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Original Article

A Comparative Study of Toxic Heavy Metals in Seminal Plasma and Whole Blood in Infertile Men

Dr Vimmi Kishore[1], Dr Roberton Gautam[2], Dr Hetandra Kumar Bhartiya[3]

[1]Assistant professor, Department of Biochemistry, Command Hospital Southern Command, Pune, MH (India)
[2]Associate professor, Department of Anatomy, Rajshree medical and Research Institute , Bareilly, UP
[3]Associate Professor, Department of Orthopaedics, Rajshree medical and Research Institute, Bareilly, UP

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Corresponding Author:

Dr Vimmi Kishore
Assistant professor, Department of Biochemistry, Command Hospital Southern Command, Pune, MH (India)

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ABSTRACT

Introduction: Male infertility is a significant health issue with a high prevalence worldwide. Heavy metals such as lead (Pb), Cadmium (Cd) and Arsenic (As) are highly toxic metals which accumulate in the human body on prolonged exposure and play an important role in causing infertility in men. The aim of the current study was to compare the levels of 3 toxic heavy metals lead (Pb), Cadmium (Cd) and Arsenic (As) in seminal plasma and whole blood of male partners of infertile couples.

Methods: A total of 150 samples of blood and semen were collected from 75 men of mean age 29.85 ± 2.51 years attending the infertility clinic of UPUMS Saifai out of which 25 were normospermic and 50 were oligospermic. Semen analysis was performed according to World Health Organization guidelines (1992). The concentrations of Pb, Cd and As were determined by Inductively coupled plasma optical emission spectrometry (ICP-OES).

Results: When the Pb and Cd level were compared in the seminal plasma of the study subject suffering from oligospermia as against the normal control the levels were found significantly increased. Moreover, in case of blood samples also the level of Pb and Cd were found to be significantly higher among the oligospermics. However, the concentration of arsenic was found absent in both cases. Significant inverse correlations were observed between blood and seminal concentrations of Pb and Cd with semen parameters especially sperm count.

Conclusion: The results of the present study suggest that Pb and Cd may be related to a moderate alteration of seminal parameters which leads to male infertility.

Keywords: *Male infertility, Lead, Cadmium, Arsenic, ICP-OES.*

INTRODUCTION

Infertility is a significant health issue with a high prevalence worldwide. According to the International Committee for Monitoring Assisted Reproductive Technology, World Health Organisation (WHO), infertility is defined as the failure to achieve clinical pregnancy after 12 months of regular unprotected sexual intercourse [1]. Infertility affects 15% couples globally, amounting to 48.5 million couples annually [2, 3]. Recent figures reveal that male infertility is responsible for approximately 30-55% of infertility cases [4].

Male infertility is a multifactorial syndrome encompassing a wide variety of disorders which lead to defective spermatogenesis [5]. Anatomic defects underlying male infertility include varicocele, vesicular

passage and ejaculatory failures, genital tract infections, gametogenesis dysfunction, molecular genetics disorders, endocrine disturbances and immunologic problems [6,7]. Additionally, factors such as lifestyle, environment and smoking have also been reported to affect gamete and embryo development [8, 9].

Lead, cadmium, mercury, and arsenic are often referred to as ‘‘heavy metals’’. These are highly toxic metals and accumulate in the human body on prolonged exposure. Exposure to lead (Pb), arsenic (As) and ption or by directly

affecting spermatogenesis [10].

Cadmium has been demonstrated to affect spermatogenesis and/or semen quality and endocrine function, by different pathogenetic mechanisms. Cd severely affects testis structure, by damaging vascular endothelium and by inducing inflammation and apoptosis within the testis. Moreover, Cd exerts direct cytotoxicity within the testis, mainly targeting the Sertoli cells and Leydig cells [11]. It induces oxidative stress in somatic and germ cells, mainly mediated by molecular mimicry and interference with essential ions, beyond apoptosis occurring in germ cells [12]. Cadmium can also interfere with the normal functions of mitochondrial enzymes [13].

Lead is a metal commonly found in the environment that is an acute and a chronic toxin. It is has been reported to be associated with reduced sperm count, poor motility and abnormal sperm morphology particularly sperm head [14]. Reproductive dysfunction has been described in men exposed to lead at the workplace, including oligozoospermia and dose-dependent asthenozoospermia [15, 16]. Other authors have also reported a reduction in spermatogenesis among battery workers as one of the findings in symptomatic lead poisoning [17]. Arsenic is a toxic metalloid known for its carcinogenic nature. In a study conducted by Sarkar et al. it was revealed that arsenic effects mainly the processes of meiosis and post-meiotic stages of spermatogenesis and acute exposure to arsenic causes rapid and extensive disruption of spermatogenesis in mice[18]. Still, the male reproductive toxicity study in relation to arsenic exposure is sparse.

