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### **Clinical studies of fluoride concentration on human beings**

Gupta Pankaj\*, Sharma Sunil Kumar, Sharma Sanjay

Department of Chemistry, Alwar Institute of Engineering and Technology  
MIA, Alwar (Raj.), INDIA.

#### **ABSTRACT**

Fluoride endemic and non endemic area were selected through ground water sample of Alwar and Dausa Districts. Three different locations containing 5.8 ppm, 9.5 ppm and 12.5 ppm fluoride were identified as fluoride endemic zone. Blood samples were collected from villagers of Alwar and Dausa Districts under supervision of medical staff. The samples were analyzed for the study of haemoglobin, TRBC and TWBC using standard techniques. The result revealed that persons residing in high fluoride endemic zone contained significantly high fluoride concentration in serum as compared to persons residing in non-endemic area. The concentration of fluoride in blood is directly proportional to the concentration of fluoride in ground water. The ascorbic acid concentration depleted significantly with increase in the concentration of fluoride in drinking water, indicative of mobilization of ascorbic acid with the increase of fluoride intake. The total Erythrocyte, packed cell volume and haemoglobin percentage declined significantly following fluoride water ingestion and decrease was found to be dose dependent. However the Leucocytes number increased to overcome from fluorosis condition in the subjects. The data suggests that the ground water of Alwar and Dausa Districts contain very high fluoride concentration which results in altered blood physiology hence not good for human health.

**KEY WORDS:** Fluoride, Blood Serum, Hemoglobin, RBC, WBC.

#### **\*Corresponding Author:**

**Dr. Pankaj Gupta**, Professor and Head,  
Department of Applied Sciences and Environmental Studies,  
Alwar Institute of Engineering & Technology,  
MIA, Alwar (Raj) INDIA  
E- mail: [pgupta1975@yahoo.com](mailto:pgupta1975@yahoo.com)

## INTRODUCTION

The World Health Organization monograph on Fluorides and Human Health contains an extensive review of studies that have reported the fluoride content of drinking water sample<sup>1</sup>. Hodge and Smith<sup>2</sup>, in 1970, estimated that 0.3 ppm to 0.8 ppm of fluoride was consumed daily from the water in a non fluoridated area. The total fluoride intake, including water, reported by Spencer et al.<sup>3</sup> in a fluoridated area, is generally 3 ppm to 4 ppm/day. Other articles from the same laboratory also reported surprisingly high fluoride intakes<sup>4,5</sup>. This study comprises drinking water from 03 sites contained a range in fluoride intake of 5.8 ppm, 9.5 ppm and 12.5 ppm in endemic zone.

## MATERIALS AND METHODS

Clinical studies of fluoride concentration on human beings was performed in Alwar and Dausa district. Alwar District is situated at Northern East of Rajasthan, it lies between 027°: 32' N latitude and 076°: 35' E longitude and Dausa district is lies between 026°: 51' N latitude and 076°: 21' E longitude.

Water samples were collected from different villages of Alwar and Dausa districts, which have different level of fluoride concentration. Finally from fluoride endemic zone three water samples were selected for study, one was drawn from Laxmangarh Tehsil (code No. 28), recorded 5.8 ppm fluoride concentration. Second sample was collected from Village Baswa of Dausa District (code no. 70), 9.5 ppm fluoride value and last was also from Village Bhansko of Dausa District (code No. 105), and 12.5 ppm fluoride level were analyzed. A controlled standard group sample was drawn from Alwar district, where RO water was used for drinking regularly contains 0.8 – 1.2 ppm fluoride concentration. Blood samples were collected by the help of trained medical staff of the villagers of Laxmangarh Tehsil of Alwar district and villages (Code No 70, 105) of Dausa district and controlled group (Standard)<sup>6,7</sup>.

Fluoridation of drinking water, the development of fluorochemical blood substitutes, and the observation of Taves<sup>6, 7, 8</sup> that human blood serum contains both inorganic fluoride and organic non-ionic fluorine have increased interest in analyzing blood for fluorine. Inorganic fluoride has been determined in blood serum or plasma spectrophotometrically<sup>9</sup>, by gas chromatography<sup>10,11</sup>, with an ion selective electrode<sup>11, 12</sup>, Spectrophotometric determinations<sup>12-16</sup>. These water samples having different concentrations of fluoride are summarized in below table-1.

Blood and urine samples were collected by the help of the trained medical staff of the subjects residing in village (Code No. 28) of Laxmangarh tehsil of Alwar and villages (Code No. 105, 70) of Dausa tehsil of Dausa district and Subjected to analysis for total R.B.C., PVC and total W.B.C. by using standard techniques.

