Analysis of DNA Damage and Oxidative Stress in Human Spermatozoa and Some Biochemical Changes in Seminal Plasma and their Correlation with Semen Quality of Infertile Men

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ABSTRACT:

BACKGROUND:

Numerous studies have shown the presence of DNA strand breaks in human ejaculated spermatozoa. The nature of this nuclear anomaly and its relationship to patient etiology is however poorly understood.

OBJECTIVE:

This study was done to investigate the relationship between nuclear DNA damage, assessed using the TUNEL assay and a number of biochemical parameters including zinc level, copper level, and the malondialdehyde (MDA) level as an indicator of the lipid peroxidation and also the standard general seminal fluid examination.

MATERIAL AND METHODS:

In this study 100 seminal samples were tested. Seventy five infertile patients aged (30.33±5.96) and 25 normal people aged (29.84±6.434) were included as a control. All tests were done to all the samples except the DNA fragmentation which was done to 60 samples only (45 patients and 15 normal).

RESULTS:

A positive relationship between DNA damage level and infertility was found. Also there were higher levels of MDA level and DNA damage in spermatozoa of infertile patients (P=0.001). We found also absence of difference in the copper level between the two groups. Regarding seminal zinc level we found that there was a low zinc level in infertile patients (P=0.001). Non-significant correlations between zinc level and the percentage of DNA fragmentation and the MDA levels were obtained.

CONCLUSION:

This study suggested that sperm DNA analysis might be better discriminator between infertile and fertile men than standard semen analysis also oxidative stress and sperm DNA damage contributed to sperms dysfunction. The infertile samples associated with high levels of oxidative stress and low seminal zinc levels.

KEY WORDS: sperm DNA fragmentation, oxidative stress, seminal zinc level.

INTRODUCTION:

Male factor infertility plays a role in approximately 50% of infertile couples [(1)]. A number of etiologies have been identified as potential causes of male infertility, which include gene mutations, infectious diseases, ejaculatory duct occlusion, varicocele, radiation, chemotherapy and erectile dysfunction [(2)].

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The trace elements are known to exist in the body at very low levels, which are lower than 0.01% of body weight. According to their physiologic function, they may be categorized as necessary, potentially necessary, and unnecessary elements [(3)]. Zinc is a cofactor for more than 200 metalloenzymes in a variety of animal species. There is evidence that Zn in seminal plasma influences sperm oxygen consumption, nuclear chromatin decondensation, and zinc deficiency causes hypogonadism and Zn is thought to be important in the stabilization of sperm chromatin [(4)].

The trace element copper (Cu) has been identified as a highly toxic element for sperm [5]. Copper in the cytosol is mainly bound to proteins, i.e. metallothioneins, which also binds the element Zn to render these elements nontoxic in case of excess. Copper is an important element for numerous metalloenzymes and metalloproteins that are involved in energy or antioxidant metabolism. In its ionic form (Cu^{+2}), the trace element rapidly becomes toxic to a variety of cells, including human spermatozoa [6].

One area of research that has been studied intensely during the past decade as a cause for male infertility is the integrity of DNA in the nucleus of mature ejaculated spermatozoa [7]. Normally, the sperm chromatin is a highly organized, compact structure consisting of DNA and heterogeneous nucleoproteins. It is condensed and insoluble in nature-features that protect genetic integrity and facilitate transport of the paternal genome through the male and female reproductive tracts [8].

For a spermatozoon to be fertile, it must be capable of undergoing decondensation at an appropriate time in the fertilization process. Infertile men manifest various nuclear alterations, including an abnormal chromatin structure, chromosomes with microdeletions, aneuploidies, and DNA strand breaks [9].

Accumulating evidence suggests that disturbances in the organization of the genomic material in sperm nuclei are negatively correlated with the fertility potential of spermatozoa, either in vivo or in vitro [10]. Some recent reports have indicated that when >30% of sperm DNA is damaged, natural pregnancy is not possible [11]. Also, it has been suggested that sperm DNA integrity may be a more objective marker of sperm function as opposed to the standard semen analysis.

Methods such as terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-nick end labelling (TUNEL) assay, which detects DNA fragmentation, annexin-V binding, detecting apoptosis-related translocation of plasma membrane phosphatidylserine and immunohistochemistry have been employed to identify germ cell apoptosis [12].

MATERIAL AND METHODS:

This study included a seventy-five of infertile patients attending the male infertility clinic at Kamal El-sameraee infertility and gynecology hospital in a period of about 6 months. All the patients were with a history of at least 1 year duration with regular unprotected intercourse. Their age (30.33±5.96 years) and they all had a normal physical evaluation and genital examination (performed by a specialist). Female partners of these men had been evaluated by a gynecologist and they had a normal reproductive and sexual history as well as normal gynecological investigations. The couples were diagnosed as "infertility because of male factor or unknown factor".

