

Excessive Fluoride in Drinking Water Alters the Trace Metal Ions in the Seminal Plasma

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Abstract

Original Research Article

Fluoride poisoning is a severe health problem in many parts of the world where fluoride contamination in drinking water is more than 1.5 ppm of fluoride. The aim of the present study was to explore the effect of fluoride in different parameters of the blood and semen in order to investigate as a marker of fluoride toxicity. In the present study, 55 male fluorosis cases were recruited for the study. The seminal profile and trace metal ions were examined in the seminal plasma of the subject and controls. The mean values of the semen volume, viability, motility, viscosity, seminal pH, sperm density and liquefaction time were found to be changed significantly in the subject as compared to the age matched controls. The concentration of Zn and Se found to be reduced while Cu and Fe were increased significantly when compared with their respective controls. The higher intake of fluoride in ground water may be associated to adverse reproductive system. The investigation of metal ions and seminal profile are snapshot of the diagnosis of the assessment of the risk of fluoride toxicity.

Keywords: Fluoride, Trace Metal ions, Seminal Plasma.

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BACKGROUND

Fluoride is widely worldwide distributed in the ground drinking water. Fluoride is required in very little amount for the development of bones and teeth [1]. Excessive fluoride ingestion affects the skeletal system and lead to cause a disease known as Fluorosis [2, 3]. It is one of the indices of chronic poisoning it may be due to long-term exposure of fluoride contaminated drinking water (fluoride >1.5 ppm) [4-7] result in accumulation of fluoride in the soft tissues and bone [8].

Fluoride has the characteristics as a strong reactive ion and powerful oxidizing agent which interact with cellular and sub cellular organelles of the cell. It is also act as enzyme inhibitor, disruptor of hormones and neurotoxicants further leads to life threatening health problems [9, 10].

Various studies have been documented that fluoride alters the neuronal ultrastructure in animals [11]. It is also evidenced that Excessive Fluoride in drinking water is also affects the cognitive behaviour and Intelligence Quotient (IQ) [12]. Moreover, Earlier it is estimated that approx. 66.6 million people in India

(about 17 states of India) including 6 million children are at risk from chronic fluoride toxicity [13], while the state of Rajasthan having all district of fluoride affected where fluoride concentration more than 1.5ppm [14].

High doses of fluoride have been repeatedly found to interfere with the reproductive system of animals and humans, which includes reduced sperm count and motility [15] and reduced rate of fertility [16]. However, the research on fluoride induced reproductive effects in humans is very limited but our previous study has documented that the fluoride induced oxidative stress is one of the mechanism of reduced semen quality [17, 18]. Trace elements in human seminal plasma play an important role in the physiological activities and regulate many endogenous antioxidant enzymes [19].

Previously, It is suggested that zinc (Zn) and selenium (Se) are essential for testicular maturity and physiology of spermatogenesis [20] while, Copper (Cu) and iron (Fe) is a naturally occurring trace element that is essential for some metabolic processes during spermatogenesis [21].

The effect of fluoride in seminal plasma and different essential trace metals is a matter of great interest to explore its mechanism of fluoride induced pathological changes in seminal plasma. Therefore, it may be positively correlated with the semen quality. On the basis of above facts, the present study was designed to investigate the metal antagonistic effect of fluoride and their relation with reduced semen quality in fluoride exposed population.

METHODOLOGY

In the present study, 55 male fluorosis cases were selected from the area where fluoride in water was more than 1.5 ppm and the mean (\pm SD) concentration of 21 random ground water samples were found to be 4.54 ± 1.85 ppm. The age matched controls were selected from the area where fluoride content in water was less than 1.5 ppm and the mean (\pm SD) concentration of 17 random ground water samples was 0.69 ± 0.41 ppm. The controls (non fluorotic) and subject (fluorotic cases) were confirmed after the performance of physical test and Dean's index [22-23], and fluoride content in the serum of control and subjects. The study was approved by the Institutional human ethical committee. A written consent of each subject was taken after explaining the aims and objectives of the study and its benefit to individual and society.

A detailed medical history and andrological examination was performed for all studied cases. Subjects currently on any medication or antioxidant supplementation were not included. Also, patients with varicocele, leucospermia, those suffering from any acute infection, smokers and alcoholic men were excluded from the study. The subjects were interviewed using personal interview and detailed information of the subjects were recorded on the pre-designed form which includes age, BMI, socio-economic status, educational level, smoking, alcohol, marital status, number of children, drug addiction and contraception.

