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Heavy Metal Pollution and Men Infertility in Al-Falluja City

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Abstract:

Infertilityis oneuof the most problemsathatufacingaadvancedunations. In the general, about halfof allacasesaof the infertility are causedby factors thaturelated toathe male partner. Propos educausesvofumalev infertility include evgeneticuand environmental factors. Blood samples from 64 infertileumen allawere living in urban its al-Fallujah city (30 azospermeiauand 34 oligospermeia) and 32 fertile men (asuthe control group) were collected. Heavy metal concentrations inusera of infertile and fertile groupswereumeasured by using Atomic Absorption Spectrophotometer. Y-chromosomemicrodeletions were detected by using PCR techniques. Significantdifferences (P≤0.05)uin the concentration ofucopper (0.0267±0.0147 and 0.0278±0.0273, for infertileuand fertile group respectively), cadmium (0.0477± 0.0038 and 0.0446±0.0059, respectively) and zinc (1.08 ± 0.16) in fertile groupamoreover wereadetected, no deletionsawere recorded in Y Chromosome in peopleuwho exposed to heavy metals in each a azospermiavor severe oligospermia groups. Spermatogenesis disruption in theamale at any phase of cell differentiationamay be increased the abnormaluof sperm count also decrease theutotalspermucount, impair the stability of sperm chromatinuordamageain the sperm DNA.

Key Words: Heavy metals, Pollution, Infertility, Falujha city, Y Chromosome.

Introduction

Male infertility is acommondisorder affecting upto 50% of infertility cases, which includes 10-15% of couples [1]. One of the main factors related to male infertility is the quantityand qualityof sperm function and sperm produced such as sperm motility [2]. The conventional reasons of male infertility arevaricocele, trauma, tumors, cysticfibrosis and genetic factor chro-mosomal abnormalities [3]. Failure of spermatogenesis is the upshotof a

multitude of causes suchas systemic diseases, endocrine disorders, malnutrition, genetic factors and environmental hazards. Genetic defects such as chromosomal abnormalitiesand mutations account for at least 30% of male infertility [4]. Many researchers and clinicians have asserted that societal progress in advanced countries andworsening of the natural environment have likely resulted in decreased male fertility. Long-reported risk factors include noise associated with manufacturing, working in high temperatures, exposure to radiation, a variety of chemical substances and electromagnetic waves [5]. Iraq was polluted with great levels dioxins and radiation, with three decades of war and neglect having left environmental ruin in large parts of the country, an official Iraqi study has found birth defects near site and higher rates of cancer [6]. Heavy and/or toxic metals are among the most public inorganic pollutants in water [7]. Several studies have compared patients with healthy subjects (normal sperm count) to male infertility (oligospermia or azoospermia) [8,9] Heavy metals may compromise male reproduction, as demonstrated by epidemiological and animal studies [8]. Cadmium (Cd) is one of the metals reflected to be potentially dangerous on an international level [9]. Acute cadmium poisoning can result from dust or breath of cadmium gases or from ingestion of heavily contaminated food or water. Cadmium can accumulate in humans body and has a long half-life (10-30 years) in tissues, mainly the kidneys [10]. Copper (Cu) was involved in suppression of spermatogenesis, while it can be poisonous at elevated concentrations, experimental implantation of copper in the vas deferens, epididymis, and scrotum of mammals has been demonstratedtoaffectfertility detrimentally. [11].The frequency of genetic anomalies (karyotype abnormalities and microdeletions) increases with the severity of the spermatogenic defect, reaching to an overall 30% (15% karyotype abnormalities and 15% of AZF microdeletions) in azoospermicmen, chromosomal microdeletions of the azoospermia factor (AZF) regions of the Y chromosome are the only common known genetic causes of spermatogenicfailure [12]. This extraoridinaryampliconic structure of the *AZF* loci renders the section as a hot spot siteforintrachromosomal ectopichomologousrecombination's andsubsequentspontaneousfrequent deletionerrors constructed a meaningful map of the*AZFc*region after sequencing the entire *AZFc*region. They found that *AZFc* consisted of three palindromes with six distinct ampliconic families [13]. The association of b2/b4 complete *AZFc* deletions (also called classical *AZFc* deletion) with spermatogenicfailureis well established as the observed phenotyperange from azoospermia to severeoligozoospermia [14,15] claimed that sperm production appeared to be stable over time in Y chromosome *AZF c*microdeletedpatients [16].

