Estimation of Leptin and Leptin Receptor Concentrations in Seminal Plasma of Primary Infertile Men

Ruqaya M. AL-Barzinji* BSc, MSc, PhD
Ahmed A. AL-Naqshbandi** BSc, MSc

Abstract:

Background: There are many sources for Leptin secretion, and it is activated by binding with its receptor known as leptin receptor, that play a role in male infertility.

Objective: To assess the levels of leptin and leptin receptors in seminal plasma among primary infertile men and its impact on semen parameters.

Patients and Methods: A case control study of 75 primary infertile males and 40 healthy individuals who were enrolled in this study during March 2013 to May 2013. Estimation of age, body mass index (BMI), semen analysis, seminal plasma leptin, leptin receptor and testosterone hormone concentration were done for all study subjects.

Results: Highly significant difference found in mean of semen parameters of infertile male compared with healthy controls. Mean concentration of seminal plasma leptin and leptin receptor of infertile men were significantly elevated, while serum testosterone concentration significantly decreased compared with healthy control.

Conclusion: There is emerging evidence that the leptin concentration negatively impacts fertility through its correlation with age, BMI, testosterone hormone and semen parameters.

Keywords: Leptin; Leptin receptor; Testosterone; semen quality.

Introduction:

Leptin, a key hormone in energy homeostasis and neuro-endocrine function, has a role in the pathogenesis of reproductive dysfunction in many states of energy imbalance [1]. Leptin is present in human Sertoli cells, seminiferous tubules, Leydig cells and germ cells. Indeed it is able to cross the testis blood barrier (TBB). Concentration of leptin in seminal plasma has a correlation with the sperm motility. By a high affinity binding protein, it’s bound with a soluble leptin receptor [2]. It is the product of the ob gene and belongs to the class 1 cytokine superfamily of receptors. Leptin receptor mRNA is expressed in the anterior pituitary, in several areas of the brain and in other tissues, also has been detected in granulose and theca cells [3]. In human, two major forms of leptin receptors (ob-R) are expressed. The short form (ob-RS) and long form (ob-RL) [4]. Leptin act via hypothalamic receptor (LEPRb) to regulate energy balance [5]. Stopping of leptin signaling due to mutation of leptin or its receptor, results in increased food intake and decreased energy expenditure phenotype reminiscent of the neuro-endocrine starvation response (including hypothyroidism and infertility) [6]. Sex steroids mainly testosterone are responsible for Leptin secretion by the adipocyte, also leptin secretion is regulated by other hormones, such as insulin and cortisol [7]. Testosterone as steroid hormones, is necessary for the maturity and maintenance of secondary sexual properties as well as beginning and maintenance of spermatogenesis [8]. A person’s weight can have a intense impact on fertility by presence correlation between body weight and sperm concentration [9]. The decrease in male fertility explained in relation to obesity, indeed obesity is considered as an etiology of male fertility [10]. There is a correlation between body weight and sperm concentration. It can disrupt the hormonal balance which is necessary for normal sperm production [11]. Semen analysis is of great importance in the initial investigation of male and its results are often taken as a surrogate measure of male infertility and pregnancy risks. It provides information on the functional status of the germ epithelium, epididymis and accessory sex glands [12]. Analysis of semen can give us information about problems in the genital organs of the male. The results of semen analysis have been used to categorize men into groups with different probabilities of achieving pregnancy within a certain time period. 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, liquidity, viscosity, volume, sperm concentration and total number of spermatozoa, sperm motility, and sperm vitality. Furthermore, differential count with respect to sperm morphology, assessment of sperm agglutination and assessment of presence of debris and other cell types in semen are also performed [13]. The aim of the study is evaluate the correlation between levels of leptin and leptin receptor in seminal plasma of primary infertile males and its effect to semen parameters.

Subjects and Methods:

Sampling Size and Area: A case control study of 75 primary infertile male and 40 healthy individual were enrolled in this study during March 2013 to May 2013. Out of 75 patients, 9(12%) from Maternity Teaching Hospital/Erbil and 66 (88%) from private clinical laboratory/Erbil. Total of 40 apparently healthy individual were selected from males without any history of infertility problems or diseases who attended private
clinical laboratory as control group. Seminal Fluid Sample Collection: The patients and healthy individuals were asked to bring semen sample which was collected in sterile, clean, wide-mouthed and labeled plastic container. Macroscopic and Microscopic Examination: After liquefaction time, according to guide line of WHO, seminal fluid was investigated for the macroscopically (appearance, volume, pH, viscosity) and microscopically (sperm count, sperm motility, sperm morphology) analysis [13]. 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 Eppendorf tube, kept at -20°C, to estimate the level of leptin and leptin receptor. Blood Sample Collection: Five ml of peripheral venous blood was collected from each patient and control group performed by using disposable syringe and collected in tubes, allowed to clot at room temperature then centrifuged at 3000 round per minute for 15 minute, the sera obtained were dispensed into labeled and sterile Eppendorf tube, kept at -20°C, to estimate the level of testosterone hormone. Quantitative Estimation of Leptin, Leptin Receptor and Testosterone Level: Enzyme linked immuno-sorbent assay (biiotek, USA) used for quantitative estimation of leptin, leptin receptor and testosterone. SPSS version 20 was used for statistical data analysis.

