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Estimation of Selective Renal Markers for the Prediction of Fluorosis Induced Nephropathy in Villages of Nellore District Andhra Pradesh, India

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Abstract: Fluorosis is become more problematic in India all over the country. Particularly in Andhra Pradesh state, next to Nalgonda, Nellore district seemed to be fluoride threaten area in India. The present study has been carried out at selective areas of Udayagiri mandal to evaluate the relation between renal failure and consumption of highly concentrated fluoride in drinking water by means of renal markers estimation. Results showed elevated levels of serum and urine fluoride contents. Control subjects showed creatinine content of 1.43 mg/dl, whereas the disordered subjects showed a value of 2.78 mg/dl, which showed a drastic increase in the serum creatinine value and the loss of renal function. Serum analysis of glomerular and tubular markers shown to be decreased when compare to their controls except B2M. Urine glomerular and tubular markers are shown to be increased drastically when compare to their controls.

Key words: Complete blood picture · Biochemical parameters · Haematological parameters · Fluoride poisoning · Water fluoride · Nellore District

INTRODUCTION

In India, fluoride and topaz are widespread and contain a high percentage of fluoride. Fluoride pollution in the environment occurs through two channels, namely natural and anthropogenic sources [1]. There is an essential relation between poverty and fluorosis as malnutrition is found to play an aggressive role in its severity [2]. Fluoride is beneficial to health if the concentration (CF) of the fluoride ion (F-) in drinking water is less than 1.5 mg/L [3]. A higher concentration causes serious health hazards. The disease caused manifests itself in three forms, namely, dental, skeletal and non-skeletal fluorosis. Dental fluorosis produces widespread brown stains on teeth and may cause pitting [4]. Skeletal fluorosis causes crippling and severe pain and stiffness of the backbone and joints [5].

Fluoride in drinking water cannot be detected by taste, sight or smell. Testing is the only way to determine the fluoride concentration. Like other trace elements, excessive quantities of fluoride can result in acute or chronic toxicity. Consumption of an excessive amount of fluoride (300 to 750 milligrams depending on body weight) in a single dose can cause acute toxicity resulting in nausea or vomiting. This level of fluoride intake would only occur as a result of some type of accidental event, such as young children consuming an overdose of fluoride supplements. The health burden of poor water quality is enormous. It is estimated that around 37.7 million Indians are affected by waterborne diseases annually (viral hepatitis, cholera, jaundice, typhoid are examples), 1.5 million children are estimated to die of diarrhoea alone and 73 million working days are lost due to waterborne disease each year.

In India, the states of Andhra Pradesh, Bihar, Chattisgarh, Haryana, Karnataka, Madhya Pradesh, Maharashtra, Orissa, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh and West Bengal are affected by fluoride contamination in water. This involves about 9000 villages affecting 30 million people [6].

Kidneys are among the most sensitive body organs in their histopathological and functional responses to excessive amounts of fluoride [7]. They are the primary organs concerned with excretion and retention of fluoride

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and thus are generally involved in chronic fluoride intoxication. In humans, only a few reports pertaining to kidney involvement in endemic fluorosis are available [8]. Kono *et al*. [9] reported impaired renal functions in fluoride-exposed workers. In contrast to cases of acute intoxication, the records of only a few autopsy reports of patients dying of chronic fluoride intoxication are traceable in the literature [8, 10]. Raised concentrations of fluoride have been found in the plasma and bone of patients with renal failure [11, 12]. As normal people absorb fluoride easily it seemed important to determine at what level of renal function fluoride excretion failed to match absorption. The fluoride ion has been shown to cross the dialysis membrane freely [13] so in patients on regular dialysis treatment fluoride from dialysis fluid might collect in the blood to be excreted.