Overnight fasting blood samples and morning first time urine samples were collected for the estimations of fluoride levels in serum and urine respectively.

Preparation of Seminal Plasma

Semen samples were collected from the subjects and controls in a clean, dry, sterilized, wide mouth, well stopper glass vial by masturbation after 2–5 days of abstinence. After liquefaction, semen samples were centrifuged at $1200 \times g$ in cold (4°C) for 20 min for the separation of seminal plasma. The supernatant (seminal plasma) was centrifuged again at $10\,000 \times g$ in cold (4°C) for 30 min to eliminate all possible contaminating cells and stored at -20°C until analyzed.

Fluoride estimation

After clinical examination of subjects and controls, 3.0 ml of blood sample was drawn under

complete aseptic condition in sample vial and was allowed to clot at room temperature for serum separation. The first urine at morning of each individual was collected. Furthermore, fluoride concentrations were measured in both serum and urine using specific fluoride electrode (Thermo Fischer, Singapore).

Trace metal investigations

The serum was treated with a mixture of HNO_3 : H_3PO_4 (6:1) till residue remained. The residue was dissolved in an appropriate amount of 0.1N HNO_3 and Fe, Cu, Zn and Se were estimated on flame atomic absorption spectrophotometer (Perkin Elmer Analyst-300). The metal contents were measured in $\mu\text{g/ml}$ serum. Known amount of each metal was processed identically so as to serve as standard control.

STATISTICAL ANALYSIS

The data were summarized as Mean \pm SD. Groups were compared by Student's t test or Mann-Whitney U test, as applicable. Pearson correlation analysis was done to assess association between the variables. Cox regression analysis was done to assess risk factors associated to fluoride concentrations in urine and serum. A two-sided ($\alpha=2$) p value less than 0.05 ($p<0.05$) was considered statistically significant.

RESULTS

The basic characteristics of two groups at admission are summarized in Table 1. The basic characteristics of two groups were similar ($p>0.05$) indicating that the two groups were comparable and may also not influence the study outcome measures (seminal profile and trace metal concentrations).

The fluoride concentrations (ppm) in urine, serum and water of two groups are summarized graphically in Fig. 1. The concentrations of fluoride in urine (1.81 ± 0.58 vs. 3.28 ± 0.34 , $Z=8.47$; $p<0.001$), serum (0.19 ± 0.02 vs. 0.66 ± 0.06 , $Z=9.06$; $p<0.001$) and water (0.69 ± 0.41 vs. 4.54 ± 1.85 , $Z=9.04$; $p<0.001$) of cases was significantly different and 45.0%, 70.8% and 84.8% higher respectively as compared to controls.

The semen profiles of two groups are summarized in Table 2. Comparing the seminal profiles of two groups, the semen volume, viability, motility and sperm density of cases was found significantly ($p<0.01$ or $p<0.001$) different and 41.5%, 14.3%, 15.8% and 15.3% lower respectively as compared to controls. However, liquefaction time, seminal pH and seminal viscosity of cases was found significantly ($p<0.001$) different and 48.6%, 10.6% and 14.8% higher respectively as compared to controls.

The concentrations of trace metals ($\mu\text{g/ml}$) in serum of two groups are summarized in Fig. 2. The concentrations of Zn and Se in cases were found significantly ($p<0.05$ or $p<0.01$) different and 14.1%

and 29.7% lower respectively as compared to controls. In contrast, the concentration of both Cu and Fe in cases was found significantly ($p < 0.001$) different and 31.3% and 35.7% higher respectively as compared to controls.

The correlation of semen profile and trace metals with fluoride concentrations in urine and serum of two groups are summarised in Table 3. The semen volume, viability, motility, sperm density, Zn and Se showed a significant ($p < 0.001$) and negative (inverse) correlation with fluoride concentrations in urine and serum. Conversely, liquefaction time, seminal pH, seminal viscosity, Cu, and Fe showed a significant ($p < 0.001$) and positive (direct) correlation with fluoride concentrations in urine and serum. Further, all variables showed high association with fluoride concentrations in serum than urine and higher in cases than controls.

