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EVALUATION OF SOME INORGANIC ELEMENTS IN THE SERUM AND SEMINAL PLASMA OF MALE PATIENTS WITH TYPE 2 DIABETES MELLITUS AND ITS RELATION TO MALE INFERTILITY STATUS

[a]Sura Ahmed Najim, Sajedah [b]Abdul Hamid Khalil, [c]Ali Yakub Majid
[a]Department of Medical Teaching Laboratories/Medical City, Baghdad, Iraq.
[b]Department of Medical Laboratory Science Technology, College of Health & Medical Technology / Middle Technical University, Baghdad, Iraq.
[c]Poisoning Consultation Center/Specialized Surgeries Hospital, Medical City, Baghdad, Iraq.

ABSTRACT
This study was conducted on 55 type 2 diabetic patients presented with infertility and 44 apparently healthy subjects is to evaluate the status of some inorganic elements (zinc, copper, magnesium) in the serum and seminal plasma of diabetic male patients presented with infertility and to assess any possible contribution of the imbalance of these elements on male infertility. The results showed ($P<0.01$) a significant decrease in the mean of serum zinc (0.059±0.009 mg/dl), and seminal plasma zinc (1.2±0.25 mg/dl) in diabetic infertile subjects compared to healthy controls (0.085±0.005 mg/dl) (2.62±0.44mg/dl) respectively. While a significant ($P<0.01$) elevation of serum copper (0.171±0.014 mg/dl), and seminal plasma copper (0.25±0.05 mg/dl) were found in diabetic infertile subjects compared to healthy non diabetics (0.118±0.01 mg/dl) (0.15±0.04 mg/dl) respectively. A significant ($P<0.01$) decrease in the serum magnesium (1.6±0.2 mg/dl), and seminal plasma magnesium (3.7±1.7 mg/dl) were found in diabetic infertile subjects compared to healthy controls (2.0±0.1 mg/dl) (5.5±1.2 mg/dl) respectively.The results of this study indicate that the homeostasis of some inorganic elements may disturb in metabolic disorders such as type2 diabetes and alternation in these elements levels in human body could have an adverse effect on male reproductive function.

KEY WORDS: Inorganic elements, Type 2 diabetes, seminal plasma, Infertility.

INTRODUCTION

Diabetes mellitus is a prevalent chronic metabolic disorder resulting from defect in insulin secretion, resistance to insulin, or both (Shaw *et al*., 2010). The most common etiological types of glycemic disorders include Type 1 diabetes (T1D), and Type 2 diabetes (T2D). T1D result from pancreatic β-cell destruction and constitutes only 10% of all diabetic cases (Belle *et al*., 2011). T2D is the most common form of diabetes which account 90% of diabetic cases and characterized by a disorder of glucose metabolism associated with a reduced ability of tissues to respond to insulin (insulin resistance) (Stumvoll *et al*., 2005). Trace elements are micro nutritive elements occurring in minute quantities of body mass and these elements are either essential elements which have multiple functional roles in human body or non essential elements which have a negative influence on living organisms even at very low concentrations (Vandecasteele and Block, 1993). An association has been observed between diabetes mellitus and trace elements in many research studies (Nourmohammadi *et al*., 2000). There is an accumulating evidence demonstrated that chronic hyperglycemia can cause significant alterations in the status of several trace elements, on the other hand, these elements might have specific roles in the pathogenesis and progress of this disease (Evliyaoglu *et al*., 2004). Infertility has been reported to have a high prevalence worldwide and male factor infertility contribute independently about 20-50% of infertile couple cases (Hamada *et al*., 2013). Type 2 diabetes affects male fertility at multiple levels, causing detrimental effects in the endocrine control of spermatogenesis and in spermatogenesis itself and as a result impairing erectile erection and ejaculation (Sexton and Jarow, 1997). Despite advances in the diagnostic methods in the field of andrology, a significant subset of sub fertile males classified as having unexplained male infertility (Abed and Jarad, 2014). Trace essential elements are essential for testicular growth and development and play a particular role in the male reproductive system including in sperm metabolism, capacitation and acrosome reaction (Chia *et al*., 2000). Disturbances in the levels of microelements may be involved in the complex processes of development of the secondary complications of diabetes mellitus, affecting many organs. Deficiency of certain essential elements in men results in reduced libido, low testosterone and sperm count and low levels of these metals has been observed in diabetes mellitus and other diseases (Rajeswari and Swaminathan, 2015). It was hypothesized that seminal plasma changes in trace elements levels were associated with poor fertilizing capacity (Omue & Dashit 1995).

