Correlation Study between Level of zinc and lead in Serum and Seminal Plasma in Infertile Patients

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Abstract

Male infertility is a serious problem all over the world. Nutritional deficiency of trace element Zinc (Zn) may play a role in male infertility as Zn plays an important role not only in normal testicular development, but also in spermatogenesis and sperm motility. Deficiency of Zn is associated with hypogonadism and insufficient development of secondary sex characteristics. In order to get a complete overview of the possible mechanism of lead-induced toxicity on the reproductive system, all important constraints like sperm parameters, morphology, and disruption in hormones, gene expressions, spermatogenesis, and steroidogenesis were analyzed from previous research findings. The normal sperm parameters were directly affected by lead exposure. It causes alteration at the reproductive axis, sperm motility and viability, acrosome reaction, chemotaxis[1]. Semen quality [2].and structural abnormality in lead treated animals [3]. Lead chloride exposure significantly repressed the motility and increased the tail anomalies and immotile sperm count [4]. The reduction of sperm motility was also found in lead chloride exposure in the human study [5]. The present study was designed to analyse the level of seminal and serum Zn and pb among different groups of infertile patients and to correlate it with sperm concentration, active, sluggish and immotile fractions of seminal parameters, with an objective to establish the role of Zn and pb in male infertility. The present study was carried out in tow- year's period from 2019 to 2020. It was a descriptive analytical study with non-probability sampling. Semen and serum examination of the patients was carried out according to the standardized method of the World Health Organization. Was estimated by atomic absorption (Flameless Atomic absorption). Data were entered using Excel Microsoft Program (2016) and analysed by using Statistical Package for Social Sciences (SPSS) version 21. Categorical data described as frequency and percentage while numerical data described means and standard deviation (SD). Chi square test was used to estimate the association between two categorical variables. While, independent sample t-test used for comparison of numerical data. Level of significance of ≤ 0.05 was considered as significant[6]. In the present study, a comparison was done for the levels of trace elements (zinc and lead in serum and semen of fertile and infertile males. The results showed a highly significant difference (p<0.05) in the level of these elements between these groups both in serum and semen. (Data were expressed as mean ± standard deviation). Conclusions: Effect of heavy elements on the parameter of semen. Heavy elements are bound together in serum and semen. Heavy elements are linked to external influences, such as smoking alcohol.

Keywords: zinc, Serum, Seminal Plasma, Infertile Patient

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Introduction

Infertility is defined as the lack of ability to conceive within one year of unprotected intercourse with the same partner. It is estimated that nearly 8–12% of couples are infertile [7], and approximately 30–40% of infertility cases are caused by male factors [8].

Several risk factors are involved in the pathogenesis of infertility, some of which include alterations in spermatogenesis due to testicular cancer, aplasia of the germinal cells, varicocele, defects in the transport of sperm, or environmental factors as well as congenital anomalies, infectious diseases, bilateral sperm ducts anomalies, pregnancy-related infections, alterations in the characteristics of semen such as a decrease in sperm motility and sperm count, the presence of antispam antibodies (ASAs), and nutritional deficiency of trace elements such as selenium and zinc (Zn) [9].

Trace elements play an important role in the male reproductive process because of their high activity at the molecular level, although they are known to exist in the body at very low levels. Zn is second only to iron as the most abundant element in human tissues. Although Zn is found in most types of foods such as red meat, white meat, fish, and milk, the World Health Organization (WHO) estimated that one-third of the world’s population are deficient in zinc. Zinc and citrate are excreted from the prostate gland as a low-molecular-weight complex; thus, it is estimated that the zinc levels in seminal plasma typically represent prostatic secretory function. After ejaculation, half of the quantity of this complex is redistributed and linked to medium- and high-molecular-weight compounds generated from the seminal vesicles [10].

The decrease in the seminal plasma zinc concentration may result from inadequate intake, reduced absorption, increased losses, or increased demand.

The commonest worldwide cause is inadequate intake as a result of a diet low in Zn or rich in phytate. Additionally, increased urinary losses can occur under conditions associated with muscle catabolism, such as sepsis, or iatrogenically from the prolonged use of drugs (Foresta et al., 2014). Furthermore, some studies have reported that a sharp decrease in zinc in the prostatic fluid may result in low zinc concentration in seminal plasma [11].

