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ANALYSIS OF LIVER FUNCTION AND BIOCHEMICAL PARAMETERS OF FLUOROSIS AFFECTED RENAL FAILURE PATIENTS IN UDAYAGIRI MANDAL, NELLORE DISTRICT, A. P., INDIA

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ABSTRACT
Fluoride in drinking water in elevated levels develops a situation known as fluorosis. In India it become more problematic in most of the areas includes Andhra Pradesh. Present study has been conducted in Udayagiri mandal, Nellore district people with renal failures under the influence of fluoride water consumption. Reports proved the elevation levels of the serum and urinary fluoride as well creatinine levels. Liver function tests were conducted to know a specific mechanism of the fluoride with renal failures. But studies showed that there are not many alterations in the liver function mechanism.

INTRODUCTION

Fluorosis, turns out to be the most widespread geochemical disease in India, affecting more than 66 million people including 6 million children under 14 years age. Though fluoride has spread its tentacles in 36,988 habitations and the number of people falling prey to fluoride poisoning have been steadily increasing, an exact exposure-health relationship is yet to be properly elucidated. There is an essential relation between poverty and fluorosis as malnutrition is found to play an aggressive role in its severity (Ozsvath, 2009; Manik Chandra and Biswapati, 2009). Fluoride is beneficial to health if the concentration of the fluoride ion (F-) in drinking water is less than 1.5 mg/L (WHO, 1994). A higher concentration causes serious health hazards. The disease caused manifests itself in three forms, namely, dental, skeletal, and non-skeletal fluorosis. The National Academy of Sciences has studied the possibility of adverse health effects from continuous consumption of fluoride over long periods of time. The Academy reported daily intake required to produce chronic toxicity after years of consumption, is 20 to 80 milligram or more per day depending upon body weight. This level of fluoride intake has been associated with water supplies containing at least 10 mg/L of natural fluoride, as in parts of India and where water consumption was high because of extreme climatic conditions.

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 (Nawalakhe and Paramasivam, 1993). It must be noted that the problem of excess fluoride in drinking water is of recent origin in most parts. Digging up of shallow aquifers for irrigation has resulted in declining levels of ground water. As a result, deeper aquifers are used, and the water in these aquifers contains a higher level of fluoride (Gupta and Sharma, 1995; Ozsvath, 2009; Manik Chandra and Biswapati, 2009).

In India it is the foremost problem in different parts of the country. Andhra Pradesh is also become popular with the curse particularly districts like Nalgonda. All 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. Any abnormalities in the liver due to the toxicants will develop the free radical mediated damage, where the detoxification can be done at liver it self. Hence we have gone for the analysis of liver function under fluoride toxicity of Nellore district fluoride threatens people (Andhra Pradesh, India).

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. 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.

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 (Bellack and Schouboe, 1958). 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 an 'One Touch Ultra' blood glucose meter (Accu Chek Glucometer, USA).

Evaluation of liver function tests (LFT)

Fasting blood samples were taken from the control and problematic subjects. Part of each serum sample was used for the usual tests performed by the preventive medicine center, and the remainder was conserved at -20ºC until assay related to the current study. Serum levels of total and conjugated bilirubin were measured by Jendrassik and Grof (1938) method. Serum alkaline phosphatase (ALP) activity was measured using the method of Bessey *et al*. (1946), serum alanine amino transferase (ALT; formerly serum glutamic pyruvate transaminase-SGPT) activity was measured using the method of Henry *et al*. (1960) and serum gamma glutamyl transferase (GGT) activity was measured by Szasz's colorimetric method (Szasz, 1969). All of the methods described were adapted for automated analysis (Automatic analyzer). Serum aspartate aminotransferase (AST; formerly serum glutamate oxaloacetic transaminase-SGOT) activity was measured by a technique derived from the technique of the Societe´ Franc¸aise de Biologie Clinique (Mathieu *et al.,* 1982). Enzyme activity values are expressed as IU/L at 30ºC.

Serum total protein and albumin were analyzed using the biuret and bromocresol green methods, respectively. In both cases, commercially available test kits, products of Randox laboratories, U.K. were used and with the manufacturers instructions strictly adhered to. Serum globulin was determined as the difference between serum total protein and albumin.

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

Udayagiri mandal of Nellore district, Andhra Pradesh, India seems to be more threaten area of fluoride toxicity in drinking water was noticed recently. 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 (1984).

