Relationship Between Oxidative Stress with Seminal Plasma Copper levels in Diyala infertile Men

[1]Ehssan Nassiaf Jassim, M.Sc† ,[2]Abdl-Monain Hamad Majeed, Ph.D.,

[1]Dep.of chemistry-Coll.of Education/Univ. of Tikrit. [2]Dep.of chemistry-Coll.of Education/Univ of Sammura.

E-mail: *Ehssan_Bioch@Yahoo.com*

Abstract

Objectives: The objective of the present study was to examine the published literature on the role of copper and malondialdehyde levels in seminal plasma of oligospermic, with normopermic men.**Methods:** The present study was conducted at Baquba Medical Hospital and a few commercial pathological laboratories in Diyala.. The patients attended the clinical with the complaint of infertility and were the male partner of married couples. The study was conducted from November 2011 to January 2012. And the infertility clinics were visited regularly to collect samples and relevant information of infertile subjects over the period at this time. 100 patients who fulfilled the inclusion criteria were selected for the study. Male subjects (23-42) years age with a mean±SDS=32.4±4.4 years There were 20 patients with oligospermia. Another twenty (25) fertile Iraqi men within a similar age group were studied as control. The control samples were treated similarly as the test samples. Copper in sample was assayed by using an atomic absorption spectrophotometric technique. Malondialdehyde was measured calorimetrically using thiobarbituric acid assay which detects thiobarbituric acid reactive substances. **Results:** Seminal plasma copper level decreased non-significantly in the oligozoospermic group compared to the control group. Whereas,. On the other hand, malondialdehyde levels which is an end product of lipid peroxidation were significantly elevated (p=0.000) in all the infertility groups studied. **Conclusions:** Inorganic elements such

as Copper work in different ways in order to maintain normal environment for spermatozoa for normal fertilization to occur.

Keywords: Inorganic elements infertility, oxidative stress, sperm dysfunction

العلاقة بين الجهد التاكسدي ومستويات النحاس في البلازماالمنويه للمرضى العقيمين في محافظة ديالى

الخلاصة

نشر دراسه جديده لمستويات النحاس والمالونداي الديهيد في البلازما المنويه للاشخاص العقيمين بالمقارنه مع الطبيعيين. الطريقه :الدراسه الحاليه انجزت في مستشفيات ومختبرات محافظة ديالى-بعقوبه.للاشخاص العقيمين الذين يعانون من نقص بعدد النطف حيث بدات من شهر تشرين اول 2011 ولغاية كانون ثاني . 2012 حيث جمعت النماذج بصوره منتظمه ودونت المعلومات المناسبه والمهمه بصوره دقيقه .وتراوحت اعمار الاشخص اللذين خضعوا للدراسه (42-23). وشملت الدراسه 20 مريض لديهم نقص بعدد النطف و 25 شخص اصحاء لديهم اطفال بعد سنه من الزواج. والذين اعتبروا مجموعه سيطره التي خضعت لنفس التحاليل والمعلومات للمجاميع المرضيه .عنصرالنحاس تم قياسه بواسطة تقنية الامتصاص الذري اللهبي . والمالونداي الديهيد تم قياسه بطريقه لونيه. النتيجه:كانت مستويات النحاس في البلازما المنويه منخفضه غير ذي اهميه في المجموعه المرضيه قليلة النطف.فيما اظهر تركيز المالونداي الديهى (كدليل تاكسدي) اظهر ارتفاعا معنويا.الاستنتاج:العناصراللاعضويه كالنحاس يعمل بطرق مختلفه للحفاظ على الحاله الطبيعيه للنطف كعامل مضادتاكسدي.

Introduction

Since the first appearance of human on earth infertility has been one the most controversial medical and social issues. Some civilizations considered it to be a punishment, while others thought of it as illness. Some blamed it on the female; others could not explain it. Infertility is a common clinical problem, leading approximately one of six couples in the UK to seek professional advice.[1] Some cases of male infertility are due to anatomical abnormalities such as varicoceles, ductal obstructions or ejaculatory disorders and others cases such as male urologic or genital infections, Drug addictions. Obesity ,Hormonal and Environmental.[21]

Relationship Between Oxidative Stress with Seminal Plasma Copper levels in Diyala infertile Men.