Materials and Methods

The case control study examined 100 infertile patients and 50 fertile controls were taken; the mean ages were between 20-45 years who attended the High Institute of Infertility Diagnosis and Assisted Reproductive Technology / AL-Nahrain University. This prospective study was accomplished through the period from October 2018 till March 2019.

Inclusion criteria

Infertile patients and all subgroups of infertility have seminal fluid analyses and they are abnormal.

Exclusion criteria

Infertile male receiving immunosuppressive and supplements drugs.

Collection and examination of samples

The collection and examination of semen were done by properly standardized procedures, as mentioned in WHO laboratory manual.
Blood collection

- Three ml blood of each patients was added to gel tube and was separate by
- Centrifuge with 3000 rpm then serum was collected in plan tube (to measure zinc and copper.
- Two ml blood of patients was added to heparin tube (to measure lead).

Storage of sample

a- Seminal plasma

After performing semen analysis, the rest of the semen samples were centrifuged at 3000 rpm for 15 to 20 minutes. The pellet was discarded, while the supernatant of the semen samples were aliquoted and stored at 4-6 °C for evaluation of seminal Zn and pb.

b- Blood

Zinc

The rest of the blood samples were centrifuged at 2 000 rpm for 15 to 20 minutes. The pellet was discarded, while the supernatant of the serum samples was aliquoted and stored at 4-6 °C Zn. Determination of seminal plasma zinc

Lead

Keeping blood in the heparin tube and keeping it in the refrigerator in a 4-6 °C.

Statistical Analysis

Data were entered using Excel Microsoft Program (2016) and analysed by using Statistical Package for Social Sciences (SPSS) version 21. Categorical data described as frequency and percentage while numerical data described means and standard deviation (SD). Chi square test was used to estimate the association between two categorical variables. While, independent sample t-test used for comparison of numerical data. Level of significance of ≤ 0.05 was considered as significant [6].

Results

4.3. Comparison of trace elements levels (Zn and Pb) in serum and semen between fertile and infertile males included in the present study:

In the present study comparison was done for the levels of trace elements (zinc and lead in serum and semen of fertile and infertile males. The results showed highly significant difference (p<0.05) in the level of these elements between these groups both in serum and semen as shown in table (4-2). (Data were expressed as mean ± standard deviation).
Table (1): Comparison of trace elements levels (Zn, Cu and Pb) in serum and semen between fertile and infertile males included in the present study:

<table>
<thead>
<tr>
<th>Element</th>
<th>Groups</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infertile</td>
<td>Fertile</td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Zn Serum (Mg/dl)</td>
<td>70.20 ± 10.71</td>
<td>96.24 ± 14.83</td>
</tr>
<tr>
<td>Pb Serum (Mg/dl)</td>
<td>26.06 ± 6.06</td>
<td>14.40 ± 2.57</td>
</tr>
<tr>
<td>Zn Seminal (Mg/dl)</td>
<td>3.21 ± 1.18</td>
<td>10.39 ± 1.85</td>
</tr>
<tr>
<td>Pb seminal (Mg/dl)</td>
<td>8.74 ± 2.71</td>
<td>4.38 ± 0.64</td>
</tr>
</tbody>
</table>

Data expressed as mean ± standard deviation.

** Highly significant difference p<0.001

4.6 normal limits of heavy elements concentration in serum and semen of infertile and control groups.

The concentration of heavy metals was measured in serum of all males included in the present study and was classified into normal and abnormal ratio according to specific limits. The normal ratio was (80-150) Mg/dl for zinc while lead normal ratio was (≤ 25Mg/dl). The concentration of these elements was considered abnormal if (<80) Mg/dl and (>25) Mg/dl for, Zn and Pb consequently. The percentage of patients with normal and abnormal concentration of heavy elements was illustrated.

In addition the concentration of heavy metals was measured in semen of all males included in the present study and was classified into normal and abnormal ratio according to specific limits. The normal ratio was (>5) Mg/dl for zinc while lead normal ratio was (≤ 5.25Mg/dl). The concentration of these elements was considered abnormal if (<5 Mg/dl) and (>5.25Mg/dl) for Zn and Pb consequently. The percentage of patients with normal and abnormal concentration of heavy elements was illustrated.

Comparison was done between the infertile and control groups of males for the presence of normal or abnormal ratio of these metals and these results recorded highly significant difference (p<0.001) between the two groups.

