Correlation of Sperm DNA Fragmentation With Some Semen Parameters Of Infertile Men In Iraqi Kurdistan Region

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1. Introduction
Infertility affects approximately 15% of couples trying to conceive and male factor contributing in roughly half of the cases. Disorders of the male reproductive system have become important public health issues as they can cause infertility, miscarriages, and abnormal outcomes in the offspring (Sergerie et al., 2005; Oehninger, 2001). The quality of sperm DNA is very important in maintaining the reproductive potential of men (Agarwal and Allamaneni, 2004). A number of studies have investigated the relationship between human sperm DNA damage and semen parameters, such as concentration, morphology, and motility (Sills et al., 2004; Ramos and Wetzels, 2001; Irvine et al., 2000). Clinical evidence showed that sperm DNA damage could be a marker of sperm quality as well as the relationships between DNA damage and male fertility status (Zribi et al., 2011; Alvarez, 2003; Evenson et al., 2002). Sperm DNA resistant to many events that occur during journey from the testis to when it reaches the oocyst for fertilization (Agarwal and Allamaneni, 2004; Aitken and Sawyer, 2003). Sperm DNA damage is clearly associated with male infertility and abnormal spermatogenesis but small percentage of spermatozoa from fertile men also possess detectable levels of DNA damage (Zini et al., 2001; Spano et al., 2000). The degree of DNA damage correlate...
with various indices of fertility such as the fertilization rate, embryo cleavage rate, implantation rate, pregnancy rate and live birth rate of the offspring (Tomsu et al., 2002). The level of sperm DNA damage is related to high levels of oxidative stress found in the semen of infertile men with this condition (Saleh et al., 2003). Testing DNA integrity is essential in fertilization and normal embryo and fetal development (Morris et al., 2002). Selection of Spermatozoa with intact DNA or with the least amount of DNA damage may help in assisted conception, When spermatozoa with extensive DNA damage are used, the embryo may fail to develop or implant in the uterus or it may be naturally aborted at later stage even when sperm with minimum DNA damage are used fetal development can be affected of later stages, resulting in a child with congenital abnormalities (Sharma et al., 2004). The routine examination of semen analysis which assess sperm concentration, percentage motility and morphology, does not identify subtle defects in sperm chromatin and sperm DNA damage (Barazani et al., 2014). Many researchers have investigated the relationship between human sperm DNA damage and sperm parameters, such as concentration, morphology and motility (Mahfouz et al., 2010). The evaluation of sperm DNA integrity in addition to routine sperm parameters could add further information on the quality of spermatozoa and reproductive potential of males (Sheikh et al., 2008; Perreault et al., 2003). Sperm DNA fragmentation has been promoted as a promising method to predict the outcome of IVF cycl (García-Ferreyra et al., 2014; Muriel et al., 2006). Sperm DNA fragmentation assessing to distinguish which couples are suitable for treatment by IVF (Check et al., 2005). SCD test sperm chromatin dispersion a simple highly reproducible and less expensive technique, yielding results highly correlated with those from other procedures like the DBD-Fish and the SCSA (Fernández et al., 2003). The objective of this study was to determine the levels of sperm DNA damage in Iraqi fertile and infertile males and it's correlation with some sperm parameters.

MATERIALS AND METHODS

The study included 40 infertile couples attended the clinic of British specialized Centre for infertility treatment and IVF in Erbil city, Iraqi Kurdistan Region throughout period from August 2012 to January 2013 and 16 healthy fertile donors proven fertility served as a control group. fertile and infertile males were divided into two groups according to their ages 35 ≥ years with mean ages 27.8 and 28.42 years for infertile and fertile males respectively while males at ages 35< years with mean ages 43.42 and 43.11 years for infertile and fertile males respectively, all infertile males were with mean duration of infertility period at least 2 ≤ years with no history of taking any medication during the last 3 months. All patients had normal genital examination.

**Semen Collection:** semen samples were collected by masturbation after 3-5 days directly into a sterile dry labeled cup in private and quite room adjacent to semen analysis laboratory. The specimen were placed in an incubator at 37°C for 30 minutes to allow liquefaction (Kvist & Björndahl, 2002) then mixed carefully for few seconds followed by microscopic examination within one hour of ejaculation, volume, concentration and motility was measured according to the guidelines of the world health organization (WHO, 2010).

