A Cross Sectional Study on Effects of Fluoride in Drinking Water on Prevalence of Dental Fluorosis and Kidney Function in Adults in Endemic Fluoride Areas in YSR Kadapa District

Dr. Sirigala Lavanya[1]
PhD student, Saveetha Dental college and hospital, Chennai, India

Dr. Prathiba Ramani[2]
Professor Saveetha Dental college and hospital, Chennai, India

Abstract:
Objective: To explore the effect of water fluoride levels on teeth and renal function in the endemic fluoride areas in YSR Kadapa district **Method:** A cross sectional study was conducted in 6 villages of YSR Kadapa district . 659 people were assessed in terms of drinking water fluoride levels and dental fluorosis. Fluoride concentrations in urine and creatinine level in serum were determined. **Results:** Water fluoride levels were ranging from 1.4mg/l to 4.2mg/l in the collected water samples in the study area in YSR district The study population consisted of 659 participants 363(55.08%)were with dental fluorosis and 296(44.91%) were without dental fluorosis. There is no much difference in levels of urinary fluoride and serum creatinine of dental fluorosis people and non dental fluorosis people residing in the study area. Serum creatinine level significantly increased in the study population than normal reference range. **Conclusion:** Our study results, prevalence of dental fluorosis and higher serum creatinine levels than reference range in the subjects suggests that long-term fluoride exposure is associated with kidney dysfunction in adults. The damage degree increases with the duration of the drinking water fluoride content. Renal damage degree is related to water fluoride concentration but not to the dental fluorosis suffering or not in the study population.

Keywords: Water fluoride, Urinary fluoride, Dental fluorosis, Serum creatinine, Renal function, YSR Kadapa district.

1. Introduction

Fluoride is beneficial to health if concentration of fluoride ion in drinking water is less than 1ppm and can cause serious health hazards at higher concentration .WHO has set a guideline for fluoride value of 1.5 mg/l[1] and BIS desirable and permissible limits are 1.0 and 1.5 mg/l in drinking water[2] respectively. Inorganic fluoride is absorbed readily in the stomach and small intestine and distributed almost entirely to bone and teeth. About 50% of the daily intake is excreted through urine .

Fluoride toxicity is more threat to the common people who are living in the endemic fluoride areas in the globe. Fluoride toxicity will affect all the parts of the human body leads to the altered life span. The disease fluorosis manifests itself in three forms namely dental, skeletal and non-skeletal forms. Dental fluorosis causes hypomineralisation of enamel of the teeth resulting in altered color and texture with increased brittleness.Skeletal fluorosis causes crippling and severe pain and stiffness of the backbone and joints.[3]

The kidney is one of the most important organs that remove fluoride from the body.About 60% of the daily fluoride absorbed by healthy adults is excreted through the kidney in the urine under normal physiological situations (Hefti, 1986).[4] Thus, the kidney is one of the most exposed organs to fluoride concentrations of all the soft tissues of human body (Dharmaratne, 2019).[5]As Kidneys are among the most sensitive body organs in their histopathological and functional responses to excessive amounts of fluoride, they are generally involved in chronic fluoride intoxication.

Many laboratory studies have shown that fluoride can cause kidney function injury, and is destructive to the different parts of kidney cortex such as glomeruli, proximal and distal curved tubules,by means of vacuolization of tubule cells, and cell infiltration (Alhusaini et al., 2018, Cárdenas-González et al., 2013, Dote et al., 2000).[6,7,6]Studies on experimental animals also have shown that fluoride causes kidney function injury through oxidative stress and apoptosis (Tian et al., 2019).[9] In cell experiments, it was found that fluoride sensitivity of cells from different organs of rats showed that kidney cells being the most sensitive type (Hongslo et al., 1980).[10]