**Table 1: Concentrations of fluoride in different areas**

Sample No.	Site Code No.	Concentration of Fluoride (ppm)	District
1	Std. Sample	0.8 -1.2 ppm	Alwar
2	Code No. - 28	5.8 ppm	Alwar
3	Code No. – 70	9.5 ppm	Dausa
4	Code No. - 105	12.5 ppm	Dausa

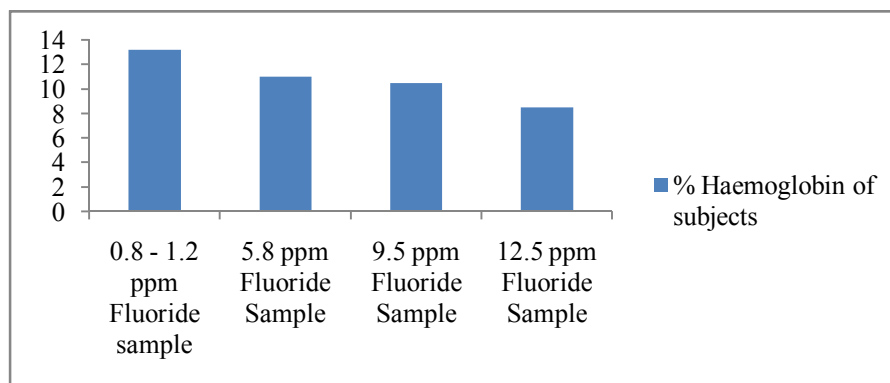
**STUDY OF HAEMOGLOBIN IN DIFFERENT SAMPLE**

Haemoglobin % was determined using Sahil’s haemoglobinometer. N/10 HCl is taken in graduated dilution tube upto mark ‘2’. Keep the dilution tube in the central column. The blood sample is taken by the help of micropipette upto mark 20 cu.mm. Transfer the blood sample in the dilution tube and mixed well with a stirrer and allow it to set for five minutes. Stir well after adding every drop of distilled water. Continue till the colour of mixture matches with the standard colour in the lateral columns. Remove the dilution tube and read the scale of haemoglobin in percentage and gms.

The entire blood samples were analyzed one by one, by above method. The analyzed data are given in Table No. 2 and also expressed as a Figure 1.

**Table 2: % Haemoglobin in different concentration of fluoride sample**

Sample No.	Site Code No.	Concentration of Fluoride (ppm)	Hb %
1	Std. sample	0.8 -1.2 ppm	13.2
2	Code No. - 28	5.8 ppm	11
3	Code No. – 70	9.5 ppm	10.5
4	Code No. - 105	12.5 ppm	8.5



**Figure 1: % Haemoglobin in different concentration of fluoride sample**

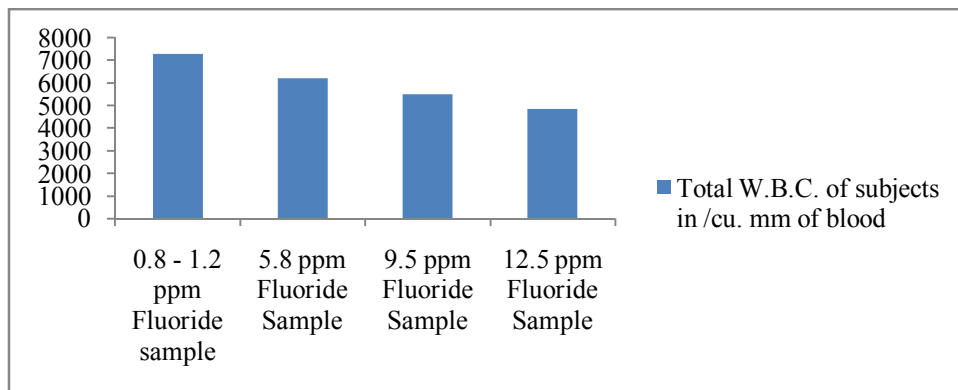
**STUDY OF TOTAL WBC IN DIFFERENT SAMPLES**

Total WBC was determined using haemocytometer. Adjusted the counting area on the Binocular microscope under low power (10X) to see central upper large square. The clean and dry coverslip was

kept over the counting area so as to rest it on two elevations and not exactly coinciding with the edge of Neubauer's Slide, but leaving a small distance at the front. Suck the blood in WBC pipette upto mark 0.5 cu mm from a picked finger of each subjects. Sucked WBC diluting fluid in the pipette upto the mark 11 cu. mm. hold the pipette horizontal between the palms of hands and kept for least one minute for thorough mixing of blood with WBC diluting fluid. The pipette was held at an angle of 45° to the horizontal and first few drops were discarded. The tip of pipette was brought close to the coverslip and a drop was just dropped. The fluid was spread by capillary action. It was kept for two minutes and then examined for uniformity of the field. Counting of WBC was done 16 small squares of left upper large square. Likewise WBC's were counted from the remaining three squares viz., left lower, right upper and right lower. The entire blood samples were analyzed one by one, by above method. The analyzed data are given in Table 3 and also expressed as a Figure 2.

