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Original Article

Dentistry Section

Biochemical Analysis of Liver Function in Individuals Affected and Unaffected by Dental Fluorosis in Endemic Fluoride Rural Areas of YSR Kadapa District, AP, India: A Cross-sectional Study

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ABSTRACT

Introduction: Fluoride is known to affect the nervous system, kidneys, liver, and gastrointestinal system, in addition to teeth and bones, when consumed in amounts exceeding 1 ppm per day over a prolonged period. YSR Kadapa district has been identified as one of the districts with endemic fluoride areas in Andhra Pradesh, India, by the Central Groundwater Board of India.

Aim: To assess the toxic effects of fluoride on liver function in individuals affected and unaffected by dental fluorosis, who consume high levels of fluoride in water, in the endemic fluoride rural areas of YSR Kadapa district.

Materials and Methods: A cross-sectional study was conducted in six villages of YSR Kadapa district, where the drinking water has elevated fluoride content. A total of 320 subjects were selected as study participants. The subjects were divided into two groups based on age and dental fluorosis: Group A, aged between 21 and 40 years, and Group B, aged between 41 and 60 years. Subjects of each group were further sub-grouped according to presence or absence of fluorosis. The selected subjects were screened for dental fluorosis, and Groups A and B were further divided into subgroups based on the presence or absence of dental fluorosis. Blood samples were collected from the participants to assess liver function using serum levels of liver function markers such as total bilirubin (direct and indirect), Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), and Alkaline Phosphatase (ALP). Water and urine samples were also tested for fluoride levels using a fluoride ion meter. Statistical significance was determined using the t-test.

Results: The mean fluoride levels in the water samples from the study areas ranged from 1.55±0.30 mg/L to 4.10±0.20 mg/L, and the urinary fluoride levels in the urine samples ranged from 0.54±0.46 mg/L to 2.13±0.845 mg/L. Among the study subjects, 60.6% (194) were affected by dental fluorosis, with 58.66% (88) belonging to Group A and 62.35% (106) belonging to Group B. There was no statistically significant difference in liver function markers in the serum between subjects with dental fluorosis and those without dental fluorosis in both groups ($p>0.05$), and the levels were within the normal reference range.

Conclusion: This study found no impaired or altered liver function in adults affected by dental fluorosis and adults unaffected by dental fluorosis who consumed water with high levels of fluoride in the YSR Kadapa district.

Keywords: Detoxifying organ, Fluorine, Liver damage, Therapeutic usage, Urinary fluoride levels

INTRODUCTION

The element fluorine is a poisonous gas that typically exists as the fluoride ion (F-) in aqueous solution [1,2]. Humans generally consume fluoride through public drinking water and their diet [1]. Fluorides are utilised in toothpaste, mouthwashes, mouth gels, and other dental products [3]. The World Health Organisation (WHO) recommends a fluoride dose of 1 ppm per day [2,4]. Consumption of doses higher than the permissible limits leads to a condition called fluorosis [1,5]. Fluorosis affects various organs in animals and humans, primarily the teeth and skeletal system, as well as the structure and functions of non-skeletal systems such as the brain, kidneys, liver, and spinal cord [5]. The liver, an important detoxifying organ responsible for processing foreign substances and metabolising biochemical and trace elements, is particularly susceptible to fluoride toxicity [6-8].

Several animal and epidemiological studies have demonstrated that excessive fluoride exposure can cause liver and kidney damage [9-11]. Fluoride affects enzymes such as serum transaminases and phosphatases, which are serum biomarkers of liver function [12]. These biomarkers have been found to be significantly elevated in adults with fluorosis [11,13]. Furthermore, some animal studies have shown histopathological changes in the hepatic parenchyma in response to high levels of fluoride exposure [9,13,14].

In light of the increasing prevalence of non-alcoholic liver diseases worldwide, attributed to multiple factors including exposure to environmental pollutants such as chlorine and arsenic in water [15], it is plausible that fluoride, among various environmental pollutants, may also have a toxic effect on liver tissue, affecting its function similarly to other pollutants like chlorine and arsenic. However, the results of various available in-vitro and in-vivo studies have yielded different conclusions. Therefore, a population-based study was undertaken to assess the toxicity of fluoride on liver function in individuals exposed to high levels of fluoride through drinking water in natural conditions.