In the epidemiological survey in general population, the investigation on chronic kidney disease of unknown etiology in Sri Lanka found that the serum and urinary fluoride concentrations of patients were significantly higher than those of the healthy control group (Nanayakkara et al., 2020)[11], which suggested that environmental fluoride exposure may have toxic effects on the kidney. Another research, concerning the effects of fluoride exposure on children's kidney injury in China showed that drinking water fluoride concentrations over 2.0 mg/L can cause kidney function injury in children, which mainly showed that water fluoride exposure was positively related to urinary N-acetyl-β-glucosidase (NAG) activity and urinary γ- glutamyltransferase (γ-GT) activity in children (Xiong et al., 2007).[12] The results of another study on the health status of children aged 8–15 years in areas of fluorosis showed a significant increase in serum creatinine, which suggested significant kidney function injury in children in fluorosis areas (Khandare et al., 2017)[13]

Three prior studies conducted in India, Japan and/or China found potential evidence of both kidney function decline in children and/or adolescents exposed to relatively high fluoride concentrations.[7,8,9] Findings of a fourth study conducted in Mexico were inconsistent.[14]Few studies conducted among young animals also demonstrated adverse renal effects of fluoride, even at low concentrations. These findings suggest that fluoride may be developmentally nephrotoxic.In endemic fluorosis areas, people are exposed to fluoride from birth, and last for decades. However, epidemiological information for the effects of long-term fluoride exposure on adult kidney function remains limited, and sensitive and robust markers to identify early kidney damage are also lacking.

YSR Kadapa district is identified by central government of India as one of the districts of Andhraprdesh where endemic fluoride areas are prevalent.[15,16]This prompted to take up the study to assess the effect of fluoride in drinking water on prevalence of dental fluorosis and renal function in endemic fluoride villages of YSR Kadapa district.

1.1 Research Question

1. Is there any prevalence of dental fluorosis, in endemic fluoride villages of YSR kadapa district ?
2. Is there any change in renal function in endemic fluoride villages of YSR kadapa district ?

2.1 Aim of the study

1. To evaluate the effect of fluoride on teeth by estimation of prevalence of dental fluorosis in the people of endemic fluoride villages in YSR Kadapa district.
2. To assess the renal function in the people of endemic fluoride villages in YSR Kadapa district.

2.3 Null hypothesis (o)

1. There is no prevalence of dental fluorosis in the people of endemic fluoride villages in YSR Kadapa district.
2. There is no effect on renal functions in people of endemic fluoride areas of YSR Kadapa district.

2.4 Null Hypothesis (a)

1. There is prevalence of dental fluorosis in the people of endemic fluoride villages in YSR Kadapa district.
2. There is effect on renal function in people of endemic fluoride areas of YSR Kadapa district.

2.5 Rationale of the study

Geologically high fluoride belt areas like YSR Kadapa district have fluoride bearing minerals like lime stone, granite rocks which is leached out to the ground water and contribute high fluoride concentration in the ground water. The Groundwater is the primary source of drinking water in this area and very few people are fed with water supply scheme. There is no available literature on the prevalence of dental or systemic flourosis, since 2000. This study has been undertaken to understand the present scenario of flourosis in the YSR Kadapa district. Thus by estimating the prevalence and severity of dental flourosis and correlating with renal functions, the possibility of the role of flouride in drinking water as a common causative factor to the above mentioned diseases in this area could be established.This could alarm the policy makers in Government of Andhra pradesh to take necessary steps to control /prevent the associated diseases .

3. Materials and Method

3.1 Research Design

3.2 Selection of Study site and population
3.3 General information Collection
3.4 Sample collection
3.5 Estimation of samples
3.6 Results and Statistical analysis

3.1 Study site and population

A cross-sectional study was conducted in the YSR Kadapa district region of Andhra Pradesh, which is geographically southern part of the India . YSR Kadapa district of south India, which seems to be one of the most fluorosis threaten area of Andhra Pradesh state as per National survey conducted as part of National fluorosis prevention program.[3]From the data of water quality studies conducted by Geology department of yogivemana university ,water samples from various fluoride affected villages were collected and sent for estimation of fluoride levels in drinking water. Thus, six villages among various villages of YSR district identified as areas of having high fluoride levels in drinking water than permissible limits of 1mg/l by WHO and BIS which were, we finally selected these six villages as our investigation sites. The participants included in our study were adults aged 21years or above, who were villagers of the villages and lived in the villages since they were born.

