The Seminal Plasma MDA Level as Bio-Marker for Oxidative Stress and Their Impact on Seminogram in Primary Infertile Males, Erbil-Kurdistan Region, Iraq

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Abstract

Male infertility is an important medical and psychosocial problem worldwide. Therefore, it is important to identify new and non-conventional factors that may play significant role in male infertility. Current study focused on detecting more risk factors and markers for primary infertility in order to develop interventions for investigation and prevent their progressions. Oxidative stress has recently been identified as an underlying mechanism of numerous sources. The aim of the present study was therefore to evaluate whether there is an association between oxidative stress and male infertility in Erbil city subjects. The present prospective case control study was carried out between March to May 2013, in Maternity Teaching Hospital/in-vitro fertilization center and private clinical laboratory. 75 infertile males represent patients group were enrolled in this study. Apparently 40 healthy individuals who have no history of clinical evidence of any disease were also joined to this study as control group. Malondialdehyde (MDA), the marker of lipid peroxidation was measured in the seminal fluid of fertile and infertile male subjects. The sperm count, sperm morphology and motility, as well as semen volume were all found significantly lower in infertile subjects compared with fertile subjects (p<0.01). In seminal plasma, the MDA level was found significantly higher in infertile group (p<0.05). Lifestyle behaviours such as varicocele repair, cigarette smoking and alcohol consumption, all enhance the generation of ROS level in the seminal plasma, such elevation associated with decreased sperm quality particularly in term of sperm count and sperm motility that leading to increase male infertility.

Keywords: MDA; oxidative stress; semen quality; primary infertile male

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Introduction

Infertility is a disease of the reproductive system defined by the failure to achieve a pregnancy after 1 year or more of regular unprotected sexual intercourse [1].

About 15% of couples do not achieve pregnancy within 1 year and seek medical treatment for infertility and less than 5% remain unwillingly childless. Infertility affects both men and women. In 50% of involuntarily childless couples a male infertility associated factor is found together with abnormal semen parameters [2].

Lifestyle factors including age, weight, alcohol intake, cigarettes smoking, industrial compound, dietary and recreational drug use impacts negatively on reproductive performance [3,4]. Male infertility can also be result of congenital and / or acquired abnormalities. They include infection of the genital tract, varicocele, developmental and anatomical abnormalities, endocrinopathies, immunological factor and genetic abnormalities [2].

Free radicals are a group of highly reactive chemical molecules consisting of one or more unpaired electrons. Free radicals derived from oxygen metabolism are designated as ROS [5]. All living aerobic cells are normally exposed to some ROS, but if ROS levels rise, oxidative stress (OS) occurs, which results in oxygen and oxygen-derived oxidants and in turn increases the rates of cellular damage. OS has been shown to be a major cause of male infertility [6].

Sources of ROS generation include: Internal source (a variety of semen components, including morphologically abnormal spermatozoa, precursor germ cells, and leukocytes) [7,8] and external source (industrial compounds, cigarette smoking, alcohol intake, exercise and elevated temperatures) [4] (Figure 1).

Reactive oxygen species cause infertility by two principle mechanisms, first ROS damage sperm membrane which in turn reduces the sperm motility and ability to fuse with the oocyte. Secondary, ROS directly damage sperm DNA [9] (Figure 2).

Spermatozoal plasma membrane lipid composition have very high levels of phospholipids, sterols, saturated and poly-unsaturated fatty acids (PUFA) therefore sperm cells are susceptible to the damage induced by reactive oxygen species (ROS) [10]. Excessive production of ROS in semen has been linked to male infertility. Estimation of malondialdehyde (MDA) is a marker of lipid peroxidation that induced by ROS [11].

Many previous studies improved that defective sperm function is associated with the presence of ROS in the seminal fluid, in which produced from many sources, and
suggested that the ROS negatively correlated with sperm count, sperm motility and sperm morphology [12, 13].

Semen is the fluid that is ejected from the penis at the time of orgasm [14]. The diagnosis of male infertility has relied upon microscopic assessment and biochemical assay to determine human semen quality [15]. Semen analysis is of great value in the initial investigation of male and its results are often taken as a surrogate measure of male fecundity and pregnancy risk. It provides information on the functional status of the germ epithelium, epididymis and accessory sex gland [16]. The aim of the basic semen analysis is to evaluate descriptive parameters of ejaculates obtained by masturbation. The qualities that are assessed are visual appearance, smell, liquefaction, viscosity, volume, sperm concentration and total number of spermatozoa, sperm motility, and sperm vitality. Furthermore and differential count with respect to sperm morphology [17]. The male infertile patient’s semen analysis still provides the fundamental information on which clinicians base their initial diagnosis [15].

