



Quantitative exfoliative cytology of squames obtained from iron deficiency anemia and healthy individuals

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Keywords

Cytomorphometry, iron deficiency anemia, quantitative exfoliative cytology, serum ferritin

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Received 12 April 2014; Accepted 02 June 2014

doi: 10.15713/ins.jcri.4

Abstract

Background: Iron deficiency anemia is the most common nutritional deficiency of world affecting people of all ages and economic groups. Previous studies on the effects of iron deficiency anemia by exfoliative oral cytology have produced conflicting results but application of quantitative parameters such as nuclear diameter (ND) and cytoplasmic diameter (CD) and nuclear to cytoplasmic ratio (N/C) has shown to be significant in the diagnosis of oral lesions. The present study was undertaken to assess the morphometric changes in CD and ND of squames obtained from buccal mucosa of patients with iron deficiency anemia and also to assess the effect of serum ferritin levels on cellular morphometry.

Materials and Methods: Thirty cases of iron deficiency anemia and 30 cases of control group were selected for the study. The control group consisted of people without clinical symptoms of anemia, normal hematological and serum ferritin levels. Scrapings were taken from the buccal mucosa of the iron deficiency anemia patients and control groups and stained with Papanicolaou stain. ND and CD were measured using image analyzer.

Results: CD and ND values of the control group were found to be in the range of 65.32-75.39 μ and 8.10-9.40 μ respectively. CD values of iron deficiency were 55.05-64.12 μ with the mean CD value of 59.77 μ and ND values were 8.69-11.24 μ with mean ND values of 9.88 μ . On correlating the serum ferritin and red cell parameters with the CD and ND values of iron deficiency anemia showed positive correlation.

Conclusions: The decrease in cytoplasmic diameter and increase in ND in iron deficiency anemia and progressive decrease in CD with decrease in serum ferritin levels suggested that iron deficiency causes significant changes in oral exfoliative cells. Cytomorphometric analysis of smears is useful in detecting the changes in iron deficiency anemia.

Introduction

Examination of cell plays a key role to our understanding of various normal and abnormal processes that affect the host. The role of exfoliative cytology in screening oral diseases has never achieved the success as it has for diagnosing cancer of uterine cervix but, the recent application of quantitative techniques has stimulated renewed interest in refining the potential role of oral exfoliative cytology, such that a reappraisal of its value as a diagnostic test is now required.

Materials and Methods

Thirty cases of iron deficiency anemia and 30 cases of control group were selected for the study based on clinical details, hematological investigations like peripheral blood smear, hemoglobin estimation (cyanmethemoglobin), total red blood cells (RBC) count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and ferritin levels in serum (using ELISA Kit, ORG 5 FE ORGENTEC Diagnostika GmbH, Germany). The

control group consisted of people without clinical symptoms of anemia, normal hematological and serum ferritin levels. Scrapings were taken from the buccal mucosa of the iron deficiency anemia patients and control groups. The smears were taken with wooden spatula and were immediately fixed in 95% ethyl alcohol for 1 hour to ensure proper fixation. The smears were then stained with prepared Papanicolaou (PAP) stain.

Nuclear and cellular diameters (ND and CD) were measured on PAP stained smears using image analyzer (Image Pro Plus Version 4.0, Media Cybernetics, USA). Twenty cells were randomly selected in a stepwise manner moving the microscope stage from left to right and then down and across in such a way as to avoid measuring the same cells again. The ND and CDs were obtained by drawing a line across the diameter using digitizer cursor in both the axes. Clearly defined cells were measured avoiding clumped or folded cells and unusually distorted nuclei and cells.

Results

The cells in the smears of the control group were mainly of superficial and intermediate type. The cells were usually sharply defined and nucleus was round to oval with a smooth peripheral outline. Nuclear pattern was found to be homogenous [Figure 1].

Iron deficiency smears comprised mainly of superficial and intermediate cells. The nucleus was regularly round to ovoid in shape with fine reticular network [Figure 2].

The mean CD and ND values of iron deficiency were compared with mean values of control group and the following observations were made.

Age and sex did not show any significant influence on the CD and ND values of iron deficiency anemia and control group.

1. Results showed that the CD and ND values of the control group to be in the range of 65.32-75.39 μ and 8.10-9.40 μ respectively. The mean CD was 69.84 μ and ND was 9.05 μ .
2. CD values of iron deficiency were 55.05-64.12 μ with the mean CD value of 59.77 μ and ND values were 8.69-11.24 μ with mean ND values of 9.88 μ .
3. The nuclear/cytoplasmic ratio (N/C) was calculated in our study for iron deficiency anemia using the mean CD and ND values in each case. The N/C ratio for control group was 0.14-0.16. The N/C ratio for iron deficiency anemia was 0.17-0.21.
4. On correlating the serum ferritin and red cell parameters with the CD and ND values of iron deficiency anemia there was positive correlation.