Table (2): Comparison of control and infertile male percentage with normal and abnormal ratio of serum heavy elements.

<table>
<thead>
<tr>
<th>Elements</th>
<th>Group</th>
<th>Count</th>
<th>%</th>
<th>Count</th>
<th>%</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn serum</td>
<td>Abnormal (&lt;80)Mg/dl</td>
<td>78</td>
<td>78.0%</td>
<td>5</td>
<td>10.0%</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td></td>
<td>Normal (80-150)Mg/dl</td>
<td>22</td>
<td>22.0%</td>
<td>45</td>
<td>90.0%</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Pb serum</td>
<td>Abnormal (&gt;25)Mg/dl</td>
<td>39</td>
<td>39.0%</td>
<td>0</td>
<td>0.0%</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td></td>
<td>Normal (≤25)Mg/dl</td>
<td>61</td>
<td>61.0%</td>
<td>50</td>
<td>100.0%</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>

Zn seminal
Abnormal (<5)Mg/dl     91     91.0%     4     8.0%   <0.001**
Normal (>5)Mg/dl      9     9.0%     46     92.0% 

Pb seminal
Abnormal (>5.25)Mg/dl 8     8.0%     48     96.0%   <0.001**
Normal (<=5.25)Mg/dl  92    92.0%     2     4.0% 

Data expressed as mean ± standard deviation.

** Highly significant difference p<0.001

Table (3): semen parameters of fertile and infertile males included in the study.

<table>
<thead>
<tr>
<th>semen parameter</th>
<th>fertile</th>
<th>infertile</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>Volume (mL)</td>
<td>4.44 ± 0.94</td>
<td>2.24 ± 1.03</td>
<td>&lt; 0.001**</td>
</tr>
<tr>
<td>Liquefaction time (minutes)</td>
<td>23.92 ± 5.78</td>
<td>40.18 ± 13.79</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>PH</td>
<td>7.67 ± 0.24</td>
<td>7.88 ± 0.30</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Sperm concentration (million/ml)</td>
<td>49.20 ± 15.15</td>
<td>44.38 ± 31.24</td>
<td>0.308NS</td>
</tr>
<tr>
<td>Progressive motile (percentage %)</td>
<td>33.52 ± 6.49</td>
<td>28.07 ± 17.45</td>
<td>0.036*</td>
</tr>
<tr>
<td>Non progressive sperm (percentage %)</td>
<td>20.69 ± 4.75</td>
<td>26.68 ± 13.70</td>
<td>0.004*</td>
</tr>
<tr>
<td>Immotile sperm (percentage %)</td>
<td>37.70 ± 8.67</td>
<td>45.49 ± 21.23</td>
<td>0.011*</td>
</tr>
<tr>
<td>Normal sperm (percentage %)</td>
<td>43.88 ± 5.71</td>
<td>27.47 ± 17.01</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Sperm agglutination (percentage %)</td>
<td>2.25 ± 0.50</td>
<td>4.44 ± 1.71</td>
<td>0.008*</td>
</tr>
<tr>
<td>Round cells (hpf)</td>
<td>6.90 ± 4.90</td>
<td>14.18 ± 13.90</td>
<td>0.04*</td>
</tr>
</tbody>
</table>

** Highly significant difference p<0.001

* Significant difference p<0.05

(NS) Non-significant difference p>0.05

Correlations between heavy metal concentration and semen parameters:

Correlations were done between serum concentration of heavy metals and semen parameters. A significant correlation (p<0.05) was noticed between serum zinc and (age, time work, infertility duration, semen volume, liquefaction time, semen PH, immotile sperm, normal morphology and agglutination). The correlation was also significant (p<0.05) between serum lead and time of work, infertility duration, liquefaction time, semen PH, percentage of immotile sperm and normal morphology percentage.