Materials and Methods

Sampling Area

A case control study of 75 primary infertile male and 40 healthy individual were enrolled in this study over the period from March to May 2013. Data were collected by interview with patients, through structural questionnaire regarding primary male infertility. Out of 75 patients, 9 (12%) from Maternity Teaching Hospital / in-vitro fertilization (IVF) center and 66 (88%) from Shayi private clinical laboratory. Total of 40 apparently healthy individual were selected from males without any history of infertility problems or diseases who attendances Shayi private clinical laboratory as control group.

Seminal Fluid Sample Collection

The patients and healthy individuals were asked to bring semen sample which collected in sterile, clean, wide-mouthed and labeled plastic container.

Macroscopic and Microscopic Examination

After liquefaction time, according to guide line of [16], seminal fluid was investigated for the macroscopically (appearance, volume, PH, viscosity, gelatinous) and microscopically (sperm count, sperm motility, sperm morphology) analysis.

Quantitative Estimation of MDA Level
After macroscopic and microscopic analysis, the remaining specimens were centrifuged at 3000 round per minute for 15 minute, to obtain seminal plasma. Seminal plasma was dispensed into labeled and sterile Eppendrof tube, kept at -20 C˚ to determine the level of MDA by using ELISA technique.

**Results and Discussion**

*A Comparison of Age, Semen Parameters and MDA Between Fertile and Infertile Men*

The age of fertile subjects ranged from 28-48 years with mean of 33.37±2.36 years; whereas the age of the infertile subject ranged from 20-43 years with mean of 30.21±0.61 years. The semen quality of infertile men was significantly lower compared to fertile. Significant difference in mean value of MDA was observed between fertile and infertile males (Table 1).

**Table 1: A comparison of age, semen parameters and mda between sterile and infertile men**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Fertile men No. 40</th>
<th>Infertile men No. 75</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>33.37±2.36</td>
<td>30.21±0.61</td>
<td>NS</td>
</tr>
<tr>
<td>Volume (ml)</td>
<td>3.12±0.09</td>
<td>2.77±0.15</td>
<td>HS **</td>
</tr>
<tr>
<td>Sperm count *</td>
<td>70.5±2.98</td>
<td>46.30±4.17</td>
<td>HS **</td>
</tr>
<tr>
<td>Sperm motility</td>
<td>71.25±2.18</td>
<td>51.46±3.06</td>
<td>HS **</td>
</tr>
<tr>
<td>Abnormal morphology</td>
<td>31.87±0.79</td>
<td>38.54±2.43</td>
<td>HS **</td>
</tr>
<tr>
<td>MDA</td>
<td>1520.1±66.60</td>
<td>1717.6±43.08</td>
<td>S</td>
</tr>
</tbody>
</table>

*P value ≥ 0.05: Non-significant, * P value < 0.05: Significant, ** P value < 0.01: Highly significant, *: x10⁶ / ml*

The mean age of infertile men was 30.21 years which is consistent with study done by [19,20,21]. It has been found that the average age of infertile men in current study seem to be younger than those participated in studies done by [22,23]. The small men age in our study due to early married age and failed to conceive, they seek treatment at early stage of marriage.

Present study recorded significantly decreased sperm quality in infertile men was compared to fertile, concordant to studies of [24, 25, 26]

Lipid peroxidation of sperm membrane is considered to be the key mechanism of ROS induced sperm damage. MDA is byproduct of lipid peroxidation and important marker for oxidative stress. The present study revealed that MDA level was markedly elevated in seminal plasma in infertile subjects. This reflects that the infertile subjects
suffer from oxidative stress induced lipid peroxidation. Several other studies also showed an elevated MDA level in the seminal plasma of infertile groups [27, 28].