Since data regarding the male reproductive capacity of metals at environmental level are still limited therefore the objective of this study was to determine the levels of Pb, Cd and As in blood and seminal plasma of males evaluated for infertility and correlate their levels with semen quality.

MATERIAL AND METHODS

Study design and study population

This is a cross-sectional study that was conducted during January 2017 to June 2018. All the male partners (age ranges 25-49yrs) from couples attending the infertility clinic of UPUMS, Saifai, Etawah with primary infertility were enrolled in the study.

Inclusion criteria:

All the male partners of infertile couples reporting to Obstetrics and Gynaecology Out-Patient Department of UPUMS Saifai, Etawah, with impaired fertility during the study duration.

Exclusion criteria:

A brief medical history of the patients was obtained before semen analysis. Subjects who had undergone pelvic surgery of hernia repair, varicocele repair, with diabetes mellitus and thyroid disturbances, genital infections, endocrine hypogonadism, and history of chemotherapy, radiation therapy, and sexual dysfunction were excluded from the study. Patients who were on antipsychotic drugs or taking alcohol, vitamin or mineral supplementation were excluded from the study.

Study consent/Ethical approval:

The study protocol was approved by the Institutional Ethical Committee of UPUMS, Saifai, Etawah. Before enrolment of the study, written informed consent from each subject was obtained in response to fully written and verbal explanation of nature of study.

Sample Collection, processing and storage:

Semen samples were collected through masturbation after an abstinence period of 3 days and for blood samples, 5ml of fasting venous blood was drawn same day and collected into EDTA vials. Semen samples were incubated for 30 minutes at 37ºC for liquefaction, followed by a routine semen analysis according to the WHO guidelines to obtain volume, sperm concentration, motility, vitality and morphology [19]. The remaining semen samples were centrifuged and the supernatant (seminal plasma) collected. Both seminal plasma and whole blood samples were stored at -20ºC until further assay.

Sample Preparation:

For estimation of metal concentrations the blood samples and the semen samples were first digested with the help of a multiwave reaction system (Multiwave 3000, Anton Paar, Perkin Elmer) with 100 mL PFA vessels, 40 bar) and pressure-temperature (p/T) sensor. 0.5mL of whole blood/semen sample was digested with 2.0mL of HNO_3, 1.0 mL of H_2O_2 and 1.5mL of H_2O in microwave digestion system, according to the digestion program. The resulting clear solutions were cooled and diluted to 5.0 mL with Milli-Q water for further analysis of heavy metals.

Determination of heavy metals:

The concentration of heavy metals Pb, As and Cd in blood and seminal plasma samples of the patients were estimated using (ICP-OES) inductively coupled plasma – optical emission spectrometer (Optima 8000, Perkin Elmer). The

standards for ICP-OES were prepared from stock solution of Pb, Cd and As obtained from Perkin Elmer. Working solutions were prepared from the stock as necessary. Calibration standards of different concentrations 0.01 mg/L^{-1} to 1.0 mg/L^{-1} were prepared from working standard solution.

Statistical analysis

All the analysis was carried out on SPSS 16.0 version (Chicago, Inc., USA). To compare categorical variables between the groups Chi-square test was used while Unpaired t-test/Mann-Whitney U test was used to compare continuous variables between the groups. The Pearson's correlation coefficient was calculated and p- value <0.05 was considered significant.

RESULTS

Demographic characteristics: Seventy-five (75) males conforming to the inclusion criteria were enrolled in the study. The males ranged in age from 25 to 40 years with a mean age of 29.85 (SD = 2.51years). A total number of 150 samples of whole blood and semen were collected. Based on sperm concentration the subjects were divided into two groups a. Normospermic (>15 million sperms/mL) which had 25 subjects; b. oligospermic (<15 million sperms/mL) which had 50 subjects. Among the normospermic group 20% were smoker and 80% non- smoker while in the oligospermic group 46% were smoker and 54% non-smoker. Table 1 shows characteristics of the participants in the study.

Table 1: Demographic characteristics and seminal parameters of subjects

Demographic characteristics	**Normospermic**	**Oligospermic**
Age	29.84±2.23	29.86±2.67
Smoking habits		
Smoker	5	23
Non-smoker	20	27
Semen Characteristics		
6 Sperm Count (×10 /mL)	56.64±16.99	7.72±3.71
Volume (mL)	2.71±0.51	2.46±0.56
Vitality(%)	63.04±11.8	55.5±6.24
Morphology(%)	63.12±9.37	49.7±10.17
Progressive motility(%)	53.64±11.95	46.2±10.6
Non-progressive motility(%)	18.4±10.27	16.16±6.9

Values are expressed as mean ± SD.