Ehssan Nassiaf Jassim, M.Sc† , Abdl-Monain Hamad Majeed, Ph.D.

One of Some causes of male infertility Reactive oxygen species (ROS) have been shown to have an important role in the normal functioning of a reproductive system and in the athogenesis of infertility. ROS may also play a role in other reproductive organ diseases. Oxidative stress develops when there is an imbalance between the generation of ROS and the scavenging capacity of antioxidants in the reproductive tract. It affects both natural and assisted fertility. Because assisted reproductive techniques are used extensively in the treatment of infertility, it is critical to understand the in-vitro conditions that affect fertilization and embryo development. Treatments that reduce oxidative stress may help infertility that is caused by this imbalance[2].

Several powerful oxidants are produced during the course of metabolism, in both blood cells and most other cells of the body[3]; these include superoxide (O2.-), hydrogen peroxide (H2O2), peroxyl radicals (ROO.) and hydroxyl radical (OH.) and are referred to as reactive oxygen species (ROS). Free radicals are atoms or groups of atoms that have an unpaired electron.[4] Chemical compounds and reactions capable of generating potential toxic oxygen species can be referred to as pro-oxidants.[5] On the other hand, compounds and reactions disposing of these species, scavenging them, suppressing their formation, or opposing their actions are antioxidants and include compounds such as nicotinamide adenine dinucleotide (NADPH), glutathione, ascorbic acid and vitamin E. In a normal cell, there is an appropriate pro-oxidant: antioxidant balance[6-7].

However, this balance can be shifted toward the pro-oxidants when the production of oxygen species is greatly increased (eg, following ingestion of certain chemicals or drugs) or when levels of antioxidants are diminished (e.g. by inactivation of enzymes involved in the disposal of oxygen species and by conditions that cause low levels of the antioxidants mentioned above[8]. This state is called “oxidative stress” and can result in serious cell damage if the stress is massive or prolonged. .Moreover, high levels of ROS are associated with sperm membrane injury through spontaneous lipid peroxidation[27] and important role in many types of cell injury, some of which can result in cell death.[9] ROS are free radicals that play a significant role in many of the sperm physiological processes such as capacitation, hyperactivation and sperm-oocyte fusion. However, they also trigger many pathological processes in the male reproductive system, and these processes have been implicated in cancer of the bladder and prostate as well as in male infertility.[9-10] Spermatozoa are sensitive to oxidative stress because they lack cytoplasmic defenses.[10-11]

Moreover, the sperm plasma membrane contains lipids in the form of polyunsaturated fatty acids, which are vulnerable to attack by ROS. ROS, in the presence of polyunsaturated fatty acids, triggers a chain of chemical reactions called lipid peroxidation.[12-13] Malondialdehyde (MDA), an end product of polyunsaturated fatty acid oxygenation, is a reliable and commonly used biomarker for assessing lipid peroxidation (18). The measurement of MDA is based on its reaction with thiobarbituric acid (TBA) to form a colored MDA-TBA adduct.[14].

Inorganic elements present in the male reproductive system have secured the attention of many investigation [15-16]. Copper is an essential trace element. It is required in the diet because it is the metal cofactor for a variety of enzymes (amine oxidase, copper – dependent superoxide dismutase, cytochrome oxidase and tyrosinase) [17] Copper accepts and donates electrons and is involved in reactions involving dismutation, hydroxylation and oxygenation.[18] However, excess copper can cause problems because it can oxidize proteins and lipids, bind to nucleic acids and enhance the production of free radicals.[18] It is thus important to have mechanisms that will maintain the amount of copper in the body within normal limits. Copper after ingested in diet is carried to the liver bound to albumin, then is taken up by liver cells, and part of it is excreted in the bile. Copper also leaves the liver attached to ceruloplasmin, which is synthesized in that organ [18-19].
The aim of this study is to find out the relation between oxidative stress and levels of copper in the seminal fluid from patients with infertility and normaspermic.

