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The effects of Pb on sperm parameters and sperm DNA fragmentation of men investigated for infertility

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Abstract:
Background: Lead (Pb) is one of the metals most prevalent in the environment and is known to cause infertility and deoxyribonucleic acid (DNA) fragmentation. This study aimed to determine the association between seminal plasma Pb and sperm DNA fragmentation in men investigated for infertility.
Methods: Male partners (n = 300) of couples investigated for infertility were recruited after informed consent was obtained. Sperm parameters were assessed according to the World Health Organization (WHO) guidelines. Seminal plasma Pb was estimated by atomic absorption spectrophotometry after digestion with nitric acid.
Results: In Pb-positive and -negative groups the sperm parameters and sperm DNA fragmentation were compared using independent sample t-test and the Mann-Whitney U-test, respectively. The mean [standard deviation (SD)] age and duration of infertility were 34.8 (5.34) years and 45.7 (35.09) months, respectively, and the mean Pb concentration was 15.7 μg/dL. In Pb positives compared to Pb negatives the means (SD) of sperm count, progressive motility viability and normal morphology were lower ($p > 0.05$) but the DNA fragmentation was significantly higher 39.80% (25.08) than Pb negatives 22.65% (11.30). Seminal plasma Pb concentration and sperm DNA fragmentation had a positive correlation ($r = 0.38$, $p = 0.03$). A negative correlation was observed between sperm DNA fragmentation and sperm concentration, progressive motility, total motility and viability. When the DNA fragmentation was ≥30% sperm concentration and viability decreased ($p < 0.05$).
Conclusions: Pb in seminal plasma had a significant effect on sperm DNA fragmentation but not with other sperm parameters.

Keywords: DNA fragmentation, Pb, sperm parameters
DOI: 10.1515/jbcpp-2019-0239
Received: September 1, 2019; **Accepted:** March 13, 2020

Introduction

Male factor infertility accounts for 50% of all cases of infertility and affects one in 20 in the general population [1]. During the past five decades, a decline in semen quality has been reported worldwide [2], [3], [4]. Occupational and environmental exposures to metals like lead (Pb) and cadmium have been reported to contribute to the decline in semen quality [5], [6], [7].

Occupational exposure to Pb occurs in smelting and refining [8], Pb-acid battery manufacturing [9], the production and use of paints [10] and the production of Pb-glazed pottery and crystal glass [11]. In Sri Lanka men also work in such industries which are rated as high risk for exposure to Pb.

There is evidence that Pb pollution in Sri Lanka is high in water, soil and food items [12]. People living in these areas are likely to be exposed through environmental sources. We anticipated that in men with no known cause of infertility possible exposure to Pb through occupational and environmental sources could cause harmful effects on sperm parameters.

As the effects of Pb on sperm parameters have not been studied in Sri Lanka this study aimed to describe the association between lead and semen parameters including sperm DNA fragmentation in men investigated for infertility.

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Materials and methods

Sample and study setting

Male partners (n = 300) of couples investigated for infertility at Vindana Reproductive Health Centre, Colombo, were recruited after informed consent was obtained. Subjects with one or more of the following were excluded.

1. A history of diabetes mellitus, mumps, tuberculosis, high blood pressure, urinary tract infection, sexually transmitted diseases and testicular injury as known causes of infertility [13].
2. Small testes (<4 × 2 cm), varicoceles or any other genital abnormalities.
3. Previous genitourinary surgery.
4. Long-term medication for systemic illnesses, hormonal treatment or vitamin supplementation.
5. Cigarette smokers.
6. Azoospermics.

Seminal fluid analysis

After a period of 3 days of sexual abstinence semen samples were collected by masturbation.

After collection, the ejaculates were left to liquefy for 30 min at room temperature. Sperm count and motility were assessed using a Makler counting chamber(Sefi-Medical Instruments, Haifa, Israel) and was counted twice. If there was more than a 10% difference between the two counts, the counting procedure was repeated. In categorizing motility the World Health Organization (WHO) criteria [14] were used.

Sperm morphology and vitality were determined using air dried smears stained with Eosin-Nigrosin. At least 200 sperms were counted in calculating the percentages of normal morphology and vitality.

When categorizing in to normozoospermic and pathozoospermic groups the WHO guidelines were used as the criteria.

The criteria for normozoospermia were a concentration of $<15 \times 10^6$/mL, with sperms of progressive motility more than 32% of spermatozoa, and normal morphology with at least 4% of the spermatozoa.

