Effect of Some Heavy Metals as Pollutant on Male Infertility in Fallujah City

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Abstract
Infertility is one of the most serious problems facing advanced nations. In general, about half of all cases of the infertility are caused by factors related to the male partner. Proposed causes of male infertility include genetic and environmental factors. Blood samples from 64 infertile men all were living in urban city (30 azospermeiau and 34 oligospermeia) and 32 fertile men (as the control group) were collected.

Heavy metal concentrations in sera of infertile and fertile groups were measured by using Atomic Absorption Spectrophotometer. The results showed that there is a significant positive correlation found between serum and semen in all parameters except total protein and RNase activity.

Keywords: Biochemical parameters, sera and semen, fertile male, Fallujah city.

Introduction

Male infertility is a common disease affecting up to 50% of infertility including 10-15% of couples[1]. A major factor associated with male infertility is sperm function and the quantity and quality of sperm production, such as sperm motility[2]. A common cause of male infertility is heavy metals[3]. Sperm failure is the result of various causes such as systemic diseases, endocrine disorders, malnutrition, genetic factors and environmental hazards. Genetic defects such as chromosomal abnormalities and mutations account for at least 30% of male infertility[4]. Many researchers and clinicians have asserted that social degradation in advanced countries and social progress may lead to a decline in male fertility. Long-term reported risk factors include manufacturing related noise, high temperature work, radiation exposure, various chemicals and electromagnetic waves[5].

Pollution with metals may affect the male reproductive system in two ways, directly and/or indirectly, the first way (directly) when they target specific reproductive organs while the second way (indirectly) when they act on the neuroendocrine system[6]. These effects can be irreversible and extend lasting if sertoli cells are disrupted through fetal development. Decreasing infertility percentage is a top priority for the WHO[7]. Over the past 50 years, human sperm concentration decreased significantly from 113 to 61 million/ml, which represents almost 50% decrease[8]. Iraq is heavily polluted by carbon dioxide and radiation, and three years of war and neglect have made the environment in most parts of the country serious.

An official Iraqi study found that perinatal birth defects and cancer incidence are higher[9]. Heavy metals and/or toxic metals are the most common inorganic contaminants in water[10]. Some studies compare patients with healthy subjects (normal sperm count) with male infertility (oligozoospermia or azoospermia)[11,12].

Heavy metals may affect male reproduction, epidemiological and animal studies[11]. Internationally, cadmium (Cd) is one of the potentially dangerous metals[12]. Acute cadmium poisoning may be caused by breathing or ingesting food or water that is heavily contaminated with dust or cadmium. Cadmium accumulates in the body and has a long half-life (10 - 30 years) in tissues, mainly in the kidneys[13]. Copper (Cu) is involved in inhibiting spermatogenesis, and elevated concentrations may be toxic. It has been shown that experimental implantation of copper in the vas deferens, epididymis and scrotum of mammals can have an adverse effect on fertility[6].

Adenosine deaminase (ADA) or adenosine aminohydrolase (EC 3.5.4.4) is an important enzyme in the metabolism of purine metabolism. It involves the breakdown of dietary adenosine and adenosine produced by nucleic acid conversion in tissues. This ubiquitous enzyme has been found in a variety of microorganisms, bacteria, plants and vertebrates, has a highly conserved amino acid sequence and is present in all mammalian cells. RNase (polyribonucleotide 2-oligonucleotide-hydrolase, (EC: 3.1.4.22) is widely distributed in nature. They all share a common characteristic, namely the 3' side RNA of cytidine and uridine. P-O5 bond cleavage.

Through the intermolecular transphosphorylation of residues[14], mammalian and other vertebrate RNase constitute a large superfamily of enzymes in addition to simple digestion. Phosphatase is classified as acid phosphatase EC (3.1.3.2) and alkaline phosphatase EC (3.1.3.1) according to its catalytic activity required for optimal pH [15,16].

Dephosphorylation and phosphorylation of seminal enzyme proteins and sperm are regulated by the opposite activities of phosphatases and kinases[17]. The aim of the present study is to examine enzymes (ADA, Alk. RNase, Acp. and AlP) activity and some metals (Cu^{+2}, Pb^{+2} and Cd^{+2}) in Male with Azoospermia and Oligospermia in blood serum and seminal plasma.