Materials and Methods:

Collection of samples

Semenalfluid was produced by masturbation after three to five days of the sexual abstinence. Samples were left for 20 to 60 minutes for liquefaction to occur, then semen quality was evaluated by using two parameters: Macroscopically and microscopic examination. All infertile male were divided into two groups according to the results of semen analysis using world health organization criteria [17]. The first group azospermia (sperm count = zero/ml), second group oligospermia (sperm count < 20 million /ml). 5ml of

blood samples were collected from two groups; 64 infertile men(30 azospermeiaand 34 oligospermeia) aged range (23-54 years) who were residing in Fallujah city, Iraq, and 32 fertile men (aged matched). A complete medical history with physical examination were done for each group. The blood sera were used for determination of heavy metals ($Cu^{2+}$and Cd^{2+}) and Zn^{2+} also blood samples were used for pcr technique.

Determination of heavy metals concentrations

The heavy metals concentration were determined by digesting 1 ml of serum sample with 5ml of an acid mixture (HNO_3: $HClO_4$) in a volume ratio of 6:1 in a glass tube. Then, the concentration of heavy metals (Cu^{+2}, Cd^{+2} and Zn^{+2}) were measured by usingatomic absorptionspectrophotometer GBC 933 plus (Shimaadzu / Japan), with air-acetylene flame and hollow cathode lamp [18].

Genomic DNA extraction

Blood samples that collectedfrom infertile and fertile male groupswere used for extraction of Genomic DNA by Wizard® Genomic DNA Purification kit (Promega, Madison, WI, USA). Then, the concentration and purity of DNA were estimated by using spectrophotometer [19].

Yq microdeletion analysis by PCR-based STS

Y Chromosome microdeletions in exposed infertile men to heavy metals were detected by using Y Chromosomemicrodeletion system version 2.0 (Promega, Madison,WI). Thepcrproductswereanalyzed by electrophoresis [19].

Results:

Theconcentration of Zn^{+2} in bloodserum Figur(1) shows lowconcentration of itinasospermia group, whereas no significant difference werenoticed in serum azospermeia and control groups.

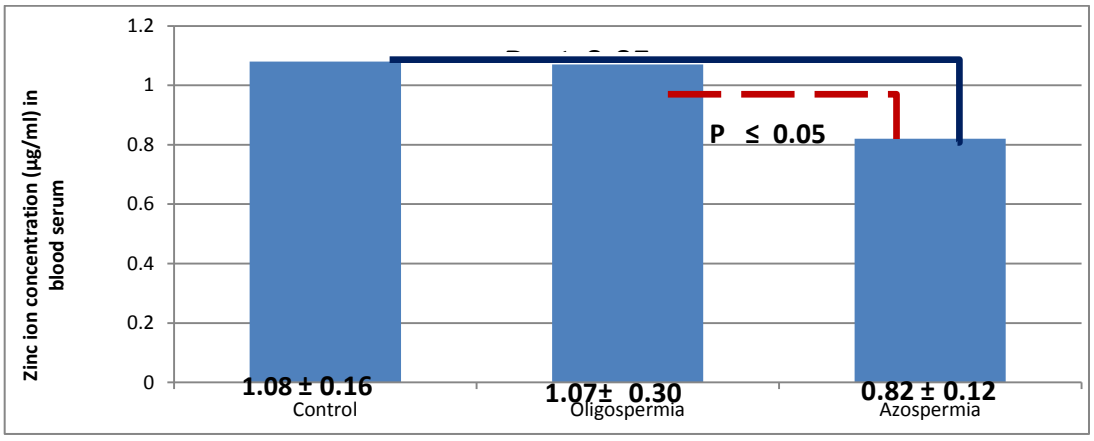

Fig.1:The zinc ion concentration in serum of patients groups and control

The 1 showed a significant increase ($p \leq 0.05$) in the concentration of $Cd^{+2}$and Cu^{+2}were 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 azospermia with oligospermia increase in Cd^{+2} were found.