MATERIALS & METHODS

Subjects

A total of 99 subjects were enrolled in this study and divided into two groups. The first group included 55 patients aged between 30-50 years who suffering from type2 diabetes and had a history of infertility. All selected patients had regular unprotected intercourse for at least 12 months without conception with their partners and their wives had no obvious causes of infertility like tubal blockage or ovulation disorders. Forty four healthy individuals with proven fertility and with no previous history of diabetes or other systemic diseases were recruited as control group.

Sample collection

A semen sample was obtained from each subject by masturbation after an abstinence period of 3-5 days and doesn't exceed five days in a dedicated room adjacent to the laboratory. The sample was collected into a clean, wide-mouth sterile graduated plastic container; the container was labeled with the patient's name and the time of collection. Once the specimen has been collected, it was placed into 37°C incubator for 30 minutes, waiting for complete liquefaction. Seminal fluid analysis was carried out in strict compliance with the WHO guidelines. After semen parameters were assessed, seminal plasma was separated by centrifugation at 3000 rpm for 10 minutes. The supernatant was transferred into a sterile plane tube and stored frozen at -20°C until trace elements analysis was done.

About 10 ml of venous blood was collected between 9:00 to 11:00 after an overnight fasting from each subject. About 2 ml of blood was dispensed into plastic tubes containing EDTA to estimate HbA1c weekly. The remaining blood part was dispensed into plane tube, allowed to clot and then centrifuged at 3000 rpm for 10 minutes to obtain sera which was transferred into another tube and stored frozen at -20°C until estimation for glucose, hormones and trace elements were performed. Fasting blood glucose and HbA1c were measured by chemical procedures using spectrophotometry. Hormonal assay were performed using Vitek Immunodiagnostic Assay System (VIDAS). Trace elements were evaluated by using atomic absorption spectrophotometer and semen parameters were examined microscopically.

Statistical analysis

Data were analyzed using Statistical Package of Social Science Software program, version 18 (SPSS). Data was summarized using mean, and standard deviation. Comparison between groups was performed using independent sample t-test for quantitative variables. Analysis of the case-control findings was reported in the term of effect size (Cohen's d) which provides information how large a difference is evident between the studied groups. Cohen defined effect sizes as weak, d = 0.2, moderate, d = 0.5, and strong, d = 0.8. Pearson correlation coefficients were calculated to signify the association between different variables.

RESULTS & DISCUSSION

Results in the table (1) are expressed as mean± SD from each parameter and represented the mean value of serum zinc, copper and magnesium in the two groups (Study and Control group). The results in the mentioned table showed a highly significant decrease in the serum zinc and magnesium in study group (0.059 ±0.009mg/dl) (1.6±0.2 mg/dl) respectively compared to that of control group (0.085± 0.005mg/dl) (2.0±0.1 mg/dl) respectively (P <0.01), while there was a significant elevation in the serum copper of study group (0.171±0.014 mg/dl), compared to that of control group (0.118±0.01 mg/dl) (P <0. 01). Table (1) also shows that the mean values of seminal plasma zinc and magnesium were significantly decreased in diabetic infertile males (1.2±0.25 mg/dl) (3.7±1.7 mg/dl) respectively, compared to that of healthy controls (2.62±0.44mg/dl) (5.5±1.2 mg/dl) respectively (P <0.01), while the mean level of seminal plasma copper was significantly higher in study group (0.25±0.05 mg/dl) compared to that of healthy subjects (0.15±0.04 mg/dl) (P<0.01).The effect of the disease on altering the status of each investigated trace element was very strong in magnitude (Cohen's d >0.8),

TABLE 1: Study-control difference in mean of serum & seminal plasma trace elements

Trace elements	Study group N= 55	Control group N= 44	Difference in Mean	P value	Cohen's d
Serum trace elements levels (mg/dl)					
Zinc	0.059±0.009	0.085±0.005	-0.026	<0.001**	-3.47
Copper	0.171±0.014	0.118±0.01	0.053	<0.001**	4.27
Magnesium	1.6±0.2	2.0±0.1	-0.4	<0.001**	-2.45
Seminal plasma trace elements levels (mg/dl)					
Zinc	1.2±0.25	2.62±0.44	-1.42	<0.001**	-4.09
Copper	0.25±0.05	0.15±0.04	0.1	<0.001**	2.18
Magnesium	3.7±1.7	5.5±1.2	- 1.8	<0.001**	-1.2

