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Original Article

Fluoride reduces semen quality at risk level in high fluoride affected regions

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ABSTRACT

Remarkably interest has occurred on potential decline in the sperm quality due to contamination of fluoride in drinking water. The aim of the present study was to investigate the possible impact of fluoride exposure on semen quality. The 113 subjects (age 25-40) were selected from the high fluoride region of Rajasthan where fluoride content in ground water was more than 2.0ppm. The age matched controls were selected from the area where fluoride content was <1.5ppm. The semen samples were collected for physiological profile and oxidative stress parameters. Significant changes in semen profiles, i.e., Semen Volume, liquefaction time, viability, motility, seminal pH, seminal viscosity, sperm density and oxidative stress parameters namely lipid peroxide levels, superoxide dismutase and catalase were observed in subject as compared with the controls following remarkably changes in serum and urine fluoride concentration. On the basis of results it may safely conclude that fluoride affect the semen quality which may further affects in human reproduction.

1. Introduction

Ground water is one of the most important sources of drinking water. Fluoride contaminations in ground water becoming a matter of great concern [1]. In India, 17 states have been confirmed endemic for fluorosis, and of these 5 states have indicated fluorosis hyperendemicity [2]. Excessive fluoride exposure over a long period of time may result in a serious health problem called fluorosis [3]. In Indian context an estimated 66.6 million people (17 states in India) including 6 million under 14 children are at risk of acquiring fluorosis [4]. Moreover in Rajasthan, people of 22 districts (out of 32) are presently consuming fluoride [5] [6], greater than permissible limit (>1.5ppm) [1]. However, the effects of fluoride on reproductive system are not fully understood. Several studies on animal model indicate that fluoride may cause adverse effects in the reproductive system of rats. Moreover, Ghosh et al., [7] reported that significant changes in sperm count and mobility occurred following sodium fluoride administered to male rats in their drinking water for 10 weeks. The mechanisms of fluoride induced such changes are still not clear. Animal studies indicated that fluoride accelerate the generation of reacting oxygen species (ROS). Different enzymatic and non-enzymatic antioxidants regulated production of ROS by normal physiological processes but excessive production of ROS may leads to oxidative stress. Interactions between fluoride and free-radical reactions have been studied in various biological systems including fluorosis [8-9].

Keeping in view the paucity of information in relation to high fluoride exposure in population residing in endemic areas and its impact on semen quality, the present study was undertaken. The significance of this study is to investigate the correlation between fluoride and semen quality and its association with the oxidative stress mechanism in Jaipur district of Rajasthan, India.

2. Materials and Method

Subject selection: A total number of 113 men with reproductive age group (between 25 and 40 years) were selected from eastern region (Rural area of Jaipur district, Rajasthan, India) where fluoride content in drinking water was more than 2.0 ppm. The

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controls and subjects were recruited using personnel interview as a tool for data collection, detail information of the subjects were collected on the predesigned proforma that includes age, educational level, socio-economic status, working schedule, duration of exposure, male contraceptive users, smoking, and other addiction history, marital status, and number of children, history of disease of the individual subjects, and his family. Moreover, the living habits, and diseases of reproductive organs (Cryptorchidism, inguinal hernia, gonorrhea, varicocele, epididymitis, chlamydia, and surgery for torsion of the testis) were also noted for the exclusion criteria of the study.

Fluoride estimations: 200 ml of drinking water was collected in a sterilized polyethylene bottle at each child's home. The fluoride levels were analyzed using fluoride ion selective electrode (Thermo Fisher Scientific Inc., Singapore). Urine and Blood sample of each subject were collected after clinical examination of subjects and controls. Sample was withdrawn from all the subjects and control under complete aseptic condition. The blood was collected in simple vial for the separation of serum. The separated serum was used for the estimation of serum fluoride levels using specific fluoride ion selective electrode (Thermo Fisher Scientific Inc., Singapore)

Sample collection: Semen samples were also collected from the subjects and controls in a clean, dry, sterilized, wide mouth, well stopper glass vial by masturbation after 3–5 days of abstinence. Physical characteristics of semen were analyzed after liquefaction of the sample. The Semen volume, Sperm density, motility, viability, motility, semen pH and viscosity were analyzed.