Dental fluorosis

To the study effect of fluoride on teeth , entire study population were considered as a single group age wise .

Renal function

To study the effect of fluoride on renal function in the study area in YSR Kadapa district, we divided the study population into 2 age groups as Group A-Individuals between 21 to 40 years and Group B-Individuals between 41 to 60 years.

3.2. General information collection

A total of 659 adults were included in the study, All participants were provided with informed consent and signed the informed consent. The study protocol was approved by the Ethics Committee of Saveetha University.Participants were given face-to-face questionnaires and a general physical examination by trained doctoral and postgraduate students.General demographic data (age, sex, education, height, weight, waist-line), socioeconomic status (family income), and disease history (hypertension) of the participants were collected. The body mass index(BMI) of the participants was further obtained by height and weight.

3.3 Oral Examination-Dental fluorosis screening

659 individuals from 6 villages of YSR Kadapa district of Andhra Pradesh State were randomly chosen for survey work. An appropriate questionnaire was administered to all the participants at the Diagnosis of dental fluorosis was performed by oral examination with the subject seated in a chair in bright daylight. The dental specialist used a mirror and a sterile dental probe for oral examination. The presence and severity of dental fluorosis were recorded. The Dean's index was used to determine the grade of dental fluorosis (Dean 1942) which was selected because of its accuracy to identify DF severity as per NPPCF.

3.4-Renal function screening

459 subjects were selected and screened for renal function after excluding persons suffering from known risk factors for renal diseases such as Diabetes, Hypertension, and other chronic diseases . We estimated blood pressure and random blood sugar levels digitally to exclude any undiagnosed cases of diabetes and thus finally , selected 459 individuals as study subjects and 190 among them were between 21 to 40years of age and 269 were between 41-60 years of age .

3.4. Sample collection

Blood samples were collected by lab technician hired . 5 ml of fasting peripheral blood samples were collected from each participant. Random blood sugar levels were measured with Accu check plus method.Serum was separated by centrifugation at 3000 rpm for 20 min . The samples were transferred to the laboratory at Kadapa in cold condition using vaccine carrier and kept at − 80 °C until further analysis.

Urine samples in the morning were collected in a polyethylene tube (50 ml) from the participants who were informed and 2 to 4 drops of toluene was added as a preservative and transported to laboratory and stored at 4 °C till analysis.

Water samples from all drinking water sources were collected from the selected villages in 500ml sterile. clean, high-density polyethylene bottles. They were labelled, coded and sent to the laboratory for fluoride estimation on the same day. All villages studied villages had both rural water supply and bore water for drinking and cooking purposes.

3.52 Estimation of serum creatinine for Renal function

Serum creatinine levels were estimated by Jaffe method in this study.

3.53 Estimation of fluoride levels in urine and water samples

Urine samples were used for determination of urinary fluoride concentrations using the National standard method Ion selective electrode method. All reference solutions for the fluoride determinations were deionized water and all chemicals used in the tests were reagents of analytical purity. Parallel samples were set for measurement and 3averages were taken.

3.6 Results

3.61Tables

Table I-Village wise levels of fluoride in drinking water ,Urine and Prevalence of dental fluorosis in study population

sno	Name of the village	Mean Fluoride level in drinkingwater	Fluoride level in drinking water range	Mean fluoride level in urine	Dental fluorosis in adults (>21years)
1	Veerapalii	3.35	3.2-3.5	1.95	74/102(72.54%)
2	Guntapalli	1.7	1.6-1.8	0.765	44/112(39.28%)
3	Guntlammayapalli	4.1	4.0-4.2	2.13±0.845	106/142(74.6%)
4	Sibyala	2.55	2.4-2.7	1.75	55/105(52.4%)
5	Rayachoty (Rural)	1.5	1.4-1.6	0.45	35/94(37.2%)
6	Indukurupalli	2.2	1.9-2.5	1.1	49/104(47.1%)
	Total				363/659(55.08%)

*spearmann'scorrelation -r=0.7500(+ve correlation)

