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INFERTILE MEN;

A COMPARATIVE STUDY OF TRACE ELEMENTS IN SERUM AND SEMINAL PLASMA IN INFERTILE MEN IN AN INDUSTRIAL AREA OF LAHORE, PUNJAB

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ABSTARCT… Objectives: The objective of this study was to compare the level of trace elements Zinc, Cadmium and lead in seminal plasma and serum of infertile men to the fertile ones. **Period:** June 2014 to August 2015. **Setting:** Pathology Department of Continental Medical Lahore, attached with Ch. Rehmat Ali Memorial Trust teaching Hospital **Methods:** A total of 400 samples of workers working in different factories for a period of 5 or more years were collected. Out of theses 80 were azoospermic. The relationship of trace elements in serum and seminal plasma of infertile men was compared with the normospermic individual controls. **Results:** The concentration of trace elements Pb and Cd was higher in seminal plasma and serum of infertile individuals while Zn level was lower. So it is suggested that high levels of Pb and Cd may affect the fertility and Zn is a crucial element for sperms and fertility in men.

Key words: Azoospermia, Normozoopermic, male infertility, seminal zinc, seminal Cadmium, seminal lead

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INTRODUCTION

Infertility in men, in the last decade is a burning health issue. During this era it is seen that quality of semen has been compromised in a way or the other. Many minor and major factors play vital role in the quality of seminal fluid. Deviation of these minor and major factors from normal values may result in severe sexual health problems and my lead to infertility.

Men infertility is a major factor that contributes to about 30-55% of infertility. Causes of Infertility in men include idiopathic male infertility, sperm motility, quantity as well as quality of sperm, and deficiency of trace elements, environmental factors and heavy metals. Different studies reveal that semen quality is being compromised by 3% each year, which indicates that more and more men are becoming infertile over time. It is hard to say exact percentage of infertility in developing countries including Pakistan but it is estimated that 8-12% face problems in conceiving during their reproductive life. This estimate indicates that millions of people are affected by it. Trace elements in semen have got vital importance recently after their significance has been realized and understanding of their important role.[1]

Different environmental factors are playing their role in causing infertility in men as well. Their main role is to decrease sperm density and increase in heavy metals concentration. Studies show that semen quality and quantity depends on age, disease, nutrition, activity and biochemistry. Cadmium is toxic to semen quality in men and it accumulates in human body over long time exposure. The biological half-life of cadmium in human body is 10-20 years.[2]

The recent exposure to cadmium is measured by its level of concentration in serum. Cadmium like other heavy metals affects human semen but how it affects is not clearly understood, no reliable data is available about its effects on reproductive health of human especially males.[3]

Copper is another element that has hazardous effects on quality of semen. It is used in a variety

of house hold and industrial products including buildings, paints, motor vehicles, tubes of copper and plastic sheet strips. It also contaminates under water soil.[4]

The function of copper regarding male fertility is not very well known. It is an important trace element that plays an important role in different metabolic processes and affects male fertility in various species. High levels of copper affect spermatogenesis. "Experimental implantation of copper in the epididymis, vas deferens, and scrotum of mammals has been demonstrated to affect fertility detrimentally. The trace element copper has been suggested as a highly toxic element for sperm and can affect sperm motility in humans". As the there is no conclusive evidence about the detrimental effects of copper, therefore this study has been designed.[5] It is inevitable to avoid lead exposure in human environment. In recent studies it is indicated that long term exposure of lead affects human semen quality. Zinc plays a vital role for sperms nuclear chromatin and cell membrane. It may have some role in capacitation, antimicrobial function for testis and sperm motility.[6]

However, present study is not about its physiological vital role but it's about the correlation of its level in seminal plasma to serum plasma in infertile males of an industrial area.[7]

MATERIALS and METHODS

This study was conducted on 400 semen samples during June 2014 to August 2015 in Pathology Department of Continental Medical Lahore, attached with Ch. Rehmat Ali Memorial Trust teaching Hospital that is 400 bedded hospitals. Out of these samples 80 were azoospermic, which were then further processes for this study. These were the individuals who were working in different industries like textile, food processing units, wood work factories, chemical industries, and paper, plastic and paints manufacturing units in the area of Manga Mandi and Raiwind road, Lahore Punjab, Pakistan. This area harbors thousands factories and small industrial units. A questionnaire was developed to know their history of smoking, drug abuse or any other disease that affects their reproductive life.

