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Human Red Blood Cell Abnormalities in Fluoride Endemic Area of Warora Tehsil, Chandrapur District, Maharashtra, India

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Abstract: The present study aimed to evaluate the effect of fluoride toxicity on structure of RBC in the population residing in fluoride endemic area of Warora tehsil, Chandrapur district, Maharashtra, India. The concentration of fluoride in water was 1.5-5 ppm. The results revealed multiple discrete blisters on the surface of red blood cells, and formation of leptocytes, stomatocytes, spherocytes, schistocytes, keratocytes, degmacytes, and dacrocytes in patients afflicted with fluorosis. The red blood cells were irregularly shaped with multiple cytoplasmic projections. There was accumulation of erythrocytes with multiple protuberances, processes, perforations, and crypt like excavations. A regularly spaced cluster of four red cells adhering side to side in a stack were observed in rouleaux formation. Morphologic abnormalities caused by fluoride ingestion included marked echinocytosis and ovalocytosis. The red blood cells were irregularly shaped with multiple cytoplasmic projections. Morphologic abnormalities caused by fluoride ingestion included various abnormalities. The majority of red blood cells of fluorotic patients revealed presence of echinocytes and crenated erythrocytes which were characterized by numerous, short, equally spaced blunt to sharp surface projections. This study has confirmed that fluoride is one of the chemical agents responsible for inducing RBC deformities.

Keywords: Fluoride toxicity, RBC deformities, Fluorosis, Stomatocytes, Spherocytes, Schistocytes, Keratocytes

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Introduction

Fluorosis caused by intake of fluoride has been recognized in India for several decades. It is wide spread in as many as 22 states of the Indian republic. In Maharashtra state 30-50% districts are facing the problem of fluoride. The worst affected districts are Nanded, Chandrapur, Solapur, Yawatmal, Bhandara, Buldhana, Jalgaon, Amravati, Akola, Wardha, and Nagpur (Madhure and Malpe, 2007; Duraiswami, 2007, 2008; Kodate

et al., 2016). Fluoride is known to affect the dental and skeletal systems (Dhurvey and Marganwar, 2013). Earlier findings from our laboratory demonstrated detrimental effects of fluoride on impairment of soft tissue functions in experimental animals (Shashi, 2002, 2003) and in humans (Shashi and Kumar, 2008). Adverse hematological effects of fluoride have been reported including damage to hematopoietic

organs (Eren *et al.*, 2005).

In order to enrich our finding regarding fluoride concentration and its impact on human body, extensive studies were undertaken to estimate specially fluoride distribution in drinking water and dental fluorosis (Marganwar *et al.*, 2013), prevalence and severity of dental fluorosis (Dhurvey and Marganwar, 2013) and Pre-monsoon assessment of groundwater estimation of fluoride level (Kodate *et al.*, 2016).

Several studies have shown that excessive ingestion of fluoride hampers haemopoiesis, alters blood parameters, and affects absorption, excretion, distribution, and retention of several minerals (Bharti *et al.*, 2007). The biochemical changes in glucose metabolism in erythrocytes have been related to the structural and functional alterations of red cells during erythropoiesis by accumulated fluoride in bones. The decreased hematocrit levels are attributed to a decrease in size of erythrocytes due to stressful conditions.

It is now known that when fluoride is ingested, it will also accumulate on erythrocyte membrane, besides other cells, tissue and organ. Raja Reddy and Shrikant (1993) stated that erythrocyte membrane turn looses calcium is pliable and is thrown into folds and attains the shape of an ameaba called echinocyte (Rewlani *et al.*, 2010). Echinnocytes undergo phagocytosis and are eliminated from circulation.

In the light of above concept and data, the present study has been carried out to find out the destruction of RBC in dental fluorotic individuals of study area.

Materials and Methods

The study was conducted on 100 patients affected with dental fluorosis (58 males, 42 females, and mean age 40.12 ± 13.30). The patients were selected randomly from high fluoride area, Warora tehsil of district Chandrapur, Maharashtra, India (water fluoride levels 1.2 - 5 mg/L). The red blood cells (in thin smear slide) were observed by using Leishman stain (Godkar and Godkar, 2003). The

study was approved by the Institutional ethics committee, Nagpur University, Nagpur, India.