According to the Central Groundwater Board of India, fluoride levels in ground water used for drinking, domestic, and agricultural purposes in YSR Kadapa district range from 2.890-3.60 mg/L [16]. The aim of this study was to assess liver function by analysing biochemical markers in the serum of individuals with dental fluorosis (who exhibit symptoms of fluoride toxicity due to high water fluoride levels) and those without dental fluorosis (who remain asymptomatic despite exposure to high water fluoride levels) in endemic fluoride

areas with varying fluoride concentrations. The objective was to compare the serum levels of liver function markers in adults residing in endemic fluoride areas with the normal range of values, thus observing any differences in liver function. The rationale behind this study was to present the results on the toxicity of fluoride to teeth and liver to the local administration, urging necessary action. Additionally, the study's findings on the impact of consuming high levels of fluoride in drinking water on liver function in adults may contribute to research on establishing safe dosages of fluoride as a prophylactic and therapeutic agent for oral diseases such as dental caries and gingivitis, as well as systemic bone disorders such as osteoporosis. The present study hypothesis was that there would be no effect of high fluoride levels in drinking water on liver function between individuals affected and unaffected by dental fluorosis in the endemic fluoride rural areas of YSR Kadapa district, Andhra Pradesh, India.

MATERIALS AND METHODS

A cross-sectional study was conducted over eight months, from September 2020 to April 2021, to assess the toxic effect of fluoride in consumable water on liver function in adults residing in rural areas of the YSR Kadapa district. The study population consisted of individuals from the endemic fluoride villages in the YSR Kadapa district, as identified by the Central Groundwater Board of India. Ethical approval for the study was obtained from the Institutional Ethical Committee of Saveetha Dental College and Hospital, Chennai, Tamil Nadu, India, with reference number SDC/PhD/05 dated 14.07.2017. Prior permission to conduct the study was also obtained from the District Medical and Health Administration, YSR Kadapa district, Andhra Pradesh, India.

Six villages were randomly selected from endemic fluoride areas in the YSR Kadapa district, and a door-to-door survey was conducted. A total of 659 adults who expressed their willingness to participate after a detailed explanation of the study objectives were included.

Inclusion criteria: Subjects aged 20-60 years, residing in the study areas for atleast 15 years, were included in the study regardless of gender.

Exclusion criteria: Subjects with hypertension, diabetes mellitus, renal diseases, or liver diseases, using medications for more than one year that may affect liver function and those who consume alcohol or tobacco were excluded from the study.

Procedure

The 320 study participants gave signed consent forms and underwent an interview, during which general demographic data such as age, sex, education, height, weight, socio-economic status, and place of residence were recorded. Additionally, information regarding the presence of diabetes, renal diseases, liver diseases, or any other chronic illnesses, as well as the use of medications, was documented. A general physical examination was performed by trained doctors and house surgeons. Dental examinations were conducted using a mouth mirror and probe under daylight conditions to screen for dental fluorosis, following Dean's fluorosis index [17]. A final screening was carried out to exclude individuals with unknown hypertension, diabetes, and liver disorders. Hypertension was assessed using a blood pressure apparatus by trained staff nurses, while random blood sugar levels were measured using the Accu check plus method to identify any individuals with diabetes using a one-step glucometer. Blood samples were also tested for hepatitis B and C using screening kits administered by trained laboratory technicians.

The study subjects were divided into two groups based on age and presence of dental fluorosis:

- **Group A:** Subjects aged between 20 and 40 years.
  - Subgroup A1: Subjects with dental fluorosis aged between 20 and 40 years.
  - Subgroup A2: Subjects without dental fluorosis aged between 20 and 40 years.
- **Group B:** Subjects aged between 41 and 60 years.
  - Subgroup B1: Subjects with dental fluorosis aged between 41 and 60 years.
  - Subgroup B2: Subjects without dental fluorosis aged between 41 and 60 years.