Inclusion Criteria

All individual included were working in theses factories for more than 5 years. All were married healthy individuals aged 25-55 years. They were not taking any treatment of infertility at that time. Patients having some primary or secondary cause were included in this study.

Exclusion Criteria

Individuals working for less than 5 years and unmarried were not included in the study. Diabetics, Thyroid disorder, herniotomy and pelvic surgery were excluded. Individuals taking any kind of medicine for the treatment of infertility were not included.

Sampling and Examination of Samples

Before collecting samples the individuals were well educated about sample collection procedure. They were advised to abstain from any kind of sexual activity for at least 3 to 6 days. All the samples were collected by masturbation and entire ejaculate was passed into graduated centrifuged container for the volume measurement.

The seminal plasma was separated from the spermatozoa by centrifugation at 3000rpm at room temperature for ten minutes. An aliquot of the seminal plasma was carefully decanted into a metal free plastic tubes (washed thoroughly with 1% nitric acid and rinsed properly with de-ionized water), and stored at -20 centigrade until required for analysis. On the basis of number of sperm, following WHO guidelines,[8] the samples were classified into A, B and C categories;

All seminal and venous blood samples collected were fresh samples. No old or overnight sample was used. The seminal plasma elements (Cd, Pd and Zn) measurements were performed by a method which includes deproteinization of seminal plasma with nitric acid, followed by spectrophotometer (frequency 50/60 HZ, U.K. England) according to the methods described by Shibata for zinc analysis.[9] The seminal plasma Cd

No. Of Sperms	Condition	Category
n > 40 x 10^6 sperm/ml	Normozoopermic	Category A
n < 40 x 10^6 sperm/ml	Oligospermic	Category B
N=0	Azoospermic	Category C

Table-I. WHO classification of Normospermic, Oligospemic and Azoospermic

Total Samples	Normospermic	Oligo spermic	Azoospermic
400	200	120	80

Table-II. WHO classifications of Normospermic, Oligospemic and Azoospermic

Elements	Sample	Normospermic (n=200)	Azoospermic (n= 80)
Cd (mg/L)	Seminal plasma	0.63 (0.19-1.71)	0.98 (0.6 – 2.9)
Cd (mg/L)	Serum	1.33 (0.95-2.4)	2.10 (1.8- 2. 15)
Pb (mg/L)	Seminal plasma	12.6 (4.5-16.9)	15.2 (6.3 - 19.1)
Pb (mg/L)	Serum	81.9 (59- 193.1)	89.1 (79 – 253)
Zn (mg/L)	Seminal plasma	197.2 (183-304.5)	163 (139 – 278)
Zn (mg/L)	Serum	7.6 (1.9- 7.9)	5.9 (0.79- 15.1)

Table-III. Median and range values of Cd, Pb and Zn in seminal plasma and serum of normosperic and azoospermic men.

and Pb measurements were performed by electro thermal atomic absorption spectrometry.[10]

STATISTICAL ANALYSIS

All samples were categorized according to WHO criteria for normospermic, oligospermic, azoospermic. All measurements were performed in duplicate and their median values were used for calculations. Statistical analysis was done by using SPSS (version 20) with level of significance $p < 0.05$.

RESULTS

All 400 samples were processed for sperm count to exclude normospermic, and oligospermic from azoospermic (infertile) using WHO criteria as cited it table-I. Out of theses samples 200 were normospermic, 120 were oligospermic and 80 were azoospermic. This is shown in table-II

All the 80 samples seminal plasma and serum of infertile men were analyzed for the level of trace elements. The results of these infertile men were then compared with normospermic subjects. The results of spectrophotometery for cadmium (Cd), zinc (Zn) and lead (Pb) were presented as median and range values. The relevant values are shown in the following table.

The values for trace elements i.e. Cadmium, Zinc and lead is shown in table-III. The values of these elements differ from one sample to another, therefore theses are shown in tabular form and presented as mean and range values. These results show that serum levels of Cd and Pb are higher in serum that in seminal plasma both normospermic and azoospermic than seminal plasma while overall Zn concentration in seminal plasma is higher than its level in serum.