**HS: highly-significant (p< 0.01)

There is an accumulating evidence demonstrated that chronic hyperglycemia can cause significant alteration in the status of several trace elements, on the other hand, these elements might have specific roles in the pathogenesis and progress of this disease (Evliyaoglu *et al*., 2004). Zinc is an essential trace element and its deficiency is related to many diseases like diabetes mellitus (Song and Chen, 2005). The cause of serum zinc levels to be decreased in diabetics may

be due to increased urinary excretion caused by interference with active transport of Zn into the renal tubular cells. Disturbed metabolism of zinc metalloenzymes and an abnormal binding of zinc to tissue proteins might also cause increased zinc urinary loss (Nsonwu *et al*., 2006). Clinical studies reported that decreased serum zinc are usually seen in T2D patients may be attributed to the impaired intestinal reabsorption of endogenous zinc and the increase in excretion of zinc into the intestine during the digestive process (Salgueiro *et al*., 2001). The reasons why low magnesium is common in diabetic patients are unclear but it may be attributed to increased urinary losses of magnesium due to glucosuria and osmotic diuresis. It was suggested that diabetes can impair the renal tubular reabsorption of magnesium from the glomerular filtrate (Quamme, 2001). Chronic hypomagnesemia being a consequence of hyperglycemia can lead to the development of macro and microvascular complications of diabetes that worsens the deficiency of magnesium (Ma *et al*., 1995). Other factors contribute to high incidence of hypomagnesemia in type 2 diabetes is related to impaired absorption of magnesium caused by gastroparesis due to autonomic neuropathies that occur in diabetes (Boulton *et al*., 2005). A possible reason that explains the elevation of serum copper in diabetic patients might be attributed to hyperglycemia, which increases glycation and stimulates the release of copper ions from copper rich compounds (Elabid and Ahmed, 2014). The release of copper ions into the blood enhances the toxic effect of metal dependent free radicals which further accelerates the oxidative stress (Abou-Seif and Youssef, 2004). Another possible explanation for these results is that that low zinc status could elevate serum copper levels (Quilliot *et al*., 2001). Low zinc levels may decrease metallothionein synthesis. Metallothionein is an intestinal protein which binds copper with high affinity and inhibits its absorption (Couzy *et al*., 1993). The role of trace elements secreted by excretory glands into ejaculates and their contribution to male infertility is poorly understood. However, many investigations were previously and still being, conducted on normal and abnormal seminal fluids in attempt to correlate alternation in trace elements levels with poor seminal fluid quality that may affect male fertility. Deficiency of certain essential elements in men results in reduced libido, low testosterone and sperm count and low levels of these metals has been observed in diabetes mellitus and other diseases (Rajeswari and Swaminathan, 2015). It was hypothesized that seminal plasma changes in trace elements levels were associated with poor fertilizing capacity (Omue and Dashit, 1995). Ali *et* al., (2005) observed that low seminal plasma zinc in infertile males should be considered as one of the primary factors involved in decreased testicular function. They also concluded that optimal seminal concentration of zinc is necessary for normal sperm function. Several factors associated with low seminal zinc level, including the accumulation of certain toxic heavy metal in testicular tissues and severe zinc depletion lead to low zinc content per ejaculate (King *et* al., 2000; Batra *et al*., 2001). Low zinc concentration in semen affects semen quality by some mechanisms including reducing antioxidant capacity and counteracting the adverse effects of other toxic heavy metals (Prasad *et al.,* 2004). Despite the lack or insufficient available literature on the role of magnesium in male infertility, it was reported that drastic reduction in the seminal plasma magnesium contributes the male reproductive disorders.

TABLE 2: Comparison of elemental content among sperm count categories in study group

Trace elements	Azoospermic (n=9)	Oligozoospermic n=(18)	Normozoospermic n=(28)	P value
Serum Zn(mg/dl)				
Mean	0.064	0.06	0.058	0.09
SD	0.011	0.008	0.009	[NS]
Seminal plasma Zn(mg/dl)				
Mean	1.22	1.21	1.2	0.84
SD	0.3	0.22	0.26	[NS]
Serum Cu(mg/dl)				
Mean	0.166	0.169	0.174	0.15
SD	0.013	0.013	0.015	[NS]
Seminal plasma Cu(mg/dl)				
Mean	0.28	0.25	0.23	0.045*
SD	0.06	0.06	0.05	
Serum Mg(mg/dl)				
Mean	1.6	1.6	1.6	0.81
SD	0.2	0.2	0.2	[NS]
Seminal plasma Mg(mg/dl)				
Mean	4.5	3.7	3.4	0.08
SD	2.1	1.6	1.6	[NS]

*Significant at $p<0.05$

The reduction in seminal magnesium might be a consequence of a defect in the active transport system that transports Mg^{+2} from blood to semen. The decreased level of magnesium in seminal fluid of diabetic infertile population suggested that magnesium might play a role in male infertility (Edorh *et al*., 2003). Elevated copper level in seminal fluid may be attributed to a deficiency of copper-binding proteins, permitting the unbound copper to circulate freely in the body which leads to the accumulation of copper in body organs and fluids (Onwuli and Ajuru, 2014). However copper has a beneficial effect on human heath but it has been suggested that high seminal copper is highly toxic for spermatozoa. It has been reported that copper can acts as a catalyst in the formation of reactive oxygen species (ROS), resulting in the damage of sperm plasma membrane (Stohs and Bagchi, 1995).