Table 2-Prevalence of Dental fluorosis in total studied population

S No	Total study population screened n(n%)	Dental fluorosis affected n(n%)	Dental fluorosis Unaffected n(n%)
1	659(100%)	363(55.08%)	296(44.91%)

Table 3-Prevalence of Dental fluorosis in the study population for renal function after exclusion of the cases suffering from known causes for kidney damage

S.No	Study Groups	Age in years	With Dental fluorosis -n(n%)	Without Dental fluorosis-n(n%)	Total-n(n%)
1	Group A	21-40	103(54.21%)	87(45.78%)	190(41.39%)
2	Group B	41-60	170(63.19%)	99(36.80%)	269(58.6%)
3	Total		273(59.46%)	186(40.52%)	459(100%)

Table 4- Comparison of Water fluoride ,Urine fluoride and Serum creatinine levels between Group A and Gropu B in the selected population

Parameter	Group A	GroupB	Normal reference range	P-value
Water fluoride mg/l	2.56±1.4	2.56±1.4	1mg/l As per WHO	>0.005(NS)
Urine fluoride mg/l	1.2±0.49	1.11±0.19	1mg/l As per WHO	>0.005(NS)
Serum creatinine in mg/dl	1.42±0.35	2.52+0.32	0.6-1.3mg/dl As per NPPCL	>0.005(NS)

Student's t Test-P Value < 0.005 significance
*The established reference interval, which has been utilized by the laboratory, is also tabulated along with the results so as to enable us to compare the results obtained from the study

Table 5- Comparison of Water fluoride , Urine fluoride and Serum creatinine levels in Group A in the selected population

Parameter	Dental fluorosis affected	Dental fluorosis unaffected	Normal reference range	*P-value
Water fluoride mg/l	2.56±1.4	2.56±1.4	1mg/l As per WHO	<0.05(S)
Urine fluoride mg/l	1.2±0.49	1.11±0.19	1mg/l As per WHO	<0.05(S)
Serum creatinine in mg/dl	1.42±0.35	1.45.±45	0.6-1.3mg/dl	>0.005(NS)

*P-value < 0.05
*Anova test to calculate P-value<0.05 significance

Table 6- Comparison of Water fluoride , Urine fluoride and Serum creatinine levels in GroupB in the selected population(41-60yrs age)

Parameter	Dental fluorosis affected	Dental fluorosis unaffected	Normal reference range	P-value
Water fluoride mg/l	2.56±1.4	2.56±1.4	1mg/l As per WHO	<0.005(S)
Urine fluoride mg/l	1.35±0.9	1.25±0.9	1mg/l As per WHO	<0.005(S)
Serum creatinine in mg/dl	2.08±0.24	2.06±0.35	0.6-1.3mg/dl As per NCCL	>0.005(NS)

Anova test - to calculate p value <0.05 significance

3.62-Observations

1. Water fluoride levels were ranging from 1.4mg/l to 4.2mg/l in the collected water samples in the study area in YSR district.(Table1).

2. Guntlammayamapalli was having maximum fluoride level in the ground water/drinking water around 4.1mg/l(mean) and rayachoty rural was having 1.5mg/l(mean).(Table1).

3. Highest urinary fluoride concentration is observed in the people of Guntlammayyapalli around 2.13±0.845mg/l and lowest 1.1mg/l in Rayachoty rural (Table1)

4. 55.08% of the study population were affected with dental fluorosis and 44.91% were unaffected. (Table2).

5. Highest prevalence of Dental fluorosis is seen in Guntlammayapalli around 74.6% followed by Veerapalli around 72.54%. Lowest prevalence 30.4%. is seen in Rayachoty rural.(Table1)

6. 48.94% in Group A and 63.19% in Group B were affected with Dental fluorosis.(Table3)

7. Group A (age between 21-40yr) showed mean urinary fluoride level about 1.20±0.49mg/l and serum creatinine level is 1.42±0.35 mg/dl and Group B (age between 41-60yr) showed mean urine fluoride level about 1.11±0.19mg/l and serum creatinine level is 2.52±0.32 mg/dl (Table4).