Material and Methods

Collection of samples: Three to five days after sexual abstinence, semen flows through masturbation. The sample was allowed to stand for 20 to 60 minutes for liquefaction and the quality of the semen was evaluated by using two parameters: visual and microscopic examination. According to the semen analysis results of the World Health Organization standard [18], all infertile men were divided into two groups: The first group of oligozoospermia (sperm number = zero / mL) and the second group of oligozoospermia (sperm number < 200,000 / mL). 5 ml blood sample was collected from two 64 infertile men (30 azospermeia and 34 oligozoospermia) (23-54 years old) and 32 fertile men (age matched) living in Fallujah, Iraq. Complete physical examinations and physical examinations were performed in each group. Serum was used to measure heavy metals (Pb^{+2}, Cu^{+2} and Cd^{+2}), also used for enzyme activity.

Determination of heavy metals concentrations: The heavy metal concentration was determined by digesting 1 ml of the serum sample with 5 ml of 6:1 volume mixture (HNO_3:$HClO_4$) in a glass tube. Then, the concentrations of heavy metals (Cu^{+2}, $Pb^{+2}$and Cd^{+2}) were measured using an atomic absorption spectrophotometer GBC 933 plus Shimaadzu / Japan air acetylene flame and hollow cathode lamp[19].

Determination of the different enzymatic activity: Alkaline RNase activity in sera and seminal plasma are expressed as units where one unit of RNase is defined as "one K unit of RNase A is the amount of enzyme required to cause an increase in absorbance of 1.0 at 260 nm at 37°C (pH 8.0) when yeast RNA is hydrolyzed to oligonucleotides. 50 units = 1 Kunitz". Adenosine deaminase (ADA) activity was determined by Giusti and Galanti method [20]. Adenosine deaminase activity was expressed using U/l where: "One U can be defined as the amount of enzyme that produces a certain amount of the enzymatic activity, that is, an amount that catalyzes a conversion of 1 micro mole of substrate per minute".

Alkaline phosphatase (Alp) activity was determined by the calorimetric method, it was expressed by kind and king unit (KAU/l)[21], which liberates 1 mg of phenol in 15 minutes at 37 °C. Acid phosphatase (Acp) activity is extremely labile at room temperature. The stabilization of the enzyme can only be achieved by acidifying with acetate buffer. Activity was determined according to a modified method[22]. Acid phosphatase activity was expressed by U/l.

Results and Discussion

The concentrations of Cu^{+2}, Cd^{+2} and Pb^{+2} in serum and semen are shown in the table (1); a significant increase ($p \leq 0.05$) in the concentration of $Cd^{+2}$and Cu^{+2} was found in the serum of infertile group that included azospermia (0.0477± 0.0038), (0.0267±0.0147) and oligospermia (0.0446±0.0059) in comparison with control group (0.0152±0.0025). Also significant value of azospermia with oligospermia increase in Cd^{+2} was found. (a)significant value with control group ($p \leq 0.05$), (b)significant value azospermia with oligospermia ($p \leq 0.05$). In the present study Pb^{+2}, seminal plasma shows a high significant increase (p= 0.00) in both azospermia group and oligospermia group compared with control. Meanwhile the comparison of Pb^{+2} between oligospermia and azospermia indicates significant increase with (p= 0.04). Table 1 shows that inverse relationship exists between lead concentration in seminal plasma and the number of sperms.

This result agrees with the study of Allouche et al[23] which showed an association between Pb^{+2} in semen and sperm count. The biological function of lead in the body is not clear; it accumulates over time with continued exposure. The concentration of lead in the body should be less than 0.1 µg/ml. An increase in its concentration in the tissues and semen is likely to cause a dysfunction of the cell receptor or a decrease in the activity of enzymes inside the cell.[24]

Table1
The concentration of Cu^{+2}, Cd^{+2} and pb^{+2} in blood serum and seminal plasma.

Group	Concentration of heavy metals in the blood serum (µg/ml)			Concentration of heavy metals in the semen plasma (µg/ml)		
	Cu^{+2}	Cd^{+2}	Pb^{+2}	Cu^{+2}	Cd^{+2}	Pb^{+2}
Azospermia N= 32	0.26 ± 0.014a	0.047 ±0.003 c	0.110 ± 0.02	0.078 ± 0.057A	0.052± 0.005A	0.112 ±0.035A, C
Oligospermia N= 34	0.28 ± 0.022b	0.044 ± 0.005b	0.104 ± 0.028	0.121 ± 0.098B	0.047 ± 0.004B	0.083 ±0.021B
Control N=32	0.052 ± 0.012	0.015 ± 0.002	0.069 ± 0.0	0.005 ± 0.002	0.04 ± 0.001	0.049± 0.008

A, a, significant azospermia group compared with control group; B, b, significant oligospermia group compared with control group; C, c, significant azospermia group compared with oligospermia group.
The capital letter is used for comparison of seminal plasma groups and the small letter for comparability of blood serum groups.
P = 0.00 highly significant, P ≤ 0.05 significant, P≥ 0.05 nonsignificant.