**B- A Comparison Between Semen Parameters and MDA Among Leuko and non-Leukocytospermic Infertile Men**

The mean value of MDA and seminal analysis (seminogram) in terms of semen volume, liquefaction time, sperm count, sperm concentration, sperm motility and sperm morphology among leuko and non-leukocytospermic infertile men show difference between them but statistically not significant (P≥0.05) (Table 2).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Leukocytospermia No. 38 Mean±SE</th>
<th>Non-leukocytospermia No. 37 Mean±SE</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period of abstinence (day)</td>
<td>3±0.00</td>
<td>3±0.00</td>
<td>ND</td>
</tr>
<tr>
<td>Volume (ml)</td>
<td>2.64±0.20</td>
<td>2.90±0.23</td>
<td>NS</td>
</tr>
<tr>
<td>Liquefaction time (minutes)</td>
<td>30±0.00</td>
<td>31.62±1.30</td>
<td>NS</td>
</tr>
<tr>
<td>Sperm count</td>
<td>45.15±5.69</td>
<td>47.48±6.18</td>
<td>NS</td>
</tr>
<tr>
<td>Sperm concentration</td>
<td>123.63±17.80</td>
<td>148.49±25.04</td>
<td>NS</td>
</tr>
<tr>
<td>Motility</td>
<td>49.07±4.26</td>
<td>55.27±4.47</td>
<td>NS</td>
</tr>
<tr>
<td>Morphology</td>
<td>48.08±4.37</td>
<td>53.42±3.43</td>
<td>NS</td>
</tr>
<tr>
<td>MDA</td>
<td>1647.0±60.68</td>
<td>1709.0±54.21</td>
<td>NS</td>
</tr>
</tbody>
</table>

P value ≥ 0.05: Non-significant, leukocytospermia: Infected men: Semen with pus >1 pus, ND: not determined *, x10⁶ / ml, **: x10⁹ / ejaculate

Leukocytospermia, also known as leukospermia, is used to designate abnormal concentrations of WBC in semen [18]. It has been considered as an indicator of male genital tract inflammation. One of the main changes during the inflammatory process is the discharge by PMN granulocytes of high level of protease such as elastase. As granulocytes are the main constituents of the WBC population in semen [29]. The presence of WBC in the semen is result in increased levels of ROS and sperm injury [4].

In our study, leukocytospermic infertile men showed decreased in sperm characteristics compared with non-leukocytospermic, similar results found by studies of [30, 31, 32, 33].
Saleh [34] found seminal leukocyte plays a role in stimulating ROS production by spermatozoa, in present study decreased ROS level found in leukocytospmia, this may due to presence compound in seminal plasma have defense mechanisms which dispose, scavenge and suppress the ROS known as antioxidant [35]. Increased ROS level found in non-leukocytospmia due to many factor responses to induce ROS [36, 37].

C- A Comparison of Semen Parameters and MDA Among Infertile Men According to Age

Men suffering primary infertility are categorized in to three groups according to age as shown in table (3). All investigated parameters reveals differences between age groups, but not significant.

Table 3: A comparison of semen parameters and mda among infertile men according to age

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Age &lt; 29</th>
<th>Age 30-39</th>
<th>Age &gt; 40</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. 39</td>
<td>No. 32</td>
<td>No. 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean±SE</td>
<td>Mean±SE</td>
<td>Mean±SE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume (ml)</td>
<td>2.64±0.16</td>
<td>3.14±0.27</td>
<td>2.50±0.28</td>
<td>NS</td>
</tr>
<tr>
<td>Sperm count +</td>
<td>47.53±5.68</td>
<td>46.84±6.59</td>
<td>30.00±19.09</td>
<td>NS</td>
</tr>
<tr>
<td>Sperm concentration ++</td>
<td>124.64±17.36</td>
<td>158.53±28.17</td>
<td>64.50±37.50</td>
<td>NS</td>
</tr>
<tr>
<td>Motility</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Motile</td>
<td>54.23±4.18</td>
<td>47.65±4.62</td>
<td>40.0±13.38</td>
<td>NS</td>
</tr>
<tr>
<td>Inmotile</td>
<td>35.25±3.50</td>
<td>41.71±4.51</td>
<td>35.0±11.72</td>
<td>NS</td>
</tr>
<tr>
<td>Morphology</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>52.35±3.79</td>
<td>49.28±4.24</td>
<td>47.50±16.13</td>
<td>NS</td>
</tr>
<tr>
<td>Abnormal</td>
<td>37.38±3.21</td>
<td>41.34±3.95</td>
<td>27.50±9.68</td>
<td>NS</td>
</tr>
<tr>
<td>MDA</td>
<td>1799.4±61.33</td>
<td>1652.1±63.12</td>
<td>1448.1±85.07</td>
<td>NS</td>
</tr>
</tbody>
</table>

P value ≥ 0.05: Non-significant, +: x10⁶ / ml, ++: x10⁶ / ejaculate

The effects of age on fertility are real and may be greater than has been thought and it’s well known that the fertility declines with age [22]. Previous studies documented that age is associated with diminished in semen volume, sperm count, sperm concentration, motility and morphology [38, 39]. Similarly, we concluded that increasing age is associated with decreased seminogram and older men have lower semen volume and sperm parameters.

