients presents an obvious negative correlation with their sperm DNA fragmentation indexes (5,6). This negative correlation shows that sperm DNA damage is one of the important factors causing sperm motility decrease and male infertility (5). By studying the correlation between infertile male sperm DNA fragmentation index and conventional semen parameters, blood microelements and seminal plasma reactive oxygen species (ROS), the present study may provide a theoretical basis for diagnosing and treating infertile males.

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Materials and methods

Sample selection. The present study included 80 infertile male patients that presented at the Department of Andrology at the Shiyuan Taihe Hospital (Hubei, China) from February 2014 to February 2016. We also recruited 20 cases of healthy male volunteers who had children as a positive control. The age range of all the study subjects was 25-39 years. The diagnostic standard of male infertility referred to the gold standard of the clinic which included the inability to get pregnant for 1 year or more despite a normal sexual life and no contraceptives. The study was approved by the Ethics Committee of Shiyuan Taihe Hospital and informed consents were signed by the patients.

Routine inspection of sperm. The study subjects were asked to refrain from any sexual activity for 3-7 days, and then ejaculate the sperm sample through masturbation. The sperm samples were placed into a disinfected plastic container. After being fully liquefied by the constant temperature dry box, a routine inspection was conducted by using the semen detecting instrument (Barui Medical Equipment Co., Beijing, China) at Huaian First People's Hospital. Each sample was selectively observed from 6 fields of view, and parameters, such as semen volume, sperm density and motility, were detected.

Detecting sperm fragmentation DNA. The sperm fragmentation DNA detection kit (Biosharp, Hefei, China) and sperm chromatin diffusion method were used to detect sperm fragmentation DNA. The fresh semen was diluted using normal saline to the target concentration ($5-10 \times 10^6/\text{ml}$), and sperm fragmentation DNA detection was operated according to kit instructions.

Detecting serum microelements and seminal plasma ROS level. Blood ($5 \mu\text{l}$) was taken from each study subject before breakfast. After the serum was separated, each microelement in the serum was detected according to the ELISA kit instructions. Within 30 min after the obtained semen sample was liquefied, the seminal plasma was obtained and an ELISA kit was utilized to detect seminal plasma ROS levels.

Statistical analysis. Experimental data were statistically analyzed using statistical software, SPSS 19.0 (Chicago, IL, USA). The association between the semen fragmentation index and conventional semen parameters, blood microelements and seminal plasma ROS were studied. Data were presented as mean \pm standard deviation (mean \pm SD). $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Analysis on sperm fragmentation DNA of infertile male patients. There were 80 male infertile patients and 20 males with children in this study. After their sperm DNA fragmentation indexes were analyzed, it was found that the sperm DNA fragmentation indexes of infertile patients were higher than the control subjects ($p = 0.008$). Data information is shown in Table I and Fig. 1.

Table I. Sperm fragmentation DNA indexes of infertile males and males who had children.

Group	DFI (%)
Infertile male patients	36.5 ± 3.87
Males who had children	16.23 ± 2.65

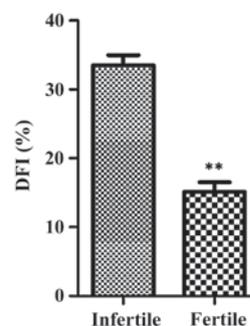


Figure 1. Sperm fragmentation DNA indexes of infertile males and males who had children, ** $p < 0.01$.

Analysis of routine data of semen. After an analysis of routine data of the semen of infertile patients, it was found that the differences between sperm DNA fragmentation indexes and each conventional semen parameter had no statistical significance ($p > 0.05$) (Table II). The study on the correlation between sperm DNA fragmentation index and each conventional semen parameter showed that the sperm DNA fragmentation index was not correlated with semen volume, sperm density, sperm motility and normal sperm formation rate ($p > 0.05$) (Table III).

Analysis on microelements in blood plasma. After the sperm DNA fragmentation index and microelements in blood plasma were analyzed, it was found that zinc and lead in blood plasma were positively correlated with sperm DNA fragmentation index ($r = 0.187$, $r = 0.078$, $p < 0.05$). Magnesium was also negatively correlated with sperm DNA fragmentation index ($r = 0.263$, $p < 0.05$). Results are shown in Table IV.

Analysis on seminal plasma ROS. After an analysis of the sperm DNA fragmentation index and seminal plasma ROS, it was found that the sperm DNA fragmentation index in the infertility group was higher than that of control group. In addition, seminal plasma ROS levels were higher than those of males who had children ($p < 0.05$). Results were shown in Table V and Fig. 2.

Discussion

Genetic information of the parental generation is passed onto the offspring through fertilization of the egg by the sperm, and therefore, the integrity of sperm DNA is important to correctly pass on genetic material to offspring (7). Correlational studies have shown that the failures of natural childbirth and assisted reproductive technology are associated with sperm DNA damage. Although the exact causes of sperm DNA damage remain to be determined, it has been suggested that there are numerous reasons that cause it. Abnormalities of environmental

Table II. Association between sperm fragmentation DNA and each parameter of semen.

Group	Semen volume (ml)	Sperm motility	Normal form of sperm (%)	Sperm density (10 ⁶ /ml)	DFI (%)
Infertile male patients	2.85±1.33	28.21±12.03	3.82±3.5	32.3±17.6	36.5±3.87
Males who had children	3.02±1.21	32.01±11.89	3.55±2.8	38.6±12.8	16.23±2.65

Table III. Study on the correlation between the sperm DNA fragmentation index and each conventional semen parameter.