Materials and methods

A case-control study was conducted at Baquba Medical Hospital and a few commercial pathological laboratories in Diyala , Iraq. After obtaining the approval of the research and ethics committee of the College of Medicine at Diyala University and written consent from the patients, 75 infertile patients were enrolled throug Male subjects (23-42) years age with a mean±SDS=32.4±4.4 years were enrolled throughout this study in the period between The study was conducted from November 2011 to January 2012.

The patients were without any treatment and had regular unprotected intercourse for at least 12 months without conception with their partners. The wives of the infertile subjects included had no obvious causes for infertility . Patients who had infertility secondary to infection, were taking medication, or had a congenital defect and had more than 10^6 leukocyte/mL in their semen analysis were excluded from this study. Also, individuals having diabetes or thyroid diseases, patients who were on antipsychotic or antihypertensive drugs, or taking alcohol, nicotine, vitamins, minerals and antioxidant supplementation within the past three months were also excluded from the study. 25 healthy donors with proven fertility and had initiated a successful pregnancy within the last year and had a normal spermiogram at the time of study were selected as controls. The patients were categorized according to their seminal fluid analysis parameters to oligozoospermic (n=20).The specimens were collected in sterile plastic containers by masturbation after an abstinence period of 48-72 hours and were analyzed within one hour of collection. After allowing the specimen liquefy for 30 minutes, seminal fluid analysis was performed to measure sperm concentration, normal sperm morphology, progressive sperm motility in accordance with the recommendations of the World Health Organization (WHO) [20] The WHO criteria for sperm normality used were as follows: sperm concentration ≥20 millions/mL of ejaculate, percentage of sperm progressive motility (a+b) ≥50% and normal sperm morphology ≥30%. Seminal plasma was separated by centrifugation at 2000 x g for 10 minutes at room temperature. The supernatant was removed immediately and kept in 20ºC.

Copper in sample was assayed by using an atomic absorption spectrophotometric technique which depends on the change in energy when the evaporation of the element as exiting the molecules by using acetylene gas for high-energy and increasing the intensity of light emitted single wavelength and passes through photo filters then falls on a photo cell and turn into an electric current and proportional with the light and with quantity of element. The basic principle is the same as that of much elements determination mentioned above except that the test wavelength copper is 324.8 nm .Seminal plasma Copper Level(SPCuL) was estimated using a pye unicam model Sp6 atomic absorption spectrophotometer (AAS). This AAS was adjusted according to the pye unicam AAS instruction.

Operating conditions for copper assessment

Element	Lamp current (mA)	Detection limit µg/ml)	Wave length (nm)	Band pass (nm)	Burner height (mm)	Air pressure (psi)	Acetylene pressure (psi)	Air flow (L/min	Acetylene flow (L/min)
Cu	5	0.003	324.8	0.2	8	30	10	5	1

Starts with aspirating the copper working solution sequentially from the most diluted to the most concentrated once, keep aspirating until the reading was stable. The resulting values are used to establish the calibration curve. Measure the absorbance of the sample and compare against the calibration curve to obtain the copper concentration in the serum.

The amount of malondialdehyde (MDA) was determined by the thiobarbituric acid (TBA) assay. 100 µl of seminal plasma was diluted with deionized water to 1ml. To each diluted sample, one-half ml of thiobarbituric acid (0.67%) was added. All tubes were heated in a boiling water bath for exactly one hour and centrifuged for 10 minutes at 1000 x g, then the supernatant was separated carefully and the absorbance of the pink color formed was measured at 534 nm against an appropriate blank [14].

Results and Discussion

The characteristics of the subjects who participated in this study with their seminal fluid parameters are listed in Table 1. Table 2 illustrates the seminal plasma copper and malondialdehyde levels in the oligozoospermia groups.