Patient with younger age showed increasing MDA level compared to normal and older age. This result supported by Venkatesh [40] who found elevated ROS levels not related to age, and this elevation may be due to present of leukocyte [41] or abnormal sperm [42].

D- A Comparison between Semen Parameters and MDA among Fertile and Infertile Men With and without Varicocele
Seminal fluid investigated for the comparison between semen parameters and MDA. Analyzing of seminal fluid samples revealed non-significant differences in semen volume and spermatozoa morphology among fertile and infertile men with and without non-varicocele, while, there was significant difference in mean value of spermatozoa count and motility between fertile and infertile men. MDA revealed significant difference between fertile and infertile males with and without non-varicocele, but there is no difference among infertile sub-group (Table 4).

**Table 4: A comparison between semen parameters and MDA among fertile and infertile men with and without non-varicocele**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Fertile men (No. 40)</th>
<th>Infertile men (No. 63)</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SE</td>
<td>Mean±SE</td>
<td></td>
</tr>
<tr>
<td>Volume (ml)</td>
<td>3.12±0.09</td>
<td>2.83±0.47</td>
<td>NS</td>
</tr>
<tr>
<td>Sperm count</td>
<td>70.50±2.98</td>
<td>45.33±11.17</td>
<td>HS**</td>
</tr>
<tr>
<td>Sperm motility</td>
<td>71.25±2.18</td>
<td>46.25±8.37</td>
<td>HS**</td>
</tr>
<tr>
<td>Abnormal morphology</td>
<td>31.87±0.79</td>
<td>37.50±2.42</td>
<td>NS</td>
</tr>
<tr>
<td>MDA</td>
<td>1520.1±66.60</td>
<td>1753.4±87.16</td>
<td>S*</td>
</tr>
</tbody>
</table>

P value ≥ 0.05: Non-significant, * P value < 0.05: Significant, ** P value < 0.01: Highly significant, Different letter means significant difference, °: x10⁶ / ml

The role of varicoceles in the etiology of male infertility continues to be controversial, due to the numerous variables that impact the outcome of varicocele repair [43]. The pathophysiology of varicocele related infertility have shown the likely influence of ultra-structural testicular changes and increased oxidative stress with implication on the seminal antioxidant capacity and sperm chromatin integrity [44].

There are two different and diametrically opposed clinical scientific fronts on correlations between varicocele and infertility. The first groups consider repair varicocele improved seminal parameters and testicular trophism [45, 46]. The second groups consider postoperative correlated negatively and leading to a reduction in semen parameters and associated with sub-fertility [47, 48, 49]. Our results are in line with second groups, varicocele affect negatively on semen parameters and have lower quality of smeniogram.
The association of testicular dysfunction with varicocele, is due to increased ROS level and seminal oxidative stress [50], which involved in the pathogenesis of sperm DNA damage [51]. This hypothesis support our results, we found elevated seminal ROS is associated with varicocele and sperm dysfunction.

E- A Comparison Between Semen Parameters and MDA Among Fertile and Infertile Men With and With non-Cigarette Smoker

Mean value of semen parameters and MDA for infertile smoker and non-smoker with fertile men are summarized in table (5). There are statistically significant differences between fertile and infertile men in ejaculate semen volume, sperm count and sperm motility. MDA also show significant difference between fertile and infertile smoker and non-smoker males.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Fertile men Mean±SE</th>
<th>Infertile men Smoker Mean±SE</th>
<th>Non-smoker Mean±SE</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (ml)</td>
<td>3.12±0.95</td>
<td>2.52±0.16</td>
<td>3.04±0.26</td>
<td>S&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sperm count</td>
<td>70.50±2.98</td>
<td>40.76±4.99</td>
<td>52.30±6.73</td>
<td>HS&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sperm motility</td>
<td>71.25±2.18</td>
<td>51.28±4.27</td>
<td>51.66±4.45</td>
<td>HS&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Abnormal</td>
<td>31.87±0.79</td>
<td>36.43±3.24</td>
<td>40.83±3.65</td>
<td>NS</td>
</tr>
<tr>
<td>morphology</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDA</td>
<td>1520.1±66.60</td>
<td>1778.3±60.48</td>
<td>1727.7±62.19</td>
<td>S&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

P value ≥ 0.05: Non-significant, * P value < 0.05: Significant,** P value < 0.01: Highly significant, Different letter means significant difference, *: x10<sup>6</sup> / ml

Cigarette smoking is a widely recognized health hazard and a major cause of mortality also affects reproductive health [52].