For controls the seminal samples of twenty five healthy fertile men were used (i.e., father of a child within the last 12 months and with no history of infertility or any abnormalities that might affect the fertility) with mean age (29.84±6.434 years).

Standard seminal fluid analysis was done to all samples after liquefaction according to the standard WHO technique (1) and seminal zinc level was tested by GIESSE DIAGNOSTIC KIT for zinc, and the seminal copper was assayed by GIESSE DIAGNOSTIC KIT for copper while the seminal MDA levels were analyzed according to the thiobarbituric acid method described by Rao and coworkers (1989) (13).

The sperm DNA fragmentation integrity was tested according to the manual supplied with TUNEL apoptosis detecting kit, DNA fragmentation/ fluorescence staining/ Bioassay ™ by US Biological, then examined under fluorescent microscope.

RESULTS:

Patients' age and results of the classical semen analysis performed by light microscopy and biochemical tests and TUNEL assay for samples of 75 infertile subjects consulting for infertility and 25 fertile donors were shown in table 1.

Table 1: Results of standard seminal analysis, biochemical tests, and TUNEL test for infertile patients and healthy fertile donors. (A probability value ≤0.05 deemed as significant and was gray highlighted).

	Infertile samples Mean± SD. (no.=75)	Healthy control Mean± SD. (no.=25)	P value
Age (years)	30.33±5.96	29.84±6.434	0.726
Sperm Count (million/ml)	25.72±21.433	56.12±20.592	0.0001
Abnormal Morphology (%)	49.07±21.129	24.20±12.22	0.0001
Progressive Motility (%)	31.93±19.064	61.00±16.137	0.0001
Seminal zinc (mmol/l)	143.27±32.396	186.92±17.197	0.0001
Seminal Copper level (mg /kg)	1.092±0.098	1.08±0.115	0.629
Seminal Malondialdehyde ($nM/10^8$)	2.365±0.5136	1.680±0.273	0.0001
DNA Fragmentation (%)	43.20±17.957 (no.=40)	24.00±5.804 (no.=20)	0.0001

Non significant correlations were obtained between sperm DNA damage percentage and seminal zinc, seminal copper, and seminal MDA and these correlations were shown in figures 1, 2, 3 respectively.

The function of zinc as antioxidant was studied through its correlation with seminal MDA. A non significant correlation was obtained also and this was demonstrated in figure 4

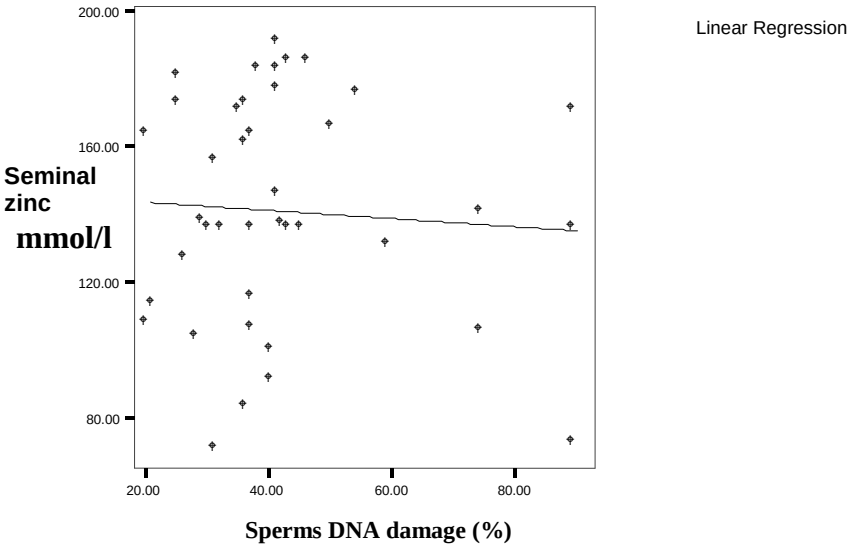

Figure 1: Correlation between seminal zinc levels and DNA fragmentation. (A non significant negative correlation; r=-0.065, *P*=0.961).