Other correlations were done between semen concentrations of heavy metals with semen parameters. For significant correlation (p<0.05) was documented between semen zinc and infertility duration, liquefaction time, PH and sperm progressive motility. On the other hand a significant correlation was recorded between lead semen concentration and age, time work, infertility duration, semen PH, progressive motility, immotile sperm percentage, normal sperm morphology and round cells number. These results were summarized in table 4.
The concentration of heavy metals in serum and in semen was evaluated and it was found that serum concentration of copper has highly significant correlation (p<0.001) with that of serum Zn and Pb the same situation was found for the concentration of heavy metals in semen.
Table (4-5): correlations between concentration of heavy metals in serum and semen

<table>
<thead>
<tr>
<th>Heavy metals</th>
<th>Zn Serum</th>
<th>Pb Serum</th>
<th>Zn Seminal</th>
<th>Pb seminal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn Serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td></td>
<td>0.000**</td>
<td>0.495</td>
<td>0.446</td>
</tr>
<tr>
<td>Pb Serum</td>
<td>0.000**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.151</td>
<td>0.405</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zn Seminal</td>
<td>0.495</td>
<td>0.151</td>
<td></td>
<td>0.000**</td>
</tr>
<tr>
<td>p</td>
<td></td>
<td></td>
<td>0.000**</td>
<td></td>
</tr>
<tr>
<td>Pb seminal</td>
<td>0.446</td>
<td>0.405</td>
<td>0.000**</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

** High statistical significance P<0.001

Data expressed as mean ±SD

Comparisons between fertile and infertile males regarding general parameters

A comparison was done between fertile and infertile males included in the present study and results showed highly significant difference (p<0.001) for type of infertility, smoking habit, surgical history, drug history and job. Also significant difference was found (p<0.05) between the two groups regarding alcohol habit.

Table (4-6): comparison of general characteristics between fertile and infertile males

<table>
<thead>
<tr>
<th>parameters</th>
<th>Group</th>
<th>Count</th>
<th>%</th>
<th>Count</th>
<th>%</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infertile</td>
<td></td>
<td></td>
<td>Fertile</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type of infertility</td>
<td>primary</td>
<td>80</td>
<td>80.0%</td>
<td>0</td>
<td>0.0%</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td></td>
<td>secondary</td>
<td>20</td>
<td>20.0%</td>
<td>0</td>
<td>0.0%</td>
<td></td>
</tr>
</tbody>
</table>
| Alcohol                 | Yes                          | 5     | 5.0% | 0     | 0.0%| 0.108
|                         | No                           | 95    | 95.0%| 50    | 100.0%|         |
| Smoking                 | Yes                          | 55    | 55.0%| 7     | 14.0%| <0.001**|
|                         | No                           | 45    | 45.0%| 43    | 86.0%|         |
| Surgical history        | Nil                          | 54    | 54.0%| 50    | 100.0%| <0.001**|
|                         | varicose veins               | 46    | 46.0%| 0     | 0.0%|         |
| Drug history            | supplements                  | 56    | 7    | 7.0%  | 0   | 0.0%    | <0.001**|
|                         | no treatment                 | 44    | 23   | 23.0% | 39  | 78.0%   |         |
| Job                     | driver taxi                  | 62    | 62.0%| 11    |     | 22.0%   |         |
|                         | employee                     |       |     |       |     |         |
|                         | worker                       |       |     |       |     |         |
|                         | soldier                      | 5     | 5.0% | 0     | 0.0%|         |
|                         | electrical engineer          | 1     | 1.0% | 0     | 0.0%|         |
|                         | study                        | 2     | 2.0% | 0     | 0.0%|         |

** High statistical significance P<0.001
(Ns) Non-statistical significance P>0.05

Discussion

Male infertility can cause by anatomical or genetic abnormalities, systemic or neurological diseases, infections, trauma, iatrogenic injury, gonad toxins and development of sperm antibodies. Several lifestyle factors and environmental issues can have a negative impact on male reproductive health [12].

Trace elements effect on human fertility and reproduction has been discussed. It has been demonstrated that the concentration at which an element is present in the body can greatly affect its function, with a potential for both positive and negative effects.

The accurate measurement of trace element concentrations in biological tissues and fluids therefore becomes very important. Such measurements can either be used to find the total trace element concentration of a particular material i.e. the sum of a trace element’s concentrations in all chemical species containing that particular trace element or can look more specifically at individual chemical species [13].

5.1. Serum and semen levels of trace elements (Zn, and Pb):

5.1.1 Comparison of serum and semen level of zinc between fertile and infertile males:

The results of the present study showed highly significant difference in the level of zinc both in serum and semen being higher in the fertile group this finding was in accordance with that of previous studies as it was recorded that Zinc plays a significant role in the physiology of sperm cells.

Although many articles have been published on the multiple functions of zinc in semen, its impacts on the structure, motility, and survival of spermatozoa are still controversial [14].