Smoke is separate into two phases: gaseous and particular phases. The major constituents that affect health are: nicotine in the particulate phase and carbon monoxide in the gaseous phase [53]. Nicotine can alter the function of the hypothalamic-pituitary axis, affecting growth hormone subsequently impact spermatogenesis [3]. Toxic components in the cigarette smoke can disrupt the testicular microcirculation and cause DNA or chromosomal damage in germinal cells [52].
Data from our results indicate that most infertile male were smokers 39(52%), that agreement with study of Al-Matubsi [54], and revealed significant low quality of sperm parameters in terms of semen volume, sperm count and motility in semen of infertile smokers. This finding is in accordance with those of some other studies [55, 56, 57].

Exposure to cigarette smoke generates high levels of oxidative stress, directly increasing seminal ROS generation, and decreasing seminal levels of antioxidant enzyme superoxide dismutase. Men who smoke also have decreased measures of sperm quality, including decreased sperm count, motility and morphologically normal sperm [4, 58]. This hypothesis support our result, a significant high seminal MDA level found, concomitantly decreased levels of antioxidants in seminal plasma of smoker.

**F- A comparison between semen parameters and MDA among fertile and infertile men with and with non-alcohol consumption**

Basic semen characteristics and MDA value stratified according to alcohol and non-alcohol consumption infertile with fertile male are shown in table (6). Significant difference found between fertile and infertile alcohol drinking males in term of sperm count, and with non-alcohol drinking infertile male in sperm motility. Statistically significance was observed in MDA among alcohol consumption infertile and fertile males.

Table 6: A comparison between semen parameters and MDA among fertile and infertile men with and with non-alcohol consumption

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Fertile men</th>
<th>Infertile men</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.40</td>
<td>No.8</td>
<td>No.67</td>
</tr>
<tr>
<td></td>
<td>Mean±SE</td>
<td>Mean±SE</td>
<td>Mean±SE</td>
</tr>
<tr>
<td>Volume (ml)</td>
<td>3.12±0.95</td>
<td>2.5±0.42</td>
<td>2.8±0.166</td>
</tr>
<tr>
<td>Sperm count</td>
<td>70.50±2.98</td>
<td>40.12±13.14</td>
<td>47.04±4.42</td>
</tr>
<tr>
<td>Sperm motility</td>
<td>71.25±2.11</td>
<td>63.75±4.40</td>
<td>50.0±3.35</td>
</tr>
<tr>
<td>Abnormal morphology</td>
<td>31.87±0.79</td>
<td>37.5±2.11</td>
<td>38.67±2.71</td>
</tr>
<tr>
<td>MDA</td>
<td>1520.1±66.60</td>
<td>1727.3±142.8</td>
<td>1716.5±45.51</td>
</tr>
</tbody>
</table>

P value ≥ 0.05: Non-significant, * P value < 0.05: Significant,** P value < 0.01: Highly significant. Different letter means significant difference, *: x10⁶ / ml

Alcohol intakes have been shown to be involved in male reproductive function impairment [59]. In our study, 8 (10.67%) of infertile men intake alcohol. Its affect fertility through interfering with function of Leydig cells, Sertoli cells, pituitary gland,
ROS production, induced apoptotic cell death in testicular germ cells and suppresses spermatogenesis through increasing the activation of caspase-3 by suppressing the activation of survival kinases which is important for cell growth [60, 61, 62]. Alcohol affect adversely on semen volume, sperm count, sperm motility and sperm morphology [63, 64, 65, 66]. Concurrent with present study, we found alcohol consumption infertile men have lower semen parameters.

Alcohol-induced oxidative stress is linked to the metabolism of ethanol involving both microsomal and mitochondrial systems. Ethanol metabolism is directly involved in the production of reactive oxygen species (ROS). These form an environment favorable to oxidative stress. It elevates malondialdehyde (MDA), cause the modification of all biological structures and consequently result in serious malfunction of cells and tissues [67]. Spermatozoa are particularly sensitive to ROS as their PUFA, which oxidizes easily [6].

Alcohol is one of exogenous compound which can be metabolized in body by enzyme cytochrome P450. This enzyme plays a very significant role in the generation of ROS. Presence of alcohol can increase the level of the enzyme, which in turn results in the generation of more ROS [68]. According to our results, infertile men with alcohol consumption have high concentration of ROS, this consistent to result of [69].

![Figure (1): Relationship of the primary pathologies of the male reproductive system, oxidative stress and infertility [70].](image-url)
Figure (2): Mechanism of oxidative stress in human semen [71].

References