Andhra Pradesh is also become popular with the curse particularly districts like Nalgonda. Al most all the relevant problems with fluoride poisoning was established by the researchers, but the people in and around the Nellore district were more repeatedly targeted by the renal failures without any other disorders like hypertensions or diabetes. To identify the relations between the increased fluoride content in the drinking water and the renal failures the study has been established [14, 15]. Studies related to exact evidence of fluoride involvement in the renal failures are no more. Most of the experiments were conducted in the renal failure patients under the supplementation of fluoride water. To know the specific mechanism of fluoride toxicity in the renal failures, studies were conducted by the estimation of glomerular and tubular markers. From this background the study was started in Nellore district (Udayagiri mandal) of Andhra Pradesh, which is geographically southern part of the India near to the Bay of Bengal.

MATERIALS AND METHODS

The study was conducted in the Nellore district region of Andhra Pradesh, which is geographically southern part of the India near to the Bay of Bengal. Nellore district is the coastal are of south India, which seems to be one of the most fluorosis threaten area of Andhra Pradesh state. From the data of water quality department as well as information from news papers, analysis has been initiated in the Udayagiri mandal of Nellore district. Among the mandal ten villages have been reported to be affected areas of fluorosis.

Selection of Samples: Five hundred individuals from 10 villages in Udayagiri mandal, Nellore district of Andhra Pradesh State were randomly chosen for survey work, which was highlighted by the local newspapers. The present study was constructed to analyze the samples that are having the renal disorders with the association of fluoride intake. Peoples suffering with regular renal failure with diabetes and hypertension were separated and omitted from the analysis.

Control Subjects: To compare each and every component or biological parameters, a group of normal healthy individuals were chosen from the same areas, who are not suffering with any of these disorders.

Analysis of Water Quality and Fluoride Content: A total of 10 samples were collected from the selected locations of each village representing the water quality of the whole area. Fluoride concentration was spectrophotometrically determined using Alizarin red-S and SPADNS reagents [16]. Sodium fluoride was used to prepare the standard solution. The main sources of drinking water in these villages are open wells, hand pumps and municipal supply.

Estimation of Serum and Urine Fluoride Content: From the selected individuals blood and urine samples were collected in non-reactive plastic containers and brought to the laboratory in an ice box. To analyze the level of fluoride in serum, blood was centrifuged and serum separated. Fluoride content of serum and urine was analyzed through SPADNS method.

Estimation of Random Blood Sugar: Random blood glucose measured routinely using 'One Touch Ultra' blood glucose meter (Accu Chek Glucometer, USA).

Estimation of Glomerular and Tubular Markers

Estimation of Transferrin: The amount of transferrin was estimated by SRID kit in the first sample was 7.5 g, we used 10.5 mg Fe and 11.44 mg anion bicarbonate.

Estimation of Ig G: The serum and urinary IgGs were measured by sandwich ELISA (Enzyme Linked Immunosorbent Assay) within one week of storage using reagents supplied by Bangalore Genei, India.

Estimation of Antitrypsin: Oxidative antitrypsin (AT) was analyzed using an ELISA with a monoclonal antibody against oxidized α_1-AT in which chloramine T-oxidized α_1-AT was the antigen [17]. The sensitivity of oxAT measurement was 1.0 ng/ml with an inter CV of <6.7%.

β2-Microglobulin Assay: The samples were analyzed by Enzyme linked immunosorbent assay (β2– microglobulin EIA kit, Immunotech, France). 2.4mg/ L was used as the upper limit, when 97% of normal values are below this cut off value.

Serum and Urine ACE Level Measurement: Serum or urine ACE level was measured by a colorimetric method (colorimetric assay kit, Fujizoki Assay, Tokyo, Japan) using p-hydroxyhippuryl- L-histidyl-L-leucine as the substrate [18].

Statistical Analysis: Statistical analysis was carried out using SPSS for windows 10.0 software (SPSS Inc.,Chicago, IL, USA) and Microsoft Excel. Values were reported as mean±standard deviation. SD was not more than 10%.

