NUCLEO-CYTOPLASMIC ALTERATIONS OF BUCCAL CELLS IN IRON DEFICIENCY ANEMIA

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Background & Objectives: Although iron deficiency is associated with constant involvement of oral mucosa, the effect of iron on cell cycle inhibitory molecules has been superficially studied. The present study was undertaken to evaluate changes in Nuclear Diameter (ND), Cytoplasmic Diameter (CD) and Nuclear / Cytoplasmic (N/C) Ratio in buccal smears in cases having iron deficiency anemia (IDA). **Methods:** The study was carried out in the Department of Physiology in collaboration with Department of Medicine at S.M.S. Medical College, Jaipur, Rajasthan. This was a case control type of study. The study group consisted thirty iron deficient cases and thirty controls of age group 20-30 years. Cytoplasmic Diameter (CD), Nuclear Diameter (ND) and Nuclear / Cytoplasmic (N/C) Ratio was assessed from the buccal mucosa and correlated with various hematological parameters. **Results:** The mean value of ND in IDA was significantly higher as compared to control cohort. Mean N/C ratio was significantly higher as compared to control cohort. On comparing Serum iron with the ND values, moderate negative correlation was found. Regression equations were assessed between Serum Iron and ND and N/C Ratio. **Interpretation and Conclusion:** The increase in ND and N/C ratio with decrease in serum iron levels suggested that iron deficiency causes significant changes in buccal mucosal cells. Further studies should be conducted to assess the influence of iron on Cell cycle kinases and inhibitors in various cells.

Key words: Nuclear Diameter, Cytoplasmic Diameter, Nuclear / Cytoplasmic (N/C) Ratio, Iron Deficiency Anemia.

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Introduction:

Iron deficiency anemia is the most common nutritional deficiency anemia of the world. In developing countries, Iron deficiency anemia is usually caused by lack of iron due to blood loss, insufficient dietary intake or poor absorption of iron from food. Iron deficiency is allied with relatively constant involvement of oral structures. The relationship between iron deficiency and oral epithelial changes still remains unclear[1,2,3].

The evolution of recent techniques of oral exfoliative cytology invites a resurgence of role of iron on cellular parameters. Moreover, the effect of Fe on cell cycle inhibitory molecules has only been ostensibly studied[4].

Scarce mention has been made of the condition of the oral mucosa in the cases so far examined, and it therefore seems highly relevant to discover whether the nucleocytoplasmic changes in buccal cells are dependent on the deficiency of Iron.

In this background and due to shortage of available literature on the histology of oral mucosa in iron deficiency, the present study was undertaken to evaluate the changes in Nuclear Diameter (ND), Cytoplasmic Diameter (CD), Nuclear / Cytoplasmic (N/C) Ratio in cytological buccal smears with isolated Iron Deficiency Anemia (IDA).

Material and Methods:

The study group consisted of thirty iron deficient cases and thirty controls in the age group of 20 – 30 years who were subjected to the inclusion and exclusion criteria.

The study was carried out in the Department of Physiology in collaboration with the Department of Medicine at S.M.S. Medical College, Jaipur, Rajasthan after obtaining approval from the Ethical

committee (No: 1392 MC/EC/2015). This was a case-control type of study.

Inclusion criteria:

- Age: 20 – 30 years, male or female.
- Isolated Iron deficient anemic patients as cases (Serum iron below 40 µg/dl)
- Who gave written informed consent for the study and were co-operative and capable of understanding the procedure.

Exclusion criteria:

- Subjects with history of any acute or chronic illness.
- Any visible oral lesions.
- Smokers, Alcoholics, Tobacco chewers.
- Subjects taking treatment for iron deficiency.

After obtaining clinical history, Hematological parameters involving hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) were performed using *Advanced Fluorescence Flow Cytometry*. Serum ferritin was assessed by *Chemiluminescent immunometric assay*, Serum iron and Total Iron Binding Capacity (TIBC) by *Spectrophotometry with Fully automated Beckman coulter AU680 analytical system* for both the groups.