Table 1: Concentrations of heavy metals (Cu^{+2}& Cd^{+2}) in serum of infertile and fertile groups

Groups	Concentration of heavy metals in the serum	
	Cu^{+2} (Mean ± SD)(µg/ml)	Cd^{+2} (Mean ± SD)(µg/ml)
Azospermeia	0.0267±0.0147[b]	0.0477± 0.0038[b]
Oligospermia	0.0278±0.0273[a]	0.0446±0.0059[a+b]
Control	0.0258±0.0127[a]	0.0152±0.0025

([a])significant value with control group ($p \leq 0.05$), ([b])significant value azospermia with oligospermia ($p \leq 0.05$).

The patients whohad high concentrations of Cd^{+2} and Cu^{+2} in their serum wereselected for molecular analysis Figures (2,3and 4).The results showed that there were nomicrodeletion in *AZF* region in Y chromosome related to Azospermia or Oligospermia .

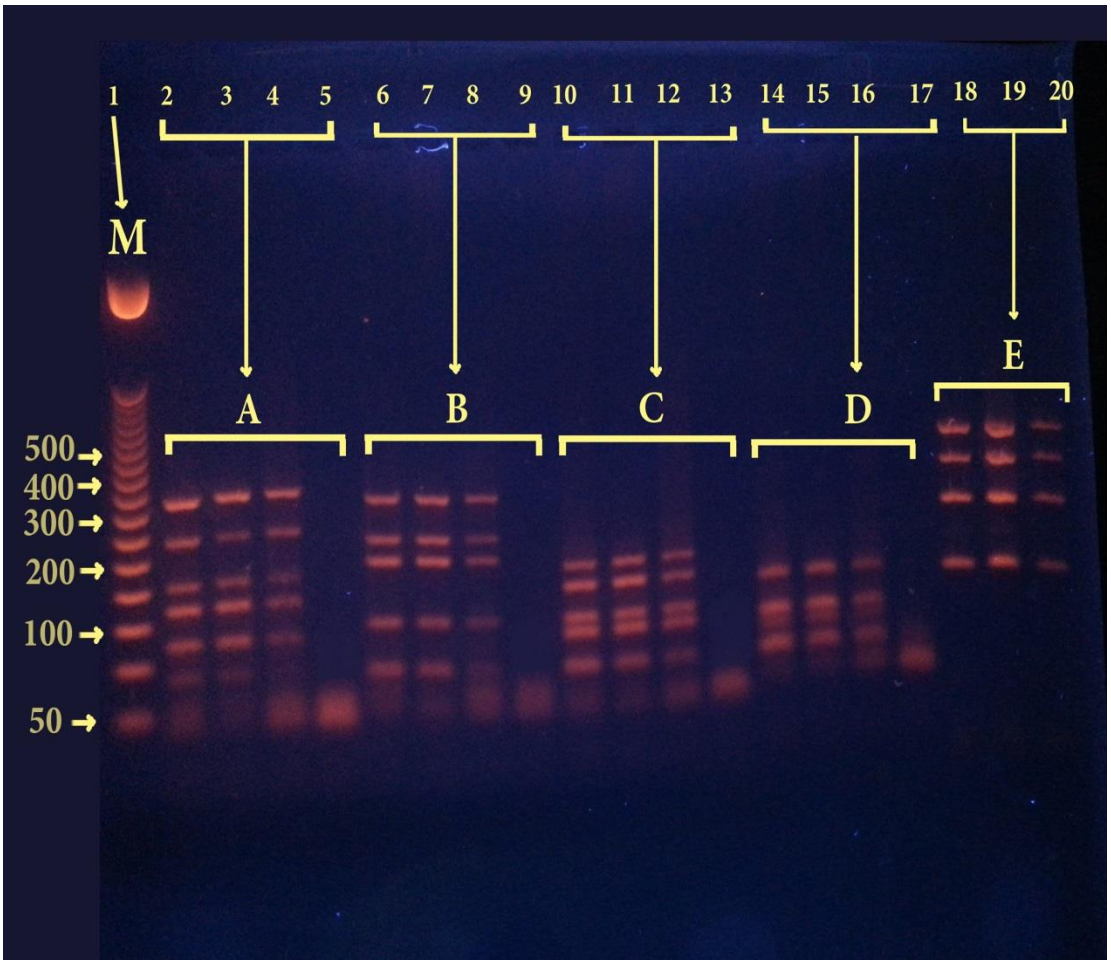

Fig. 2: Amplification of genomic DNA of infertile men exposed to pollution with Cd+2 analyzed by multiplex Master Mix kit(Promega, Madison,WI). Lane 1: (M) represents 50 bp DNA ladder. Lane2-5 :(A);6-9(B);10-13(C);14-17(D);18-20(E) represents the control primer pair that amplifies a fragment of x-linked SMCX. No 2= patient no. one(with high concentration of Cd $^{+2}$); no 3= patient no. two (with a high concentration of Cd $^{+2}$; no 4= positive control ; no 5= negative control. Similar in order similar (B, C, D, E). The DNA products were electrophorized on 1.7% agarose gel at 5 V/cm for 1.5 hrs, stained with ethidium bromide.