Lead, Cadmium and Arsenic levels in blood: Cadmium was found to be significantly higher (p = 0.001) among oligospermic (0.13 ± 0.11µg/dL) as compared to normospermic (0.02 ± 0.01µg/dL). Lead was also found to be significantly higher (p = 0.0001) among oligospermic (17.51 ± 9.73µg/dL) compared to normospermic (5.04 ± 2.11 µg/dL) while Arsenic was not detected in both normospermic and oligospermic.

Lead, Cadmium and Arsenic levels in seminal plasma: In seminal plasma also we observed similar trend. Cadmium in seminal plasma was found to be significantly higher (p = 0.001) among oligospermic (0.06 ± 0.02µg/dL) as compared to normospermic (0.01± 0.01µg/dL). Similarly Pb was also found to be significantly higher (p = 0.0001) among oligospermic (13.18 ± 8.69µg/dL) as compared to normospermic (3.20± 5.45µg/dL) whereas As was not detected in both the groups.

Heavy metals concentration in both blood and seminal plasma is shown in Table 2.

Correlation of level of heavy metals in blood with semen parameters: Table 3 shows the Pearson's correlation analysis of level of heavy metals in blood with seminal parameters. Significant inverse correlation was observed between level of Pb and sperm count (r = - 0.59, p = 0.0001). We also observed significant moderately negative correlations of Pb levels with semen volume (r = -0.38, p = 0.001), vitality (r = 0.37, p = 0.001) and morphology (r = -0.36, p < 0.002). There was significant moderately negative correlation between level of Cd in blood and sperm count (r = -0.40, p = 0.001), vitality (r = -0.41, p = 0.001) and morphology (r= -0.37, p = 0.004) among the subjects.

Correlation of level of heavy metals in seminal plasma with semen parameters: Table 4 shows the Pearson's correlation analysis of level of heavy metals in seminal plasma with seminal parameters. Significant inverse correlations were found between Pb concentrations and sperm count (r = -0.52, p = 0.0001) and sperm morphology (r = -0.42, p = 0.0001). We also observed significant moderate negative correlations of Pb with semen volume (r = -0.37, p = 0.001) and sperm vitality (r = -0.37, p = 0.001). Cadmium concentrations showed a significant mild negative correlation with semen parameters especially sperm count (r = -0.37, p = 0.001), sperm vitality (r = -0.36, p = 0.001) and sperm morphology (r = 0.34, p = 0.003).

Table 2: Sem metals in sub	inal plasma and jects	blood levels	of heavy		Seminal plasma	3.20±5.45	13.18±8.69	0.0001*
					Cd(µg/dL)			
Parameter	Normospermic	Oligospermic	p-value		Blood	0.02±0.01	0.13±0.11	0.001*
Pb(µg/dL)								
					Seminal plasma	0.01±0.01	0.06±0.02	0.001*
Blood	5.04±2.11	17.51±9.73	0.0001*					

As(µg/dL)		
Blood	0.00±0.00	NA
Seminal plasma	0.00±0.00	NA

Values are expressed as mean ± SD. *statistically significant, p <0.05 NA- not applicable

DISCUSSION

Although with recent advancements in technology and immense research, various causes of male infertility have been identified till date, still there remains a subset of infertile males with unknown aetiology. The root cause of male infertility is sperm dysfunction and semen quality is used as a surrogate measure of male fecundity. Recent trends in male reproductive health have shown the association of decline in semen quality and fertility with occupational and environmental chemical exposure based on animal and human studies [20]. Although there are inconsistent reports of association of Pb, Cd and As with the decline in semen quality [21], it is credible that several environmental factors may cause male infertility, including exposure to heavy metals as their toxicity in humans is well documented [22]. However, it is consistent in the literature that male infertility is variable with a multitude of influencing geographical differences, including environmental and lifestyle factors [20].

In the present study we observed significantly higher levels of Pb and Cd in whole blood samples of oligospermic males as compared to normospermic males. A similar trend was seen in the seminal plasma samples where we observed significantly higher levels of Cd and Pb (table-2) in seminal plasma of oligospermic males as compared to normospermic males. This may be associated with adverse reduction in the basic semen parameters: sperm concentration, motility, vitality and morphology observed in the current study. This result is consistent with the studies of Telisman et al. [23] and Xu et al. [24] that indicate deleterious effects of Pb and Cd on male fertility. Arsenic is a cell toxicant, As^{3+} can block cell respiration by easily binding to sulfhydryl to form a stable complex, block cellular metabolism and finally inhibit some enzymes [25]. However in our study As was not detected in both whole blood and semen samples; which may be due to its presence in very small traces.

