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Biochemical and Haematological Investigations on Fluorosis Threaten Patients at Nellore District, Andhra Pradesh, India

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Abstract: Fluoride is beneficial to health if the concentration of the fluoride ion in drinking water is less than 1.5 mg/L. Excess levels of fluoride in drinking water leads to the development of fluorosis. 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 fluorosis in means of biochemical and haematological parameters. Results showed three villages seem to be high levels of water fluoride content. Fluoride consumption leads to the development of altered haematogram. But not much effected when compared to the healthy individuals.

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

INTRODUCTION

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 [1]. 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 [2-4].

Fluoride is beneficial to health if the concentration (CF) of the fluoride ion in drinking water is less than 1.5 mg/L [1]. A higher concentration causes serious health hazards. The disease caused manifests itself in three forms, namely, dental, skeletal and non-skeletal fluorosis [2]. Skeletal fluorosis causes crippling and severe pain and stiffness of the backbone and joints [2]. Even though extensive studies have been conducted, there seems to be no effective cure for these diseases. Therefore, it is desirable to drink water having a fluoride concentration less than certain value. Hence, drinking water with CF > 1.5 mg/L (1 mg / L in India) needs treatment [4]. As a result, deeper aquifers are used and the water in these aquifers contains a higher level of fluoride [5, 6].

Fluoride toxicity is the more abundant threat to the common people who are living in the content areas in the globe. Fluoride toxicity will affect all the parts of the human system leads to the altered life span. 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. 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 related to clinical and haematological studies, it has been established.

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 specifically clinical and haematological alterations, the study has been designed. From this background the study was started in the Nellore district region of Andhra

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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.

Analysis of Water Quality and Fluoride Content: A total of 10 samples was 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 [7]. 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.

Hematological Findings: Only those who volunteered were included. An early morning visit was made on a mutually agreed date by a laboratory technician. Physical details were recorded and blood samples were drawn by venipuncture from each member and placed in a heparinized tube. The whole blood was transported at 4°C to the laboratory whereas all the analyses were conducted on the day of blood collection. The hematological parameters were estimated on a Coulter Counter ZF6 with a hemoglobinometer attachment. Blood smears were prepared from fresh blood and air dried. The red cell morphology was assessed after appropriate staining. Hemoglobin (Hb) was determined spectrophotometrically (540 nm) using cyanomethemoglobin method and expressed as g/100ml. Red blood cell counts (RBC) were made in Neubauer chamber with Hayem dilution solution. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated according to Ranzani - Paiva [8]. The red cells were separated from the plasma by centrifugation and washed twice with cold physiological saline. Complete blood picture (CBP) was got through the Semi auto analyzer (Model CHEM 400), Electronics India, India.

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 the more threaten area by fluoride toxicity 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 [9].

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: Analysis of the hematological (complete blood picture) parameters of the normal and fluoride affected peoples

	WBC	RBC	Hb%	HCT	MCV	MCH
Control (n=50)	5.20±1.89	4.76±0.49	12.65±1.79	41.92±10.82	92.16±7.59	26.51±1.61
Fluoride affected (n=90)	6.05±1.84	4.66±0.53	11.91±1.93	38.46±5.11	81.97±4.94	25.15±2.40
SEM	0.403	0.105	0.382	2.308	1.619	0.345
Significance	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001

Table 3: Analysis of the hematological (complete blood picture) parameters of the normal and fluoride affected peoples

	MCHC	PLT	RDW-SD	RDW-CV	MPV	P-LCR
Control (n=50)	28.86±1.62	151.47±65.27	48.19±4.63	14.81±0.78	11.66±1.23	40.95±10.47
Fluoride affected (n=90)	30.85±1.47	198.22±76.43	42.16±2.33	13.83±2.67	10.15±3.23	31.78±15.81
SEM	0.345	13.916	0.987	0.168	0.588	2.868
Significance	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001

Table 4: Analysis of the hematological (complete blood picture) parameters of the normal and fluoride affected peoples

	LYM%	MXD%	NEUT	LYM#	MXD#	NEUT#
Control (n=50)	38.62±12.36	2.57±1.80	41.50±11.93	2.04±0.79	0.14±0.12	2.25±1.36
Fluoride affected (n=90)	44.26±11.74	8.58±4.70	37.64±17.46	2.73±0.77	0.51±0.28	2.51±1.70
SEM	2.635	0.462	5.659	0.169	0.0294	0.378
Significance	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001

After knowing this, studies were further extended to know the haematological alterations in the selected individuals. Complete blood picture can provide a clear picture of the cellular as well as chemical components of the living system. Hence, studies were conducted in the control as well fluoride affected people. Selected objects blood samples were used for the analysis. Table 2 shows some of the important haematological parameters like white blood cells (WBC), RBC, Hb percentage, haematocrit (HCT), MCV and MCH. Results showed that there was a slight increase in WBC content, shows a significant ($p<0.001$) increase. But in the case of other parameters there was a different response. Except WBC reaming all mentioned parameters showed to be significantly ($P<0.001$) decreased. In the case of HCT and MCV there was a much difference when compared to that of controls. In case of controls HCT and MCV showed 41.92 and 92.16, respectively, but in treated the values decreased to 38.46 and 81.97, respectively (Table 2).