Discussion

Oral exfoliative cytology provides a simple, relatively pain free, non-invasive diagnostic test. Cowpe^[1] advocated the idea of using morphometry for improved diagnosis of oral lesions using cytological techniques. Computer assisted morphometric analysis of exfoliative cytology have improved the ability to measure various cell parameters. This study was carried out to

determine whether oral exfoliative cytology with morphometric analysis of cells is useful in detecting the changes in iron deficiency anemia.

The mean CD and ND values of the study group were compared with the control group values. Age and sex did not show any significant influence on the CD and ND values of iron deficiency anemia and control group.

On comparison of RBC parameters with the CD and ND values of iron deficiency anemia, positive correlation was found between the red cell parameters (hemoglobin, RBC, MCV, MCH, MCHC), ferritin levels and CD and ND values. Hence it can be suggested that the changes in the red cell parameters and serum ferritin levels may be correlated to the changes in the CD and ND values.

Serum ferritin is a protein that helps to store iron in our body. Estimation of serum ferritin levels indicates amount of iron storage in the body. On correlating the serum ferritin levels in iron deficiency anemia with CD and ND values, the decrease in CD value was more when the serum ferritin levels were less and progressively increased with increase in ferritin levels. The ND values, which were significantly reduced, compared to the ND values of control group were almost constant. These findings

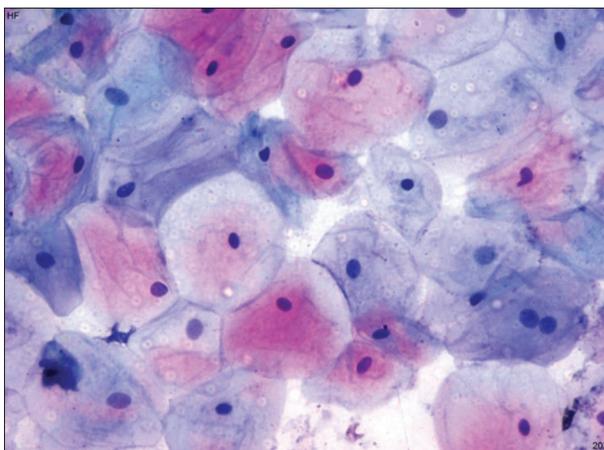


Figure 1: Papanicolaou stained smear from control group

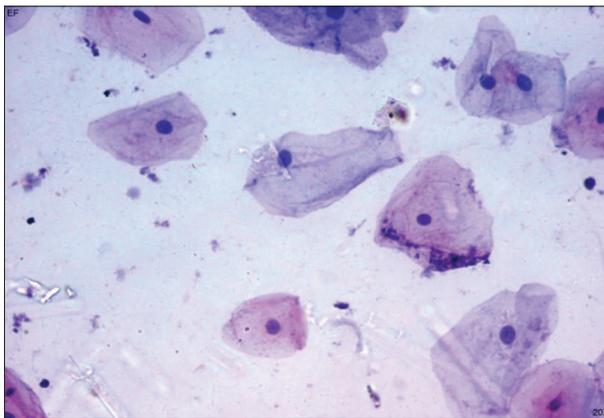


Figure 2: Papanicolaou stained smear from iron deficiency anemia

suggest that the CD values are more sensitive to the serum ferritin levels than ND values [Table 1].

Our result showed that the CD and ND values of the control group to be in the range of 65.32 μ -75.39 μ and 8.10 μ -9.40 μ respectively. The mean CD was 69.84 μ and ND was 9.05 μ .

Ramaesh *et al.*, (1998)^[2] evaluated that the mean CD and ND values in normal smears varies from 43.29 μ -59.18 μ and 7.00-9.2 μ respectively using calibrated eye piece for measurement. The values of CD in our study were slightly more. This may be due to different methods of measurement.

Cowpe *et al.* (1991)^[3] compared the values obtained by plan metric method with that of image analysis system and found that there was no significant variation in nuclear size but there was a significant elevation in the measurement of cytoplasmic size.

The CD values in iron deficiency anemia were 55.05 μ -64.12 μ with the mean CD value of 59.77 μ and ND value were 8.69-11.24 μ with mean ND values of 9.88 μ . On comparing the mean CD and ND values of iron deficiency anemia with that of the control group, there was highly significant ($P < 0.001$) decrease in mean CD [Table 2] and highly significant increase ($P < 0.001$) in mean ND [Table 3].

Results obtained in our study is in agreement to those obtained by Boddington (1959)^[4] and Monto *et al.* (1961)^[5] who found decrease in cell size and an increase in nuclear size in iron deficiency anemia.