RESULTS AND DISCUSSION

Recently, it was noticed that Udayagiri mandal of Nellore district andhra Pradesh, India seems to be more threaten area of fluoride in drinking water. A sum of total ten fluoride affected villages has been find out with the help of water control department and the water samples has been taken for the analysis of water fluoride content. Water samples from different bore wells of ten villages showed a maximum range of 2.37 to 6.74 ppm by SPADNS method (Table 1). Among the selected ten villages three are showing high levels of fluoride content in their drinking water (ranges 4-7 ppm). Particularly Varikunta padu showing a maximum fluoride content of 6.74 ppm. These three villages namely, Varikunta padu (6.74 ppm), Kolangadi palli (5.12 ppm) and Gangireddy palli (4.43 ppm) were take for the further entire study. Almost all the selected villages are higher than the permissible level of 1 ppm according to WHO [3].

Analysis of the samples showed the fluoride content in abnormal range both in urine and serum (Table 2). The generally accepted average normal serum fluoride value is 8 μM (0.15 ppm.) as found by Singer and Armstrong [19]. Incase of urine fluoride acceptable point is 1 mg per liter. But, in the case of the selected objects it seems to be more when compared to the normal value. Particularly Kolangadi palli people showed a maximum of 2.27 ppm of serum fluoride and in case of urine fluoride Varikuntapadu people are showing a maximum range of 4.00 mg where the normal values of serum and urinary fluoride are 0.15 ppm and 1 mg, respectively.

After screening of the data we have selected a sum of 90 people, who are never suffered with hypertension or diabetes. Later we have collected their urine and serum samples for the assay of fluoride content. Even though the selection was done specifically remove the hypertension and diabetic people, again a cross check has been made to know the random blood glucose levels as well as blood pressure of the selected 90 fluoride threaten individuals (Table 3). Blood pressure was measured with the help of a local rural medical practitioner. Random blood glucose level was assayed with the help of one pick gluometer. These results showed that the values are not significant and there was not much change when compared to that of control value. Mean value of RBS showed to be 175 mg/dl, whereas control mean value is 173 mg/dl (Table 3). Similar types of studies under fluoride toxicity with reference to dental and skelaetal fluorosis has been made by several workers [19-24].

Control subjects showed the creatinine content of 1.43 mg/dl, whereas the disordered subjects showed a value of 2.78 mg/dl, which shows a drastic increase in the serum creatinine value and the loss of renal function. From the results we can observe a significant ($p<0.001$) increase in the serum creatinine content, almost doubled with the control value indicates the loss of renal function and symptoms of renal failure (Table 3).

Table 1: Flouride contents in water samples of the selected ten villages in and around Udayagiri Taluk (Nellor edistrict, A.P., India)

Name of the village	Flouride content in water
Turkapalli	4.01±0.83
Pakeerpalem	4.00±0.66
Varikunta padu	6.74±1.24
Bijjam palli	2.92±1.02
Masi peta	2.37 ±0.98
Singa reddy palli	2.98 ±1.31
Boda banda	3.47±0.88
Kolangadi palli	5.12±1.56
Gangireddy palli	4.43±1.98
Basine palli	3.12±1.22

Table 2: Flouride contents in serum and urine samples of the selected ten villages people in and around Udayagiri Taluk (Nellor edistrict, A.P., India)

Name of the village	Flouride content in Serum	Flouride content in Urine
Turkapalli	1.47±0.61	2.13±0.89
Pakeerpalem	2.10±0.95	2.22±1.02
Varikunta padu	2.2±0.45	4.00±1.85
Bijjam palli	1.50±0.35	2.12±0.42
Masi peta	1.91±0.97	1.07±0.62
Singa reddy palli	1.05±0.33	2.10±0.15
Boda banda	2.19±0.21	1.23±0.67
Kolangadi palli	2.27±0.49	2.26±0.89
Gangireddy palli	2.13±0.61	2.00±0.46
Basine palli	2.10±0.29	2.12±0.51

Table 3: Analysis of the blood pressure, random blood sugar and serum creatinine of the normal and fluoride affected peoples