A fasting venous blood sample of 5 mL was collected from each participant by a laboratory technician. The samples were then transferred to the Institutional laboratory under cold conditions using a vaccine carrier and stored at -80°C until further analysis. After centrifugation of the blood samples at 3000 rpm for 20 minutes, the serum was used to analyse liver function markers. Automated analysers in the Institutional laboratory were utilised to measure Liver Function Tests (LFTs) including total bilirubin, direct bilirubin, indirect bilirubin, ALP, SGOT, and SGPT [Table/Fig-1] [18].

Liver Function Tests (LFT)	Reference Normal range [18]
Total bilirubin	Upto 1 mg/dL
Direct bilirubin	Upto 0.2 mg/dL
Indirect bilirubin	0.2-0.8 mg/dL
ALP	42-115 IU/L
SGOT/ALP	8-40 IU/L
SGPT/ALT	5-35 IU/L

[Table/Fig-1]: Reference values considered for LFT tests [18].
ALP: Alkaline phosphatase; SGOT: Serum glutamic oxaloacetic transaminase or ALP: Aspartate aminotransferase; SGPT: Serum glutamate pyruvate transaminase or ALT: Alanine aminotransferase

The reference values for LFTs were determined based on the Erba company biochemical analyser and the reagents used in the Institutional Laboratory for LFT analysis at the biochemistry laboratory, RIMS Medical College, Kadapa.

Water samples were collected in sterile, clean, high-density polyethylene bottles, each containing 500 mL, from various sources such as bore wells and public water supply taps used for drinking and cooking purposes in all study villages. These samples were properly labeled, coded, and transported to the laboratory for fluoride estimation.

Urine samples were collected from the participants in 50 mL polyethylene tubes. To preserve the samples, 2 to 4 drops of toluene were added, and the samples were transported to the laboratory and stored at 4°C until analysis.

Fluoride ion levels in both the collected water samples and urine samples were estimated using the National standard method (Ion selective electrode) with the assistance of a fluoride ion meter. This estimation procedure is similar to the one followed in the National fluorosis prevention program in India [19].

STATISTICAL ANALYSIS

Statistical analysis was performed using IBM SPSS Statistics for Windows. Descriptive statistics were used to determine the percentage distribution of the variables. Mean, median, and standard deviation were calculated for quantitative variables. A t-test was employed to calculate the p-value, with a significance level of <0.05 considered statistically significant.

RESULTS

The mean fluoride levels in the water samples collected from the study areas ranged from 1.55±0.30 mg/L to 44.10±0.20 mg/L. The urinary fluoride levels in the urine samples ranged from 0.54±0.46 mg/L to 2.13±0.845 mg/L [Table/Fig-2].

Out of the total 320 study subjects, 150 belonged to Group-A (age 21-40 years) and 170 belonged to Group-B (age 41-60 years). Among the study subjects, 194 (60.6%) were affected by dental

S. No.	Name of the village	Fluoride level in water (mg/L) Mean±SD	Fluoride level in urine (mg/L) Mean±SD
1	Veerapalli	3.35±0.21	1.95±1.02
2	Guntapalli	1.70±0.20	0.77±0.24
3	Guntlammayapalli	4.10±0.20	2.13±0.85
4	Sibyala	2.55±0.15	1.75±0.91
5	Rayachoty Rural	1.55±0.30	0.54±0.46
6	Indukurupalle	2.20±0.31	1.12±0.67

[Table/Fig-2]: Fluoride levels in water and urine samples in the study areas in YSR Kadapa district.

fluorosis, with 88 (58.66%) belonging to Subgroup-A1 (age 21-40 years with dental fluorosis) and 106 (62.35%) belonging to Subgroup-B1 (age 41-60 years with dental fluorosis) [Table/Fig-3].