Seminal plasma obtained by centrifugation was stored in metal-free, labeled polypropylene plastic tubes at −20 °C [15].

Pb analysis

Pb analysis was done by graphite furnace atomic absorption spectrophotometry (GFAAS) (Varian Spectra 250; Australia). A volume of 0.5 mL of seminal plasma was digested in 5 mL of ultrapure grade concentrated nitric acid (HNO_3) and 2 mL of 30% hydrogen peroxide (H_2O_2) in a Teflon digestion vessel (XP 1500 Plus) placed in a microwave oven (Mars 907511, CEM Corp., Matthews, NC, USA) for 30 min. The filtrate was diluted using deionized water. Both control and blank samples were treated in a similar manner for each batch of tests. The GFAAS was calibrated using 10, 20 and 40 μg/L standards (Inorganic Ventures; Lakewood, NJ, USA). Blank and test samples were aspirated into GFAAS by using a 283.3 nm wavelength. Both test and control samples were analyzed in duplicate and the average was taken as the result. The minimum detection limit for Pb was 0.32 μg/L.

Sperm DNA fragmentation assessment

Sperm DNA fragmentation was assessed using the Halosperm kit (Halotech DNA; Madrid, Spain) in a sub sample of randomly selected Pb-positive (n = 20) and -negative (n = 20) men. Fragmentation levels above 30% and below 30% were considered as the high fragmentation group and low fragmentation group, respectively [16].

Statistical analysis

Statistical analysis was done using SPSS (Version16). Means of sperm parameters of Pb-positive and Pb-negative groups and Pb concentration (mean) of normozoospermic and pathozoospermic groups were compared using an independent sample t-test. The correlation between sperm parameters and Pb in seminal plasma was analysed by Spearman's correlation. The means of sperm DNA fragmentation of Pb positive and Pb negative men was compared using the Mann-Whitney U-test.

Results

Characteristics of subjects

In the total population of 300 subjects the mean age, body mass index (BMI) and duration of infertility were 34.8 (5.34) years, 24.3 (4.28) (kg/m^2) and 45.7 (35.09) months, respectively.

The age, BMI and duration of infertility were similar in Pb-positive and Pb-negative subjects (Table 1).

Table 1: Characteristics of Pb-positive and Pb-negative men.

Characteristics of subjects [mean (SD)]	Pb positive (n = 115)	Pb negative (n = 185)	p-Value[a]
Age, years	34.9 (5.30)	34.8 (5.37)	0.9
BMI, kg/m^2	24.8 (3.87)	24.0 (3.7)	0.1
Duration of infertility, months	47.3 (36.9)	44.7 (33.9)	0.5

[a]Independent sample t-test.

Seminal plasma Pb in the study population

Lead was detected in seminal plasma of 38.3% (n = 115) of men investigated for infertility. In the total population the mean Pb concentration was 15.7 μg/dL. The mean Pb concentration of Pb-positive men was 41.1 μg/dL with a range of 2–123 μg/dL. Pb-negative men did not have Pb in their seminal plasma and therefore the Pb concentration was 0. The mean (SD) Pb concentration in seminal plasma was higher in pathozoospermics [17.2(3.02)] than the normozoospermics [15.0(1.70)] although the difference was not statistically significant.

The association between sperm parameters and Pb

The means of semen volume, sperm concentration, progressive motility, total motility, normal morphology and viability were not significantly different in Pb-positive and Pb-negative men ($p > 0.05$) (Table 2).

Table 2: Sperm parameters of Pb-positive and Pb-negative men.

Semen parameter [mean (SD)]	Lead positive (n = 115)	Lead negative (n = 185)	p-Value[a]
Volume, mL	2.5 (1.17)	2.8 (1.11)	0.60
Sperm concentration, million/mL	59.98 (53.45)	63.54 (54.13)	0.58
Progressive motility, %	40.75 (16.45)	41.23 (19.60)	0.8
Total motility, %	47.24 (17.27)	47.07 (20.31)	0.9
Normal morphology, %	35.64 (17.76)	35.76 (17.54)	0.95
Viability, %	51.37 (18.51)	52.78 (20.28)	0.54

[a]Independent sample t-test.

