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Influence of fluoride exposure on hypothalamic pitutary gonadal axis hormones and semen quality

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Abstract

The main objective of the present study was to explore the influence of fluoride exposure on reproductive hormones in different stages of fluorosis. Since, fluoride has been reported to be a causative factor for reduced semen quality. However, limited scientific literature is available on this aspect of effect of fluoride on clinical samples. In the present study, we recruited 75 fluorotic patients (age 25-35) and categorize in three groups namely mild, moderate and severe. Each group was included 25 patients. The controls were selected from the Jaipur district, Rajasthan India, where fluoride content in ground water was <1.5ppm. Fasting blood samples was collected for the estimations of follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone (TT) and prolactin. Semen samples were also collected for the investigation of sperm physiology. The results of the present study showed significantly ($p<0.05$) reduced semen volume, liquefaction time, viability, motility and semen viscosity in all groups of fluorotic patients as compared with the controls. On the other hand, we observed markedly ($p<0.05$) increased serum FSH and prolactin and decreased LH and testosterone levels as per the severity of disease. On the basis of results it may conclude that excessive fluoride exposure accelerate the severity of disease along with the altered hormonal profiles and reduced semen quality. A positive correlation was observed between fluoride, hypothalamic gonadal axis and semen quality.

INTRODUCTION

The endocrine system consists of a set of glands and the hormones they produce that help guide the development, growth, reproduction and behavior of human beings. It is evident that there is little evidence to suggest that endocrinopathy is the primary cause of infertility in fluoride exposed population. The three components are hypothalamus, anterior pituitary and gonads have exclusively regulatory functions, mediated by its hormones. This hypothalamic -pituitary-gonadal axis is finely tuned system controlled through a classic negative feedback mechanism[1,2] Gonadotropin releasing hormone (GnRH) secreted by the hypothalamus elicits the release of gonadotrophins namely follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary gland. FSH stimulates spermatogenesis through binding with receptors in the sertoli cells. While, LH induces the production of testosterone in leydig cells, which in turn may act on the Sertoli and peritubular cells of the seminiferous tubules and stimulates spermatogenesis. The failure of pituitary to secret FSH and LH will result in disruption of testicular function leading to infertility.

Fluoride is abundantly found in the ground drinking water in worldwide and very little amount is required to humans. Fluorosis is one of the manifestations of chronic poisoning from long-term exposure to high levels of fluoride, and is a serious health problem in many parts of the world where drinking water contains more than 11.5-ppm of fluoride. Epidemiological investigations indicate that fluoride may cause adverse effects in the reproductive system of males living in fluorosis endemic areas [3]. It is reported that, fluoride causes deformities in the cellular structure of spermatozoa leading to abnormality in count, viability and motility [4] [5]. Investigation shows that thyroid markedly effects the testicular development and its abnormal function alters semen quality and male fertility following testicular size, sperm motility and ejaculate volume. It is hypothesized that fluoride could interfere in the functioning of pituitary gland further may alters the secretion of follicle stimulating hormone (FSH) and luteinizing hormone (LH).

MATERIAL AND METHODS

Subject selection: A total of 75 fluorotic patients (reproductive age group between 25 and 35 years) were recruited from rural area of Jaipur district, Rajasthan and categorized in three groups as per the clinical examination and fluoride concentration in serum namely mild, moderate and severe. The healthy controls were selected from the area where fluoride content was <1.5ppm. The controls and subjects were selected using personal interview as a tool for data collection, detailed information of the subjects were recorded on the predesigned proforma that includes age, educational level, socio-economic status, working schedule, duration of exposure, male contraceptive users, smoking, addiction history, marital status, and number of children, history of disease of the individual subjects, and his family. Moreover, the clinical examination of testes was also carried out in terms of diseases of reproductive organs (previous or current genital diseases such as cryptorchidism, inguinal hernia, varicocele, epididymitis, gonorrhea, chlamydia and surgery for torsion of the testis).

Fluoride estimations: A sample of 200 ml of drinking water was collected in a sterilized polyethylene bottle at each subject's home for fluoride estimation. 5.0 ml blood and urine sample were also taken for fluoride estimations. 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.