S. No.	Study groups	Age in years	Dental fluorosis affected n (%)	Dental fluorosis unaffected n (%)	Total n (%)
1	Group-A	21-40	88 (58.66%) (subgroup-A1)	62 (41.33%) (subgroup-A2)	150 (41.39%)
2	Group-B	41-60	106 (62.35%) (subgroup-B1)	64 (37.64%) (subgroup-B2)	170 (58.6%)
3	Total		194 (60.6%)	126 (39.37%)	320 (100%)

[Table/Fig-3]: Prevalence of dental fluorosis in the study population age group wise in Group-A and B.
Figures in parenthesis indicate percentage; Students t-test *p-value of <0.05 significant

In Group-A, the serum levels of total bilirubin, direct bilirubin, indirect bilirubin, ALP, SGOT, and SGPT were comparable between subjects in subgroups A1 and A2. All these levels were within the normal reference range. There was no significant statistical difference in liver function marker parameters between subjects of subgroup-A1 and A2 in Group-A (p>0.05) [Table/Fig-4].

Parameter	Subgroup-A1 (Dental fluorosis affected)	Subgroup-A2 (Dental fluorosis unaffected)	*p-value
Serum total bilirubin (mg/dL)	0.811±0.02	0.791±1.01	0.302
Serum direct bilirubin (mg/dL)	0.158±0.02	0.160±0.04	0.091
Serum indirect bilirubin (mg/dL)	0.701±0.01	0.698±0.05	0.079
ALP (IU/L)	91.23±0.21	94.01±1.04	0.234
SGOT (IU/L)	31.31±2.88	32.83±1.92	0.162
SGPT (IU/L)	29.88±3.20	31.21±2.01	0.24

[Table/Fig-4]: Comparison of liver function markers between dental fluorosis affected and unaffected subjects in Group-A (21- 40 years).
Students t-test *p-value of <0.05 significant

Similarly, there was no significant statistical difference in liver function marker parameters between subjects of subgroup-B1 and B2 in Group-B (p>0.05) [Table/Fig-5]. The observed values for these parameters were within the normal reference range. No significant difference in liver function marker parameters was found in dental fluorosis-affected subjects between Group-A and Group-B (p>0.05) [Table/Fig-6].

Parameter	Subgroup-B1 (Dental fluorosis affected)	Subgroup-B2 (Dental fluorosis unaffected)	p-value
Serum total bilirubin (mg/dL)	0.829±0.08	0.818±0.09	0.331
Serum direct bilirubin (mg/dL)	0.167±0.03	0.168±0.03	0.112
Serum indirect bilirubin (mg/dL)	0.664±0.06	0.654±0.79	0.081
ALP (IU/L)	95.14±2.84	96.04±3.04	0.362
SGOT (IU/L)	30.18±3.43	31.71±3.21	0.15
SGPT (IU/L)	27.98±3.31	30.20±3.67	0.29

[Table/Fig-5]: Comparison of liver function markers between dental fluorosis affected and unaffected subjects in Group-B (41-60 years).
Students t-test *p-value of <0.05 significant

Parameter	Subgroup-A1 (Dental fluorosis affected)	Subgroup-B1 (Dental fluorosis affected)	p-value
Serum total bilirubin (mg/dL)	0.811±0.02	0.829±0.08	0.45
Serum direct bilirubin (mg/dL)	0.158±0.02	0.167±0.03	0.07
Serum indirect bilirubin (mg/dL)	0.701±0.01	0.664±0.06	0.091
ALP (IU/L)	90.14±25.84	95.14±2.84	0.289
SGOT (IU/L)	31.32±2.88	30.18±3.43	0.132
SGPT (IU/L)	29.88±3.20	27.98±3.31	0.062

[Table/Fig-6]: Comparison of liver function markers between dental fluorosis affected subjects in Group-A (21-40 years age) and Group-B (41-60 years age).
Students t-test*p-value of <0.05 significant

DISCUSSION

In the present study, it was observed that mean levels of fluoride in the collected water samples from the villages ranged from 1.5 mg/dL to 4.1 mg/dL. These levels are significantly higher than the safe limit of 1 mg/dL as declared by the WHO [4]. The most reliable method to confirm an individual's exposure to fluoride is by measuring urinary fluoride levels in their urine samples [20]. In this study, all the urine samples from the study subjects showed the presence of urinary fluoride, confirming their exposure to fluoride.