Preparation of Seminal Plasma:. After liquefaction, semen samples were centrifuged at 1200 × g in cold (4 ◦C) for 20 min for the separation of seminal plasma. The supernatant (seminal plasma) was centrifuged again at 10 000 × g in cold (4 ◦C) for 30 min to eliminate all possible contaminating cells and stored at −20 ◦C until analyzed. All biochemical estimations were carried out on seminal plasma.

Biochemical Parameters: Seminal plasma protein was measured by the method of Lowry et al, [10] using bovine serum albumin (BSA) as standard. The concentration of fructose (mg/ml) was measured according to the standardized method of Foreman (1979) using spectrophotometer [11]. The lipid peroxide (LPx) levels were measured by the method of Okhawa et al [12]. The thiobarbituric acid reacting substances (TBARS) of the sample were estimated spectrophotometrically at 532 nm and expressed as nmole of MDA /mg protein. The superoxide dismutase (SOD EC 1: 15.1.1) activity was determined from its ability to inhibit the reduction of NBT in presence of PMS according to the method of McChord and Fridovich [13]. The reaction was monitored spectrophotometrically at 560nm. The SOD activity was expressed as U/mg protein (1 unit is the amount of enzyme that inhibit the reduction of NBT by one half in above reaction mixture). Catalase (CAT, EC 1.11.1.6) activity was assayed as per the method of Aebi et al [14] using hydrogen peroxide as substrate; the decomposition of H_2O_2 was followed at 240nm on spectrophotometer. The CAT activity was expressed as U/mg protein.

3. Results

Table 1 shows demographic indicators of control and subjects. Age, BMI were insignificant change in control and subjects. The socioeconomic status was considered only lower class. All the subjects and control were married with 100% (H.Sc. passed). There is only 3-4% alcoholic in both groups. Both control and subjects were not drug addicted, also they were not using contraceptives. Fluoride content was measured in water, urine and serum samples of subjects and controls. The significantly ($p<0.001$) increased amount of fluoride content were present in subjects when compared with the controls (fig.1)

Table 1: Profiles of fluoride exposed and controls based on questionnaire data.

	Control (100)	Exposed (113)
Age	32.5±6.8	31.8 ±7.2
Socio-economic status	Lower (100%)	Lower (100%)
Married	100%	100%
Literacy (H.Sc.)	100 %	100 %
Smokers	58%	51%
Alcoholic	4% (occasionally)	3.5% (occasionally)
Drug addicted	Nil	Nil
Male Contraceptive	Nil	Nil

Data are expressed as mean ± SD (age) and rest of the parameters in percentage for control and subjects

Figure 1: -The concentration of fluoride in water (A), serum (B) and Urine (C) expressed as mean ± SD for control and subjects.

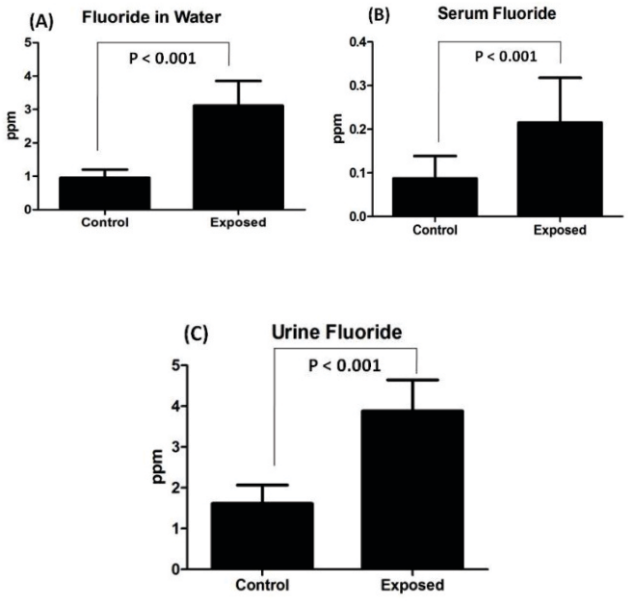

Table 2 described the semen profile of the fluoride exposed and controls. The liquefaction time was found to be significantly ($p<0.05$) elevated by 41% in subjects as compared with the control. The motility was remarkably ($p<0.05$) reduced by 14% in subjects when compared with the controls. On other hand seminal viscosity and density were found to be reduced significantly ($p<0.05$ and $p<0.001$) by 17% and 18% as compared with the control respectively.