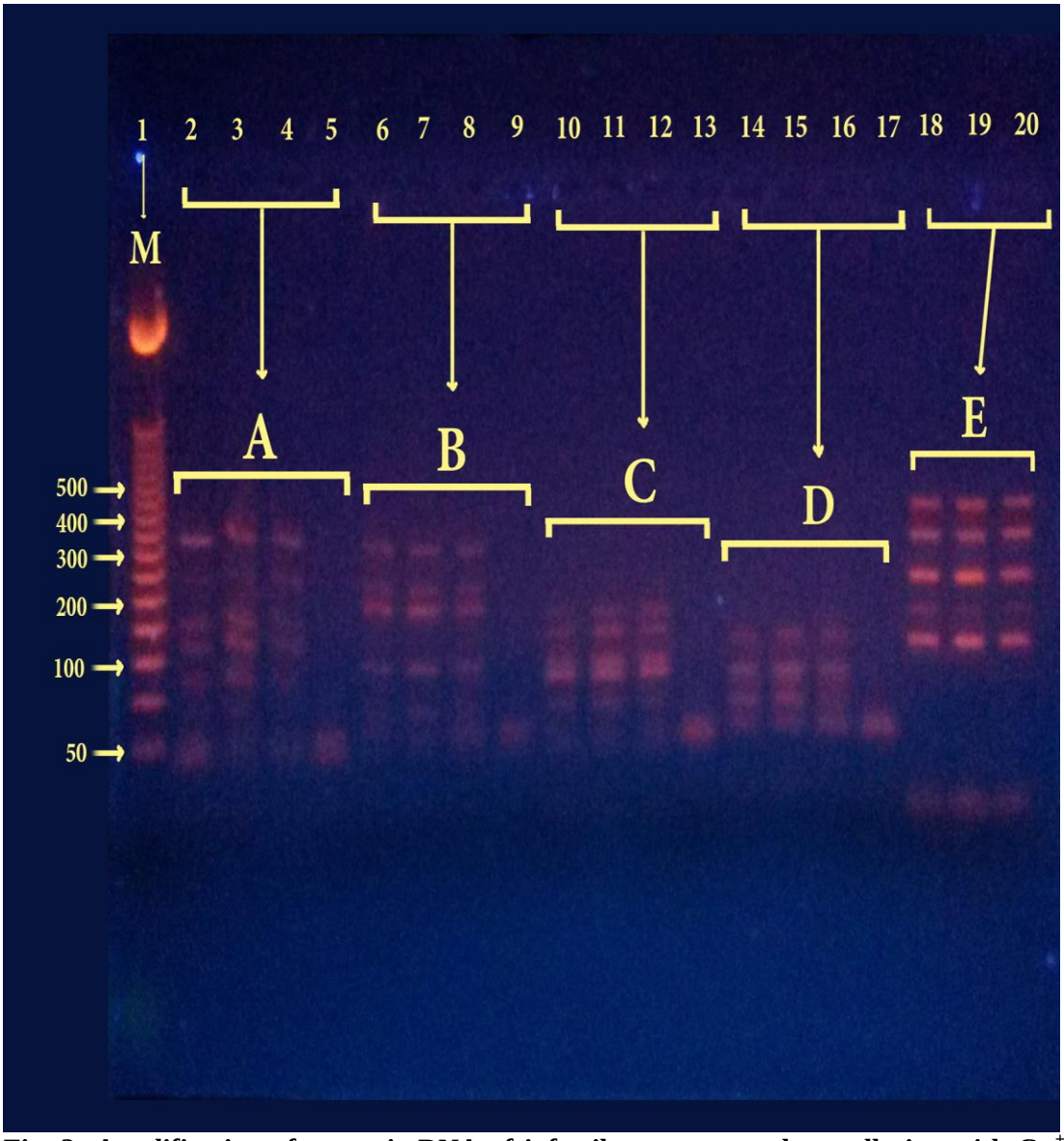

Fig. 3: Amplification of genomic DNA of infertile men exposed to pollution with Cu^{+2} analyzed by multiplex Master Mix kit(Promega, Madison,WI). Lane 1: (M) represents 50 bp DNA ladder. Lane2-5: (A);6-9(B);10-13(C);14-17(D);18-20(E) represent the control primer pair that amplifies a fragment of x-linked SMCX. No 2= patient one(with high concentration of serum Cu^{+2}; no 3= patient no. two (with high concentration of serum Cu^{+2}); no 4= positive control ; no 5= negative control . Similar in order similar (B, C, D, E) The DNA products were electrophorized on 1.7% agarose gel at 5 V/cm for 1.5 hrs, stained with ethidium bromide.