Semen analysis: 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. 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.

Hormonal analysis in serum:

Serum testosterone (TT), Luteinizing hormone (LH), follicle stimulating hormone (FSH) and prolactin (PRL) were measured using a double antibody radioimmunoassay (RIA) method by Gamma Counter[6] [7]. Intra and inter assay coefficients of variation were 10.0, 14.0, 8.5 and 12.5%, respectively.

RESULTS

Semen profile

The semen profiles were carried out in control and subjects and data are presented in figure-1. The semen concentration was found to be significantly ($p<0.001$) reduced in all the groups of subjects. The maximum reduction was observed in severe group by 43% and minimum 24% in mild when compared with controls. The severe fluoride group comprises 25% and 20% reduction compared to mild and moderate respectively. Liquefaction time found to be markedly ($p<0.001$) increased by 25%, 39% and 43% in mild, moderate and severe respectively as compared to the controls, while liquefaction time increases in severe group by 35% and 23% when compared with mild and moderate respectively. On the other hand viability was maximum reduced by 33% in severe and least in mild (5%) as compare to controls. The viability in severe group reduced by 30% and 21% as compared to mild and moderate respectively. Motility was reduced by 33%, 16% and 14% in severe, moderate and mild, when compared with the controls. While, the severe fluorotic patient was exhibit reduction by 22% and 20% as compared with mild and moderate fluorotic patients respectively. Seminal pH were found to significantly ($p<0.05$) increased by (5%) as compared with the controls. The seminal viscosity were markedly ($p<0.01$) reduced by 25%, 18% and 15% in severe, mild and moderate respectively, as compared to controls. While the severe group exhibited reduction by 11% and 9% as compared with moderate and mild respectively.

Hormonal Profile

The hormonal profile were investigated in serum and data (mean + S.D.) are presented in figure 2. The FSH were found to be significantly ($p<0.001$) increased by 265%, 62% and 49% in severe, moderate and mild group of fluorotic patients when compared with the controls. FSH in the severe group was increased by 144% and 125% when compared with mild and moderate respectively. LH were found to be markedly ($p<0.001$) reduced by 38%, 23% and 16% in severe, moderate and mild group when compared with controls. On the other hand, 144% and 125% reduction were observed in severe as compared with moderate and mild group respectively. The testosterone levels were significantly ($p<0.001$) reduced by 44%, 28% and 25% in severe, moderate and mild group when compared with the controls. A 25% and 22% reduction was observed in severe as compared with mild and moderate group respectively. The concentration of prolactin was found to be increased significantly

Table 1. Demographic data of control and fluorotic patients

Parameters	Control	Mild	Moderate	Severe
Age	31.6 ± 5.4	32.1± 6.3	29.8± 4.1	32.2 ± 6.1
BMI	21.7± 1.2	23.1± 0.8	22.4 ± 0.6	23.4 ± 1.0
Socio-economic status	Lower (100%)	Lower (100%)	Lower (100%)	Lower (100%)
Literacy	100 %	100 %	100 %	100 %
Smokers	58%	51%	47%	52%
Alcoholic (Occasionally)	4%	3.5%	3.2%	4.2%
Drug addicted	Nil	Nil	Nil	Nil
Male Contraceptive	Nil	Nil	Nil	Nil