Table-2: Semen profile (mean ± SD) of the control and subjects

Seminal Profile	Control (100)	Exposed (113)	p-value
Semen Volume (ml)	3.88 ± 0.5	3.57 ± 0.5	0.1022
Liquefaction time (min)	14.7 ± 2.4	20.7 ± 3.7	0.0012
Viability (%)	67.3 ±14.4	63.4 ± 12.9	0.0557
Motility (%)	73.3 ±12.8	63.2 ± 12.0	0.0156
Seminal pH	7.45 ± 0.25	7.52 ± 0.3	0.4375
Seminal viscosity (mm)	2.45 ± 0.53	2.03 ± 0.34	0.0245
Sperm density (million/ml)	130.8 ± 8.1	107.7 ± 17.3	0.0009

Data are expressed as mean ± SD in control and subjects

Table 3 described the biochemical parameters in semen of the fluoride exposed and controls. The concentration of protein, superoxide dismutase and catalase were found to be significantly ($p<0.001$) reduced by 22%, 37% and 16% in exposed as compared with controls respectively. Fructose and lipid peroxide levels were markedly ($p<0.001$) increased by 83% and 37% in subjects when compare with controls respectively.

Table-3: Semen biochemical profile (mean ± SD) of the control and subjects

Seminal biochemical Profile	Control (100)	Exposed (113)	p-value
Total protein (mg/ml)	87.3 ± 8.5	67.8 ±11.3	0.0006
fructose (mg/ml)	0.6 ±0.2	1.1 ± 0.4	0.0001
Lipid peroxide levels (nmole MDA/ mg protein)	2.7 ±0.4	4.6 ± 0.8	0.0002
Superoxide dismutase (unit / mg protein)	7.6 ±1.1	4.8 ± 0.8	0.0001
Catalase (unit / mg protein)	8.1 ± 0.9	6.8 ± 0.9	0.0046

Data are expressed as mean ± SD in control and subjects

4. Discussion

Several reports are documented about decreasing human sperm quality due to the impacts of environmental factors such as environmental and occupational exposure to trace elements, environmental pollution, and pesticides. In the present study we selected subjects from fluoride exposed population for this study. Sperm quality is one of the most important indexes of physiology of male reproductive function. Many studies have been documented alterations in sperm quality induced by fluoride in vivo and in vitro in animal models [15-16]. In the present study, we observed significant change in sperm quality in terms of semen volume, liquefaction time, viability, motility, seminal viscosity and sperm density in fluoride exposed population. However, some experimental results indicate that fluoride does not affect sperm quality in rats [17-18]. On the other hand many experimental studies [19-22] support to the finding of the present studies.

There is much evidence that oxidative stress is an important mediator of F induced reproductive toxicity [23]. In the present study, increased lipid peroxidation, caused by reactive oxygen species (ROS), were evaluated by estimating seminal malondialdehyde content, SOD and CAT. However, when mean levels of all oxidative stress markers in subjects were compared with the control were found to be markedly changed. It is suggested that that the over-production of ROS in the male reproductive tract may be a potential cause of sperm dysfunctioning. The small amounts of ROS are essential for regulation of normal sperm functions but at high levels they have potential toxic effects on sperm quality and function [24]. Sperm plasma membrane has a high concentration of polyunsaturated fatty acids which makes it susceptible to lipid peroxidation by ROS, this can leads to loss of membrane fluidity and integrity, as a result of this the spermatozoa lose their competence to participate in the membrane fusion events associated with fertilization. Also they can attack DNA, induced strand breaks and oxidative stress damage in spermatozoa [25]. Reddy et al [26] reported that significant difference in lipid peroxidation, glutathione and vitamin C in blood of human fluorotic patients from endemic fluorosis area. Moreover, Inkielewicz et al. [27] also reported a reduced activity of catalase and -SH groups and increased concentration of thiobarbituric acid reactive substances. It is suggested that severe pathologic changes due to overproduction of ROS in the testicular tissue may play a role in the mechanism of testicular degeneration associated with infertility.

5. Conclusion

In conclusion, fluoride was found to decrease the sperm quality with elevated oxidative stress apparently being one of the pathways that lead to decrease the quality of semen. There are more study with more sample size is required to understanding the mechanism of reproductive toxicity of fluoride in population.

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