**Sperm DNA Fragmentation (SDF):** Fresh semen samples was collected and sperm DNA fragmentation assay performed immediately once the sperm sample has obtained. SDF was assisted by halosperm G2, a kit supplied by halotech DNA company depending on sperm chromatin dispersion test(SCDT) using agarose microgel on a pretreated slide .the principle of
this method was using unfixed fresh sperm, an initial acid treatment denatures DNA in sperm cells, fragmented DNA following with lysis solution to remove most of the nuclear proteins, nucleoids with large haloes of spreading DNA loops, emerging from a central core, are produced. The nucleoids with large haloes of spreading DNA loops emerging from a central core are produced. The nucleoids from sperm with fragmented DNA either do not show a dispersion halo or the halo is minimal.

**Statistical Analysis:** comparisons between fertile and infertile males were tested by student's t-test using SPSS11. Correlation between SDF and ages, sperm motility, sperm concentration and ejaculate volume were evaluated using Pearson correlation coefficients. P≤0.05 was considered as statistically significant.

**RESULTS**

The current study revealed the differences in some seminal fluid parameters between fertile and infertile males according to their ages. There were significant decrease in ejaculate volume, sperm concentration and sperm motility of infertile males 35 ≥ years with means reaches to 2.70±0.240 ml, 22.36 ± 3.626 106 sperm/ml and 46.78 ± 1.676 % to each parameter respectively compared to fertile males at the same ages as shown in (Table 1). Also there are significant decrease in sperm concentration of infertile males 35< years that reaches 37.04± 4.224 106 sperm /ml compared with fertile males from the same ages 52.22 ± 2.961 106 sperm /ml accompanied by significant decrease in sperm motility to 37.04 ± 1.406% vs 51.33 ± 2.094% in fertile males (Table 2). Results of sperm chromatin dispersion test showed that the percentage of SDF in infertile males was significantly higher than fertile males (28.368± 2.149 vs 16.85 ± 1.737 %) in males 35 ≥ years and (31.09± 2.468% vs 15.88± 2.502%) in males 35< years as shown in (Table 3). Our data referred to presence of significant negative correlation between infertile male ages with sperm motility at (r = -.702) as well as a negative correlation with sperm concentration and ejaculate volume at r = -.360 and r = -.349 respectively. Also SDF increases with advancing age in a positive correlation at r = .052 as illustrated in (Table 4). No significant correlation was found between SDF and infertile male ages (Figure 1). In addition, there was a significant negative correlation between SDF and sperm motility at r = -.420 (Figure 2) accompanied with negative correlation of SDF with sperm concentration at r = -.150 (Figure 3). No correlation was observed between SDF and ejaculated volume in infertile males (Figure 4).

**Table 1: Some Seminal Fluid Parameters In Fertile and Infertile Males 35 ≥ Years**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean±SE Fertile</th>
<th>Mean±SE Infertile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>27.8 ±1.036</td>
<td>28.42±1.109</td>
</tr>
<tr>
<td>Volume (ml)</td>
<td>*2.70±0.240</td>
<td>3.80± 0.262</td>
</tr>
<tr>
<td>Ejaculation</td>
<td>22.36 ± 3.626</td>
<td>58.85 ± 3.120</td>
</tr>
<tr>
<td>Sperm Concentration (106/ml)</td>
<td>46.78 ± 1.676</td>
<td>58.00 ± 4.380</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE

Differences significant at (p* <0.05)

**Table 2: Some Seminal Fluid Parameters In Fertile and Infertile Males 35 < Years**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Mean±SE Fertile</th>
<th>Mean±SE Infertile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>43.42±1.221</td>
<td>43.11±1.567</td>
</tr>
<tr>
<td>Ejaculation Volume (ml)</td>
<td>2.32±0.194</td>
<td>2.73± 0.184</td>
</tr>
<tr>
<td>Sperm Concentration (106/ml)</td>
<td>37.04± 4.224</td>
<td>52.22 ± 2.961</td>
</tr>
<tr>
<td>Sperm Motility (%)</td>
<td>1.406 *</td>
<td>2.094</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE

Differences significant at (p* <0.05)
Table (3) Percentage of SDF in fertile and infertile males failed in IVF Program Values are expressed as mean ± SE

<table>
<thead>
<tr>
<th>Males</th>
<th>35 ≥ years</th>
<th>35 &lt; years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>% SDF</td>
</tr>
<tr>
<td>Fertile</td>
<td>7</td>
<td>16.85 ± 1.737</td>
</tr>
<tr>
<td>Infertile</td>
<td>19</td>
<td>28.368 ± 2.149*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE
* Differences significant at (p < 0.05)

