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The Impact of Serum Ferritin on Exfoliated Cells Morphology from Buccal Mucosa among Iron Deficiency Anemia Patients: Morphometric Study

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Abstract

Iron deficiency anemia is the world's most common dietary deficiency-affecting people of all ages and economic classes. The current research was performed to assess the quantitative changes in the cytoplasmic diameter CD, nuclear diameter ND, and nuclear to cytoplasmic ratio (N/C) of squamous cells collected from iron deficiency anemia patients' buccal smears and also to evaluate the effects of serum ferritin concentrations on cellular morphometry. This was a prospective cross-sectional study. The research group consisted of twenty-five cases of iron deficiency and twenty-five cases of apparently healthy controls. Scrapings were taken from both group's buccal mucosa and stained with Papanicolaou stain. The ratio ND, CD, and N/C were measured using the Optika camera microscope. The results of this study revealed a highly significant and positive correlation between serum ferritin and ND, and a significant inverse correlation with ND and N/C ratio, as well as significant differences in anemic patients and controls in CD, ND, and N/C ratio. The significant reduction in serum ferritin from anemia with iron deficiency contributes to morphometric changes in exfoliated buccal mucosal cells.

Key words: Ferritin, Exfoliated Cells, Buccal Mucosa, cytoplasmic diameter, nuclear diameter, nuclear-cytoplasmic ratio.

Introduction

Iron deficiency is a disease that is caused by too little iron in the body (Aboud et al., 2019). Iron deficiency is a major deficiency in nutrition and the leading cause of anemia (Edris et al., 2011). The terms anemia, iron deficiency, and anemia with iron deficient are also used interchangeably but equivalently. Iron deficiency varies from depleted iron stores with no functional or health deficiency to iron deficiency with anemia, which affects the functioning of several organs (Nivia et al., 2015). Health is on a continuum—one does not arrive at good health accidentally (Faraj et al., 2019). Quality of life includes the mental, physical, emotional and social feeling of well-being, and reflects patients' mental evaluation of their status of health and their response to it (Marzangi et al., 2018; Sundus et al., 2018).

The normal oral epithelium is a stratified layer of squamous. The inner layer is the layer of basal cells, and then comes the layer of prickle cells and then the layer of cornified surfaces. The granular layer will be larger if overlying keratin exists. ND decreases when we go from the basal layer to the superficial layers, and CD increases. The nucleus of basal cells is comparatively smaller. The cells of the prickle are bigger than the basal cells but the nucleus is smaller than the base cells. The physiological activity of the nucleus decreases as the epithelium matures, and the nucleus condenses onto the surface. When the cells are cornified, the nucleus disappears (Yip, 1994).

During negative iron balance, iron in all tissues is gradually reduced, and a wide range of non-erythroid changes have been identified in humans and animals, including nail spoon shape, atrophic gastritis, and alterations in the oral epithelium. The oral changes are among the most frequent. Epithelial thickness was not significantly reduced but the absence of melanin and the presence of a chronic inflammatory infiltrate were identified. In most cases, it was impossible to distinguish between normal and iron-deficient biopsies by using histological techniques alone, but the biopsy procedure may have hidden structural or morphological anomalies. Although the weight of the evidence available supports iron deficiency as the primary etiological factor in the development of epithelial lesions, the mechanism through which it is regulated is unknown (Lu, 2016).

Iron is important to cell development. Many studies have reported a very significant reduction in the total epithelial thickness, particularly the thickness of the maturation compartment, and low levels of enzymes in iron-deficient patients' buccal epithelium. Prolonged ID leads to lower levels of Hb, which bring inadequate oxygen to oral mucosa and eventually contribute to mucosal

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atrophy. All of these studies suggest that ID, whether functioning locally or by systemic mechanisms, may have a major impact on oral pathogenesis (Wu, 2014).

Serum ferritin is the recommended initial diagnostic test Though, despite being iron-deficient (false negatives), individuals can have ferritin in the normal range, as ferritin is an acute-phase reactant may be elevated in a wide variety of inflammation including infection. A ferritin test is therefore significant when scoring abnormally low but less important when the calculation is normal (Johnson-Wimbley et al., 2011; Bermejo and Garcı´a-Lo´pez, 2009; Killip et al., 2007).

The objective of this research is to assess cytomorphometic modifications in exfoliated buccal mucosa cells obtained from Sudanese patients with iron deficiency anemia.

Material and Methods

This prospective cross-sectional analysis was conducted to assess the cytomorphological differences in buccal smears from Sudanese patients with iron deficiency anemia who visited Khartoum State Private Clinic seeking medical advice and comparing their findings with apparently healthy individuals. Fifty Sudanese patients with a healthy as well as anemic iron deficiency were the key targets for this research.