8. Group A showed mean urine fluoride level about 1.2±0.49 and serum creatinine level about 1.42± 0.35 in dental fluorosis affected subjects and mean urine fluoride level about 1.11±0.19 and serum creatinine level about1.45 ±0.45.(Table5)

9. Group B showed mean urine fluoride level about 1.35± 0.9 and serum creatinine level about 2.08± 0.24 in dental fluorosis affected subjects and mean urine fluoride level about 1.25±0.9and serum creatinine level about 2.06± 0.35.(Table6)

3. Discussion

Our study aimed to assess the effect of fluoride on teeth by estimation of prevalence and severity of dental fluorosis in all the studied population in YSR Kadapa district and effect if any on kidneys by estimation of renal function marker that is serum creatinine in selected population in the study areas.

The kidney function can be evaluated by several indexes.[17] Measuring serum creatinine is a useful, inexpensive and reliable marker for renal function for evaluating renal function.[17,19]Creatinine is a non-protein waste product of creatinine phosphate metabolism by skeletal muscle tissue. Creatinine production is continuous and is proportional to muscle mass. Creatinine is freely filtered and therefore the serum creatinine level depends on the Glomerular Filtration Rate (GFR).[20] Accepted normal serum creatinine levels for adult men is 0.74 to 1.35 mg/dL and for adult women is 0.59 to 1.04 mg/dL as per NCLLS.[21,22]

Renal dysfunction diminishes the ability to filter creatinine and the serum creatinine rises. If the serum creatinine level doubles, the GFR is considered to have been halved. A threefold increase is considered to reflect a 75% loss of kidney function.[20.,23]

Effect of fluoride on Teeth

In the present study, it is observed that water fluoride levels were ranging from 1.4mg/l to 4.2mg/l in the collected water samples in the study areas. Among the selected six villages , Guntlammayamapalli was having maximum fluoride level in the ground water/drinking water around 4.1mg/l(mean) and rayachoty rural was having 1.5mg/l(mean).Almost all the selected villages are higher than the permissible level of 1 ppm according to WHO (1984) (Table 1).This range of fluoride in water is classified as areas under strata I and Strata II as per National Programme for Prevention and Control of Fluorosis (NPPCF-2018) .[3]

In the present study , estimation of fluoride content in the urine samples of the study population shows abnormal range of fluoride confirms they are consuming the water containing fluoride. Acceptable point for urine fluoride is 1 mg per liter as mentioned in National Programme for Prevention and Control of Fluorosis (NPPCF-2018) .[3.] Highest urinary fluoride concentration is observed in the people of Guntlammayyapalii around 2.13± 0. 845mg/1 and lowest 1.1mg/1 in Rayachoty rural (Table 1) and is proportional to the levels of fluoride in the drinking water that is high fluoride in drinking water and high concentration of fluoride in the urine.As urine is the most important metabolic pathway for elimination of fluoride from the body, it has been considered as a useful biomarker for contemporary fluoride exposure at a population- but not individual level.[24,25]Urine fluoride concentration among the biomarkers of fluoride exposure is generally accepted as the best indicator of fluoride exposure because it can be recollected noninvasive and systematically reflects the burden of fluoride exposure from drinking water. Hence, special attention has been given to it as a biomarker, and is used as an indirect indicator of fluoride intake.[17],[24]

In our study , effect of fluoride on teeth is established as we observed 55.08% prevalence of dental fluorosis in studied areas in YSR Kadapa district (Table2).Highest prevalence is seen in

Guntlammayapalli around 74.6% followed by Veerapalli (72.5%),lowest prevalence 30.4%. seen in Rayachoty rural(Table1) .It is observed that prevalence of dental fluorosis correlates with the water fluoride level that high fluoride in drinking water reflects in high prevalence of dental fluorosis. All the study areas are having the similar temperatures around 28℃ to 45 ℃ . So, all the studied population consume water in the same frequency and same quantity.[26]Our study results correlates with the similar findings in the studies of Mella et al (1994)[27]in chille and Gopalakrishnan et al (2012)[28]in Tamilnadu.