Detection index	r	P-value
Semen volume	0.067	0.106
Sperm density	0.815	0.533
Sperm motility	-0.114	0.068
Normal form rate of sperm	-0.089	0.093

Table IV. Association between sperm DNA fragmentation index, conventional parameters and microelements in blood plasma.

Parameters	Zinc	Iron	Calcium	Magnesium	Lead	Copper
DFI (%)	0.187 ^a	-0.037	0.016	-0.263 ^a	0.078 ^a	-0.132
Sperm density	-0.013	-0.021	-0.042	0.053 ^a	0.079	0.018
Sperm motility	-0.223 ^a	-0.178	-0.087	0.285 ^a	-0.045	-0.057
Sperm motility rate	-0.029 ^a	-0.022	-0.027	0.267 ^a	-0.028	0.124

^ap<0.05.

Table V. Association between sperm DNA fragmentation index and seminal plasma ROS level.

Group	DFI (%)	ROS level
Infertile male patients	36.5±3.87	548.9±108.2
Males who had children	16.23±2.65	416.3±95.5

ROS, reactive oxygen species.

factors can lead to variation of genes and chromosomes, and because the DNA damage leads to the destruction of the integrity of genetic information, this may result in the occurrence of infertility. Additionally, leukocytes in human tissue may produce excessive ROS because of an increase in infectious diseases in the reproductive system, which causes further damage to sperm DNA (8). Under normal physiological status, ROS participates in processes such as regulating immunological surveillance, signal transduction and cell growth, and is therefore an important medium of signal transduction. ROS plays an important role

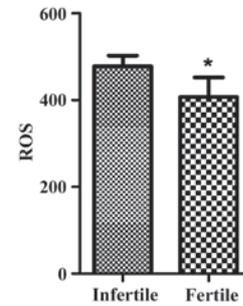


Figure 2. Seminal plasma ROS level of infertile males and males who had children, *p<0.05. ROS, reactive oxygen species.

in regulating body function and increasing probability of fertilization (9,10). The main components of ROS are free radicals, peroxide, oxygen ions and are mainly produced by leukocyte and sperm in semen. The reactivity of the oxygen in ROS may cause damage to the sperm of the cytomembrane, thus resulting in the damage of sperm DNA and impacting sperm motility, which may finally affect fertility (11).

By analyzing the infertile male sperm DNA fragmentation index and conventional semen parameters, this study has shown that the conventional semen parameters were not correlated with sperm DNA fragmentation index, and therefore, sperm DNA detection can be added to the diagnosis and treatment of infertile patients in clinic in addition to the routine semen analysis, which may further completely reflect male fertility (12). After an infertile male's sperm DNA fragmentation index and blood microelements were analyzed, it was found that zinc and lead in serum were positively correlated with the sperm DNA fragmentation index. The function of zinc is that it mainly has an effect on the thalamus-pituitary gland-testis axis (13), and through the regulation of the sex hormone secretion and controlling gonad development, it may have a great effect on semen quality and cause an issue of spermatogenesis, which may lead to male infertility (14). An excessive lead ion concentration in blood can produce toxic effects on sperm (15), and impact sperm motility, which may cause the decrease of male sperm quality and result in infertility. Magnesium in serum was found to be negatively correlated with sperm DNA fragmentation index. Study results also show that when the content of magnesium ion in the serum rises, sperm motility rates and rectilinear motion sperm motility rates notably increase, at the same time, sperm density also increases. The results of the present study on the association between infertile male sperm DNA fragmentation index and seminal plasma ROS levels have shown that the ROS levels in the infertility group were apparently higher than those of the normal childbirth group (p<0.05). The main reason of an increase in seminal plasma ROS levels causing sperm damage

and thus resulting in infertility is that the sperm cytomembrane is damaged to varying degrees by the existence of a superoxide anion and oxygen free radicals (16-18). Additionally, correlational research data have shown that seminal plasma ROS levels have an effect on both sperm forward movement and sperm activity rate, which may cause a decrease in its activity rate (19). Therefore, in clinic, male fertility ability can be improved by reducing seminal plasma ROS levels. Sperm DNA chain break causes sperm DNA damage, leading to chromatin crosslinking, which is the main mechanism causing an increase in ROS levels and subsequently causing infertility. At the same time, it can reduce the production of the spermatoblast ATP, reduce the sperm motility rate, damage sperm cytomembrane and cause infertility (20-22).

In conclusion, infertile male's sperm DNA fragmentation index is not correlated with conventional semen parameters but is positively correlated with blood microelements zinc, lead and seminal plasma ROS, and is negatively correlated with magnesium. The results above provide enough theoretical bases for diagnosing and treating infertile males in clinic.

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Availability of data and materials

The datasets analyzed during the current study are not publicly available due to the protection of patient privacy but are available from the corresponding author on reasonable request.

Authors' contributions

DX and XJ designed and conducted the study and analyzed the data. CL detected the sperm parameters like density and motility. YZ detected the sperm fragmentation DNA. SZ and EJY detected the serum microelements and seminal plasma ROS level. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Shiyan Taihe Hospital (Shiyan, China) and informed consents were signed by the patients.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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