Scrapings were obtained with the help of a wooden spatula from the right buccal mucosa and spread on a glass slide which was immediately fixed with 95% alcohol. The slides were stained with H & E stain and mounted on the same day. Images of the buccal mucosal cells were acquired, assessed and labeled using TSview7 software.

Twenty cells for each patient were randomly selected in a zigzag manner. The Nuclear Diameter and Cytoplasmic Diameter of the buccal mucosal cells were obtained by drawing a line across the diameter using digitizer cursor in both the axes. The mean and standard deviation of the cytoplasmic diameter (CD), nuclear diameter (ND) and the nuclear/cytoplasmic (N/C) ratio was calculated for each case.

Statistical Analysis:

Statistical analysis was performed with the SPSS, Version 23 software. Variables were compared using unpaired t- Test and Relationship between variables in the groups was assessed by using Pearson's correlation coefficient and linear regression. Probability P value <0.05 was considered as statistically significant.

Result:

Age and sex did not show any significant influence on the CD, ND and N/C ratio values of iron deficiency anemia and the control cohort.

The mean CD and ND values of Iron deficiency were compared with the control group and the following findings were observed (Table -1):

Table -1

	Group	Mean ± SD	T	P
Serum Ferritin (ng/ml)	**IDA**	15.53 ± 11.527	7.444	0.000 *
	Control	72.00 ± 39.917		
Serum Iron (µg/dl)	**IDA**	27.33 ± 6.535	-9.077	0.000*
	Control	77.05 ± 29.281		
Serum TIBC (µg/dl)	**IDA**	352.67 ± 52.340	5.015	0.000 *
	Control	300.17 ± 23.421		
Mean CD (µm)	**IDA**	73.15 ± 4.051	-7.734	0.000*
	Control	79.64 ± 2.178		
Mean ND (µm)	**IDA**	13.02 ± 0.455	8.167	0.000*
	Control	12.12 ± 0.397		
N/C Ratio	**IDA**	0.178 ± 0.0099	12.135	0.000*
	Control	0.152 ± 0.0062		

The mean values of Cytoplasmic diameter in iron deficiency anemia was significantly lower as compared to controls while mean values of Nuclear diameter and Nucleo cytoplasmic ratio in iron deficiency anemia were significantly higher as compared to controls.

Table 2: Correlation between Serum Iron & Cytological Parameters:

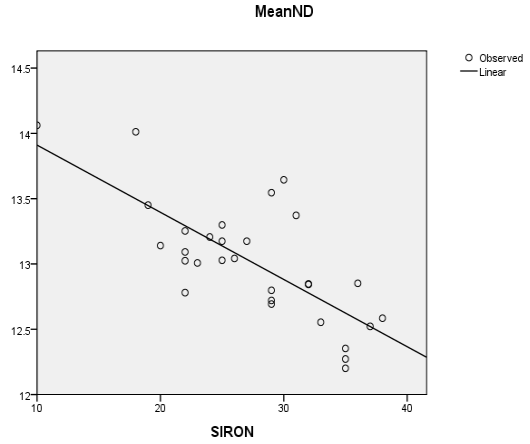
		Mean CD (µm)	Mean ND (µm)	N/C Ratio
Serum Ferritin (ng/ml)	**IDA**	0.078	-0.590**	-0.437*
	Control	-0.100	0.199	0.222
Serum Iron (µg/dl)	**IDA**	- 0.047	- 0.739**	- 0.410*
	Control	- 0.300	0.090	0.274
Serum TIBC (µg/dl)	**IDA**	0.193	0.196	-0.070
	Control	0.084	0.300	0.183
**. Correlation is significant at the 0.01 level (2-tailed).				
*. Correlation is significant at the 0.05 level (2-tailed).				

On comparing values of Serum iron in Iron deficiency anemia, a moderate negative correlation was found with the nuclear diameter values (r= -0.739**, p=0.000) and nucleocytoplasmic ratio (r= -0.410**, p=0.000).

A moderate negative correlation (-0.590**, p=0.000) was found in iron deficiency anmia between serum ferritin and nuclear diameter. On comparing Serum ferritn with the nuceocytoplasmic ratio in Iron deficiency anemia, moderate negative correlation (r= -0.437*, p=0.000) was found.