The cytological alteration of the cells would suggest a maturation defect with interference of RNA and DNA synthesis according to Monto *et al.* (1961).^[5] Cell kinetic studies in iron deficiency by Rennie and MacDonald (1984b)^[6] described a reduction in the time taken for DNA synthesis (T_s) in iron deficiency anemia. This reduction in T_s occurred in the presence of unchanged labeling index but there was reduction in the

duration of the 'S' phase suggesting an increased cell production. Further investigations are required to evaluate this on a cytochemical basis.

A variety of tissue changes have been described in patients suffering from iron deficiency anemia and although it is generally assumed that these result from derangement of intracellular iron metabolism, no satisfactory evidence of this relationship has been established by Jacobs (1969).^[7]

Jacobs (1961)^[8] in a study of the epithelial changes in iron deficiency noted decreased levels of the iron containing enzyme cytochrome C in buccal mucosa from anemic patients. Dagg *et al.* (1966)^[9] observed similar findings in patients with iron deficiency anemia, but no correlation between the epithelial atrophy, the symptoms and degree of enzyme depletion was found by either author.

Ranasinghe *et al.* (1985)^[10] studied the effect of iron deficiency on respiration of hamster cheek pouch epithelium *in vitro*. He concluded that iron deficiency anemia affects cell respiration directly, by interfering with the amounts and functions of iron containing enzymes or cytochromes in the electron transport chain.

The N/C ratio was calculated in our study as quoted by Sandler (1962)^[11] using the mean CD and ND values in each case. The N/C ratios for control group were 0.14 and 0.16. On comparing the N/C ratios of 0.17-0.21 in iron deficiency anemia with controls, there was slight increase in N/C ratio. Statistical analysis showed that there is a highly significant ($P < 0.001$) difference in the N/C ratio of control group when compared to iron deficiency anemia [Table 4].

Monto *et al.* (1961)^[5] showed alteration of N/C ratio in iron deficiency anemia. Macleod *et al.* (1988)^[12] assessed that N/C ratios increased due to significant nuclear enlargement in vitamin B₁₂ deficiency, while Graham and Rheault. (1954) reported an increase in both cell and nuclear area in deficiency of vitamin B₁₂.

The pathological processes affecting the cells has two significant morphologic changes occurring as activity of the cell increases regarding the relationship of the nucleus to the cytoplasm. There is less ability for cytoplasm to mature into its most mature cell type, so that there is greater immaturity to the cytoplasm of the cell with greatly increased activity. Additionally, the amount of cytoplasm the cell makes decreases relative to the amount of nucleoplasm so that the N/C ratio increases.¹³

There was a positive correlation between the CD and ND values in the control group and in iron deficiency anemia, suggesting that there was decrease in CD with decrease in ND values.

Cowpe *et al.* (1985)^[14] showed positive correlation when comparing the nuclear and cellular mean values of normal oral

Table 1: CD and ND values at different ferritin levels

Ferritin (mg/mL)	CD (in μ)	ND (in μ)
4-6	56.59	9.92
6-8	57.51	9.41
8-10	59.73	9.95

ND: Nuclear diameter, CD: Cellular diameter

Table 2: Comparison of mean CD values in the study group and control group

Group	Mean \pm SD	Mean \pm SD	t value	Significance
Control versus iron deficiency anemia	69.84 \pm 3.92	59.77 \pm 3.07	11.06	$P < 0.001$

CD: Cellular diameter, SD: Standard deviation

Table 3: Comparison of mean ND values in the study group and control group

Group	Mean \pm SD	Mean \pm SD	t value	Significance
Control versus iron deficiency anemia	9.05 \pm 0.34	9.88 \pm 0.57	6.742	$P < 0.001$

ND: Nuclear diameter, SD: Standard deviation

Table 4: Comparison of N/C ratios in the study group and control group

Group	Mean \pm SD	Mean \pm SD	t value	Significance
Control versus iron deficiency anemia	0.14 \pm 0.006	0.19 \pm 0.01	20.28	$P < 0.001$

N/C ratio: Nuclear/cytoplasmic ratio, SD: Standard deviation

squames, a positive correlation between cellular and nuclear area has also been reported for normal oral mucosal cells by Lee *et al.* (1973).^[15]

Ramaesh *et al.* (1998)^[2] showed that there is positive correlation of the mean values of ND and CD for normal cells. However the mean ND and CD values of cells obtained from lesions with epithelial dysplasia and squamous cell carcinoma showed poor correlation.

Conclusion

The decrease in cytoplasmic diameter and increase in ND in iron deficiency anemia and progressive decrease in CD with decrease in serum ferritin levels suggested that iron deficiency causes significant changes in oral exfoliative cells. Cytomorphometric analysis of smears is useful in detecting the changes in iron deficiency anemia.

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How to cite this article: Vanishree M, Kale A, Hallikerimath S, Thakur B. Quantitative exfoliative cytology of squames obtained from iron deficiency anemia and healthy individuals. *J Adv Clin Res Insights* 2014;1:10-13.