	Blood Pressure	Random Blood Sugar	Serum Creatinine
Control (n=50)	120/80±10	173.58±15.83	1.43±0.35
Fluoride affected (n=90)	130/90±10	175.59±18.06	2.78±0.24
SEM	NS	3.475^{NS}	0.412
Significance	P<0.001	P<0.001	P<0.001

NS: Non-significant

Table 4: Analysis of the glomerular and tubular markers in the serum of the control and test objects

	Transferrin	IgG	α-1-Antitrypsin	Beta-2 MG g/ml	ACE
				3.03±0.99	
Control (n=50)	1313.92±179.87	1742.86±160.51	222.33±17.99	2.60 normal	44.97±8.72
Fluoride affected (n=90)	1232.89±224.93	1364.96±299.73	221.67±16.81	10.60±2.08	37.07±12.68
SEM	43.288	57.684	3.234	0.400	2.441
Significance	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001

Table 5: Analysis of the glomerular and tubular markers in the urine of the control and test objects

	Transferrin in urine	IgG in urine	α-1-Antitrypsin	Beta-2 MG	ACE
Control (n=50)	195.50±29.30	34.54±2.37	36.61±8.38	1.24±0.98	11.46±0.84
Fluoride affected (n=90)	221.43±49.48	45.41±7.71	39.96±6.54	3.64±0.97	13.77±1.46
SEM	9.352	1.457	1.236	0.183	0.276
Significance	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001

Table 4 shows the analysis of serum glomerular and tubular markers in the control and test samples. Transferrin is a plasma protein that transports iron through the blood to the liver, spleen and bone marrow. The blood transferrin level is tested for diverse reasons like to determine the cause of anemia, to examine iron metabolism (for example, in iron deficiency anemia) and to determine the iron-carrying capacity of the blood. Low transferrin can impair hemoglobin production (since to make hemoglobin, you have to have iron) and so lead to anemia. Low transferrin can be due to poor production of transferrin by the liver (where it's made) or excessive loss of transferrin through the kidneys into the urine. Here in this present study the level of transferring seems to be low when compared to that of control (Table 4). That indicates the chance of anemia due to fluoride toxicity. Low levels of IgG occur in macroglobulinemia. In this disease, the high levels of IgM antibodies suppress the growth of cells that produce IgG. Other conditions that can result in low levels of IgG include some types of leukemia and a type of kidney damage (nephrotic syndrome). Here we can find the low levels of serum IgG, but with in the normal range indicating the altered renal function (Table 4).

Alpha 1-antitrysin (A1AT) is produced in the liver. Accumulation of this in liver causes lower levels of AlAT in blood results in the development of liver cirrhosis. Excessive excretion of AlAT through urine indicates the loss of renal function. In present case there is no difference with the control value. It seems to be almost equal, that indicates the normal functioning of liver (Table 4). Beta 2-microglobulin is a protein found on the

surface of many cells. Testing is done primarily when evaluating a person for certain kinds of cancer affecting white blood cells including chronic lymphocytic leukemia, non-Hodgkin's lymphoma and multiple myeloma or kidney disease. In our study it was very interestingly rapid enhancement of B2M was noticed.

The control subjects showed 3.03 g/ml, whereas the fluoride affected people showed a maximum increase of B2M to 10.60 g/ml. That shows a drastic increase which indicates the altered renal activity in the fluoride affected people (Table 4). There was a significant ($P<0.001$) change was noticed compared to the normal. This altered range is more supportive for further analysis for the fluoride mediated renal damage. The angiotensin-converting enzyme test is used to measure the blood level of angiotensin-converting enzyme, which converts angiotensin I to angiotensis II and controls blood pressure. Angiotensin-converting enzyme and ACE2 are highly expressed in the kidney. The role of ACE in the development of renal damage is generally accepted. Here in the present study the ACE level seems to be decreased when compared to that of control individuals (Table 4). Control individuals having a concentration of 44.97 and fluoride affected people are showing a concentration of 37.07 indicating a significant ($P<0.001$) decrease. This indicates the accumulation of angiotensin I.

