



## Detection of cadmium and cobalt levels in blood and seminal plasma of infertile men in Baghdad city

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### Abstract

Estimation of cadmium and cobalt concentrations were measured in patients' seminal plasma and sera (oligozoospermia, asthenozoospermia, and zoospermia) compared to healthy males as (control group). One hundred and one (73 infertile males and 28 fertile males) their sera and seminal plasma levels were detected (motility, concentrations and antisperm antibodies by tray agglutination test (TAT)). As well as, the concentration of cadmium and cobalt in patients and control groups' sera and seminal plasma were detected. The results showed that cell count and motility are  $59.89 \times 10^6$  and 57.01% in the normospermia, while in the patients groups they are  $(14.32, 26.37, \text{ and } 0.00) \times 10^6$  and 18.91, 23.11 and 0.00% in the oligozoospermia, asthenozoospermia and azoospermia, respectively. Antisperm antibodies results showed 49 (67.13%) negative ASA and 24 (32.87%) positive ASA, there was significant difference ( $P < 0.05$ ) between positive and negative ASAs. The mean levels of cadmium in sera and seminal plasma showed significant difference ( $P < 0.05$ ), (6.43, 7.42, and 7.92)  $\mu\text{g/l}$  in sera, while (4.21, 4.96, and 5.31)  $\mu\text{g/l}$  in the oligozoospermia, asthenozoospermia and azoospermia, respectively, compared to control group which were (5.27 and 3.19)  $\mu\text{g/l}$ . The mean concentration of cobalt in the sera and seminal plasma showed no significant difference ( $P > 0.05$ ), it were (14.38, 15.11, and 16.85) in the sera, while it were (12.17, 12.99, and 14.89)  $\mu\text{g/l}$  in the seminal plasma in oligozoospermia, asthenozoospermia and azoospermia, respectively, compared to control group which were (13.71, and 10.92)  $\mu\text{g/l}$ .

Keywords: Blood serum, Semen, Infertile male, Antisperm antibodies, Cadmium, Cobalt.

### Introduction

Infertility can be defined as the failure to conceive (regardless cause) within one year of unprotected intercourse (DeMelo-Matin, 2002). Infertility affects about 8-12% of the world's population and in about half of cases man are either the single cause or contribute in the couples infertility (Barbary, 2003).

Infertility is complex and has many causes and consequences depending on gender, sexual abnormalities and environmental factors (Haymond and Gronowski, 2006). Human semen contains high concentrations of trace elements (minerals) like magnesium, copper, selenium, zinc, chromium, cobalt, cadmium, lead and other metals; these trace elements play very vital role in affecting various parameters of semen (Sorensen *et al.*, 1999). Certain cases of male infertility are due to congenital causes such as varicocele, absent vasa differentia and undescended testes, or acquired caused such as endocrine disorders, chronic or acute illness, trauma and surgery, other acquired causes of male infertility include erectile

dysfunction and life style (Balen and Jacob, 2003).

Cadmium ( $\text{Cd}^{++}$ ) has molecular homology with zinc and calcium, and compensates with them for resorption to the body (Foulkes, 1985). The major source of inhalative cadmium intoxication is smoking, and the human lung resorbs 40-60% of the cadmium content in cigarette smoke (Elinder *et al.*, 1976). As a result, smokers receive dose of cadmium daily and contaminated drinking and food, particularly cereals, such as rice, wheat and also potato and green leafy vegetables (El-Dars *et al.*, 2013; Gold *et al.*, 2006).

Several factors can increase this uptake, such as low intake of vitamin D, calcium and iron, it has been demonstrated that cadmium uptake in people with anemia and habitual iron deficit, such as children or menstruating women in higher than in other people (Flanogan *et al.*, 1978).

A higher level of cadmium intake, more than standard level has a significant adverse effect on growth rate (Monsefi *et al.*, 2010), but its toxic effect on tissues are not the same in all tissues, i. e., it varies from tissue to another and are seen

primarily in sensitive tissues such as liver, kidney, ovary and especially testes (Orisakwe, 2014).