Results:

Table 1: Comparison of Semen Parameters, Leptin, Leptin Receptor and Testosterone between Fertile and Infertile men

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Fertile men n=40</th>
<th>Mean±SE</th>
<th>Infertile men n=75</th>
<th>Mean±SE</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>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume (ml)</td>
<td>3.12±0.09</td>
<td>2.77±0.15</td>
<td>HS **</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sperm concentration*</td>
<td>70.5±2.98</td>
<td>46.30±4.17</td>
<td>HS **</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sperm motility</td>
<td>71.25±2.18</td>
<td>51.4±3.06</td>
<td>HS **</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal morphology</td>
<td>31.87±0.79</td>
<td>38.5±2.43</td>
<td>HS **</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>268.3±11.59</td>
<td>314.8±24.57</td>
<td>HS **</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptin receptor (pg/ml)</td>
<td>1270.0±95.84</td>
<td>2250.5±14.88</td>
<td>S *</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
<td>11.64±0.66</td>
<td>7.62±0.30</td>
<td>HS **</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P value ≥ 0.05: Non-significant, * P value < 0.05: Significant, ** P value < 0.01: Highly significant, x10^6 / ml

Table 2: Comparison of Semen Parameters, Leptin, Leptin Receptor and Testosterone between Seminal Classes of Leukocytespermic Infertile Men

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normozoospermia n=24</th>
<th>Mean±SE</th>
<th>Oligozoospermia n=10</th>
<th>Mean±SE</th>
<th>Azozoospermia n= 4</th>
<th>Mean±SE</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (ml)</td>
<td>2.91±0.28</td>
<td>2.05±0.35</td>
<td>2.54±0.28</td>
<td>NS</td>
<td>2.54±0.28</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Sperm concentration*</td>
<td>67.16±4.89</td>
<td>10.4±2.39</td>
<td>0.00±0.00</td>
<td>HS **</td>
<td>0.00±0.00</td>
<td>HS **</td>
<td></td>
</tr>
<tr>
<td>Sperm motility</td>
<td>60.0±3.41</td>
<td>44.5±2.03</td>
<td>0.00±0.00</td>
<td>HS **</td>
<td>0.00±0.00</td>
<td>HS **</td>
<td></td>
</tr>
<tr>
<td>Abnormal morphology</td>
<td>38.5±2.30</td>
<td>313.0±56.21</td>
<td>327.8±73.93</td>
<td>NS</td>
<td>327.8±73.93</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>2615.4±257.30</td>
<td>1982.4±299.54</td>
<td>2172.6±426.51</td>
<td>NS</td>
<td>2172.6±426.51</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Leptin receptor (pg/ml)</td>
<td>8.92±1.07</td>
<td>8.59±1.97</td>
<td>7.24±1.38</td>
<td>NS</td>
<td>7.24±1.38</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

P value ≥ 0.05: Non-significant, ** P value < 0.01: Highly significant, Different letters means significant difference, x10^6 / ml

Table 3: Comparison of Semen Parameters, Leptin, Leptin Receptor and Testosterone between Seminal Classes of non-Leukocytespermic Infertile Men