The study found that 60.63% of the subjects in the study population had dental fluorosis, while 39.37% did not have dental fluorosis. The subjects with dental fluorosis exhibited symptoms of chronic fluoride toxicity, which may be attributed to their inherent susceptibility to fluoride toxicity compared to the subjects without dental fluorosis residing in the same area and exposed to the same levels of fluoride. This observation is supported by a study conducted by Everett ET et al., which compared the susceptibility to fluoride among 12 different inbred mouse strains. The study found that the A/J mouse strain was highly susceptible, showing rapid and severe development of dental fluorosis compared to the other tested strains. On the other hand, the 129P3/J mouse strain was least affected, showing minimal dental fluorosis. These results suggest the contribution of a genetic component in the pathogenesis of dental fluorosis [21].

A higher prevalence of dental fluorosis was observed in Group-B subjects, who belonged to a higher age group (62.35%), compared to Group-A subjects, who were in a younger age group (58.66%). This finding differs from the results of a study conducted by Idon PI and Enabulele JE where the prevalence of dental fluorosis was observed to decrease with increasing age. The previous study suggested that the decrease in prevalence might be due to a decrease in the desire for improved aesthetics among the younger age group [22].

The probable reason for the higher prevalence of dental fluorosis in adults in this study, particularly in subjects aged between 41-60 years (Group-B), compared to those aged between 21-40 years (Group-A), could be explained by the fact that adults in the age group of 41-60 years might have been exposed to higher levels of fluoride during their childhood compared to individuals in the 21-40 years age group. Dental fluorosis in humans typically occurs when individuals are exposed to high levels of fluoride during tooth development in childhood [1,3].

Other possible reasons for the higher prevalence of dental fluorosis in the older age group could be improved public water supply or environmental factors such as increased rainfall in the study areas, which might have altered the quality of groundwater and resulted in decreased fluoride exposure for the younger age group during their childhood. Additionally, it is worth noting that there may have been fewer opportunities to screen more adults in the 21-40 years age group on the study day, as many of them may have migrated to other places for education and employment reasons.

In this study, liver function in the study subjects was assessed through LFTs, including serum levels of total bilirubin, direct bilirubin,

indirect bilirubin, ALP, SGOT, and SGPT [13]. The present study results, which showed no significant changes in the levels of liver function markers within the normal reference range in individuals exposed to fluoride levels ranging from 1.5 mg/L to 4.2 mg/L in the study areas, even after consuming fluoride water for 15 years or more, are consistent with the findings of Wan GM et al., in China [23]. Their study also found no changes in LFTs, even at fluoride levels of 23 mg/L in the water supply.

Similarly, Jaganmohan P et al., observed no significant difference in hepatic function markers between individuals residing in high fluoride areas and normal fluoride areas in Udayagiri Mandal, Nellore district, Andhra Pradesh, India [24]. Ahmed I et al., studied the effects of drinking water with high fluoride levels on hepatic functions in people from two villages in Pakistan and reported no evidence of decreased hepatic function in subjects consuming high fluoride water, as assessed by bilirubin, Aspartate Aminotransferase (AST), and Alanine Amino-Transferase (ALT) levels [25].

This study also found no statistical difference in the levels of liver function markers between subgroups of dental fluorosis-affected and unaffected subjects in both Group-A and Group-B study groups, which consisted of individuals of different ages. Furthermore, all the levels were within the normal reference range and were statistically insignificant. These results are consistent with the findings of Liang CH who observed no statistically significant difference in hepatic biochemistry between people affected and unaffected by fluorosis, as assessed by liver function analysis in six different high fluoride areas in China [11].

The observations in this study differ from the findings of Shivashankara AR et al., in Karnataka, India, who reported impaired liver biochemistry in cases of skeletal fluorosis, with elevated levels of AST, ALT, and ALP [8]. Another study by Shashi A and Bhardwaj M in India, conducted in regions where fluorosis was endemic, noted differences in hepatic enzymes, with an increase in ALP levels in all subjects with elevated serum fluoride levels. Only fluorosis-affected females were found to have increased levels of AST and ALT, suggesting that fluoride exposure intensifies the activities of hepatic function enzymes in osteofluorosis, potentially due to cellular damage in the liver [26].