Cobalt ( $\text{Co}^{++}$ ) is an important component of vitamin  $\text{B}_{12}$ . Approximately 4.5% of molecular weight of vitamin  $\text{B}_{12}$  (Cynocobalamin) is composed of elemental cobalt. The need of cobalt for thymine synthesis, which is required for DNA synthesis, explains the biological role of cobalt for cell division, growth and reproduction (Judson, 1997).

Infertility is likely to arise as a secondary consequence of debilitating condition such as severe cobalt deprivation, sign of cobalt deficiency include delayed uterine involution, irregular estrous cycle and decreased conception rate, dietary cobalt requirement for lactating cow is 0.1 ppm of the ration dry matter intake (Satish, 2003).

Cobalt leads to pernicious anemia, severe fatigue, shortness of breath, low thyroid, angina, panic-anxiety attacks, asthma, cardiomyopathy, congestive heart failure, polycythemia, neurological problem, skin rash, dermatitis and infertility (Umesh *et al.*, 2014).

Trace elements play vital role in the metabolic process of the body, either by acting as co-factor in metabolic pathways or they may be an integral part of enzyme system that catalyze specific biochemical reaction (Donatus and Gospel, 2014).

This study aims to evaluate cadmium and cobalt levels in blood serum and seminal plasma of fertile and infertile males.

### Materials and Methods

**Study Groups:** One hundred and one including 73 patient suffering from infertility and 28 healthy apparently as control group. Their ages were 22-63 years. Patients were married, infertile with their fertile female partner, which were for one year or more unproduction intercourse.

**Seminal Fluid Specimens:** Specimens of semen were collected through masturbation after 3 days abstinence. Samples were incubated at  $37^{\circ}\text{C}$  for 30min. for liquefaction. A routine semen analysis was performed upon liquefaction. According to (WHO, 2010), the volume, pH, sperm motility, sperm concentration, viscosity, morphology, and viability were measured. The remaining semen samples were centrifuged at 1000 rpm for 10 min. the seminal plasma was separated, and then stored at ( $-20^{\circ}\text{C}$ ) in Eppendorf tubes.

Wave lengths of cadmium (228.9nm) and of cobalt (240.7nm) were made for each sample. The accuracy and precision of analytical methods were tested with standard reference materials.

**Serum Metal Analysis:** Two millimeters of whole blood were drawn from both infertile patients and

control groups, and then centrifuged. The sera samples frozen for later trace elements then lysed (cadmium and cobalt) by freezing and thawing. Determination of these elements was carried out according to (absorption spectrophotometer Shimadzu, Japan) instructions. Antisperm antibodies in both sera and seminal plasma in patients and control groups were done using tray agglutination test (TAT). The semen specimen and sera of patients and control groups were collected during (July-December, 2014)

**Statistical Analysis:** The results were expressed in mean and standard deviation. The comparison of two of subjects was done using the student's t-test to determine whether the differences are statistically significant or not. The results were considered statistically significant when P is less than 0.05 ( $P < 0.05$ ).

### Results and Discussion

Table (1) show the results of cell count and percentage of motility. It was found that the mean count is  $(59.89 \pm 7.3) \times 10^6$  sperm cell/ml, which agrees with WHO recommendations count ( $20 \times 10^6$ ) sperm cell/ml. Whereas, it is (14.32, 26.37, and  $0.00 \times 10^6$ ) sperm cell/ml in oligozoospermia, asthenozoospermia, and azoospermia, respectively. The percentage of motility is  $(57.01 \pm 6.9)\%$ , which in agreement with (WHO, 2010) percentage (50)% and above, while it is (18.91, 23.11, and 0.00)% in oligozoospermia, asthenozoospermia, and azoospermia, respectively. WHO provides a guide for healthy sperm count and motility, normal count is equal to or more than 20 ( $\geq 20$ ) million per one millimeter, and motility 5% and above, if these large numbers and startling, one conclusion could be quickly drawn which is generally requires large numbers of sperm count to achieve pregnancy, these numbers are capable of making the long and difficult journey through cervix to fallopian tube to the waiting egg (WHO, 2010). The present finding in patients groups (oligozoospermia, asthenozoospermia, and azoospermia) shows decreased count and motility compared to control group, which could be belong to presence of inflammatory cells (peroxidase-positive granulocytes), in addition, exposed to seminal vesicular fluid show decreased motility, survival and protection of the sperm chromatin (Jequier, 2006), indicating that an abnormal sequence ejaculation can cause decreased sperm function, reduced sperm motility, which can, therefore, be a symptom of disorders related to male accessory sex gland secretion and to the sequential emptying of these glands (Bjorndahl, 2010).