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 (1977). 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.

A detailed questionnaire has been prepared and data has been gathered from the villagers of selected three villages (Questionnaire not enclosed). This includes the personal details like age, sex, duration of stay in the specified area, drinking water source, and parental history and present or

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	113.58 ± 15.83	1.43 ± 0.35
Fluoride affected (n = 90)	130/90 ± 10	125.59 ± 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

previous experience of diseases like diabetes and hypertension. It helped us to omit the people suffering with hypertension or diabetes, whereas they may be chance of getting the renal failures with the hypertension or diabetes. After screening of the data we have selected a sum of 90 people, who are never suffered with hypertension or diabetes.

After selection we have collected their urine and serum samples for the assay of fluoride content. While collecting the samples we have noticed their details like height, weight, waist and hip ratio and other details (Data entry sheet enclosed). 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 ($p < 0.001$) and there was not much change when compared to that of control value. Mean value of RBS showed to be 125 mg/dl, whereas control mean value is 113 mg/dl (Table 3).

Similar types of studies under fluoride toxicity with reference to dental and skelaetal fluorosis has been made by several workers (Saralakumari and Ramakrishna Rao, 1993; Shivashankara *et al.,* 2000).

Measuring serum creatinine is a useful and inexpensive method of evaluating renal dysfunction. Creatinine is a non-protein waste product of creatine 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). 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. Present study reveals that there was a drastic increase, almost doubled with the control value indicates the loss of renal function and symptoms of renal failure (Table 3). 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.

Table 4 explains about the detailed analysis of LFTs. Here we can find a minute enhancement with the control values. But there was no one parameter showing drastic change. Al most all parameters are in normal range reference values. SGOT and SGPT enzyme activities were found to be a slight decreased when compared to control values. Serum bilirubin is also in normal range both in control and treated subjects. Protein content incases of albumin and globulin seems to be slightly increased (Table 4). That indicates fluoride is not showing much toxicity in the liver and even enzymatic activities were also seems to be not much elevated. Thus LFT results indicated there was not much significantly ($p < 0.001$) altered in the fluoride affected people. Fluoride is known to inhibit protein synthesis, mainly due to impairment of peptide chain initiation (Hoerz and McCarty, 1971) and by interfering with peptide chains on ribosomes (Ravel *et al*., 1966). That indicates fluoride is not showing much toxicity in the liver and even enzymatic activities were also seems to be not much elevated. Thus LFT results indicated there was not much significantly ($p < 0.001$) altered in the fluoride affected people. The increased levels of serum transaminases in fluorotic individuals suggest alteration in liver function. These levels increase several times if cellular damage occurs in the liver, so these enzymes are markers for assessing liver function.

Table 4: Analysis of the liver function tests of the normal and fluoride affected peoples

	Control (n = 50)	Fluoride affected (n = 90)	SEM	Significance
Total Bilirubin (mg/dL)	0.818 ± 0.09	0.829 ± 0.08	0.0147	$p < 0.001$
Indirect (mg/dL)	0.168 ± 0.03	0.167 ± 0.03	0.00606	$p < 0.001$
Direct (mg/dL)	0.654 ± 0.79	0.664 ± 0.06	0.0121	$p < 0.001$
ALP (U/L)	144.04 ± 30.42	155.14 ± 25.84	4.973	$p < 0.001$
SGOT (IU/L)	31.71 ± 3.21	30.18 ± 3.43	0.660	$p < 0.001$
SGPT (U/L)	30.20 ± 3.67	27.96 ± 3.31	0.637	$p < 0.001$
Total Protein(g/L)	6.63 ± 0.49	6.87 ± 0.45	0.0871	$p < 0.001$
Serum Albumin (g/L)	3.07 ± 0.53	3.69 ± 0.39	0.0745	$p < 0.001$
Serum Globulin (g/L)	3.49 ± 0.74	3.40 ± 0.86	0.166	$p < 0.001$
A/G Ratio	0.92 ± 0.28	1.14 ± 0.17	0.0327	$p < 0.001$

Hence from this study we can conclude that the fluoride content of the selected villages seems to be very high and much influencing the serum as well as fluoride content. But there was not many alterations in the liver function tests, which indicate fluoride is not influencing the liver activity in the fluoride, threaten people.

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