In a study conducted by Malin AJ et al., in the USA, examining renal and hepatic function in adolescents with fluoride exposure, it was suggested that this exposure may lead to altered levels in renal and hepatic-related parameters. The authors also noted that their study was cross-sectional, which could introduce the possibility of reverse causality, as altered renal and/or hepatic function may impact fluoride absorption and metabolic processes in the body [27].

In contrast, this study found no significant difference in the serum levels of liver function markers among dental fluorosis-affected subjects between Group-A and Group-B. Furthermore, the levels of these markers in the serum were within the normal reference range. Thus, there appears to be no difference in the effect of fluoride on liver function, even among symptomatic individuals of fluoride toxicity (subjects with dental fluorosis), regardless of age. This finding contrasts partially with the conclusions of Xiong X et al., who studied fluoride-exposed children and suggested that consuming water with fluoride levels exceeding 2.0 mg/L can cause liver and kidney damage in children. The study also implied that dental fluorosis is independent of liver damage but not kidney damage [28].

Animal studies conducted by Sashi A and Thaparb SP in rabbits [9] and Anjum KM et al., in domestic chickens [14] with high fluoride exposure observed elevated levels of ALP, AST, ALT, and bilirubin, which are liver function markers and indicators of liver function. These studies concluded that fluoride has an impact on liver biochemistry.

Dental fluorosis is not solely a cosmetic issue, but it also serves as a biomarker for chronic fluoride toxicity in individuals exposed to high fluoride levels during tooth development in childhood. It provides insight into the effects of fluoride on vital organs. Individuals with dental fluorosis may exhibit symptoms of fluoride toxicity due to their inherent susceptibility to fluoride toxicity, compared to asymptomatic individuals (those without dental fluorosis) exposed to the same fluoride environment. This likelihood is supported by a study conducted by Everett ET et al., which concluded that there is a genetic component to dental fluorosis susceptibility based on the difference in the prevalence of dental fluorosis in the f2 generation of mice produced by crossing A/J (DF-susceptible) and 129P3/J (DF-resistant) inbred mice [29].

Similarly, it is plausible that vital tissues such as renal tissue and hepatic tissue in individuals with dental fluorosis may be more sensitive to fluoride toxicity, resulting in impaired liver and renal function compared to unaffected individuals residing in the same fluoride environment and exposed to the same levels of fluoride for the same duration [30]. This study assessed hepatic function in individuals with dental fluorosis and those without dental fluorosis residing in the same environment with high fluoride exposure, comparing the observed results to the normal range of liver markers. However, the results obtained were within the normal reference range and showed no difference in liver function between individuals with dental fluorosis and those without dental fluorosis, despite higher fluoride exposure in endemic fluoride areas in YSR Kadapa. This suggests that there is no difference in the effect of fluoride on liver tissue.

Limitation(s)

This study had several limitations. Firstly, it was a cross-sectional investigation, which provides incomplete evidence to fully explain the toxicity of fluoride on liver function. More longitudinal studies are needed to establish a stronger causal relationship. Secondly, there are various influencing factors that are difficult to control or fully understand, due to individual differences. This is a disadvantage of population research compared to animal experimental research. Despite these limitations, the authors made efforts to collect important covariates to minimise confounding effects. Lastly, the small sample size in this study was also a limitation, which may decrease the statistical power and limit the generalisability of the findings.

CONCLUSION(S)

This research concludes that fluoride is not hepatotoxic at the mean fluoride levels ranging between 1.5 mg/L to 4.1 mg/L in drinking water, even after long-term consumption of 15 years or more. The study found no impairment or difference in liver function among the study subjects who consumed high-fluoride water in endemic fluoride areas with mean fluoride levels ranging between 1.5 mg/L to 4.1 mg/L in rural YSR Kadapa district. This was observed in both dental fluorosis-affected and unaffected adults, with the levels of liver function markers falling within the normal reference range. However, larger studies are needed to further investigate potential hepatic derangement at the cellular level in fluorotic endemic areas with varying levels of fluoride in drinking water. These studies would contribute to a conclusive decision and aid in the safe prescription of fluoride for preventive and therapeutic use in dental diseases and bone disorders.

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