Although a negative correlation between lead in seminal plasma and semen volume (r = −0.25, p = 0.007) sperm count (r= −0.04, p = 0.6), progressive motility (r= −0.07, p = 0.45), total motility (r = −0.08, p = 0.4) viability (r = −0.001, p = 0.9) and normal morphology (r = −0.07, p = 0.4) was observed, it was a weak correlation (Table 3).

Table 3: Correlation between seminal plasma Pb concentration and different parameters of the total population, normozoospermics and pathozoospermics.

Parameter	Total Pb positive (n = 115) Spearman's rho		Pb positive normozoospermics (n = 77) Spearman's rho		Pb positive pathozoospermics (n = 38) Spearman's rho	
	(r)	(p)	(r)	(p)	(r)	(p)
Semen volume, mL	−0.25	0.007[a]	−0.22	0.05	−0.307	0.06
Sperm concentration, million/mL	−0.04	0.6	0.006	0.9	−0.007	0.9
Progressive motility, %	−0.07	0.4	−0.13	0.3	−0.057	0.7
Total motility, %	−0.08	0.4	−0.114	0.3	−0.095	0.6
Viability, %	−0.001	0.9	0.05	0.6	−0.013	0.9
Normal morphology, %	−0.07	0.4	−0.012	0.9	−0.178	0.3

[a]p < 0.05.

The association between Pb, sperm DNA fragmentation and sperm parameters

Sperm containing the halo around the head showed sperm with unfragmented DNA (Figure 1A) while sperm without a halo around the head showed sperm with fragmented DNA (Figure 1B). A significant positive correlation (r = 0.38, p = 0.03) was found between seminal plasma Pb level and sperm DNA fragmentation. The mean (SD) DNA fragmentation of Pb-positive men [39.80% (25.08)] was higher (p = 0.03) than Pb-negative men [22.65% (11.30)].

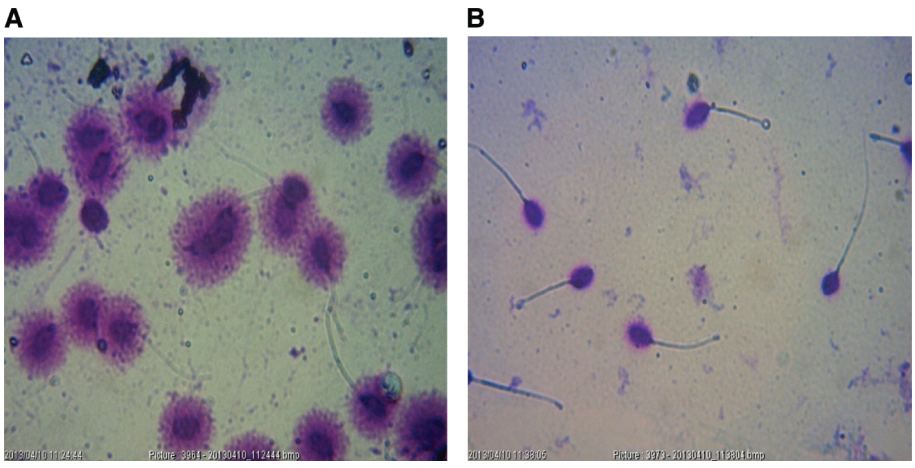

Figure 1: (A) Sperm with halo showing unfragmented DNA. (B) Sperm without halo showing fragmented DNA. Figure 1 showing unfragmented sperm with halo and fragmented sperm without halo

Men with a DNA fragmentation of 30% or more had a significantly higher Pb concentration when compared to those with a lower DNA fragmentation (p = 0.001). The means of all the sperm parameters of men with a DNA fragmentation of 30% or more were lower when compare to subjects with a DNA fragmentation less than 30% with a significant difference in sperm concentration and sperm viability (Table 4).

Table 4: Pb concentration and sperm parameters of men with high DNA fragmentation and low DNA fragmentation.

Parameter [mean(SD)]	Sperm DNA fragmentation ≥30% (n = 16)	Sperm DNA fragmentation less than 30% (n = 24)	p-Value[a]
Pb, µg/dL	37.62 (35.38)	9.29 (8.93)	0.001[b]
Sperm concentration, million/mL	33.17 (32.34)	70.36 (68.20)	0.01[b]
Progressive motility, %	35.00 (16.82)	43.04 (14.64)	0.1
Total motility, %	41.75 (17.83)	48.45 (14.68)	0.2
Normal morphology, %	19.56 (7.07)	22.87 (10.46)	0.3
Viability, %	41.93 (15.90)	52.66 (12.23)	0.02[b]

[a]Mann-Whitney U-test; [b]$p < 0.05$.