Exclusion criteria

Any patient with a history of any acute or chronic disease, noticeable oral lesions, drinkers, alcoholics, tobacco chewers, iron deficiency therapy subjects were excluded from this study. To achieve good results, we identified three fields from which individual cells were selected that showed a good nuclear topographic region and outstanding delineation; furthermore, separate cells were targeted for optimum enumeration apart from overlapped rejoin.

Methods:

Data were collected randomly from any patients admitted to the lab or any outpatient who visited the clinic and suspected as IDA. The smears were taken with a wooden spatula and were immediately fixed in 95% ethyl alcohol for a half-hour to ensure proper fixation. The smears were then stained with prepared Papanicolaou (PAP) stain as follow

Papanicolaou staining (Pap stain)

Using tongue depressors, exerting gentle pressure, cells were scraped from clinically normal-appearing buccal mucosa. Scrapping was smeared on clean labeled slides. Slides were fixed in 95% alcohol for 30 minutes. Air drying of the smears was avoided as it leads to alterations in the cellular morphology, Smear was stained using conventional pap stain. The technique is done through hydration of the smear in different concentrations of alcohol, 90% ethanol for 3 minutes, 70% alcohol for 3 minutes, distilled water for 2 minutes. Then applied of the nuclear stain Harri's hematoxylin for 4 minutes, `bluing for 4 minutes, counterstain with OG6 (orange G6) for 4 minutes, differentiated with 95%alcohol, followed by cytoplasmic stain with EA50 (eosin azure 50) for 4 minutes, differentiated with 95%alcohol just rinse, finally dehydrate, clear and mounting. The Papanicolaou stained cells were examined under x40.

Cytomorphometric Analysis

The samples were done in a stepwise manner, moving the slide from the left upper corner to right and then down to avoid measuring of the same cell. The cells were identified based upon the morphology and staining characteristics. All quality control measures were adopted during specimens' collection and processing. All stained smears showed fair quality. Nuclear and cellular diameters (ND and CD) were measured on PAP stained smears using the OptikaB1digital microscope camera (Optika digital innovation).

Ten 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 repeat measuring the same cells again. The ND and CDs were obtained by drawing a line across the diameter using a digitizer cursor in both the axes. Clearly defined cells were measured avoiding clumped or folded cells and unusually distorted nuclei and cells.

In each smear, ten cells were selected by moving the microscope stage in the "Z" shape to avoid recounting of the same cell. Optika camera software program user interface, nuclear and cellular diameters were obtained, and then mean cellular diameter (CD), nuclear diameter (ND), and the nuclear/cytoplasmic ratio were calculated for each case.

Statistical analysis

Data were analyzed with the statistical package of social science (SPSS) version 22. Independent samples test was used to differences of morphometric parameters in gender and age groups among patients and control, however, Pearson correlation was utilized to reveal the correlation between serum ferritin and morphometric parameters.

Ethical consideration

This study was approved by the ethical committee of the National University – Faculty of medical laboratory science. Every patient enrolled in this study approved to be part of the study and verbal consent has been obtained from the participant after a good discussion with them and the result of the study would be utilized for their benefits.

Results

A total of 50 participants took part in this study, they were divided equally into two groups, the first group is patients suffering from anemia with iron deficiency and the other group is the control group and they are healthy and have no diseases.

The participants were divided into two age groups, aged 20-29 years and the other group, aged over 30 years. The first group made up the largest number of 21 (84 %) participants. As for the sex groups, there were 12 females (48%) who formed while the males formed 52% as shown in Table 1.

As for the second table, it indicates the relationship between the gender and morphometric parameters, and the results of all relationships are considered to be highly significant. The table also contains the age groups that exhibited a significant relationship ($P \geq 0.05$) with the morphometric parameters.

As for the third table, it describes the significant positive correlation ($P \geq 0.05$) between the serum ferritin concentration and the cytoplasmic diameter CD, that is, low serum ferritin concentration leads to decrease in the diameter of the cytoplasm, and the correlation between the serum ferritin, ND, and N/C ratio is an inverse relationship.

The relationship between hematological and morphometric parameters between patients and control was presented in Table 4, and both were considered statistically significant ($P = 0.05$).

Figure 1 shows the morphology of normal cells exfoliated from the buccal cavity and Figures 2, 3, and 4 indicate variations in morphology in iron deficiency anemia patients. Figure 5 demonstrates modern software for calculating cell cytoplasm diameter (CD) and nucleus diameter (ND) and nucleus-to-cytoplasm ratio (NC).