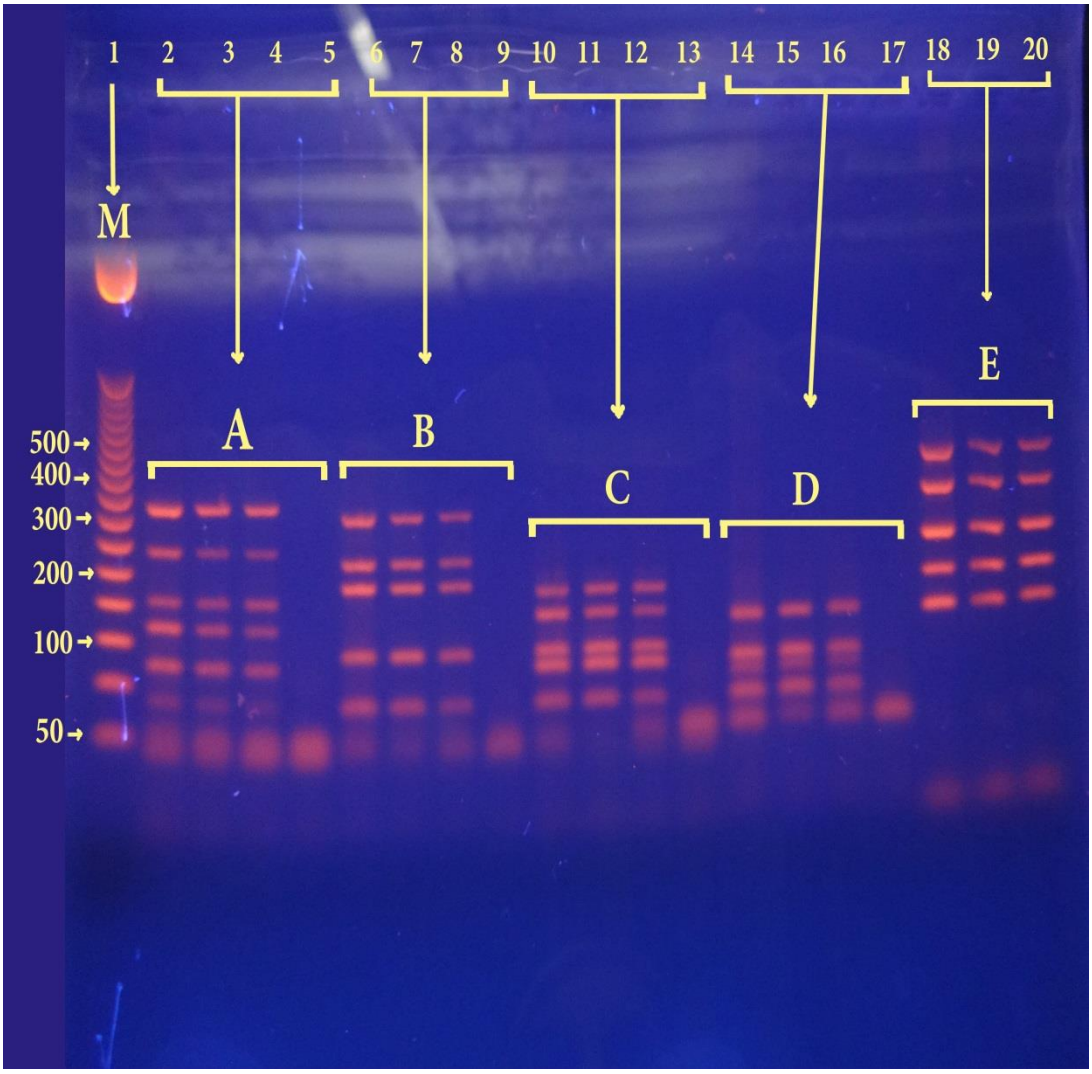

Fig. 4: Amplification of genomic DNA of fertile male analyzed by multiplex Master Mix kit(Promega, Madison,WI). Lane 1: (M) represents 50 bp DNA ladder. Lane2-5: (A); 6-9(B);10-13(C);14-17(D);18-20(E) represent the control primer pair that amplifies a fragment of x-linked SMCX. No 2= control no. one(with high concentration of serum Zn $^{+2}$); no 3= control no. two (with high concentration of serum Zn $^{+2}$); no 4= positive control; no 5= negative control . Similar in order similar (B, C, D, E) The DNA products were electrophorized on 1.7% agarose gel at 5 V/cm for 1.5 hrs, stained with ethidium bromide.

Discussion:

Rivalry between the cadmium ion and the zinc ions for the samebinding sites in each of enzymes, proteins and transporters,may be changing the enzymeactivity, that affect the structureand function of cell membranes, bring oxidative stress and apoptosis on the other hand that can be inhibit RNA and DNA synthesis [20]. In the present study, a significant increasing ($p \leq 0.05$) in the concentration of the cadmium ions werefoundinseraof each azospermia and oligospermeia malein comparison with the control group, this may have serious consequences on cell growth, differentiation and development. While, essentialmetals such as zinc may decreasetheabsorption and retentionoftoxic metalsand preventtheir toxic effects. Also, Zn^{+2} have an important role in theantioxidant system, adaptive responseand genetic repairsystem. Therefore, the interaction between many toxic or/and essential