But no correlation could be found between serum iron and serum ferritin when compared with mean cytoplasmic diameter in IDA.

Figure 1: Scatter plot:- Correlation of Serum iron and Nuclear diameter (In IDA):



Regression equation for Serum Iron and ND (in IDA):

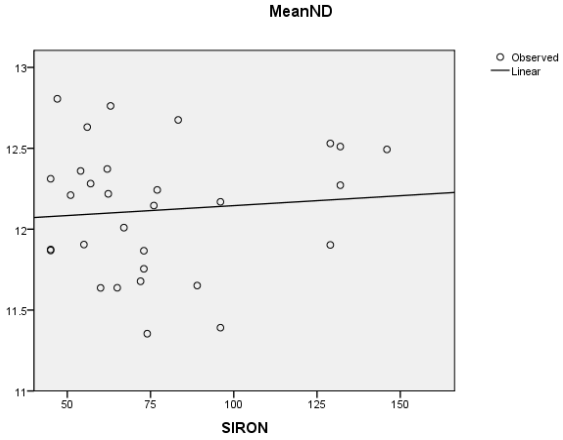
$Y = a + bX = 14.425 – 0.051X$ (R^2 =0.546)

Where Y = Dependent Variable = ND

X = Independent Variable = S. Iron

Regression Equation and Coefficient of Determination clearly indicate that if S. Iron decreases, than ND significantly increases.

Figure 2: Scatter plot:- Correlation of Serum iron and Mean Nuclear diameter (control group)



Regression equation for Serum Iron and ND in Control group:

$Y = a + bX = 12.023 + 0.001X$ (R^2 =0.008)

Where Y = Dependent Variable = ND

X = Independent Variable = S. Iron

Regression Equation and coefficient of determination are clearly indicated that S. Iron and ND are not significantly correlated.

Regression equation for Serum Iron and CD in IDA:

$Y = a + bX = 73.941 – 0.029X$ (R^2=0.062)

Where Y = Dependent Variable = CD

X = Independent Variable = S. Iron

Regression Equation and coefficient of determination clearly indicate that Serum Iron and Cytoplasmic Diameter are not significantly correlated.

Regression equation for Serum Iron and CD in control:

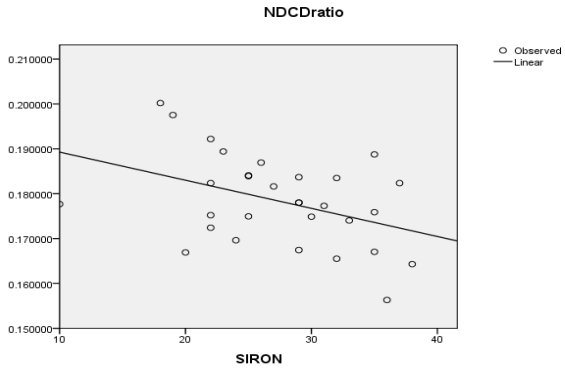
$Y = a + bX = 81.36 – 0.022X$ (R^2 =0.09)

Where Y = Dependent Variable = CD

X = Independent Variable = S. Iron

Regression Equation and coefficient of determination clearly indicate that S. Iron and CD are not significantly correlated.

Figure 3: Scatter plot:- Correlation of Serum iron and ND/CD Ratio (In IDA):



Regression equation for Serum Iron and N/C Ratio (IDA):

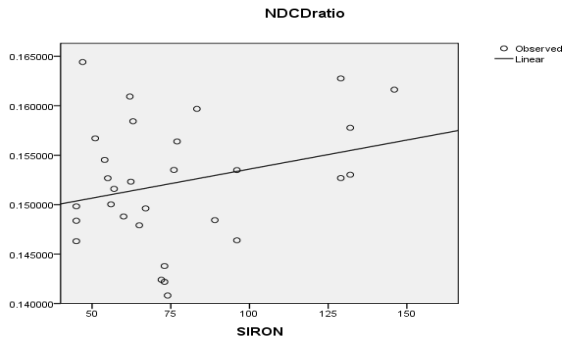
$Y = a + bX = 0.196 – 0.001X$ (R^2 =0.168)

Where Y = Dependent Variable = N/C ratio

X = Independent Variable = S. Iron

Regression Equation and coefficient of determination clearly indicate that if S. Iron decreases, than N/C ratio significantly increases.