Table 4: Correlation of male age with SDF and some semen parameters

<table>
<thead>
<tr>
<th>Male (Years)</th>
<th>SDF (%)</th>
<th>Sperm motility (%)</th>
<th>Sperm Concentration (106 sperm/ml)</th>
<th>Ejaculation Volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>35 &lt; years</td>
<td>29.8 ± 0.10</td>
<td>41.67 ± 8.38</td>
<td>30.07 ± 19.04</td>
<td>2.50 ± 0.97</td>
</tr>
<tr>
<td>35 ≥ years</td>
<td>.39</td>
<td>.702**</td>
<td>.360*</td>
<td>-.349</td>
</tr>
<tr>
<td>Fertile</td>
<td>.05</td>
<td>p&lt;0.01</td>
<td>p&lt;0.05</td>
<td>p&lt;0.05</td>
</tr>
<tr>
<td>Infertile</td>
<td>.215</td>
<td>NS</td>
<td>.640**</td>
<td>.634**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE
Differences significant at (p < 0.05)
Differences significant at (p < 0.01)

DISCUSSION
Sperm chromatin dispersion test (SCDT) are currently available to assess sperm DNA integrity which measures the susceptibility of sperm DNA to denaturation (Fernández et al., 2003; Evenson et al., 1999). From the results of our study we demonstrated a negative correlation between sperm motility and sperm concentration with sperm DNA fragmentation, these results were agreement with (Sun et al., 1997) who demonstrated a significant negative correlation between semen parameters and DNA damage in sperm and supported by (Sheikh et al., 2008) which assure the presence of negative correlation between SDF and sperm motility at r = -0.263. Fragmentation of sperm DNA may be attributed to intrinsic factors such as oxidative stress, aging and varicocele or due to extrinsic factors such as medication and environmental factors (Singh and Agarwal, 2011). Ejaculated sperm DNA fragmentation might result of spermatozoa to mature normally. Oxidative stress and excessive production of ROS has been shown to affect the integrity of sperm.
chromatin (Sharma et al., 2004; Aitken et al., 2003; Saleh et al., 2003) and incomplete maturity of gametes play an important role in SDF due to affecting the activity of topoisomerase II (Sailer et al., 1995; Manicardi et al., 1998) as well as changes in genetic materials due to Apoptotic process (Richburg, 2000; VanDyke et al., 2000; Sinha Hikim et al., 1997; Furuki et al., 1996). Varicoceles are associated with the abnormal retention of sperm cytoplasmic droplets and that these retained droplets are correlated with sperm DNA damage in infertile men (Fischer et al., 2003; Zini et al., 2000). SDF has been frequently observed among infertile couples with unexplained aetiologies and with recurrent pregnancy failures and high abortion rates (Carrell et al., 2003; Host et al., 2000). Inflammation of the epididymis and testis is another factor associated with SDF which result in leukocytospermia and have been associated with increased levels of reactive oxygen species and subsequent sperm DNA damage (Erenpreiss et al., 2002). Testis temperature play an important role in sperm DNA integrity (Paul et al., 2008). Sperm DNA damage had a significant effect on fetal development after implantation in ICSI (Borini et al., 2006) that the pregnancy rate was decreased in ICSI if the sperm DNA fragmentation in infertile males is exceeded 27-30% (Larson-Cook et al., 2003) because of impairment of blastocyst formation due to sperm DNA fragmentation and as a result failed IVF cycle (Ahmadi and Ng, 1999) this results was agreement with (Virro et al., 2004) but disagreed with (Boe-Hansen et al., 2006) who stated that 27% of sperm DNA fragmentation in infertile males did not prevent IVF success (Boe-Hansen et al., 2006) therefore we can use SDF test as a diagnostic tool of male infertility which is aid in selection of spermatozoa with less amount of fragmentation in assisted reproductive techniques (ART) because SCDT is very simple, high reproducible and precise whereas the Comet and Tunel assays lack the consistency and reliability needed in a clinical setting (Sheikh et al., 2008; Evenson and Wixon, 2006). Medications and radiation therapy affect sperm DNA integrity and male infertility depending on both duration and dose of exposure because of their cytotoxic effect on spermatogenic epithelium (Morris, 2002; Sailer et al., 1995). Exposure to air pollution may result in sperm DNA fragmentation and affecting male infertility (Specht et al., 2012; Rubes et al., 2005; Evenson and Wixon, 2005). Smoking is another factor affect the sperm DNA integrity because smoking causes increased production of leukocyte-derived reactive oxygen species, which has adverse effects on mature sperm therefore SDF can be considered as an independent parameter with prognostic value in the treatment of male infertility (Potts et al., 1999).

REFERENCES


Saleh, R., A.A. Agarwal, R.K. Sharma, et al., 2003. Evaluation of nuclear DNA damage in spermatozoa from...