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normozoospermia n=23</th>
<th>Mean±SE</th>
<th>Oligozoospermian=10</th>
<th>Mean±SE</th>
<th>Azozoospermia n= 4</th>
<th>Mean±SE</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (ml)</td>
<td>3.06±0.3</td>
<td>2.64±0.47</td>
<td>2.75±0.52</td>
<td>NS</td>
<td>2.75±0.52</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Sperm concentration*</td>
<td>69.26±5.57</td>
<td>16.4±7.86</td>
<td>0.00±0.00</td>
<td>HS **</td>
<td>0.00±0.00</td>
<td>HS **</td>
<td></td>
</tr>
<tr>
<td>Sperm motility</td>
<td>62.60±3.93</td>
<td>57.0±7.31</td>
<td>0.00±0.00</td>
<td>HS **</td>
<td>0.00±0.00</td>
<td>HS **</td>
<td></td>
</tr>
<tr>
<td>Abnormal morphology</td>
<td>35.0±2.30</td>
<td>38.5±2.79</td>
<td>0.00±0.00</td>
<td>HS **</td>
<td>0.00±0.00</td>
<td>HS **</td>
<td></td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>327.2±50.45</td>
<td>360.3±46.17</td>
<td>433.5±193.21</td>
<td>NS</td>
<td>433.5±193.21</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Leptin receptor (pg/ml)</td>
<td>2333.9±136.63</td>
<td>2363.1±292.33</td>
<td>2764.2±762.03</td>
<td>NS</td>
<td>2764.2±762.03</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
<td>9.96±1.51</td>
<td>8.43±2.53</td>
<td>7.99±1.33</td>
<td>NS</td>
<td>7.99±1.33</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

P value ≥ 0.05: Non-significant, ** P value < 0.01: Highly significant, Different letters mean significant difference, x10^6 / ml
Discussion:
The mean age of primary infertile men was 30.21 years which is consistent with study done by Gowri et al. (2010)[14]. It has been found that the average age of infertile men in current study seem to be young; this may due to seeking for treatment at early stage of marriage. Significant decreased sperm quality in infertile men was found when compared to fertile men, concordant to study of Al-Dakhly et al. in 2007[15].Zorn et al. in 2007 demonstrated that leptin level was increased in infertile males and adversely affected sperm quality through regulation of Leydig cell function[16]. This result concordant to Zorn and co-worker, we found increasing concentration of leptin in infertile men with worse quality of seminal parameters. Moreover, Leptin can exert their function through expression of functional leptin receptor [17]. Similarly the study detected increased leptin receptor concentration in semen of infertile men. Testosterone is necessary for normal sperm development. The present results showed that there was a significant decline testosterone level in sera of infertile men associated to poor sperm quality compared to healthy control and this is in agreement with result of Khatoon et al. (2012)[18].

Other number of patients with normozoosperma were recorded in both leukocystospermic and non-leukocystospermic infertile male concordant to Al-Dakhly et al. (2007) [15]. These results revealed significant differences in the seminogram between seminal classes in terms of sperm concentration, motility and morphology in leukocystospermic and non-leukocystospermic infertile men, same conclusion observed by Masroor et al. (2013) [19]. Normal leptin secretion is necessary for normal reproductive function, in this study, a trend was observed for non-significant higher leptin concentration in seminal plasma of azoospermic men, that may be caused by circulating leptin that is capable of crossing the blood-testis barrier by an leakage[20]. Jorsaraei et al. (2010) also reported that the source of leptin in seminal plasma is likely from seminal vesicle, prostate tissue or testis tissue in addition to spermatozoa [21]. This finding is similar to conclusion of Ma et al. [22], who showed that sperm negative patient, has a higher leptin level than that of sperm positive patient. Normozoospermic infertile men accompanied by rise in testosterone level more than oligozoospermic and azoospermic subject is concordant with Sheikh et al. (2005) [23]. 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 [24]. Previous studies documented that age is associated with diminished in semen volume, sperm concentration, motility and morphology [25]. Similarly, this study concluded that increasing age is associated with decreased semenogram and older men have lower semen volume and sperm parameters. Older age in current study revealed an increasing leptin level and its receptor in which associated with decline in testosterone level [26]. Obesity is defined when the BMI greater than 30 kg/m², it cause many pathophysiological problems and adversely affects male fertility by endocrine, thermal and genetic mechanisms. In obese men, more androgen is converted to estrogen via aromatization in peripheral fat. Consequently, serum testosterone level is reduced. In obese men, the scrotum remains in closer contact with surrounding tissue, predisposing to increased scrotal temperature that may adversely affect semen parameters [27]. Results of this study indicated negative effect of BMI on sperm quality and obese men have lower sperm parameters, this finding is supported by other researchers [28] [29].Obese men in current study associated with increased leptin and leptin receptor level, concordant to Roberts et al. (1997)[30]. Increasing in leptin and its receptor level associated with decline in testosterone level[31].In this result, infertile obese men were associated with decreased testosterone, consistent with results of Pauli et al. (2013)[32].
Conclusion:  
Semen plasma concentrations of leptin and their receptor play a vital role in infertility and inverse effect on semen parameters.

Author contribution:  
Ruqaya M. Ghareeb Taher Al-Barzinji: Supervisor  
Ahmed Abdul-Qader Abdul-Salam Al-Naqshbandi: MSc Student

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