5.1.2 Comparison of serum and semen level of lead between fertile and infertile males:

The results of the present study documented highly significant difference between serum and semen level of lead being higher in infertile group as shown in These results were in agreement with previous studies which concluded that higher levels of lead were associated with male infertility as detected by changes in hormonal profile and seminal parameters.

Environmental exposure to Pb may adversely impact on fertility in men. Smoking habits and anemia are considerable factors in male infertility. Kidney functions and reproductive hormonal profiles are deleteriously impacted in males exposed to Pb in a regular manner [15].

5.2 Comparisons of seminal fluid parameters between fertile and infertile males:

Semen analysis results were compared between fertile and infertile groups which showed highly significant difference in semen volume, liquefaction time, pH, and normal morphology, significant difference was found in motility grades, agglutination percentage, and round cells number but no significant difference was documented in sperm concentration.

There was extensive overlap between the fertile and the infertile men. Although each of the sperm measurements helped to distinguish between fertile and infertile men, none was a powerful discriminator. This finding was in accordance with previous studies [16].
5.3. Comparison of percentage of males with normal trace elements limits in serum and semen between fertile and infertile groups:

The normal limit of copper concentration was 80-150 mg/dl and <10 in serum and semen respectively while the abnormal limit was >150 mg/dl and >10 mg/dl in serum and semen respectively. Regarding zinc the normal limit was 80-150 mg/dl and >5 mg/dl in serum and semen respectively while abnormal limits was <80 and <5 respectively. While that of lead the normal limits was ≤ 25mg/dl and ≤ 5.25mg/dl in serum and semen respectively and if >25 mg/dl in serum or >5.25 mg/dl in semen it was considered abnormal. These limits values depended on previous studies [17].

When comparison was done between fertile and infertile groups for percentage of males with abnormal trace elements limits in serum and semen all of them recorded highly significant difference indicating the important effect of these trace elements on male fecundity and fertility and this was documented in previous studies [18].

5.4 Correlations between serum and semen concentration of trace elements and seminal fluid analysis parameters:

5.4.1 Correlations between serum and semen concentration of Copper and seminal fluid analysis parameters:

The concentration of copper was measured in serum and semen of all males included in the study and correlation was done with all parameters of seminal fluid analysis. There was no significant correlation between serum copper and seminal fluid analysis parameters except percentage of normal sperm morphology there was highly significant (p<0.001) correlation. In addition the results of the present study recorded no significant correlation between semen concentration of copper and seminal fluid analysis parameters except liquefaction time which showed highly significant (p<0.001) correlation.

These results are not in agreement with a study was done by Wong et al. (Wong et al., 2001), who mentioned that there was a positive connection between blood copper concentrations and sperm motility. Another study documented that an important connections between semen copper level and sperm concentration, progressive motility, and normal morphology [19].However, Aydemir et al. (Aydemir et al., 2006) announced higher levels of plasma copper in a subfertile male group, contrasted with a fertile male group [20].

In the given study, which is in agreement with that of the present study there was no alliance was found between copper level and sperm volume, count, motility, and normal morphology [21].This variance between the results of the above-mentioned studies may be due to the redox activity of copper [22]. On one hand, copper plays a role as a trace element essential for the activity of several metalloenzymes and metalloproteinase engaged in energy or antioxidant metabolisms [23].On the other hand, copper is known to be a catalyst for Fenton and Haber Weiss reactions which generate hydroxyl free radicals from hydrogen peroxide and superoxide ion radicals [24].

Copper not only boosts ROS formation but also can bind directly to the free thiol groups of cysteine. In consequence, copper can lead to oxidation and becomes linked between proteins, thus inactivating enzymes or impairing structural proteins [25].In consequence, the ionic form of copper has been specified as a highly toxic element. Since spermatozoa include high concentrations of polyunsaturated fatty acids and [26],generate reactive oxygen types, mainly superoxide anion and hydrogen peroxide, they are especially liable to per-oxidative damage [27],including lipid peroxidation. Damage to lipid membranes prompted by reactive oxygen types has been proposed as one of the major causes of human male infertility [28]. Consistently, Sakhaee and others (Sakhaee et al., 2012) displayed reduced sperm concentration, motility, and viability in rats poisoned with copper. The current study displays high levels of ROS value in the Cu-H group compared to the Cu-L group [14].