Table (1): Total count and motility percentage in fertile and infertile groups semen

Parameter	Infertile Groups (No. 73 $\pm$ S.D)				P. value
	Normospermia (Control) No.=28	Oligozoospermia	Asthenozoospermia	Azoospermia	
Count $\times 10^6$	59.89 $\pm$ 7.3	14.32 $\pm$ 3.9	26.37 $\pm$ 5.1	0.00	P<0.05
Motility%	57.01 $\pm$ 6.9	18.91 $\pm$ 4.1	23.11 $\pm$ 3.7	0.00	P<0.05

Table (2) shows 24(32.87)% positive results of antisperm antibodies in sera and seminal plasma, while shows 49(67.13)% negative ASAs result. The distribution in antisperm antibodies, which was detected by tray agglutination test on patients' sera and seminal plasma, was (32.87)%, this result agrees with (Marbeen *et al.*, 2007), who found 25.8% ASA positive in seminal fluid of infertile patients, as well as (Abdulla, 2009), who found (29.9)% ASA positive in serum of infertile males.

Table (3) shows the mean sera and seminal plasma of cadmium and cobalt levels for the control and infertile groups. The sera and seminal plasma cadmium level of the oligozoospermia, asthenozoospermia, and azoospermia is (6.43, 7.42, and 7.92), (4.21, 4.96, and 5.31)  $\mu\text{g/ml}$  compared to control group which is (5.27, and 3.19) $\mu\text{g/ml}$ , respectively. The level of cadmium in sera and seminal plasma shows significant difference ( $P<0.05$ ) than those of control group, while the level of cobalt in sera and seminal plasma of oligozoospermia, asthenozoospermia, and azoospermia is (14.38, 15.11, and 16.85), (12.17, 12.99, and 14.89) $\mu\text{g/ml}$  compared to control group in which the level is (13.71, and 10.92) $\mu\text{g/ml}$  respectively, that shows no significant differences between patients groups group and control ( $P>0.05$ ).

Some authors reported an increase in cadmium concentration in infertile men compared to fertile ones (Jockenovel, 1990). This study agrees with (Oluyemi *et al.*, 2006), which shows a significant difference ( $P<0.05$ ) in cadmium level in both sera and seminal plasma levels in the three groups (oligozoospermia, asthenozoospermia, and azoospermia) compared to control group.

Dant *et al.* (2003) demonstrated an increase in

lead and cadmium levels in the seminal plasma of infertile men, they also reported significant negative correlation of those toxicants with sperm motility and concentration in oligozoospermic men. The role of increased seminal plasma trace metals concentration is poorly understood in the regulation of reproductive function in the occupationally unexposed male (Benoff *et al.*, 2000).

Environmental discharge of cadmium is due to the use of petroleum products, combinations of fossil fuels (petroleum, and coal), and municipal refuse contribute in airborne cadmium pollution (DeRosa *et al.*, 2003).

Cigarette smoking is an important factor when we consider the effect of cadmium exposure on human health; a single cigarette has been reported to contain (1.5) $\mu\text{g}$  of cadmium (Chia *et al.*, 1994). Moreover, one tenth of the metal content of a cigarette is inhaled (Elinder *et al.*, 1985).

In this study, sera and seminal plasma levels of cobalt in infertile, and control groups did not show a significant difference ( $P>0.05$ ), although higher level of cobalt was observed in the infertile groups (oligozoospermia, asthenozoospermia, and azoospermia) compared to fertile group (Control), but the levels were not statistically significant.