We also observed a significant negative correlation of level of Pb in seminal plasma with sperm count, vitality and morphology (table-4). Similarly there was a significant negative correlation of level of Pb in blood with sperm count and a mild negative correlation with sperm morphology and vitality (table-3). This is in consonance with the studies of other authors [23,26].

Table 3: Pearson's correlation of level of heavy metals in blood with semen parameters

Semen Parameters	Pb		Cd		As	
	R	p-value	r	p-value	R	p-value
Sperm Count	-0.59	0.0001*	-0.40	0.001*	--	--
Volume	-0.38	0.001*	-0.26	0.02*	--	--
Vitality	-0.37	0.001*	-0.41	0.001*	--	--
Morphology	-0.36	0.002*	-0.37	0.004*	--	--
PR	-0.25	0.03*	-0.20	0.07	--	--
NR	-0.15	0.19	-0.04	0.69	--	--

*Statistically significant correlation at $p < 0.05$

Table 4: Pearson's correlation of level of heavy metals in seminal plasma with semen parameters

Semen Parameters	Pb		Cd		As	
	r	p-value	r	p-value	R	p-value
Sperm Count	-0.52	0.0001*	-0.37	0.001*	--	--
Volume	-0.37	0.001*	-0.23	0.04*	--	--
Vitality	-0.37	0.001*	-0.36	0.001*	--	--

Morphology	-0.42	0.0001*	-0.34	0.003*	--	--
PR	-0.33	0.004*	-0.20	0.08	--	--
NR	-0.07	0.50	-0.03	0.74	--	--

*Statistically significant correlation at $p < 0.05$

There is considerable agreement that high or even moderate concentrations of Pb cause fertility problems in humans. Fatima et al. reported a decline in sperm count, sperm motility and morphology with >40 µg/dL of lead in blood [27]. Telisman and colleagues also showed significantly lower sperm density and motility with high blood lead concentrations (36.7µg/dL) [23]. This shows that high concentrations of Pb seem to be clearly associated with sperm damage.

However, there are conflicting results about the effect on semen quality at low lead concentrations. Hernandez- Ochoa and colleagues found that even low lead concentrations in seminal fluid (0.2µg/dL) were associated with impaired semen quality, motility, morphology, and sperm concentration [28]. In contrast, some studies have reported no significant associations between low Pb and Cd concentrations and semen parameters [10, 29, 30]. In our study we observed a significant negative correlation between blood and seminal plasma concentrations of Cd and sperm count, vitality and morphology among the subjects (table-3). This result is in accordance with the studies of other authors [23, 31]. This indicates that Cd might have an important role in the decline of semen quality which is possibly due to the oxidative impact of Cd on spermatogenesis and its deleterious effect on testicular structure and function as reported in previous studies [11, 12, 13].

As seen with Pb, there is no agreement on the effect of low concentrations of cadmium on semen quality. Telisman et al. [23] found that even low concentrations of cadmium (<1µg/dL) in whole blood were associated with pathologic sperms. Benoff [31] and colleagues concluded that sperm concentration, motility, and morphology are affected even with low seminal plasma concentrations of cadmium (0.028µg/dL). Mendiola [10] and colleagues also found that low concentrations of cadmium in seminal plasma (0.085µg/dL) were morphology or sperm concentration. Equally, Hovatta et al. [29] showed no correlation between higher cadmium concentrations in seminal fluid (0.15µg/dL) and sperm concentration. Chia and colleagues [32] did not find any impairment of morphology and motility with low concentrations of cadmium in whole blood (0.095µg/dL). Similarly, Mendiola et al. [10] showed that cadmium measured in whole blood and blood plasma did not impaired morphology, motility, or sperm count. Moreover, Meeker et al. [30] reported no effect of low cadmium concentrations in whole blood (0.04µg/dL) on sperm density and motility.

Taken together, statistically significant inverse correlations were found between the sperm concentrations, morphology and vitality and the levels of Pb and Cd in the present study. One limitation of this study was the limited number of subjects included in the study due to a very short duration of the study which calls for conducting further evaluations.

CONCLUSION

The overall findings of the present study indicated that lead and cadmium may have a negative impact on various semen parameters especially sperm count which may ultimately lead to male infertility. Future work will involve examining these suggestive relationships with a larger sample size. Additional human epidemiologic studies, as well as mechanistic studies, are needed to confirm these findings.

Conflict of Interests

Authors have no conflict of interests.

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