There was a significant negative correlation between sperm DNA fragmentation and sperm concentration ($r = -0.45$, $p = 0.003$) progressive motility ($r = -0.43$, $p = 0.006$), total motility ($r = -0.35$, $p = 0.03$), and viability ($r = -0.37$, $p = 0.02$).

Discussion

The goal of this study was to describe the association between Pb and semen parameters including sperm DNA fragmentation in men investigated for infertility, after excluding known causes of infertility which is a main difference when comparing other studies on Pb and sperm parameters.

In the current study semen Pb concentration among men investigated for infertility with low sperm concentration was high and similar results have been reported previously [17]. A negative correlation between seminal plasma lead concentration and sperm concentration in oligoasthenozoospermic men in a general population has been reported [18], while some have reported negative correlation between the seminal plasma lead and normal sperm morphology [19]. Similar to our study many have reported no significant association between seminal plasma lead and semen quality [15], [20].

In the current study, a significant positive correlation was observed between Pb in seminal plasma and sperm DNA fragmentation. The mean DNA fragmentation of Pb-positive men were significantly higher ($p = 0.03$) when compared to Pb-negative men and similar results have been reported [21].

DNA fragmentation in sperm cells is caused by aberrant chromatin packaging during spermatogenesis [22], defective apoptosis before ejaculation [23], [24], excessive production of reactive oxygen species (ROS), or a reduction in the total antioxidant capacity [25]. Accordingly, impaired chromatin packaging, apoptosis and ROS alone can induce DNA damage in human spermatozoa and ROS can result in single- and double-strand DNA breaks.

The suggested mechanisms by which Pb damage sperm DNA is either by affecting DNA synthesis in precursors of the spermatozoa or by interfering with the normal replacement of nuclear histones by cysteine-rich protamines during sperm chromatin condensation. In an experimental animal study, Pb has been shown to bind firmly with thiol groups present on cysteine residues in protamine – a protein that exerts a protective function on DNA [26]. In humans, zinc contributes to sperm chromatin stability and binds to protamine 2. Pb competes with zinc and binds human protamine 2 causing conformational changes in the protein [27]. This decreases the concentration of DNA protamine 2 binding which probably leads to alterations in sperm chromatin condensation [28].

In our study with sperm DNA fragmentation, the sperm concentration, progressive motility, total motility and viability were significantly reduced not sperm morphology. These findings are consistent with the results of other studies [29], [30], [31], [32], although the techniques used to detect DNA fragmentation differ among the studies.

Further, in men with higher index of DNA fragmentation (>30%) the sperm concentration and viability were significantly lower. Yilmaz et al. reported similar results with sperm concentration but not with sperm viability [16]. In the current study the high Pb concentration in the seminal plasma of men with a high DNA fragmentation may have contributed to the observed reduction in sperm count and viability.

The negative correlation between DNA fragmentation and sperm viability is a new finding reported in this study. As viability assesses the ability of sperm to survive in the female reproductive tract, this is an important parameter for fertilization under natural or assisted conditions.

Although the sperm parameters routinely assessed were normal, the failure to fertilize in this group of men investigated for infertility could be due to the effects of Pb on the sperm function. As reported by Benoff et al.

Pb in the tissue could affect hormone receptor kinetics, enzyme activities and hormone secretion [33]. Hence, it will be worthwhile to explore the effects of Pb on sperm function specially when the failure to fertilize is not due to the known causes. Further the high sperm DNA fragmentation in men with Pb in seminal plasma needs to be considered when offering assisted reproductive techniques to these couples in the management of infertility.

Conclusion

Pb in seminal plasma had a significant effect on sperm DNA fragmentation but not with other sperm parameters.

Acknowledgments

University Grants commission (UGC) of Sri Lanka is acknowledged for providing the research grant.

Research funding: This study was supported by the research grant from the University Grants Commission (UGC) of Sri Lanka (UGC/ICD/RG 2011/05).

Author contributions: All authors have accepted responsibility for the entire content of this submitted manuscript and approved its submission.

Competing interests: The authors declare that they have no competing interests.

Informed consent: The study purpose and procedures were explained to all subjects and written informed consent was obtained from all individuals included in this study.

Ethical approval: Ethical approval was obtained from the Ethical Review Committee of the Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka (511/10).

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