Table 1: shows the distribution of Socio-demographics (n =50)

Variables	Patients (n =25)		Controls (n=25)		Total	
	Frequency	Percent	Frequency	Percent	Frequency	Percent
Age in years						
- 20-29	21	84.0	15	60.0	36	72.0
- >30	4	16.0	10	400	14	28.0
Gender						
- Female	12	48.0	14	56.0	26	52.0
- Male	13	52.0	11	44.0	24	48.0

Table 2: Morphometric parameters differences in gender and age groups among patients and control.

Morphometric parameters (µm)	Gender	Patients Mean ± SD (n=25)	Control s Mean ± SD (n=25)	T - value	Sig.
CD	- Female	36.7 ± 5.7	48.1 ± 11.7	3.09	0.005
	- Male	38.3 ± 8.2	50.9 ± 12.8	2.92	0.008
ND	- Female	9.0 ± 2.4	6.3 ± 1.4	3.67	0.001
	- Male	8.9 ± 1.7	6.5 ± 1.3	3.70	0.001
N/C ratio	- Female	0.1 ± 0.2	0.1 ± 0.1	2.95	0.007
	- Male	0.21 ± 0.04	0.13 ± 0.04	5.08	0.000
Age group in years					
CD	- 20-29	36.48 ± 7.16	47.95 ± 12.54	-3.48	0.001
	- >30	42.9 ± 2.3	51.4 ± 11.5	-1.45	0.172
ND	- 20-29	9.1 ± 2.0	6.4 ± 1.4	4.44	0.000
	- >30	8.4 ± 2.1	6.4 ± 1.3	2.14	0.053
N/C ratio	- 20-29	0.2 ± 0.1	0.1 ± 0.0	4.39	0.000
	- >30	0.2 ± 0.0	0.1 ± 0.0	3.90	0.002

Table 3: Correlation between serum ferritin and morphometric parameters

Parameters	Serum Ferritin	CD	ND	N/C ratio
Serum Ferritin (ng/ml)	1			
CD (µm)	0.587(**)	1		
ND(µm)	- 0.527(**)	-.154	1	
N/C ratio (µm)	- 0.498(**)	-.134	.206	1

** Pearson correlation is significant at the 0.01 level (2-tailed).

Value of r range (-1.0-1.0)

Table 4 The differences between hematological and morphometric parameters

Morphometric parameters (µm)	Hematological parameters	Patients Mean ±SD (n=25)	Controls Mean ±SD (n=25)	T-value	P-value
CD	RBCs(cell x109/L)	3.75 ± 0.7	4.93 ± 1.20	-4.26	0.001
ND		8.96 ± 2.02	7.10 ±1.84	5.30	0.000
N/C ratio		0.20 ± 0.05	0.13 ± 0.04	5.32	0.000
CD	PCV (%)	37.5 ± 7.0	49.3 ± 12.0	-4.26	0.000
ND		8.96 ± 2.0	6.4 ± 1.3	5.30	0000.
N/C ratio		0.20 ± 0.05	0.13± 0.05	5.32	0.000
CD	Hb (g/dl)	37.5 ± 7.0	49.3 ± 12.0	-4.26	0.000
ND		8.96 ± 2.0	6.39 ± 1.4	5.30	0.000
N/C ratio		0.20 ± 0.05	0.13 ± 0.5	5.32	0.000
CD	Serum ferritin (ng/ml)	37.8 ± 7.1	47.2 ± 12.3	-3.08	0.003
ND		8.96 ± 2.0	6.4 ± 1.3	5.30	0.000
N/C ratio		0.20 ± 0.05	0.13 ± 0.05	5.32	0.000