It is observed that 48.94% in group A were affected with Dental fluorosis and 63.19% in Group B were affected with dental fluorosis.Analysis of the urine samples showed that the fluoride content is in abnormal range in urine samples in the study population. (Table 3)

Effect of fluoride on Renal function

There is enough medical evidence worldwide to prove that there is a direct connection between high fluoride levels in drinking water and kidney disease.Adults who had renal damage due to fluoride in childhood are more vulnerable to CKD by continuing to consume water from the same source. [18]

We examined the relationship between water fluoride concentrations,urinary fluoride and serum creatinine levels , marker of kidney function in this cross-sectional study of endemic fluorosis in YSR Kadapa district.

Further, our study results showed that both Group A and Group B subjects showed increase in serum creatinine levels than the normal range of serum creatinine though the results are not statistically significant (Table 4).This states that fluoride has effect on the renal function and this correlates with the study results of xianzhi xiong et al studies in china (2007)[12] and P.Jaganmohan et al studies in Nellore (2010).[20] But, their study results showed both significant increase in renal function markers and their results were statistically significant.This difference could be explained as the renal markers observed in Xiong et al studies[12] where enzyme markers and in Jaganmohan et al studies the subjects were known renal disease patients.[20].

It is further observed that there is no much difference in Serum creatinine levels between Dental fluorosis affected and unaffected persons in group A and group B. (table5&6). Our study results agrees with the study results of Xianzhi xiong et al studies in china (2007)[12] suggesting that kidney function markers were similar between the same level of drinking water fluoride suggesting that damage to kidney functions was mainly related to the concentrations of drinking water fluoride but not with symptoms of dental fluorosis.

Our findings showed that serum creatinine levels are higher in Group B than Group A, though both levels are more than the normal range of serum creatinine levels in the similar age groups as per reference range .(Table4,5 &6) Thus, supports that age modified the association between fluoride exposure and serum creatinine levels.This result coincides with Liaowei Wu et study in China (2021)[29] who states that there is an increased risk of injury to the function of kidneys with long term exposure to fluoride in drinking water.[29] slight increase in the serum creatinine suggests the sub clinical changes in the kidneys leading to decreased filtration of serum creatinine from the blood and thus results in increased serum creatinine.

Our study agrees with the statements reported by other researchers,that infants and children who are exposed to high levels of fluoride through drinking water and diet are exposed to more

fluoride retention in the body, thereby weakening their kidneys and becoming more vulnerable to kidney diseases in their adulthood .[18,30.31.32] Children are vulnerable to fluoride as low as 2 ppm in drinking water, due to their ability to retain absorbed fluoride as higher as 80% in the body/kidney. Therefore, if continue to consume water from the same source, could result in the child becoming an adult with a sick kidney, prone to ending up as a CKD patient.[18] our study results also of the opinion that long-term fluoride exposure is associated with kidney dysfunction in adults as the study subjects were found to be residing in these villages in YSR Kadapa district since birth.

4. Conclusion

In the present study, both the prevalence of Dental fluorosis and increase in serum creatinine levels suggests that, there is effect of fluoride in drinking water on teeth and kidneys and increased chances of development of chronic kidney disease in endemic fluoride areas in YSR Kadapa district .The most positive point about this study is that our study population is a natural population, not patients with clinical kidney disease. The study examined the relationship between fluoride exposure and kidney damage, and the damage is more likely to be expressed as early (subclinical) damage.This needs the policy makers to iniate steps to provide fluoride free water to these areas in YSR Kadapa district.

6.Limitations of the study

This study had several limitations. Our study was a cross-sectional investigation and is incomplete adequacy as evidence to explain the kidney toxicity of fluoride as we have studied only serum creatinine as a renal function marker while other indices need to be assayed, and more longitudinal studies are needed. In our study, known factors for kidney function injury were not all collected for covariate control, which makes our results likely to be questioned by other researchers. However, this is also the disadvantage of population research compared with animal experimental research, that is, the influencing factors caused by individual differences are difficult to be controlled artificially or fully understood by researchers. Fortunately, we did our best to collect important covariates.
Further because of covid pandemic it became very difficult to increase the sample size.