Table 1. Characteristics of Patients and Sperm parameters

Characteristics of Patients	Oligozoospermia (n=22)	*p value*	Normozoospermia (n=25)	*p value*
Age (year)	32.4±4.4	0.259	290.30±2.30	0.840
Seminal fluid volume (*mL*)	3.01±0.88	0.31	4.01±1.10	0.611
Sperm count (*million/mL*)	11.10±6.03	0.000	85.85±25.41	--
Sperm progressive motility (a+b)%	63.80±5.41	0.140	67.21±12.11	--
Sperm normal morphology (%)	75.20±18 .21	0.113	65.75±23.78	--

Table 2. Mean Seminal Plasma Copper and Malondialdehyde levels:

	p	Oligospermic (n=22 *value*	Normspermic (n=25)	*p value*
Copper (µg/mL)	163.52±14.77	0.370	172.29±18.30	0.000
MAD (µmole/L)	13.56±0.88	0.000	9.30±1.53	0.000

Seminal plasma copper levels correlated positively but not significantly (r=0.21, 0.34; p>0.05) with normal sperm morphology in the control and oligozoospermic groups respectively, (Figs.1,2). Malondialdehyde levels showed significant elevation (p=0.000) over normal control values in the oligozoospermic seminal plasma samples.

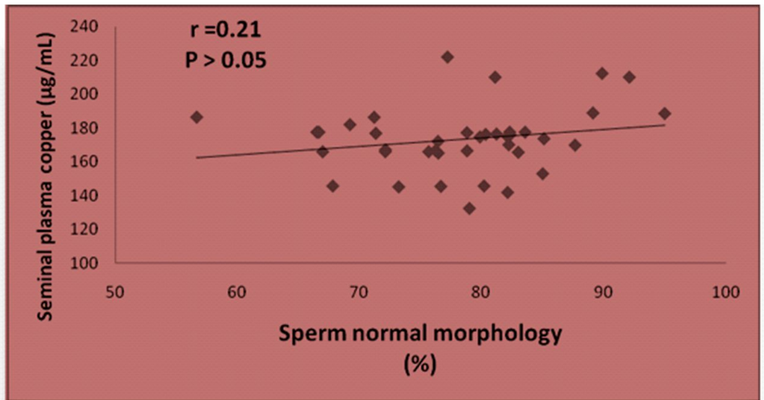

Figure 1. Pearson's Correlation Plot of SeminalPlasma Copper levels versus Normal SpermMorphology in control group

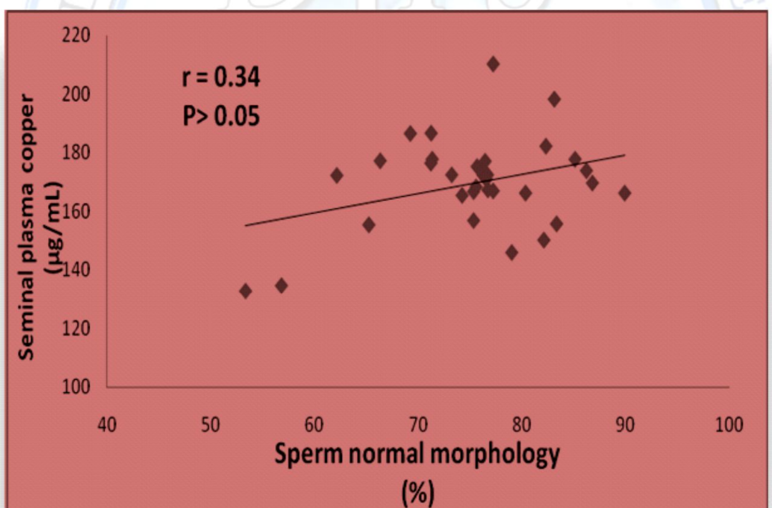

Figure 2. Pearson's orrelation plot of seminal plasma copper levels versus normal sperm morphology in patients with oligozoospermia

Copper is involved in oxidation- reduction reactions and has a dominant role in divers proteins such as cytochrome oxidase and cytoplasmic superoxide dismutase. [22]