Table 3 shows the continuation of haematological parameters like MCHC, platelets (PLT), standard deviation for red cell distribution width (RDW-SD), coefficient variation of red cell distribution width (RDW-CV), mean platelet volume (MPV) and preliminary results of platelet larger cell ratio (P-LCR). Here also similarly there was significant ($p<0.001$) increase in the case of MCHC and PLT i.e. 28.86 and 151.47 were observed in controls, whereas treated subjects showed 30.85 and 198.22, respectively. Remaining all parameters were shown to be decreased (Table 3). Table 4 shows the continuation of parameters of haematology like lymphocyte (LYM) percentage, mixed cell count (MXD) percentage, neutrophylls (NEUT), lymphocytes number, MXD number and NEUT number. Here also we can find increase in some of the parameters like LYM percentage, MXD percentage, lymphocytes number, MXD number and NEUT number. Particularly drastic increase was observed in MXD percentage i.e. 2.57 in control whereas fluoride affected persons showed 8.58, which shows a significant ($P<0.001$) increase. Next to this there was a significant ($P<0.001$) increase noted in case of LYM percentage. Control value shows 38.62 whereas fluoride threatens subjects showed 44.26 (Table 4).

Even though the knowledge of science has been improved rapidly, the studies related to the fluoride toxicity and development of renal failure is not much focused area. Most of the fluoride toxicity related to tooth and bone has been exploited. But, studies related to this area are scanty. To fill the scientific gap present study has been developed. The present study has been conducted in the Nellore district which was recently noted by the government as well as newspapers as most threaten area. Nellore is the geographically south part of the country very near to Bay of Bengal. Particularly the sub areas in this district like Udayagiri mandal become more susceptible to fluoride poisoning. Especially people

belongs to this area are getting frequent renal problems and admitting to the hospitals and are even deaths also noted. This information was initiated us to conduct a research work on the fluoride mediated renal damage and its involvement with several biological parameters. The study was started with 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). Similar type analysis in the drinking water fluoridation has been earlier reported by several workers [10-14]. 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.

Complete blood picture can provide a clear picture of the cellular as well as chemical components of the living system. Selected objects blood samples were used for the analysis. Table 2 shows some of the important haematological parameters like WBC, RBC, Hb percentage, HCT, MCV and MCH. There are only a few reports in the literature of anemia in fluorotic individuals. Haemoglobin levels in the endemic villages were low, compared to those in the control population. Though the difference between mean values was not significant, individual values in the endemic population showed great fluctuation. Though fluoride is capable of causing anemia, the haemoglobin level is also governed by the individual's nutritional status. Decreases in haemoglobin and erythrocyte count were also reported in camels near a super-phosphate factory [15]. Results showed that there was a slight increase in WBC content, shows a significant ($p<0.001$) increase. But, in the case of other parameters there was a different response. Except WBC reaming all mentioned parameters showed to be significantly ($P<0.001$) decreased. In the case of HCT and MCV there was a much difference when compared to that of controls. In case of controls HCT and MCV showed 41.92 and 92.16, respectively, but in treated the values decreased to 38.46 and 81.97, respectively (Table 2). Table 3 shows the continuation of haematological parameters like MCHC, PLT, RDW-SD, RDW-CV, MPV and P-LCR. Here also similarly there was significant ($p<0.001$) increase in the case of MCHC and PLT i.e. 28.86 and 151.47 were observed in controls, where treated subjects showed 30.85 and 198.22, respectively. Remaining all parameters were shown to be decreased (Table 3).

Table 4 shows the continuation of parameters of haematology like LYM percentage, MXD percentage, neutrophylls, lymphocytes number, MXD number and NEUT number. Here also we can find increase in some of the parameters like LYM percentage, MXD percentage, lymphocytes number, MXD number and NEUT number. Particularly drastic increase was observed in MXD percentage i.e. 2.57 in control where as fluoride affected persons showed 8.58, which shows a significant ($P<0.001$) increase. Next to this there was a significant ($P<0.001$) was noted in case of LYM percentage. Control value shows 38.62 whereas fluoride threatens subjects showed 44.26 (Table 4).

The various biochemical parameters investigated in this study are useful indices of evaluating the toxicity of fluoride in human beings [16, 17]. Assessment of haematological parameters can be used to determine the extent of deleterious effect of fluoride on the blood of an animal [18, 19]. It can also be used to explain blood relating functions of a metal or its chemical products [16]. Such analysis is relevant to risk evaluation as changes in the haematological system have higher predictive value for lower animals or human toxicity, when the data are translated from animal or human studies [20]. The non-significant effect of the fluoride on the RBC may be an indication that the balance between the rate of production and destruction of the blood corpuscles (erythropoiesis) was not altered. MCHC, MCH and MCV relates to individual red blood cells while Hb, RBC, PCV, LUC and RCDW are associated with the total population of red blood cells. Therefore, the absence of significant effect of fluoride on RBC, Hb, MCH, MCHC and RDW could mean that neither the incorporation of haemoglobin into red blood cells nor the morphology and osmotic fragility of the red blood cells was altered [21]. The decreased MCV and the elevated levels of PLT and MXD percentage in the fluoride affected people further suggest selective toxicity of the fluoride and its components. The significant increase in the lymphosites and MXD could possibly suggest enhancement in the ability of the blood component to phagocytose. Lymphocytes are the main effector cells of the immune system [21, 22]. The enhancement in the lymphocytes in this study may affect the effector cells of the immune system. All these alterations suggest selective toxicity of the drinking water fluoride on the haematological parameters investigated in this study.

From this study it was concluded that, among the ten villages Varikunta padu, Kolangadi palli and Gangireddy palli drinking water have high fluoride content.

Biochemical and haematological findings of the three selected village peoples were affected by the fluoride concentration.

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