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

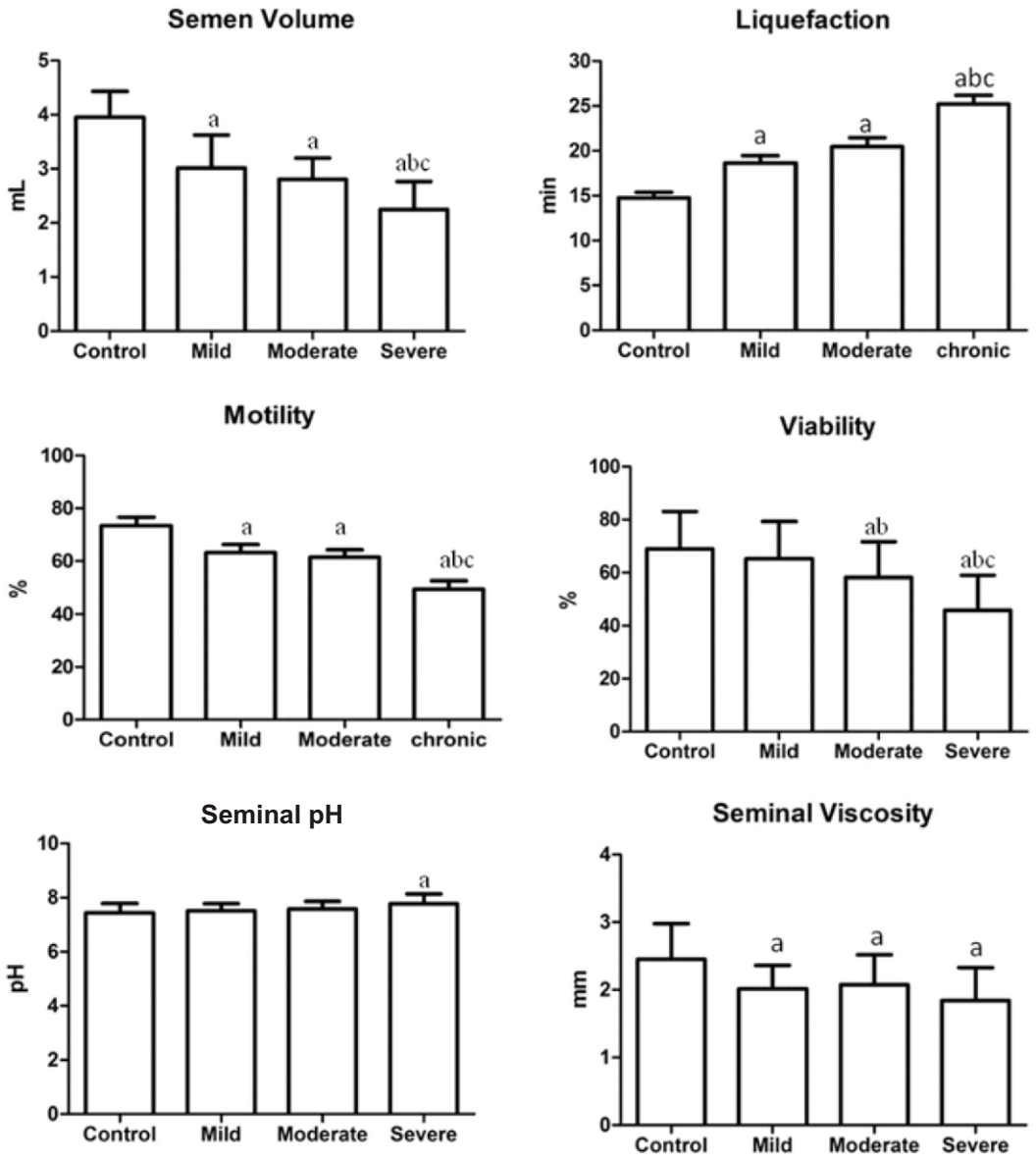

Figure 1. The semen profile are expressed as mean ± SD for control and subjects. Significance comparison was carried out using One way ANOVA followed by dunnett test and minimum significant was consider as $p<0.05$ when compared with control vs subjects.

by 24% and 9% in severe and moderate as compared with the controls. Severe group comprises with increment by 27% and 14% when compared with mild and moderate fluorotic group respectively.

DISCUSSION

In the present study, we observed significant deterioration in semen quality in the fluoride exposed populations. There are three groups of subjects namely mild moderate and severe fluorotic patients. The semen profiles were investigated and compared with each other and with the control healthy individuals. Semen volume was found to be reduced in fluorotic patients. The low volume of semen is called hypospermia it may be due to hormonal changes or an obstruction of the ejaculatory duct. Other cases may result from retrograde ejaculation, infection or hormone problems. The increased liquefaction time was observed in subjects. Delayed liquefaction of semen indicates disorders of accessory gland function. The Increased semen viscosity, which is unrelated to the coagulation-liquefaction phenomenon, signifies a disorder of accessory gland function and may affect the accuracy of assessment of both sperm density and motility. It is only clinically relevant when there are very few sperm in the post coital test. Fluoride has also reduces the viability and motility. Sperm motility becomes critical at the time of fertilization and it is one of the biological characteristics of the spermatozoa. The poor motility is called asthenozoospermia which is associated with reduced viability of spermatozoa. Non-motile spermatozoa are called necrozoospermia. It is characterized by total absence of moving sperm [8]. It is suggestive that fluoride interferes with the sperm physiology, it may be due to direct contamination of