A positive connection between copper levels and TOS values was also shown. These results elucidate that some toxic effects of copper may also occur in infertile males. In light of this, the mechanisms for preserving the balance between essential and toxic levels of copper are very important for good sperm quality[29].

In fact, the copper ions in semen are mainly bound to proteins. Thus, copper existing in seminal plasma from fertile males is catalytically inactive [30].

Besides, antioxidant protection at positions of gamete production, maturation and storage and embryo implantation has been developed [31].

Although, the function of copper in male reproductive capacity evidence to be largely unknown, this metal evidences to be related in spermatozoa motility and it may also act at the pituitary receptors which dominance the release of LH [32]. Jockenhövel and others explained a weak but important positive connection between blood copper concentrations and sperm motility [33].

The recent study was not in agreement with both the above studies. Wong and other colleges [34], Semen volume, morphology and motility are shown an important variation between normal and abnormal with but unimportant with Cu signal that the higher the volume of semen the more will be the concentration of Cu the seminal fluid. This finding agrees with the observation of Kanwal [35].

5.4.2 Correlations between serum and semen concentration of zinc and seminal fluid analysis parameters:

Correlation between serum zinc and seminal fluid analysis parameters except sperm concentration, progressive motility, agglutination and round cells number there was significant correlation. In addition the results of the present study recorded no significant correlation between semen concentration of zinc and seminal fluid analysis parameters except liquefaction time, pH and progressive motility which showed significant the concentration of zinc was measured in serum and semen of all males included in the study and correlation was done with all parameters of seminal fluid analysis. There was significant p-value Volume (0.037), p-value Liquefaction time (0.049), p-value PH (0.043), p-value non-progressive sperm (0.04) p-value Immotile sperm (0.045), p-value Normal sperm (0.02) for serum, P-value Liquefaction time (0.028), p-value PH (0.02) for seminal Correlation These results were in accordance with previous studies which recorded that high zinc concentration is related to poor sperm quality, whereas other studies showed no important social reported that Zn concentration in seminal plasma should be considered as one of the factors responsible for decreased testicular function in infertile male subjects[36].

While other studies were not in agreement with present study like that of WHO declared that zinc influences sperm motility by improving sperm oxygen uptake and controlling energy utilization through the ATP system[37] Zinc may also increase the activities of zinc-dependent enzymes, such as sorbitol dehydrogenase and lactate dehydrogenase, which play an important role in the servicing of sperm motility [38].

Zinc plays a significant role in the physiology of sperm cells. Although many articles have been published on the multiple functions of zinc in semen, its impacts on the structure, motility, and survival of spermatozoa are still controversial [39].

Some studies signalize that a high zinc concentration is linked with normal morphology and increased sperm cell motility and density. Consistently higher zinc levels were found in fertile men, compared to those who were infertile, and zinc supplementation was declared to be an effective method for treatment of the infertile males with chronic prostatitis [40].
Ali et al., 2007 have announced that zinc plays a vital role in the physiology of spermatozoa and spermatogenesis and an essential nutritional ingredient [41]. Omu and associates (1998) had explained that Zn therapy results in an important improvement in sperm quality with increases in sperm density, progressive motility, and improved conception and pregnancy result [42]. Prostate-specific antigen, zinc, and activity ultra α-glucosidase in seminal plasma were found to be correlated with abnormal semen viscosity (Holmes, 2020). Some trace amounts of metals are essential for physiological homeostasis [43].

5.4.3 Correlations between serum and semen concentration of lead and seminal fluid analysis parameters:

The concentration of lead was measured in serum and semen of all males included in the study and correlation was done with all parameters of seminal fluid analysis. There was significant p-value PH (0.031), p-value non-progressive sperm (0.031), p-value Immotile sperm (0.038), p-value Normal sperm (0.023) in serum correlation between serum lead and seminal fluid analysis parameters except semen volume, sperm concentration, progressive motility, agglutination and round cells number which was not significant. In addition the results of the present study recorded no significant correlation between semen concentration of lead and seminal fluid analysis parameters except PH, progressive motility, immotile sperm normal sperm morphology percentage and round cells count which showed significant p-value PH (0.011), p-value Progressive motile (0.043), p-value Immotile sperm ( 0.04), p-value Round cells ( 0.043)in seminal correlation Higher levels of lead were associated with male infertility as detected by changes in hormonal profile and seminal parameters. Environmental exposure to Pb may adversely impact on fertility in men. Smoking habits and anemia are considerable factors in male infertility. Kidney functions and reproductive hormonal profiles are deleteriously impacted in males exposed to Pb in a regular manner [44].