To see the effect of fluoride concentrations in urine and serum on seminal profile, Cox regression analysis was done and summarised in Table 4 and 5, respectively. The Cox regression analysis (unadjusted or crude) revealed a significant ($p < 0.01$ or $p < 0.001$) 3.67, 2.84, 2.07 and 2.73 fold risk (odds ratio) associated to fluoride concentrations in urine on semen volume, liquefaction time, viability and seminal pH respectively and 3.71, 2.87, 2.09 and 2.77 fold risk respectively when adjusted to age and BMI. Similarly, a significant ($p < 0.01$ or $p < 0.001$) 6.24, 3.89, 3.16 and 2.91 fold risk (unadjusted) was found associated to fluoride concentrations in serum on semen volume, liquefaction time, viability and seminal pH respectively and 7.32, 3.91, 3.23 and 2.96 fold risk respectively when adjusted to age and BMI.

Table-1: Basic characteristics of two groups

Characteristics	Controls (n=55) (%)	Cases (n=55) (%)
Age (yrs)	33.67 ± 5.09	33.84 ± 5.52
BMI (kg/m ²)	21.42 ± 2.01	21.44 ± 2.28
Socio-economic status	Lower (100%)	Lower (100%)
Literacy (H. Sc.)	100.0%	100.0%
Smokers	58.0%	51%
Alcoholic	4.0% (Occasionally)	3.5% (Occasionally)
Married	99.0%	96.0%
Child at least One	100.0%	87.0%
Drug addicted	Nil	Nil
Male contraceptive	Nil	Nil

H. Sc- higher secondary

Table-2: Seminal profiles of two groups

Seminal Profile	Controls	Cases	p value
Semen Volume (ml)	3.36 ± 0.22	1.97 ± 0.62	<0.001
Liquefaction time (min)	14.85 ± 5.82	28.87 ± 6.53	<0.001
Viability (%)	50.29 ± 12.68	43.09 ± 11.24	<0.001
Motility (%)	47.18 ± 15.90	39.73 ± 14.32	0.007
Seminal pH	6.97 ± 0.29	7.80 ± 0.24	<0.001
Seminal viscosity (mm)	1.68 ± 0.23	1.98 ± 0.49	<0.001
Sperm density (million/ml)	132.85 ± 16.89	112.58 ± 15.19	<0.001

The data are expressed as Mean ± SD and compared by Mann-Whitney U test.

Table-3: Correlation of seminal profile and trace metals with fluoride concentration in urine and serum of controls and cases

Variables ^{***}	Controls (n=55)		Cases (n=55)	
	Urine	Serum	Urine	Serum
Semen volume	-0.62	-0.60	-0.84	-0.84
Liquefaction time	0.69	0.49	0.77	0.78
Viability	-0.83	-0.73	-0.88	-0.88
Motility	-0.79	-0.71	-0.85	-0.85
Seminal pH	0.54	0.60	0.88	0.86
Seminal viscosity	0.69	0.67	0.80	0.79
Sperm density	-0.74	-0.54	-0.91	-0.93
Zn	-0.87	-0.96	-0.96	-0.96
Cu	0.98	0.90	0.95	0.94
Se	-0.86	-0.95	-0.97	-0.95
Fe	0.86	0.89	0.94	0.91

***- $p < 0.001$

Table-4: Unadjusted and adjusted effect of fluoride concentrations in urine on seminal profile by Cox regression analysis

Seminal profile	Unadjusted			Adjusted		
	OR	95% CI	p value	OR	95% CI	p value
Semen volume	3.67	1.52-5.69	0.001	3.71	1.55-5.74	0.001
Liquefaction time	2.84	0.78-3.90	<0.001	2.87	1.79-4.02	<0.001
Viability	2.07	1.03-3.11	0.001	2.09	1.07-3.16	0.001
Motility	1.01	0.99-1.03	0.378	1.01	0.99-1.03	0.374
Seminal pH	1.73	1.51-2.98	<0.001	1.77	1.54-3.01	<0.001
Seminal viscosity	1.00	0.41-2.44	1.000	1.00	0.41-2.45	0.997
Sperm density	1.01	0.98-1.04	0.507	1.01	0.98-1.04	0.446

Table-5: Unadjusted and adjusted effect of fluoride concentrations in serum on seminal profile by Cox regression analysis