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References

1. World Health Organization (WHO) (2002) Environmental health criteria-fluoride. WHO, Geneva, p 227
2. Bureau of Indian Standards (BIS) (2012) Indian standard drinking water specifications (Second Revision) BIS 10500. BIS, New Delhi
3. National programme for prevention and control of fluorosis (NPPCF) revised guidelines (2018) Directorate General of Health services Ministry of Health & Family welfare Government of India
4. Hefti ASchweiz Monatsschr Zahnmed -Fluoride metabolism. (1984) 1986 Jan;96(1):305-16
5. Dharmaratne R. Exploring the role of excess fluoride in chronic kidney disease: A review. Human & Experimental Toxicology. 2019;38(3):269-279.
6. Alhusaini, A.M., Faddah, L.M., El Orabi, N.F. and Hasan, I.H., 2018. Role of some natural antioxidants in the modulation of some proteins expressions against sodium fluoride-induced renal injury. BioMed Research International, 2018.
7. Cárdenas-González, M.C., Del Razo, L.M., Barrera-Chimal, J., Jacobo-Estrada, T., López-Bayghen, E., Bobadilla, N.A. and Barbier, O., 2013. Proximal renal tubular injury in rats sub-chronically exposed to low fluoride concentrations. Toxicology and applied pharmacology, 272(3), pp.888-894.
8. Dote, T., Kono, K., Usuda, K., Nishiura, H., Tagawa, T., Miyata, K., Shimahara, M., Hashiguchi, N., Senda, J. and Tanaka, Y., 2000. Toxicokinetics of intravenous fluoride in rats with renal damage caused by high-dose fluoride exposure. International archives of occupational and environmental health, 73, pp.S90-S92..
9. Tian, X., Feng, J., Dong, N., Lyu, Y., Wei, C., Li, B., Ma, Y., Xie, J., Qiu, Y., Song, G. and Ren, X., 2019. Subchronic exposure to arsenite and fluoride from gestation to puberty induces oxidative stress and disrupts ultrastructure in the kidneys of rat offspring. Science of the Total Environment, 686, pp.1229-1237.
10. Hongslo, C.F., Hongslo, J.K. and Holland, R.I., 1980. Fluoride sensitivity of cells from different organs. Acta Pharmacologica et Toxicologica, 46(1), pp.73-77.
11. Nanayakkara, S., Senevirathna, S.T.M.L.D., Harada, K.H., Chandrajith, R., Nanayakkara, N. and Koizumi, A., 2020. The Influence of fluoride on chronic kidney disease of uncertain aetiology (CKDu) in Sri Lanka. Chemosphere, 257, p.127186.
12. Xiong, X., Liu, J., He, W., Xia, T., He, P., Chen, X., Yang, K. and Wang, A., 2007. Dose–effect relationship between drinking water fluoride levels and damage to liver and kidney functions in children. Environmental research, 103(1), pp.112-116.
13. Khandare, A.L., Gourineni, S.R. and Validandi, V., 2017. Dental fluorosis, nutritional status, kidney damage, and thyroid function along with bone metabolic indicators in school-going children living in fluoride-affected hilly areas of Doda district, Jammu and Kashmir, India. Environmental monitoring and assessment, 189, pp.1-8.
14. Jiménez-Córdova MI, Cárdenas-González M, Aguilar-Madrid G, et al. Evaluation of kidney injury biomarkers in an adult Mexican population environmentally exposed to fluoride and low arsenic levels. Toxicol Appl Pharmacol 2018; 352: 97–106.
15. Water quality standards -States Wise Details of Partly Affected Districts with Select Contaminants in Ground Water of India ** Updated as on 2019 Central Ground Water Board (CGWB)Ministry of Jal Shakti, Department of Water Resources, River Development and Ganga Rejuvenation, Government of India
16. Central Ground water Board. Ministry of water resources Government of India Ground water brochure YSR District (kadapa), Andhra pradesh (AAP-2012-13)