The seminal plasma MDA level measured in this study was 9.26 ±1.53 μM in control group, 12.56±0.88 μM in the oligozoospermia group. These results were different from those obtained by Zarghami et al. in 2005 and by Hsieh et al. in 2006.[23-24] Oligozoospermia were associated with higher seminal plasma MDA activity (p=0.000). Increased MDA activity could represent the pathologic lipid peroxidation of the spermatozoa membrane and the following inhibition of sperm motility and viability.[25] A positive correlation was found in this study between the seminal plasma MDA concentration and sperm concentration (r=0.206; p>0.05) in the oligozoospermic group and this finding was incompatible with the studies of Geva et al. in 1996, Fraczek et al. in 2001 and Kobayashi et al. in 1991.[25-26] Also from this study, there was no significant correlation between seminal plasma MDA and sperm progressive motility (data not listed) and this was inconsistent with the study conducted by Suleiman et al. in 1996.[27] The positive association (r=0.21, 0.34 ; p>0.05) between seminal plasma copper and normal sperm morphology in the control and oligozoospermic groups respectively may indicate that copper ion is essential for maximal superoxide dismutase activity which is considered as the principal antioxidant enzyme that may lead to less free radical formation during the spermatogenesis process and to increased normal sperms formed in morphology.

Conclusions

- Elevated oxidative stress is involved in the pathogenesis of male infertility.
- Inorganic elements such as Copper work in different ways in order to maintain normal environment for spermatozoa for normal fertilization to occur.

Recommendations

1.Study each one of the minerals in details alone to know it's metabolic Pathway to get it useful in detection and Treatment.

2.Study the influence of some medicine that used to treat many Pathogenesis cases as anemia, hypertension, diseases of digestive System, kidneys, heart, and liver, on the level of these minerals in the blood and other biological fluids.

3.Study the level of these minerals in both male & female in different ages.

4.Study the role of copper in biological processes because it is yet been clearly established.

5.Evaluation of the oxidative stress in testicular tissues of in infertile animal model.

Statistical analysis

All values were expressed as means ±SDS. The data were analyzed by using of computer SPSS program. Student's t–test was used to examine the differences between different groups [16].

Acknowledgements

The authors thank Al-Ebtehal Clinic's pathological laboratories in Diyala for its support of this study.

References

1. Mohammed E,Mohammed H. Reactive oxygen species and antioxidant in seminal plasma and their impact on male fertility.2009.3(3):87-110.
2. Agarwal A and Allamaneni SS: Role of free radicals in female reproductive diseases and assisted reproduction.Reporod Biomed online9 2004 (3)338-472.
3. Agarwal A and Allamaneni SS (2004):: Role of free radicals in female reproductive diseases and assisted reproduction.Reporod Biomed online9 (3)338-47.
4. Aitken RJ, Clarkson JS, Hargreave TB, (1989): .Analysis of the relationship between defectivesperm function and the generation of reactive oxygen species in cases of oligozoospermia. JAndrol 110:214-220.
5. Akiyama, M., 1999. In vivo scavenging effect of ethylcysteine on reactive oxygen species in human semen. Nippon Hinyokika Gakkai Zasshi 90 (3), 421–428.