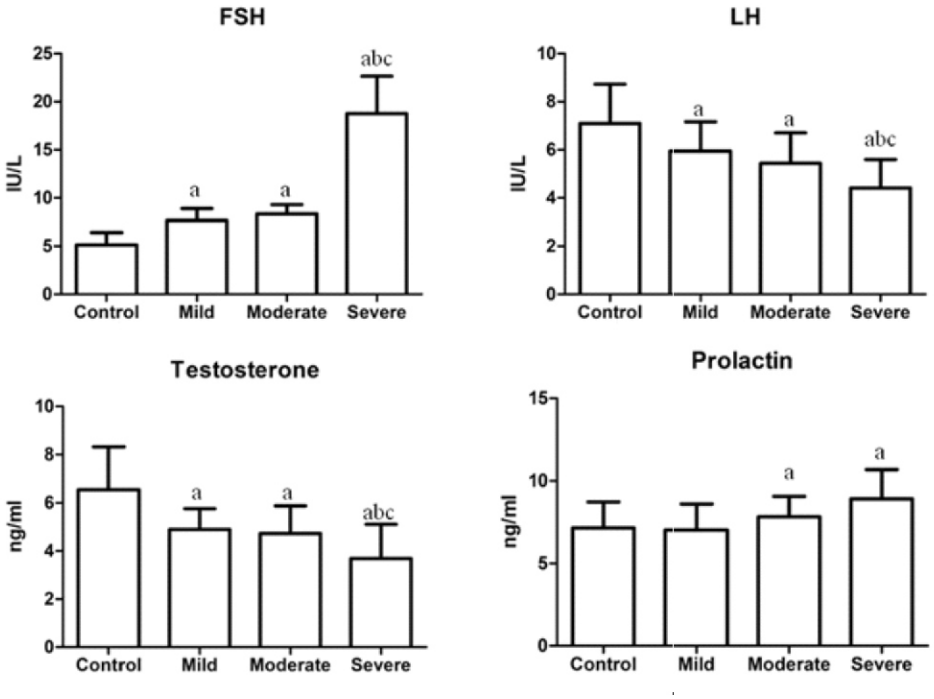

Figure 2. The serum hormones levels are expressed as mean ± SD for control and subjects. Significance comparison was carried out using One way ANOVA followed by dunnett test and minimum significant was consider as $p<0.05$ when compared with control vs subjects.