This result agree with (Abou-Sharkra *et al.*, 1898), who reported that environmental exposure to heavy metals such as lead do not significantly contribute to male infertility. However, as with most metals, excessive levels of cobalt may be toxic to reproductive cells as reported by (Elbetieba *et al.*, 2004), when an individual is exposed to toxic levels of any metal, the body burden of the metal will increase and it will most likely affect the metabolic processes especially reproduction.

Table (2): Distribution of infertile males according to ASAs in sera and seminal plasma

ASAs Result		No.	%	Titer of ASA	* P. Value ( $P \leq 0.05$ )
Serum	-Ve	49	67.13 <sup>A</sup>	-	Sig.
	+Ve	24	32.87 <sup>B</sup>	1/32	Sig.
Seminal Plasma	-Ve	49	67.13 <sup>A</sup>	-	Sig.
	+Ve	24	32.87 <sup>B</sup>	1/64	Sig.

Different letters: significant difference ( $P < 0.05$ ).

Table (3): Levels of cadmium and cobalt in fertile and infertile groups' sera and seminal ( $\mu\text{g/ml}$ )

Age range group/Year		No.	Infertile groups+S.D No.=(73)			P. Value
			Oligozoospermia No.=(28)	Asthenozoospermia a No.=(31)	Azoospermia No.=(16)	
Cadmium	Serum	5.27±0.58	6.43±0.61	7.42±0.52	7.92±0.81	$P > 0.05$
	Seminal plasma	3.19±0.47	4.21±0.51	4.96±0.49	5.31±0.57	$P > 0.05$
Cobalt	Serum	13.71±0.98	14.38±1.17	15.11±1.08	16.85±1.22	$P > 0.05$
	Seminal plasma	10.92±0.81	12.17±1.12	12.99±0.92	14.89±1.17	$P > 0.05$

### Conclusion

High cadmium level is significant ( $P < 0.05$ ) in the sera and seminal plasma of infertile males compared to fertile group, as well as, high cobalt level is not significant ( $P > 0.05$ ) in the sera and seminal plasma of infertile males, were observed in this study.

### References

- Abdulla, S., 2009. The effect of serum and semen antisperm antibodies on the post costal test and semen fluid parameters among infertile couples in Erbil governorate. M.Sc. thesis, Hawler Med. Univ. Erbil.
- Abou-Shakra, F. R.; Ward, N. I.; and Everard, D. M. 1989. The role of trace metals in male infertility. *Fertil. Steril. J.*, 52 :307-310.
- Balen, A. and Jacob, H. 2003. Infertility in practice. 2<sup>nd</sup> ed., Churchill-Livingstone, Edingurg.
- Barbara, B. 2003. Men contribute to and suffer from infertility. *Family health international*, 23 :17-20.
- Benoff, S.; Jacob, A., and lam, R. H. 2000. Male infertility and environmental exposure to lead and cadmium. *Human Reprod. Update* 6 :107-121.
- Bjorndahl L. 2010. The usefulness and significance of assessing rapidly progressive spermatozoa. *As. J. Androl.*, 12 :33-35.
- Chia, S. E.; Xu, B.; Ong, C. N.; *et. al.*, 1994. Effect of cadmium and cigarette smoking on human serum quality. *Intl. J. Fertil. Menopausal Studies*, 39 :292-298.
- DeMelto-Martin, I. 2002. On Cloning human being. *Bioethics*, 16 :246-265.
- DeRosa, M.; Zarrilli, S.; Paersano, L. *et. al.* 2003. Traffic pollutants affect infertility in men. *Human Reprod.*, 18 :1055-1061.
- Donatus, O. O. and Gospel, 2014. Cobalt and copper levels in the seminal plasma of infertile men living in Harcourt metropolis. *Intl. J. Clin. Nut.*, 2(2): 27-31.
- El-betieba, A.; Thai, A. S.; Al-Thani, R. K.; *et. al.* 2004. Chronic Exposure to cobaltous chloride caused adverse effect on fertility of male mice. *Toxicol Appl. Pharmacol.*, 173(3): 351-355.
- El-Days, F. M. S.; Baker, M. H. M.; and Gabre, A. M. 2013. Reduction of COD in resin production waste water using three types of activated carbon. *J. Environ. Treat. Tech.*, 1(3) :126-136.
- Elinder, C. G.; Kjellstram, T.; Friberg, L.; and Linnman, B. L. 1976. Cadmium in kidney cortex, liver, and pancreas from Swedish autopsies. *Arch. Environ. Health*, 31(6) :292-302.
- Elinder, C. G.; Kjellstram, T.; Hogstedt, C.; *et. al.* 1985. Cancer mortality cadmium workers. *Br. J. Indus. Med.*, 42 :651-655.