Results

In the present study the subject affected with dental fluorosis were selected, various stages of crenation were investigated. Echinocytes were morphologically altered crenated red blood cells, characterized by numerous short, more or less evenly spaced, blunt to sharp surface projections, and have a serrated outline and irregular edges. The cells have deformed and angulated cell periphery with spicule formation. Spiny knobs were regularly dispersed over the cell surface (Figs. 1a, b). There is formation of rouleaux. Rouleaux formation (Pseudoagglutination) is a linear arrangement of red blood cells (coinstack) caused by an increased blood concentration of fibrinogen, globin, or paraproteins. These are prominent in blood smear of fluorotic patients. A regularly spaced cluster of four to five red cells adhering side to side in a stack was observed (Figs. 1c, d).

Study revealed crenated spiny cells ad small tear drop cell piokilocytes and the length tail vary from cell to cell and alsoknown as dacrococytes (Figs. 1e, f). In the present investigation knizocytes were observed. Knizocytes "Pinched" cells are triconcave red cells that have a central bar of haemoglobin and clear spaces on either asides (Fig. 2g). Leptocytes were observed in dental fluorotic individual in the present study. Leptocytes are thin, flattened red cells with excessive surface area compared to its content. These cells tend to fold and appear as folded bowl-shaped cells (Fig. 2h). Some RBCs undergo structural changes and form central V shape. Three-dimensional appearance of a red cell showed characteristic central V- shaped hemoglobinized thick rim surrounded by an area of pallor (Fig. 2i).

In the present investigation, RBC of dental fluorotic individual shows outcome of spherical bodies from RBCs. The surfaces of many red cells

Fig. 1 a-f: Photomicrograph of various forms of pathologic erythrocytes from fluorotic patients. a-b: echinocyte (100x), c-d: Rouleux formation (100x), e: crenated erythrocyte (100x), f: dacrocyte (100x).

are studded with small spherical bodies. The cells showed small protrusions on the cell surface (Fig. 2j). The results revealed that the red blood cell from fluorotic individuals exhibited presence of many poikilocytes such as ovalocytes (Fig. 2k).

Torocytes were observed in the dental fluorotic individuals from study area. Torocytes were ring-shaped red cells with a sharply defined clear central area and a thickened peripheral ring of haemoglobin. These are resulted from the

Fig. 2 g-l: Photomicrograph of various forms of pathologic erythrocytes from fluorotic patients. g: Knizocyte (100x), h; Leptocyte (100x), i: red blood cell with central 'V' shaped rim (100x), j: red blood cell with spherical bodies (100x), k: Ovalocyte (100x), l: Torocyte (100x).

peripheral redistribution of haemoglobin (Fig. 2l).

Discussion

It is well known that erythrocyte membrane is the primary site of action of fluoride intoxication. The structural alteration of red blood cell is well correlated with the kinetics of fluoride toxin in mice (Jacyszyn and Marut, 1986; Machalinska *et al.*, 2002) and in human (Machalinski *et al.*, 2000).

Earlier study reported that the fluoride when consumed in excess concentration affects virtually in every phase of human metabolism. It can readily penetrate cell membrane including those of erythrocyte and disturb the structure of cell membrane of RBC (Sarala Kumari and Rao, 1991). In the present study the red blood cells are irregularly shaped with multiple cytoplasmic projections in fluorotic individuals. This is in

agreement with observations of Kamble and Velhal (2010) and Samanta and Bandyopadhyay (2012) in experimental animal.

Fluoride causes disruptive effect on erythropoiesis, enhanced production of superoxide radicals and lipid peroxidation that lead to alterations in erythrocytes cell membrane function and structure in humans. The observations presented here exhibit morphologic abnormalities in the shape of human red blood cell during fluoride toxicity.

The present investigation reveals multiple discrete blisters on the surface of red blood cells, and formation of leptocytes, echinocytosis, ovalocytosis and dacrocytes in patients afflicted with fluorosis. A regularly spaced cluster of four red cells adhering side to side in a stack are observed in rouleaux formation. Similar results have been reported by Fischer (1986); Sarala Kumari and Rao (1991); Rawlani *et al.* (2010); Samanta and Bandyopadhyay (2012) and Shashi and Meenakshi (2012) in human fluorotic individuals.

High fluoride concentration may disturb the anion channel of erythrocytes membrane, which leads to hemolysis and swelling of cells (Grabowska *et al.*, 1991). The correlation between fluorosis and RBC membrane destruction has been explained by Shantakumari and Subramanian (2007). Therefore, it is concluded that excessive fluoride causes toxic effect on membrane of red blood cells of human being and alter the structure and functions of the cell.

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