**Table 3: Total WBC count in different concentration of fluoride sample**

Sample No.	Site Code No.	Concentration of Fluoride (ppm)	Total WBC
1	Std. sample	0.8 -1.2 ppm	7300
2	Code No. - 28	5.8 ppm	6200
3	Code No. - 70	9.5 ppm	5500
4	Code No. - 105	12.5 ppm	4850



**Figure 2: Total WBC count in different concentration of fluoride sample**

### **STUDY OF TOTAL RBC IN DIFFERENT SAMPLE**

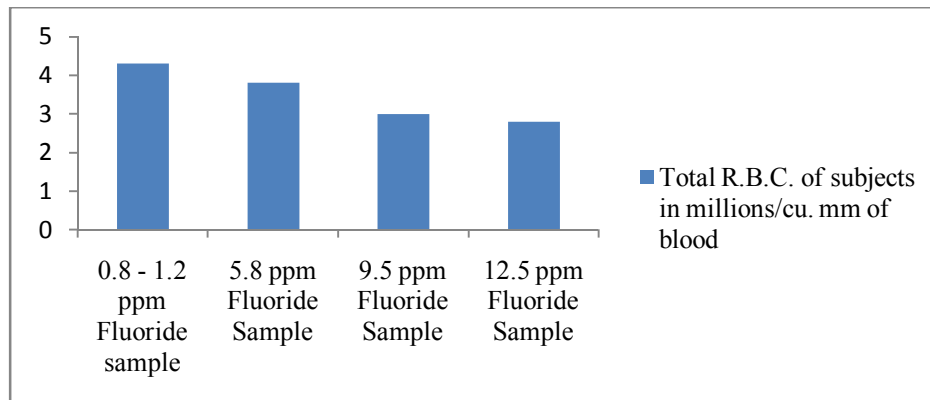
Haemocytometer containing Neubauer's counting chamber, RBC pipette and cover slip, Binocular Microscope with 10X and 40X eye piece, RBC diluting fluid, spirit, pricking needle.

Total RBC was determined using haemocytometer. Adjusted the counting area on the Binocular microscope under low power (10X) to see central large square. The clean and dry coverslip was kept over the counting area so as to rest it on two elevations and not exactly coinciding with the edge of

Neubauer’s Slide, but leaving a small distance at the front. Sucked the blood in RBC pipette upto mark 0.5 cu mm from a picked finger of each subjects. Sucked RBC diluting fluid in the pipette upto the mark 101 cu. mm. hold the pipette horizontal between the palms of hands and kept for least one minute for thorough mixing of blood with WBC diluting fluid. The pipette was held at an angle of 45° to the horizontal and first few drops were discarded. The tip of pipette was brought close to the coverslip and a drop was just dropped. The fluid was spread by capillary action. It was kept for two minutes and then examined for uniformity of the field. Counting of RBC was done from 5 squares, four corners and centre<sup>14,15</sup>.The entire blood samples were analyzed one by one, by above method. The analyzed data are summarized in Table 4 and also expressed as a Figure 3.

**Table 4: Total RBC count in different concentration of fluoride sample**

Sample No.	Site Code No.	Concentration of Fluoride (ppm)	Total RBC
1	Std. sample	0.8 -1.2 ppm	4.3
2	Code No. - 28	5.8 ppm	3.8
3	Code No. – 70	9.5 ppm	3
4	Code No. - 105	12.5 ppm	2.8



**Figure 3: Total RBC count in different concentration of fluoride sample**

## RESULT AND CONCLUSION

The values of all parameters were found to be higher or below than permissible limit which may leads to various health problems called Fluorosis. Data of hemoglobin (Table 02) and TWBC (Table 03) reveals that the fluoride contaminated water makes the villagers are very prone to attack of many diseases. Similarly TRBC count (Table 04) shows that those who are using RO water regularly are much strong and healthy against the diseases. Analysis shows that the fluorosis is also lead to leukemia, anemia, blood hemorrhage and also decrease the power of immunity, because of decreasing

RBC and WBC count. The data suggests that the ground water of study area of Alwar and Dausa Districts contain very high fluoride concentration which results in altered blood physiology hence not good for human health<sup>16</sup>.

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