metalscould be essentially importantfor the health outcomesof heavy metal exposure. These interactionsdue to inter individual differences in susceptibility to opposing effects of metals in men [21]. Cadmium replaces Zn^{2+}leading to reduced activity of superoxide dismutased (SOD), this will be manifested and lead toenzymestructuraldistortion. Viability of spermatozoa was also reduced in cadmium exposed [22]. The high concentration of copper ion was noticed in sera of aozspermeia in compression with oligospermeia and the control group ($p \leq 0.05$). Copper canact as both a pro-oxidant and an antioxidant. Free radicalsoccur naturally in the body this will be lead to damagecell membrane, contribute to the development of a numeral of health troubles, diseases, and act together with genetic material. As an antioxidant, Cu^{+2} is neutralize as free radicals, or as scavenges and may reduce or help prevent some of the damage they cause [23]. Copper toxicity in humans, possibly due to redox cycling and the generation of reactive oxygen species that damage the DNA [24]. Copper in current study has an important role as toxic metal for sperm, heavy metals may affect the male reproductive system indirectly, when they act on the neuroendocrine system or directly when they target specific reproductive organs. These effects canabe long lasting and irreversibleif sertoli cells are disrupted through fetal development. Also the trace element like copper has been suggested as a highly toxic element for sperm and can affect sperm motility in humans [11]. The numeral ofsertoli cells controls the number of sperm produced in adult-hood, because all sertoli cells can support only a limited number of germcells that develop into sperm. According to Sharpe *et al*, Sertoli cells prolif rate during the fetal, neonatal and prepubertal period, and each of these periods is particularly sensitive to the adverse effects of heavy metals [25]. The disruption of spermatogenesis in men at any phase of cell differentiation can increase the abnormal sperm count, decrease the total spermacount, impair the stability of sperm chromatin or damage sperm DNA [26]. The Y chromosome is essential not only for human sex determination but also for maintenance of sperm cells and their development. The regions of the Y chromosome responsible for maleinfertility are located on the long arm of the chromosome as well as are termed (AZF: azoospermia factor), AZFa, AZFb and AZFc [27]. Microdeletions in AZF are dealing with male infertility. As the spermatogenesis severity increases, the frequency of the microdeletionsalso increases[27]. The present study was not shown any differ between studying groups (azospermeia, oligospermeia and the control) in profile of Y chromosome, in all group absence of deletion in Y chromosome, the increase heavy metal in serum did not effect on the genetic level.

Conclusion:

It can be concluded that the increasing of Cd^{+2} and Cu^{+2} in sera of infertile male did not effecton the geneticlevel (Y chromosome); theexposure to heavy metalmay be not cause any *AZY* microdeletions in the infertile male Y chromosome. But this increasing may be causdecrease in the fertility because the effect of the heavy metal on male reproductive system.

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التلوث بالعناصر الثقيلة وعقم الرجال في مدينة الفلوجة

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الخلاصة :

العقم هو احد اخطرالمشاكل ألأجتماعية التي تواجة الأزواج، بشكل عام حوالي نصف حالات العقم يكون سببها الذكور، ومن اهم اسباب العقم هي الأسباب الوراثية والأسباب البيئية. جمعت عينات الدم من 64 حالة مرضية توزعت بين 30 حالة عديمي النطف و34 حاله قليلي النطف بالأضافة الى 32 حالة من الأشخاص الطبيعيين كمجموعة سيطرة، جميع العينات في الدراسة يعيشون في مدينة الفلوجة وضواحيها، قيست العناصر النزرة في مصل الدم بستخدام جهاز المطياف الذري. استعمل جزء من الدم للكشف عن مورث الذكورة باستخدام تفاعل البلمرة التسلسل للعينات التي اظهرت اعلى مستوى من التلوث بالعناصر النزرة ولمجموعة السيطرة للعينات التي اظهرت اعلى مستوى من تركيز الزنك. أظهرت النتائج وجود فروق معنوية بين المجاميع لعنصر النحاس والكادميوم كما لم يسجل أي خلل في المورثة الخاصة بالذكور. أي خلل يحدث في عملية تكوين النطف ممكن ان يؤدي الى ظهور نطف غير سليمه، كما ان انخفاض العدد الكلي للنطف ممكن ان يكون سببه خلل في المورثة الخاصة بالذكور.

الكلمات المفتاحية: التلوث ، العناصر الثقيلة، العقم ، مدينة الفلوجة، الكروموسوم Y