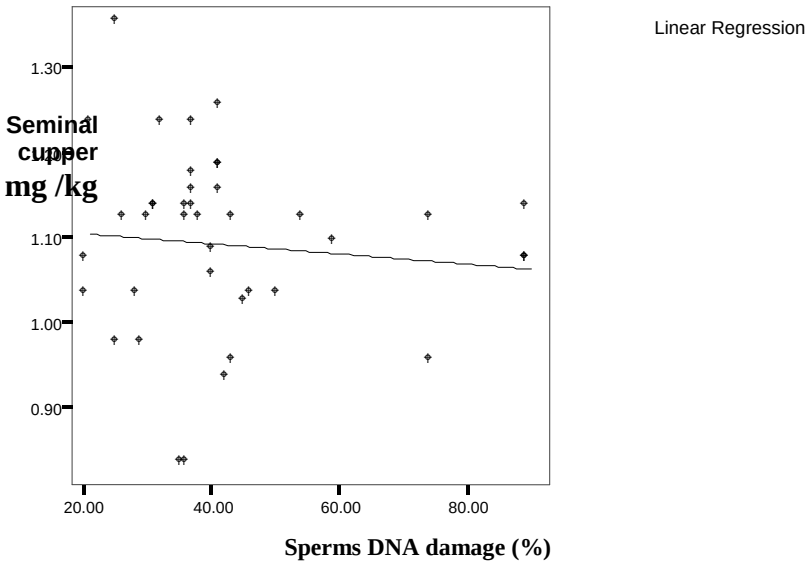

Figure 2: Correlation between seminal cupper levels and DNA fragmentation. (A non significant negative correlation; r=-0.101, *P*=0.537).

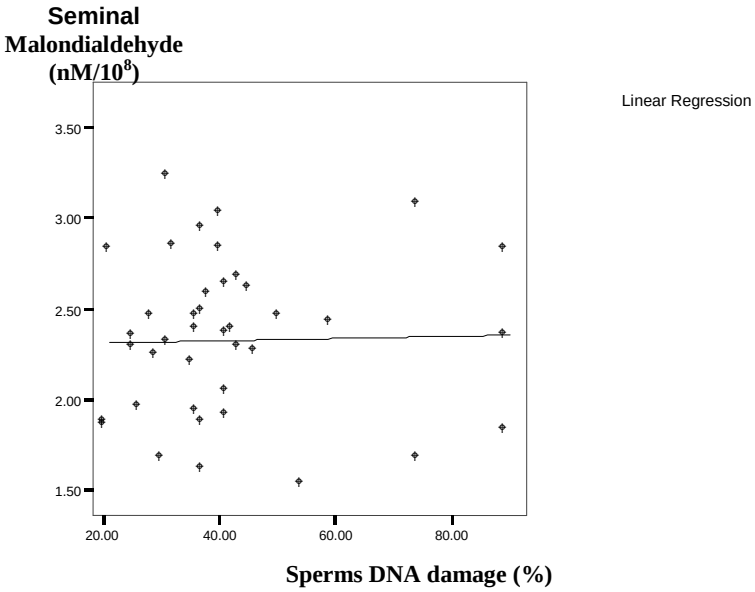

Figure 3: Correlation between MDA levels and DNA fragmentation. (A non significant positive correlation; r= 0.026, *P*=0.871).

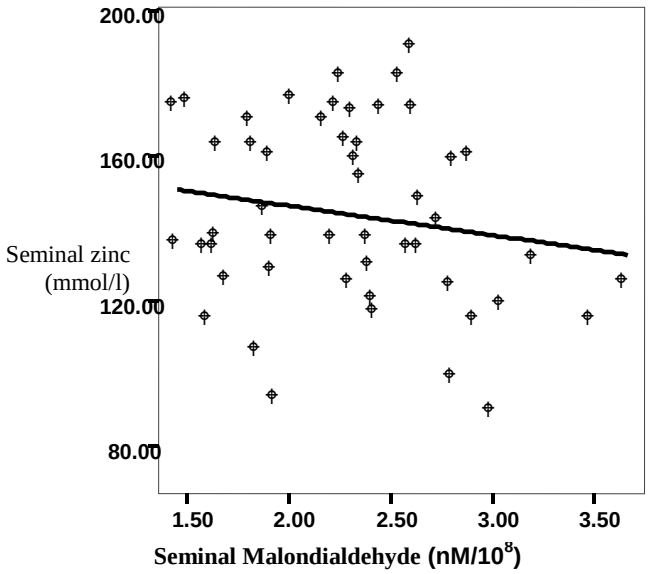

Figure 4: Correlation between seminal zinc levels and seminal MDA. (A non significant negative correlation; r=-0.128, *P*=0.273).

DISCUSSION:

Different studies have been proposed to explain the origin of DNA damage in mature spermatozoa from infertile men, including defective sperm chromatin packaging, apoptosis and oxidative stress [14]. Other studies tried to correlate the seminal plasma contents with the male factors of infertility.

This study shows also that infertile samples had a low level of seminal plasma zinc than the fertile sample (P=0.0001). There are several mechanisms by which zinc might interfere with spermatogenesis and sperm function.