The lead was inversely associated with semen parameters as, ejaculate volume, sperm count, sperm concentration, live sperms, and rapid mobility [45]. Semen analysis is the single most useful and fundamental inquiry of males’ infertility [46]Evidence of various studies proposes that cigarette smoking may have a hurtful action on male fertility by reduction of sperm creation, Motility, and increasing abnormal shapes. Smokers are 60% more prone to be infertile than non-smokers. Smokers also have high levels of serum estradiol and low levels of LH, FSH, and prolactin than non-smokers, all these findings can negatively impact spermatogenesis [47] There was an important increase in serum creatinine and blood urea in the study group when compared to the observation group. These results are comparable to previous literature, where a persuasive relation between abnormal renal function and blood lead levels (BLLs) <10 μg/dL was announced [48] registered that, Pb could negatively impact the male reproduction, by disruption of hypothalamic-pituitary-gonadal axis or by direct negative impact on spermatogenesis, leading to impairment of semen quality [49].

A propensity towards the reduction of quality has been proved in males exposed to heavy metals [50].

There is the obvious agreement that high or even moderate values of lead led to fertility problems in males. Radin and others presented that >40 μg/dL of lead in the blood leads to a reduction in sperm count (Radin et al., 2012). In addition, they announced lower motility (<50%) with levels >35 μg/dL in whole blood (Vidaeff, Alex C Sever, Lowell E)[50].

Telisman also displayed that high lead concentration in blood (36.7μg/dL) remarkably lowers sperm density and motility (51)High levels of lead seem to be clearly associated with sperm damage (Callaway, O’Callaghan and David McIntyre, 2009). After adjusting for confounding variables (e.g. age, smoking, alcohol, blood cadmium, serum copper,
zinc increase in blood lead was markedly) linked with reducing percentages of morphologically healthy and subnormal sperm and with increasing percentages of slow sperm and overly wide sperm [52]

In another study, the seminal plasma lead values of males not exposed to lead in their work or environment were found to negatively coordinate with fertilization ability of sperm acrosome reaction and the fertilization rate when using the IVF procedure, and also with seminal plasma zinc levels [53]

As a result, the previous trials propose that lead may remarkably reduce the quality of semen, even at low-levels that is usual for common people all over the world. many trials including lead labours have demonstrated that paternal blood levels of lead of about 30–40 μg/dL are a most likely threshold for the high rate of spontaneous abortions, decreased rate of live births and prolonged time to pregnancy(Fuller, 2018). Although changeable findings or minor incompatibility were also revealed blood plasma lead and were inversely coordinated with the rate of fertilization [54] [55]. The split of lead in whole blood between the erythrocyte and plasma fractions varies considerably [56] and appears dependent upon plasma protocol [57].

Since the fragment of whole blood lead in plasma is more readily available for distribution to other organs, therefore, should be considered the biologically relevant measure [58]. However, the use of blood plasma lead levels in the study of human infertility is limited by the current unpredictability as to baseline blood plasma lead levels in normal individuals. Positive relationships between blood lead levels and seminal plasma lead levels have been announced after both work exposures and environmental exposures to lead [59].

This suggested that a mechanism must exist in which lead exposures were readily conveyed to the male reproductive tract. Therefore, in the current study, it is checked the relationship between seminal plasma lead levels and male infertility. Seminal lead levels were unforeseen as, according to questionnaire responses, none of the subjects was engaged in work likely to produce exposure to metal ions [60].

We also report that the level of lead in seminal plasma is negatively correlated with male fertility potential, as measured seminal plasma lead levels can occur without detectable effects on male reproductive endocrine function, and that increased lead intake may be weakly associated with decreased sperm concentration, decreased normal morphology and decreased sperm motility [61].

5.5 Comparisons of general characteristics between fertile and infertile males

In table 4-6 some environmental and social factors which were suspected to have an effect on male fertility were summarized and compared between fertile and infertile males. It was found that alcohol drinking, smoking; surgical treatment for varicocele, drug history and heavy work was more in infertile males which indicates the effect of these factors on male fertility.

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