Alteration in enzyme activity may be an indication of the toxic of heavy metal and other pollutants. Therefore, one of the present study aims is to evaluate the effect of metal pollution on the activity of some enzymes involved in purine metabolism in blood serum and seminal plasma of men living in the pollutant region. The current study was carried on in order to check the effect of metal pollution on the activity level of alkaline phosphatase, acid phosphatase, alkaline Ribonucleases, and adenosine deaminase in blood serum and seminal plasma of fertile and infertile individual male.

Activity of Alkaline Ribonucleases (RNase): Seminal plasma Ribonucleases RNase might be acting directly against an extracellular ribonucleic acid (RNA) including extracellular RNA involved in cell-cell communication[25]. The activity of alkaline ribonucleases (Alk. RNase) in blood serum and seminal plasma is shown in table 2.

RNase in blood serum can be defined as key molecule in intracellular regulatory where it has an important role in gene expression but also may be used as a biomarker of many pathological conditions like lung, colorectal and prostate cancer[26].

Activity of Adenosine Deaminase (ADA): The result is presented in table 3 for each of the seminal plasma and blood serum. The enzyme activity in seminal plasma (Table 3) shows a significant increase in patient groups compared with that of the control group where the activity in azospermia group was mean ± SD, 96.229 ± 12.576 (U/l) and in oligospermia was mean ± SD, 53.827 ± 29.148 (U/l) with highly significant increase ($P \leq 0.001$) and ($p=0.000$) respectively compared with control group. The comparison between azospermia group and oligospermia group shows nonsignificant difference in these enzyme activities. The results of the current study disagree with Du et al[27] who reported that ADA is absent in seminal plasma.

This study examind the activity of one of an enzyme that affects the metabolism of adenosine, particularly adenosine deaminase, in the human reproductive system. It is clear from the result in the table 3 that there is a negative relationship between ADA activity in seminal plasma and the total sperm count.

Table 2
Activity of alkaline RNase in seminal plasma and blood serum of all groups

Groups	Activity of RNase in seminal plasma (U/l) (mean ±SD)	Activity of RNase in blood serum (U/l) (mean ±SD)
Azospermia (n=32)	13.509 ±2.929	11.756± 7.239
Oligospermia (n=34)	14.262 ± 3.53	14.935± 7.995
Control (n=32)	10.38 ± 3.035	7.005 ± 2.408
P≤ 0.05	N.sg	a, b

(a) significant azospermia group compared with the control group
(b) significant oligospermia group compared with the control group
The capital letter is used for comparison of seminal plasma groups and the small letter for comparability of blood serum groups. $P \leq 0.00$ highly significant, $P \leq 0.05$ significant, $P \geq 0.05$ nonsignificant. The result of the comparison of this activity in seminal plasma is shown in table 2. The comparison of the activity between each azospermia group and oligospermia group with the control group as well as between azospermia group and oligospermia group was nonsignificant ($P \geq 0.05$).

Table 3
Activity of ADA in seminal plasma and blood serum of all studied groups

Groups	Activity of ADA in seminal plasma (U/l) (mean ±SD)	Activity of ADA in blood serum (U/l) (mean ±SD)
Azospermia (n=32)	96.229 ± 12.576	91.874 ± 10.793
Oligospermia (n=34)	53.827 ± 29.148	66.165 ± 27007
Control (n=32)	5.276 ± 1.367	18.856 ± 3.441
P≤ 0.05	A, B	a, b, c

A,a significant azospermia group compared with the control group; B,b significant oligospermia group compared with the control group; c significant azospermia group compared with oligospermia group
The capital letter is used for comparison of seminal plasma groups and the small letter for comparability of blood serum groups. $P \leq 0.00$ highly significant, $P \leq 0.05$ significant, $P \geq 0.05$ nonsignificant.

Environmental pollution such as increase in heavy elements leads to disorders in coordination between immune operations and metabolic processes that cause reduction in the balance within the body of the organism and the activities of antioxidants as well as metabolic processes in which ADA has an important role in the immune system; inhibition the activity of this enzyme lead to death of cell as a result of the accumulation of adenosine in cell and toxic operations also inhibit the synthesis of RNA[27,28].