Zinc exerts an in vitro effect on oxidative changes in human semen and is considered as a scavenger of excessive O_2 production by defective spermatozoa and/or leukocytes after ejaculation [15]. It modulates the stability of biological membranes by inhibiting lipid peroxidation through influence on phospholipases, resulting in fluidity changes [16]. Such correlation between seminal zinc and the marker of oxidative stress could not be obtained in this study.

Therefore, several studies in the last decades have tried to correlate zinc levels with semen quality parameters, although pathologic conditions of the prostate gland do not necessarily implicate interference with sperm function. It also has to be considered that ejaculated spermatozoa represent spermatogenesis over several months. The results of these studies have been controversial, and overall no marked association was found. Whereas some studies suggested some relationship of zinc levels with standard variables such as sperm motility [17] and/or sperm count [18]. While Sorensen and coworkers [19] could not prove the same relationship.

This study shows that there was no correlation between seminal zinc levels and the DNA damage percentage. These findings were disagreed with Keel and Webster[4] who proved that seminal zinc is important for the stability of sperm chromatin.

We tried also to correlate the level of seminal copper of the infertile patients with healthy normal seminal fluid. It was found that there was no correlation between the seminal copper level among the samples examined (P value>0.05). This result was in common with the findings of Huang and coworkers [20] which show that seminal plasma copper level did not correlate with any of semen parameters. While Aydemir and coworkers [21] found that copper levels in serum and seminal plasma in infertile male group were significantly higher than those in the fertile male group.

Concerning oxidative stress (OS), it is obvious from the results of this study that infertile patients had significantly increased OS as compared to

fertile donors, verified by highly significantly increased "Seminal malondialdehyde concentration" (MDA) in these patients than in control donors (P=0.0001).

Among the various methods for detection of lipid peroxidation, we chose to measure spontaneous MDA production, which reflects the peroxidation of polyunsaturated phospholipids, the major components of sperm membrane[22]. MDA measurements are physiological and relevant because major loss of sperm function may occur with minimal damage to the membranes that envelop the sperm and/or divide key intracellular sperm compartments. Geva and coworkers [23] demonstrated that the high MDA production in the male with low fertilization rates in their previous IVF cycles. They also found that the reduction of MDA by using antioxidant therapy was correlated with the improvement of fertilization rates.

Levels of MDA in this study articulated with results of several other workers. High levels of ROS were spotted by Moustafa and coworkers in patients with abnormal semen parameters compared to donors [14].

It has been recently reported that ROS (endogenously generated or provided as an exogenous stimulus) can cause an increase in DNA fragmentation in human spermatozoa[24]. In the present study, seminal samples of infertile patients had higher levels of sperm DNA fragmentation (TUNEL positive) than healthy samples (P=0.0001). But a non significant correlation was obtained between seminal OS and sperms DNA damage percentages. This result goes with Verit and coworkers [25] who did not find any relationship between sperm DNA damage and oxidative stress in infertile men.

Sakkas and coworkers[7] proposed 2 theories to describe the origin of DNA damage in mature spermatozoa. The first arises from studies performed in animal models and is linked to the manner in which mammalian sperm chromatin is packaged, while the second attributes the nuclear DNA damage in mature spermatozoa to apoptosis. Those individuals with high rates of apoptosis may have an increased percentage of sperm with genetic damage and may have a higher number of sperm that are immotile. A third theory was correlating the oxidative stress (elevated level reaction oxygen species or diminished antioxidant defense mechanism) result in DNA damage [26].

The cause that, the healthy seminal samples had low levels of TUNEL positive sperms might be that the apoptotic cells are believed to undergo heterophagic elimination by phagocytes without releasing pro-inflammatory mediators or ROS. It has been hypothesized that a similar mechanism probably provides for senescent or abnormal sperm removal [27].

Regarding possible correlation between percentage of apoptosis and age of patients, we did not find any correlation in the various groups studied. This was as expected since the subjects under examination were young adults; only four were in their forties (40–41 years old). This is not an absolute finding and relates only to the patients in our study.

Results of TUNEL assay shows that about 43% of sperm in infertile samples were found to be TUNEL positive, a value similar to 44% found by Al-Hashimi [28] in 40 infertile Iraqi patients. Donnenlly and coworkers [29] also showed that 40% of sperm from semen and 20% of sperm from fraction (after swim up separation) with high sperm motility from infertile men had DNA fragmentation using TUNEL assay. These results were higher than that reported by Shen and coworkers [30] which were around 15%.

CONCLUSION:

The sperm DNA study was a good way to differentiate between infertile and fertile man. Higher levels of seminal MDA which is an indicator of oxidative stress were found in infertile samples and this might lead to morphological and functional disturbance but not correlated with sperm DNA damage levels and further studies are recommended to find the origin of DNA damage and the factors that lead to increase or decrease its levels. The function of seminal zinc as antioxidant was not proved in this study.

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