Table (2) shows the trace elements levels in study group according to sperm count categories. The study group was classified according to their sperm count (million/ml) into three subgroups (WHO 2010 guidelines) (WHO, 2010), azoospermic=0 sperm (n=9), oligozoospermic<15(10^6/ml) sperm (n=18) and normospermic>15(10^6/ml) sperm (n=28). The results from this table revealed that the mean seminal plasma copper concentration was highest in cases with the most form of sperm count anomaly (azoospermic=0.28 ±0.06mg/dl) and lowest among those cases with normal sperm count (normozoospermic=0.23± 0.05mg/ dl).This positive trend of seminal plasma copper with increasing sperm count abnormality was statistically significant (P_{value}= 0.045).The remaining trace elements showed no obvious statistical significant difference between the three ordered categories of sperm count.

The most common manifestation of male infertility is a low sperm count and more than 90% of male infertility cases are due to low sperm count or poor sperm quality (Lamb, 2010). This finding of seminal plasma copper agrees with that of Eidi *et al***.** (2001) who showed a significantly higher seminal plasma copper concentration in azoospermic and oligozoospermic males compared to normospermic males presented with infertility. Although copper is an essential trace element that plays a critical role in several enzymes but at suboptimal or high level of this element, it becomes toxic to a variety of cells, including human spermatozoa (Wong *et al.,* 2001). However the role of copper in male reproductive capacity seems to be largely unclear, but it has been reported that copper can act on FSH receptors, interfering with spermatogenesis (Weibe *et al.,* 1997). It has been also identified that copper has a highly toxic effect on the seminiferous epithelium; this could explain the highest level of copper in the azoospermic group (Rebrelo *et al.,* 1996).

Results in the table (3), shows the correlation between the selected trace elements in the serum and seminal plasma of diabetic males and their serum sex hormones. This study revealed that only a significant weak negative linear correlation between serum copper and prolactin hormone (P<0.05).In addition to that, serum magnesium was negatively correlated with serum testosterone, while the remaining elements were correlated in small magnitude with serum hormones and showed no statistical significance.

TABLE 3: Linear correlation coefficient between serum hormones and trace elements among cases

Trace elements	FSH (mIU/ml)	LH (mIU/ml)	Prolactin (ng/ml)	Testosterone (ng/ml)
Serum trace elements levels (mg/dl)				
Zinc	0.24	0.01	0.14	0.22
Copper	0.07	0.15	0.27*	0.20
Magnesium	0.14	0.01	0.01	0.29*
Seminal plasma trace elements levels (mg/dl)				
Zinc	0.04	0.01	0.24	0.12
Copper	0.04	0.10	0.02	0.06
Magnesium	0.15	0.22	0.15	0.19

*Significant at p< 0.05

The interplay of hormones in the male axis is extremely sensitive to external perturbations at any level, from the hypothalamic pulse to testicular function. Trace metals exposure can alter the male hormonal status by disrupting the hypothalamic pituitary axis. The inverse correlation between serum copper and prolactin hormone was demonstrated previously. Meeker *et al***.** (2009), showed that serum copper were inversely correlated with prolactin hormone. It has been reported that elevated copper may cause a neural hyperdopaminergic activity (Pfeiffer and Mailloux, 1987). This could explain that excess copper might have indirect inhibitory effect on prolactin. Animal studies has been reported that high doses of copper caused significant elevation in serum corticosterone level which lead to reducing prolactin release (Broikes *et al*., 1991; Chattopadhyay *et al*., 2002).

There are limited data demonstrating the relationship between magnesium and testosterone. Although this current study showed an inverse correlation between serum magnesium and testosterone hormone levels, it is worth to refer that another study demonstrated a positive correlation between the two previous mentioned parameters (Cinar *et al*., 2011). The inverse correlation between Mg^{+2} and testosterone could be explained by an evidence reported that magnesium may mediated variation in the testosterone-SHBG affinity (Excoffon *et al*., 2009). Sex hormone binding

globulin (SHBG) is a major carrier glycoprotein of sex steroids particularly testosterone, each monomer of SHBG contains three metal-binding sites. Magnesium binds to SHBG in a non specific manner which leads to an inhibition in testosterone binding to SHBG. This could lower the level of total testosterone in circulation (Avvakumov *et al*., 2009).

CONCLUSION

The mean levels of all the selected trace elements (Zn, Cu, and Mg) showed a highly significant difference in the serum and seminal plasma of diabetic infertile subjects compared to healthy non diabetics. These results concluded that diabetes mellitus can alter the metabolism of several trace elements which can aggravate its complications including affecting male fertility potential.

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