6. Allen, R.G. and Balin, A.K. (1989). Oxidative influence on the development and differentiation: An overview of a free radical theory of development. Free Radi. Biol. Med., 6: 631-661.
7. BAKER, J., Brindle, D. S., Irvine and R. J. Aitken, (1996): Protective effect of antioxidant on the impairment of sperm motility by activated polymorphonuclear leukocytes. Fertil. Steril. 652: 411-419.
8. Bauer, R.O.; Demeter, I.; Hasemann, V. et al. (1980). Structural properties of the zinc sites in Cu, Zn, superoxide dismutase: Perturbed angular correlation of gamma rays spectroscopy on the Cu-Cd superoxide dismutase derivative. Biochem. Biophys. Res. Commun., 94: 1296-1302.
9. Bell, M.,Wang, R.,Mellstron,WJG.,Sikka, SC (!993). Effect of cry preservation protocol on sperm membrane liquid peroxidation and recovery of motile human sperm. J. Androl., 14: 472-478.
10. Agarwal A, Prabakaran SA, Said TM. Prevention of oxidative stress injury to sperm. J Androl 2005 Nov-Dec;26(6):654-660.
11. Saleh RA, Agarwal A. Oxidative stress and male infertility: from research bench to clinical practice. J Androl 2002 Nov-Dec;23(6):737-752.
12. Agarwal A, Hamamah S, Shekarriz M. Reactive oxygen species and fertilizing capacity of spermatozoa. Contracept Fertil Sex 1994;22:327-330.
13. Zalata AA, Ahmed AH, Allamaneni SS, Comhaire FH, Agarwal A. Relationship between acrosin activity of human spermatozoa and oxidative stress. Asian J Androl 2004 Dec;6(4):313-318.
14. Sheu JY, Ku HP, Tseng WC, Chen MT, Tsai LY, Huang YL. Determination of thiobarbituric acid adduct of malondialdehyde using on-line microdialysis coupled with high-performance liquid chromatography. Anal Sci 2003 Apr;19(4):621-624.
15. Marshall WJ, Bangert SK. The gonads. In: Marshall WJ, Bangert SK, editors. Clinical Chemistry. 6th ed. Mosby Elsevier, 2008. p. 200.
16. Swerdloff RS, Wang C. The testis and male sexual function. In: Goldman L, Ausiello D, editors. Cecil textbook of medicine. 22nd ed. WB Saunders, 2008. p. 1472 – 1483.

17. Skandhan KP, Makada MT, Amith S. Levels of cadmium, nickel, manganese and lead levels in normal and pathological human seminal plasma. Urologia 2005;72(4):461-464.
18. Murray RK. Plasma proteins and immunoglobulins. In: Murray RK, Granner DK, Rodwell VW, editors. Harper's illustrated biochemistry. 27th ed. Lange Medical Books/ McGraw-Hill, 2006. p. 596,597.
19. Crook MA. Vitamins, trace elements, and metals. In: Crook MA, editor. Clinical Chemistry & Metabolic Medicine. 7th ed. Edward Arnold (Publishers) Ltd. 2006. p. 230.
20. World Health Organization. WHO laboratory manual for the examination of human semen and sperm- cervical mucus interaction. 4th ed, Cambridge University press, 1999.
21. F. O. Olayemi. A review on some causes of male infertility. African Journal of Biotechnology2010. 9(20) : 2834-2842.
22. Roychoudhury S, Slivková J, Bulla J, Massányi P. Copper administration alters fine parameters of spermatozoa motility in vitro. Folia Vet 2008;52:64-68.
23. Zarghami N, Khosrowbeygi A. Seminal plasma levels of 15-F 2α- isoprostane, malondialdehyde, and total homocysteine in normozoospermic and asthenozoospermic males. Indian J. Clin. Biochem. 2005;20(2):86-91 doi:10.1007/BF02867406.
24. Hsieh YY, Chang CC, Lin CS. Seminal malondialdehyde concentration but not glutathione peroxidase activity is negatively correlated with seminal concentration and motility. Int J Biol Sci 2006;2(1):23-29.
25. Verma A, Kanwar KC. Effect of vitamin E on human sperm motility and lipid peroxidation in vitro. Asian J Androl 1999 Sep;1(3):151-154.
26. Kobayashi T, Miyazaki T, Natori M, Nozawa S. Protective role of superoxide dismutase in human sperm motility: superoxide dismutase activity and lipid peroxide in human seminal plasma and spermatozoa. Hum Reprod 1991 Aug;6(7):987-991.
27. Mohammad S,Anthony J,Ashok A. A Method of human Semen Centrifugation to Minimize the latrogenic Sperm Injuries Caused by Reactive Oxygen Spcies.*European Urology* 1995,28:31-35.