After identifying the glomerular and tubular markers in the serum studies has been made to know the status of the same in the urine to confirm the fluoride mediated renal failures. From the table 5 it is clear that transferrin levels are hiked in the fluoride affected people. Control people are showing a value of 195.50 and the fluoride affected are showing 221.43. From the earlier table it is clear that transferrin concentration seems to be low in the serum and now it increases in the urine indicates the loss of renal function (Tables 4 and 5). Similar results were found in the case of IgG in the urine as well as in serum. Here also we can find the decreased concentration of serum IgG and increased levels of urinary IgG indicates the renal alterations. The control urinary IgG seems to be 34.54 and in the threaten people it reaches to 45.41. This shows a significant ($P<0.001$) increase (Table 5). But, in the case of AlAT it was changed, where the serum AlAT is not having any significant change. But here we can clearly find altered values of AlAT.

The control individuals are showing 36.61 AlAT, where the affected people are showing an increased value of 39.96 indicates the increase in the excretion of AlAT due to renal failure (Table 5). Here B2M also showing similar pattern of over excretion. Here we can find 3.64 in the treated people where the control value is 1.24. It was found to be drastically increased in the serum as well as in urine of the affected people. The same was also found with ACE levels here the control value is 11.46 and the treated people are showing 13.77, which means over excretion indicates the renal problems (Table 5).

Thus studies were conducted to evaluate the glomerular and tubular marker in urine as well as serum of the control and fluoride affected people. Table 4 shows the analysis of serum glomerular and tubular markers in the control and test samples. Transferrin is a plasma protein that transports iron through the blood to the liver, spleen and bone marrow. The blood transferrin level is tested for diverse reasons like to determine the cause of anemia, to examine iron metabolism and to determine the iron-carrying capacity of the blood. Low transferrin can impair hemoglobin production and so lead to anemia. Low transferrin can be due to poor production of transferrin by the liver or excessive loss of transferrin through the kidneys into the urine. Here in this present study the level of transferring seems to be low when compared to that of control (Table 4). That indicates the chance of anemia due to fluoride toxicity. Low levels of IgG occur in macroglobulinemia. In this disease, the high levels of IgM antibodies suppress the growth of cells that produce IgG. Other conditions that can result in low levels of IgG include some types of leukemia and a type of kidney damage (nephrotic syndrome). Here we can find the low levels of serum IgG, but with in the normal range indicating the altered renal function (Table 4).

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In glomerular kidney disease, the glomeruli can't filter it out of the blood, so levels increase in the blood and decrease in the urine. In tubular kidney disease, the tubules can't reabsorb it back into the blood, so urine levels rise and blood levels fall. After a kidney transplant, increased blood levels may be an early sign of rejection.

Increased urinary levels are found in people with kidney damage caused by high exposure to the heavy metals cadmium and mercury. Periodic testing of workers exposed to these metals helps to detect beginning kidney damage. B2m is normally cleared by the kidneys at a rate comparable to GFR [25, 26], then reabsorbed and catabolized in the tubules and serum levels are inversely related to GFR [27]. Clearance by conventional dialyzers is negligible as these membranes are impermeable to β2m. Production of β2m in normals is 9 mg/ hr/70 kg [27]. Production may be increased in proliferative disorders [28] and rheumatoid arthritis [29] as indicated by high serum levels in the presence of normal renal function.

The routine classical evaluation of nephropathy (any type of renal problems) includes the identification of glomerular and tubular markers in the patient's serum and urine. The normal individual doesn't contain this content elevated in their urine or in serum samples. These glomerular and tubular markers include: transferring, Ig G, antitrypsin, β-2-microglobulin and angiotensin converting enzyme (ACE). Recent studies also have demonstrated that, there were tubular components in renal complications of disease conditions as shown by the detection of renal tubular enzymes and low molecular weight proteins in the urine as well as in serum. In fact, tubular involvement may precede glomerular involvement because several of these tubular proteins and enzymes are detectable even before the appearance of microalbuminuria and rise in serum creatinine [30].

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