17. World Health Organization. Basic methods for assessing renal fluoride excretion in community prevention programmes for oral health Geneva, Switzerland: World Health Organization, 2014.
18. Dharmaratne, R.W. Fluoride in drinking water and diet: the causative factor of chronic kidney diseases in the North Central Province of Sri Lanka. Environ Health Prev Med 20, 237–242 (2015).
19. Perrone, R.D., Madias, N.E. and Levey, A.S., 1992. Serum creatinine as an index of renal function: new insights into old concepts. Clinical chemistry, 38(10), pp.1933-1953.
20. Jaganmohan P, Narayana SVL, Sambasiva R (2010) Prevalence of high fluoride concentration in drinking water in Nellore district, Anadra Pradesh, India: a biochemical study to develop the relation to the rental failures. World J Med Sci 5:45–48
21. Villanova, P.A., 2000. National Committee for Clinical Laboratory standards: how to define, determine and utilize reference intervals in the clinical laboratory: proposed guidelines. NCCLS document.
22. Verma, M., Khadapkar, R., Sahu, P.S. et al. Comparing age-wise reference intervals for serum creatinine concentration in a "Reality check" of the recommended cut-off. Indian J Clin Biochem 21, 90–94 (2006).
23. Gowda S, Desai PB, Kulkarni SS, Hull VV, Math AA, Vernekar SN. Markers of renal function tests. N Am J Med Sci. 2010;2(4):170-173.
24. Idowu OS, Azevedo LB, Valentine RA, Swan J, Vasantavada PV, Maguire A, et al. (2019) The use of urinary fluoride excretion to facilitate monitoring fluoride intake: A systematic scoping review. PLoS ONE 14(9)
25. Del Carmen AF, Javier FH, Aline CC. Dental fluorosis, fluoride in urine, and nutritional status in adolescent students living in the rural areas of Guanajuato, Mexico. J Int Soc Prev Community Dent. 2016;6(6):517-522.
26. García-Escobar TM, Valdivia-Gandur I, Astudillo-Rozas W, et al. Moderate and Severe Dental Fluorosis in the Rural Population of Anantapur, India: Change in Their Biological Susceptibility?. Int J Environ Res Public Health. 2022;19(18):11293. Published 2022 Sep 8.
27. Mella, S., X. Molina, and E. Atalah. "Prevalence of endemic dental fluorosis and its relation with fluoride content of public drinking water." Revista medica de Chile 122.11 (1994): 1263-1270.
28. Gopalakrishnan, S.B., Viswanathan, G. & Siva Ilango, S. Prevalence of fluorosis and identification of fluoride endemic areas in Manur block of Tirunelveli District, Tamil Nadu, South India. Appl Water Sci 2, 235–243 (2012).
29. Liaowei Wu, Chenlu Fan, Zaihong Zhang, Xin Zhang, Qun Lou, Ning Guo, Wei Huang, M ,Association between fluoride exposure and kidney function in adults: A cross-sectional study based on endemic fluorosis area in China, Ecotoxicology and Environmental Safety,Volume25,2021,112735,
30. Ludlow, M., Luxton, G. and Mathew, T., 2007. Effects of fluoridation of community water supplies for people with chronic kidney disease. Nephrology Dialysis Transplantation, 22(10), pp.2763-2767.
31. Dharma-Wardana, M.C., Amarasiri, S.L., Dharmawardene, N. and Panabokke,C.R., 2015. Chronic kidney disease of unknown aetiology and ground-water ionicity: study based on Sri Lanka. Environmental geochemistry and health, 37, pp.221-231.
32. Nanayakkara, S., Senevirathna, S.T.M.L.D., Karunaratne, U., Chandrajith, R., Harada, K.H., Hitomi, T., Watanabe, T., Abeysekera, T., Aturaliya, T.N.C. and Koizumi, A., 2012. Evidence of tubular damage in the very early stage of chronic kidney disease of uncertain etiology in the North Central Province of Sri Lanka: a cross-sectional study. Environmental health and preventive medicine, 17(2), pp.109-117.