Figure 4: Scatter plot:- Correlation of Serum iron and ND/CD Ratio (In Control group):



Regression equation for Serum Iron and N/C Ratio (Control group):

$Y = a + bX = 0.148 + 0.00005X$ (R^2 =0.075)

Where Y = Dependent Variable = N/C ratio

X = Independent Variable = S. Iron

Regression Equation and coefficient of determination clearly indicate that S. Iron and N/C ratio are not significantly correlated.

Discussion:

Iron deficiency anemia is a global problem and develops due to dearth of iron for hemoglobin formation[5,6]. A wide variety of non erythroid changes in IDA were described in man and animals by Daliman (1974)[7] and reviewed by Jacobs (1982)[8] and include nail changes, atrophic gastritis and changes in oral epithelium. Paterson (1919)[9] earliest described the microscopic changes of oral mucosa in anemia.

Oral mucosal abnormalities are frequent in IDA, and may occur before significant alterations in red cell morphology or hemoglobin level are noted[10]. Although the weight of available evidence supported iron deficiency as the prime etiological factor in the development of oral lesions, the underlying mechanisms remained unclear.

Our study showed a significant increase in nuclear diameter, decrease in cytoplasmic diameter and increase in nucleo-cytoplasmic ratio ($p < 0.001$) in iron deficient group when compared with the control group. These results are in agreement to those reported by Boddington (1959)[11] whose findings were later confirmed by Monto et al (1961)[12] by examining buccal mucosa cells before, during and after Iron therapy. These changes were reverted by iron therapy, further accentuating the

role of iron in nucleo-cytoplasmic alterations. However Gururaj et al (2004)[13] reported a significant increase in CD, ND and N/C ratio in anemic patients as compared to control group.

Rennie and Macdonald (1984)[10] described a reduction in the time taken for DNA synthesis (Ts) in iron deficiency anemia and iron deficiency without anemia. They suggested that as the Iron deficiency develops there is an increased rate of new cell production but decrease in the size of epithelial cells. Jacobs (1961)[14] further noted decreased levels of iron containing enzyme Cytochrome C in buccal mucosa in anemic patients.

Our study showed negative correlation of serum Iron with Mean nuclear diameter(r= -0.739**, p=0.000) and Nucleo-cytoplasmic ratio (r= -0.410**, p=0.000) But no correlation could be found between serum iron and Mean Cytoplasmic diameter (r= -.047**, p=0.000). however, a positive correlation was observed by Ramaesh et al. (1998)[15] among the mean values of ND and CD for normal cells.

It also showed A negative correlation of Serum ferritin with mean nuclear diameter (r= -0.590**, p = 0.000) and nucleo-cytoplasmic ratio (r= -0.437*, p=0.000). Sumanthi et al (2012)[16] were compared RBC parameters & serum ferritin with CD & ND values in iron deficiency anemia and reported negative correlation. However, Vanishree et al (2014)[4] observed a positive correlation with CD and ND in Iron deficiency anemia.

Iron (Fe) plays a crucial role in cellular proliferation and as the greatest demand occurs during the late G1/S phases of the cell cycle, so its deficiency impedes G1/S phase and results in apoptosis. This has been partly attributed to the activity of iron requiring enzyme of DNA synthesis, Ribonucleotide reductase[17,18].

However, the precise role of Fe in cell-cycle control still remains unclear. Fu and Richardson 2007[19] observed that Fe depletion increased the mRNA of the universal cyclin-dependent kinase inhibitor, *p21CIP1/WAF1*, while its protein level was not found to be elevated. This observation is unique to the G1/S arrest seen after Fe deprivation.