Activity of Alkaline phosphatase (ALP): The result are presented in table 4. It is clear from table 4 that the activity of ALP in seminal plasma shows a highly significant increase (P=0. 00) in oligospermia when compared with the corresponding control group while significant decrease is observed (P=0. 01) in this activity in azospermia patients with the corresponding control group. A highly significant increase (P=0. 00) is obvious to show presence of oligospermia group when compared with azospermia group. Despite extensive research on alkaline phosphatases in the male reproductive tract, their role in reproductive physiology is not clear. The mechanism of action on spermatozoa is generally assumed to be inhibition of sperm phosphodiesterase activity, resulting in elevation of complementary adenosine monophosphate levels in spermatozoa[29]. The significant differences of ALP activity in seminal plasma refer to sperm number only, not for the sperm motility or activity. The existence of a positive relationship between sperm counts and the activity of this enzyme in the semen samples has been suggested.

The ALP activity in blood serum is shown in Table 4 It is clear from this table that the activity of ALP shows a nonsignificant difference between patient groups (azospermia and oligospermia) with their corresponding control groups as well as between oligospermia and azospermia group. The ALP is a zinc; metalloenzyme; also, it is activated by divalent ion like magnesium ion[30]. The normal blood serum ALP was 3-13 KAU/dl[31]. It is clear from the result of enzyme activity in blood serum that its level activity was within this range in all groups.

Activity of Acid Phosphatase (ACP): Acid phosphatases (ACP) are a family of enzymes classified as hydrolytic enzymes. They are specifically grouped together because of the shared ability to catalyze the hydrolysis of orthophosphate monoesters under acidic conditions[32].

The current study measured the enzyme activity (Table 5) in each of the seminal plasma and blood serum.

Table 4
Activity of ALP in seminal plasma and blood serum of all groups

Groups	Activity of ALP in seminal plasma (KAU/dl) (mean ±SD)	Activity of ALP in blood serum (KAU/dl) (mean ±SD)
Azospermia (n=32)	16.316 ± 7.474	6.226 ± 1.626
Oligospermia (n=34)	28.472 ± 14.147	6.704 ± 1.340
Control (n=32)	25.133 ± 3.409	7.697 ± 1.539
P≤ 0.05	A, B, C	N. sg

A,a significant azospermia group compared with the control group; B,b significant oligospermia group compared with the control group; C,c significant azospermia group compared with oligospermia group
The capital letter is used for comparison of seminal plasma groups and the small letter for comparability of blood serum groups. P ≤ 0.00 highly significant, P ≤ 0.05 significant, P≥ 0.05 nonsignificant.

Table 5
Activity of Acp in seminal plasma and blood serum of all groups

Groups	Activity of ACP in seminal plasma (U/l) (mean ±SD)	Activity of ACP in blood serum (U/l) (mean ±SD)
Azospermia (n=32)	14224.941 ± 8743.912	72.531 ± 33.945
Oligospermia (n=34)	5299.294 ± 3554.536	76.391 ± 14.750
Control (n=32)	23703.03± 6592.016	88.306 ± 15.902
P value	A, C	N. sg

A,a significant azospermia group compared with the control group; B,b significant oligospermia group compared with the control; C,c significant azospermia group compared with oligospermia group
The capital letter is used for comparison of seminal plasma groups and the small letter for comparability of blood serum groups. P ≤ 0.00 highly significant, P ≤ 0.05 significant, P≥ 0.05 nonsignificant.

The physiological function of ACP may be associated with the liquefaction process of semen plasma[33]. The comparison between the activity of ACP in the seminal plasma azospermia group with control indicates a highly significant decrease (p≤ 0.01) while the comparison between oligospermia and control nonsignificant (P ≥ 0.05) but the comparison between oligospermia and azospermin shows a highly significant decrease (P=0.00). Human ACP has had a considerable impact as tools of clinical investigation and intervention. High levels of acid phosphatase are found in

prostatic phatologies as hypertrophy, prostatitis or carcinoma. The result of ACP activity in blood serum is shown in table 5, it is clear from the comparison about the activity of ACP in blood serum between each azospermia, oligospermia, and control. A nonsignificant difference is present ($P \geq 0.05$). Decreased serum acid phosphatase has no clinical significance [(34)].

Conclusion

Increased exposure to pollutants such as heavy metals (lead, cadmium and copper) are likely to lead infertility in male.

1. Seminal plasma biochemical parameters upon in fertilization occur locally so that study of such parameter in blood plasma cannot be used as an indicator of what is happening in seminal plasma.

2. Fallujah city is contaminated with high level of heavy metals.

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