DISCUSSION

The results of our study show that the level of trace elements zinc, cadmium and lead are higher in seminal plasma as well as serum of infertile men as compared to normospermic ones that have been used as control. The findings of our study are close to the findings of a similar study conducted by tawfiq et al in Iraq.[1] Zn is the most crucial trace element required for the normal production, function and transfer of the sperm. In our study the seminal plasma and serum levels of zinc are higher in normospermic patients than azoospermic patients. These results are similar to the study conducted by Altaher et al.[5] Another study conducted by Ali et al also supports our study that the level of zinc was lower in seminal plasma as well as serum of azoospermic individuals than

in Normospermic ones.[11] Zinc plays central role in sperm motility, the number of sperm, viability of sperms and as antioxidant to the enzymes. Zinc sulphate is used in autoimmune diseases of sperms, so adequate concentration of zinc is required for normal production and function of sperm.[12, 13]

Factory workers exposure to heavy metals like Pb and Cd have been studied in different places in the world and stated that theses detrimental metals do accumulate and in human reproductive system and may cause infertility in men. In a review article Benoff et al described that cadmium and lead levels of seminal plasma and serum were higher in infertile men that in normal individuals. This supports our study. However, it is also stated that it is not conclusive indicator of infertility. Similarly in our study the seminal plasma and serum levels of Cd and Pb are higher in oligospermic patients than in the Normospermic controls.[11] Another article written by sheineir states that individual exposure to heavy metals for a long time affects its reproductive ability and heavy metals like lead and cadmium levels of infertile men was higher in seminal plasma and serum of these individuals than the Normospermic ones.[15] These findings are close to our study and support our study. The results of our study do suggest that theses heavy metals after their long exposure do affect the sperm quality in men and affect male fertility.

CONCLUSION

The findings of our study present that the serum and seminal plasma levels of zinc, cadmium and lead levels do affect the fertility in men. Lead and cadmium levels were higher in infertile men than normosperic while zinc level was lower than normal individuals.

This study covers a small industrial area of the city and do not represent all workers of factories where they get exposed to these heavy metals. Therefore a large scale study should be conducted to the real picture. There should be strict monitoring and guidelines to reduce the exposure of workers to these metals working in these industrial units. The workers should be well educated about hazardous effects of theses metals and advise them proper precautionary measure.

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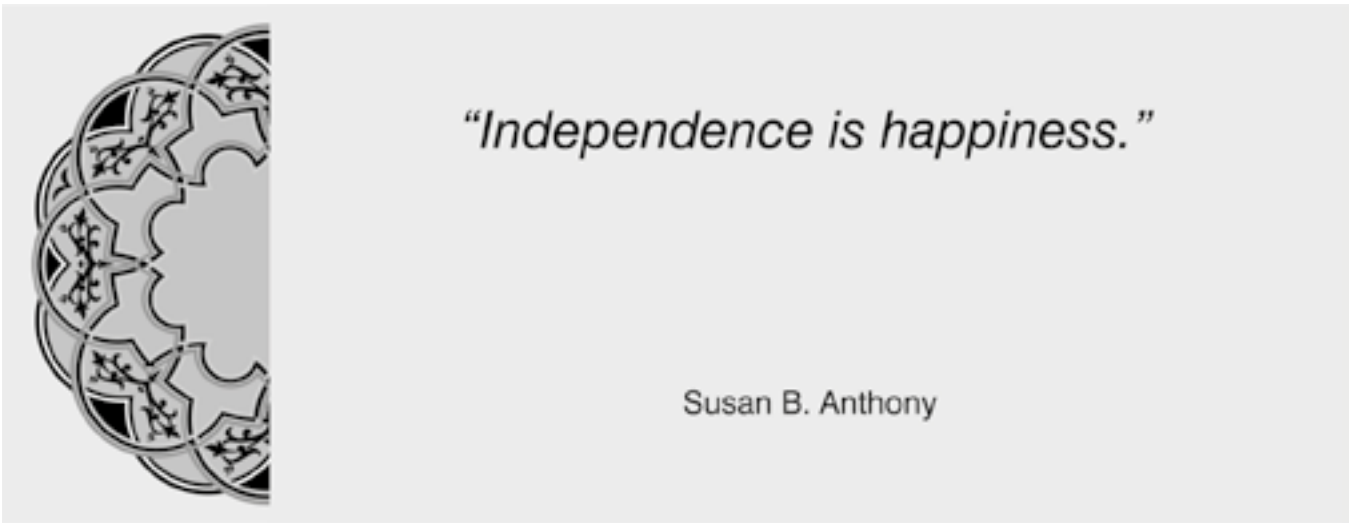

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