- Flangan, P.; McLellan, J.; Haist, J. *et. al.* 1978. Increased dietary cadmium absorption in mice and human subjects with iron deficiency. *Gastroenterol.*, 74(5): 841-846.
- Foulkes, E. 1985. Interactions between metals in rat jejunum implications on the nature of cadmium uptake. *Toxicol.*, 37(1): 117-125.
- Gold, J; Scheid, F.; Gross, C.; *et. al.* 2006. The toxicology of cadmium and resulting hazards for human health. *J. Occup. Med. Toxicol.*, 1(22): 1-6.
- Haymond, S. and Gronowski, A.M. 2006. Reproductive related disorders in Teitz textbook of clinical chemistry and molecular diagnosis edited by burtis *et. al.*, Elsevier Saunders, :2079-2152.
- Jequier, A. M. 2006. The importance of diagnosis in the clinical management of infertility in male. *Reprod. Biomed.*, 13 :331-335.
- Jockenhovel, F; Bals, P. M.; and Nieschlag. 1990. Seminal lead and copper in fertile and infertile men. *J. Andrologia*, 22 :503-511.
- Judson, G. J. 1997. Vitamin B<sub>12</sub> responses to cobalt pullets in beef cows. *Aust. Vet. J.*, 75 :660-662.
- Marbeen, M. I.; Bra, D. H.; and Ghalib, A. K. 2007. The incidence of seminal antisperm antibodies in infertile males in Kirkuk governorate. *J. Zankoy Sulaimani.*, 10(1): 15-20.
- Marbeen, M. I.; Bra, D. H.; and Ghalib, A. K. 2007. The incidence of seminal antisperm antibodies in infertile males in Kirkuk governorate. *J. Zankoy Sulaimani.*, 10(1): 15-20.
- Monsefi, M.; Alae, S.; Moradshahi, A.; Rohani, A. 2010. Cadmium-induced infertility male mice. *Environ. Toxicol.*, 25(1) :94-102.
- Oluyemi, A.; Ayodele, O. A.; Olayiwola, B; and John, I. A. 2006. Cadmium toxicology: a possible Cause of male infertility in Nigeria. *Reprod. Biol.*, 6(1): 17-30.
- Orisakwe, O. 2014. Lead and cadmium in public health in Nigeria: physicians neglect and pitfall in patient management. *N. Am. J. Med. Sci.*, 6(2): 61.
- Pant, N.; Upadhyay, G.; Pandey, S.; *et. al.* 2003. Lead and cadmium concentration in the seminal plasma of men in the general population: correlation with sperm quality. *Reprod. Toxicol.*, 17: 447-450.
- Satish, K. 2003. Management of infertility due to mineral deficiency in dairy animals. In proceeding of ICAR summer school on advance diagnostic techniques and therapeutic approaches to metabolic and deficiency diseases.
- Sorensen, M. B.; Bergdahl, N. H.; Hjollund, J. P.; *et. al.* 1999. Zinc, magnesium, and calcium in human seminal fluid: relations to other semen parameters and fertility. *Mol. Hum. Reprod.*, 5: 331-337.
- Umesh, S.; muneendra, H. K.; and Harjit, K. 2014. Role of micronutrients in immunity and fertility of animal. *Nat. Dairy Res. Institute.*
- World Health Organization (W.H.O). 2010. Laboratory manual for the examination and processing of human semen 5<sup>th</sup> ed.