One of the main reasons why doubt has existed about the effect of iron deficiency upon the oral mucosa has been the lack of adequate assessment of the iron status of the study group. Too often the hemoglobin level in conjunction with the clinical appearances and an isolated serum iron level are the only investigations. Studies lacking full hematological and biochemical assays of controls and experimental groups often give inconclusive results.

Conclusion:

The increase in nuclear diameter and nucleo-cytoplasmic ratio with decrease in serum iron levels suggested that iron deficiency causes significant change in buccal mucosal cells. Further studies should be conducted to assess the influence of iron on cell cycle kinases and inhibitors in buccal mucosal cells and various other cells of the body.

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References:

1. Rajendran. R, Diseases of the blood and blood forming organs: Shafer, Hine, Levy. Shafer's textbook of oral pathology. 6th ed. Philadelphia: Elsevier; 2009. p. 754-5.
2. Lozoff B, Corapci F, Burden MJ, Kaciroti N, Angulo-Barroso R, Sazawal S, et al. Preschool aged children with iron deficiency anemia show altered affect and behavior. J NUtr 2007; 13:683-9.
3. Brugnara C. Diagnostic approaches for iron deficiency and erythropoiesis. Clin Chem 2003;10:1573-8.
4. M.Vanishree, Alka Kale, Seema Hallikerimath, Balaramsingh Thakur. Quantitative exfoliative cytology of squames obtained from iron deficiency anemia and healthy individuals. Journal of Advanced Clinical & Research Insights,2014;1,10-13.
5. Lokeshwar MR, Mehta M, Mehta N, Shelke P, Babar N. Prevention of iron deficiency anemia: How far have we reached? Indian J Pediatr 2010;78:593-602.
6. Baynes RD, Bothwell TH. Iron deficiency. Annu Rev Nutr 1990;10:133-48.

7. Daliman P In: Iron in Biochemistry and Medicine. Eds. A Jacobs & M Worwood. Academic Press, London & New York; 1974; pp 437-476.
8. Jacobs A: Non-haematological effects of iron deficiency. Clin Haematol. 1982; 11: 353-364.
9. Paterson D R, Journal of Laryngology 1919; 34, 289-291
10. Rennie JS, MacDonald DG, Dagg JH. Iron and the oral epithelium: a review. Journal of the Royal Society of Medicine. 1984;77(7):602-607.
11. Boddington MM. Changes in buccal cells in the anaemias. J Clin Pathol 1959;12:222-7.
12. Monto RW, Rizek RA, Fine G. Observations on the exfolative cytology and histology of the oral mucous membranes in iron deficiency. Oral Surg Oral Med Oral Pathol 1961;14:965-74.
13. Gururaj N, Sivapathasundharam B, Sumathy N. Cytological findings in iron deficiency anemia. Indian J Dent Res. 2004; 15(4):126-28.
14. Jacobs A. Iron-containing enzymes in the buccal epithelium. Lancet 1961;2:1331-3.
15. Ramaesh T, Mendis BR, Ratnatunga N, Thattil RO. Cytomorphometric analysis of squames obtained from normal oral mucosa and lesions of oral leukoplakia and squamous cell carcinoma. J Oral Pathol Med 1998;27:83-6.
16. Sumanthi J, Reddy GS, Anuradha CH, Sekhar PC, Prasad LK, Reddy BV. A study on cytomorphometric analysis of exfoliative buccal cells in iron deficiency anemia patients. Contemp Clin Dent 2012; 3: S156-9.
17. N.T. Le, D.R. Richardson, The role of iron in cell cycle progression and the proliferation of neoplastic cells, Biochim. Biophys. Acta. 2002; 1603:31–46.
18. J.A. Cotruvo, J. Stubbe, Class I ribonucleotide reductases: metallocofactor assembly and repair in vitro and in vivo, Annu. Rev. Biochem. 2011; 80: 733–767.
19. Dong Fu and Des R. Richardson, Iron chelation and regulation of the cell cycle: 2 mechanisms of posttranscriptional regulation of the universal cyclin-dependent kinase inhibitor p21CIP1/WAF1 by iron depletion. American Society of Hematology; Blood: 2007;110(2):752-761.

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