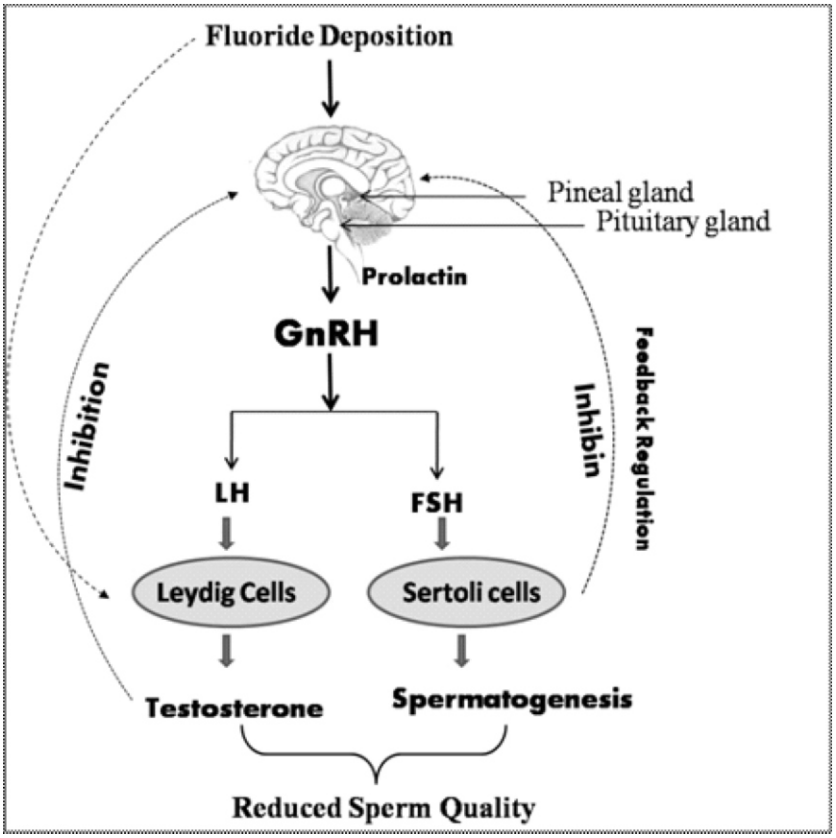

Figure 2. Schematic diagram of the hypothalamic-pituitary-gonadal axis showing fluoride induced dysregulation of neural systems that regulate GnRH secretion and feedback of gonadal steroid hormones at the level of the hypothalamus and pituitary.

fluoride in testicular organelles. The decline in sperm viability and motility as in results are concomitant with finding of Ghosh et al., [9]. They showed fluoride induced testicular toxicity in rats. Several impaired physiological and inflammatory processes increased seminal pH. This might have been influenced by seminal vesicles and undesirable secretions from several different glands results infection should be suspected if the pH exceeds 8.00 or below 7.2 [10]

It is reported that fluoride disrupts various hormones involved in male reproductive function and is clearly associated with the alterations in various male hormones namely LH, FSH, testosterone and prolactin. In the present study we attempt to correlate fluoride exposed population and their effect in semen profiles and their regulatory hormones. The LH and FSH are dimeric protein which is secreted by the pituitary gonadotropes. It acts on the gonad for the initiation of sexual maturation by sequential and synergistic manner [11]. Alteration in FSH, LH, testosterone and prolactin is the main indicator of the dysfunctioning of spermatogenesis. For initiation of spermatogenesis and maturation of spermatozoa, FSH is necessary. It is reported that higher concentration of FSH is considered to be a reliable indicator of germinal epithelial damage and associated with azoospermia and severe oligozoospermia [12]. Moreover, it is reported that elevated levels of serum FSH with increasing severity of seminiferous epithelial destruction. In the present study, FSH levels were significantly elevated in fluorotic patient. It was gradually increased in mild to severe condition. It has been reported that fluoride modify spermatogenesis by modifying G-protein coupled receptors [13] [14]. Therefore, fluoride induced modification of G-proteins may inhibit the release of testosterone which further initiated the spermatogenesis. It is reported that fluoride is known to accumulate in the pineal gland [15] and to inhibit the release the antigoanadotropic chemical like melatonin [16]. It is suggestive that reduced levels of gonadotropic hormones would result in decreased testosterone levels. As evident in the present study, testosterone levels were found to be gradually reduced as the increased exposure of fluoride. Moreover, Susheela et al. [17], has reported earlier that F causes significant degenerative changes and alterations in the diameter of leydig cells. These alterations in them would decrease the ability of leydig cells to synthesize testosterone. In the present study we observed increased level of prolactin which reflects the over production of reproductive hormones. The role of serum prolactin in male infertility is still unclear but the elevated prolactin levels in men are usually the result of overactive prolactin cells in the pituitary gland. Elevated prolactin called Hyperprolactinemia, which inhibits the secretion of the gonadotrophins releasing hormone (GnRH) results it may causes decreased pulsatile release of FSH, LH and Testosterone further it may lead to spermatogenic arrest and altered sperm quality (figure-3) [18].

CONCLUSION

The present study indicates preliminary evidence that severity of fluorosis may induce endocrine disruption over the hypothalamic-pituitary-testis axis by influencing regulation of reproductive hormone resulting reduced sperm quality. A positive correlation was observed between fluoride, hypothalamic gonadal axis and semen quality. However, further in depth studies is required to understand the pathophysiology of fluorosis and its association with reduced semen quality.

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