Seminal profile	Unadjusted			Adjusted		
	OR	95% CI	p value	OR	95% CI	p value
Semen volume	6.24	3.29-11.82	<0.001	7.32	3.69-14.50	<0.001
Liquefaction time	3.89	2.83-7.96	0.002	3.91	2.91-8.16	0.002
Viability	3.16	1.02-6.10	0.002	3.23	1.09-6.34	0.002
Motility	1.01	0.98-1.03	0.498	1.01	0.99-1.03	0.481
Seminal pH	2.01	1.01-3.04	<0.001	2.06	1.09-3.27	<0.001
Seminal viscosity	1.71	0.71-4.11	0.234	1.73	0.72-4.17	0.224
Sperm density	0.98	0.95-1.01	0.124	0.98	0.95-1.01	0.125

DISCUSSION

Human seminal plasma contains several trace metals that play an important role in the semen functions, including in sperm capacitating, metabolism, and the acrosome reaction and as a cofactor for antioxidant enzymes. In the present study, we observed significant change in the sperm morphology and concentration of trace metals (Cu, Zn, Fe and Se) in the seminal plasma of subject when compared with control group. Therefore, it is suggestive that fluoride could be one of the causes of reduced quality of sperm. Since, these transitional metals are implicated in a number of physiological, toxicological, and pathological processes due to their capacity to undergo changes of oxidation states involving electron transfer [24].

Since, the quality of Sperm is an important index of impaired reproductive function, as we were found a statistically significant decrease of the semen volume, sperm count and motility in subjects. Similar results were reported that significant decrease in the motility of cauda epididymal spermatozoa of fluoride treated animals [25-27]. Moreover, various experimental studies have conducted to explore the effect of fluoride accumulation which is correlated with the oxidative stress correlates in animals [28-31].

Zinc is one of the most important micronutrient for human health particularly sperm physiology [32] and responsible for semen ejaculation [33] and superoxide dismutase activity [34]. In the present study, reduced concentration of Zn in seminal

plasma of fluorotic subjects, suggested that impaired defence mechanism against reacting oxygen species (ROS). Moreover, reduction in Zn levels can also be explained by increased sperm ROS production and loss of superoxide dismutase in subject group results arising the harmful effects of ROS to sperm cells which are associated with abnormal sperm parameters [35] and it is indicative of poor semen quality [36].

Cu and Fe both are essential due to their high redox potential property and importance as cofactors for a variety of metabolic proteins, such as cytochrome C oxidase and superoxide dismutase [37]. In the present study, the concentration of Cu and Fe were found to be increased significantly in the subjects. Physiologically, both Fe and Cu regulate overall pro oxidant and antioxidant balance, since they are involved in the production of hydroxyl radical through regulation of Fenton reaction [38]. On the other hand, Cu together with zinc is responsible for dismutation of superoxide radicals to hydrogen peroxide by superoxide dismutase (SOD) and ceruloplasmin [39]. Therefore, increased concentration of reactive oxygen species may potentiate increased rate of lipid peroxidation and have a negative impact on the sperm concentration and motility [40-42].

The reduced concentration of Se in seminal plasma investigated in fluorotic patients as compared with the controls. Se is the essential for the antioxidant defence system, thyroid functioning, immune modulation and spermatogenesis. Se is a cofactor for metalloenzyme glutathione peroxidase which

detoxifying peroxides and hydroperoxides [43]. This mechanism was evidenced by the significant reduction in the levels of glutathione and glutathione peroxidase enzyme in our previous study [44-45].

The semen profiles were also investigated and compared with the control healthy individuals. Semen volume, delayed liquefaction, increased semen viscosity, and altered sperm motility observed in our study. There were no significant changes in pH value of the semen. In this study we examined a possible correlation of fluoride and seminal plasma trace elements namely Zn, Cu, Se and Fe. Various studies have demonstrated that fluoride-induced oxidative stress and reduced antioxidant. Recently it is reported that fluoride alters the Cu and Zn which are the integral part of the SOD [46].

CONCLUSION

On the basis of results it may conclude that the trace metal Cu, Zn, Se and Fe exhibited antagonistic effect on fluoride exposure. These are the important for the semen physiology and sperm morphology. Furthermore, metals are important cofactors of the majority of antioxidant enzymes and their presence is required for a proper seminal prooxidant-antioxidant balance. However, further study will also require with more samples and other seminal profiles to understanding the mechanism of action of fluoride.

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