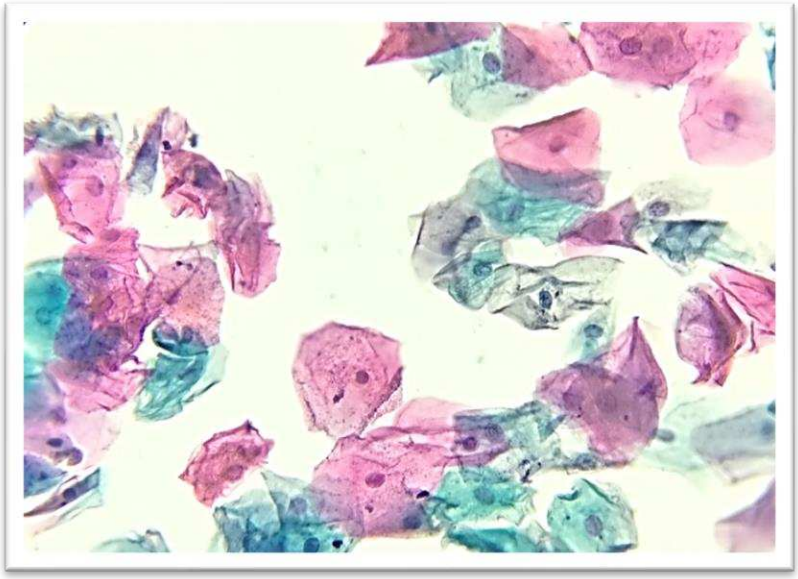

Figure 1 Shows Normal buccal smear show both superficial and intermediate cells

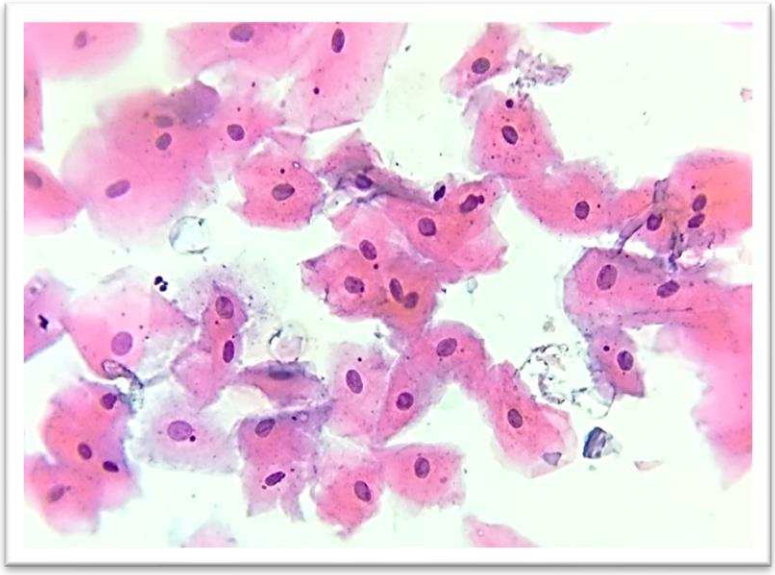

Figure 2 Illustrates IDA smear Iron deficiency smears comprised mainly of superficial cells and few intermediate cells. The nucleus was regularly round to ovoid.

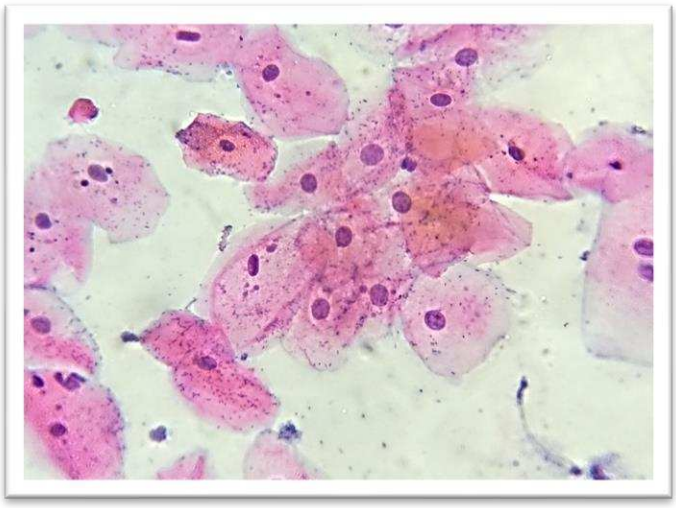

Figure 3 Explains IDA smear shows superficial cells with an increased nuclear diameter and binucleated cell.

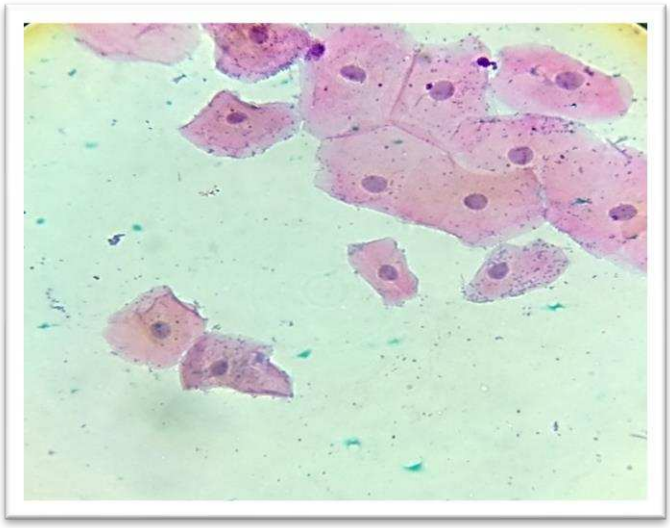

Figure 4 IDA smear show superficial cells with an increased nuclear diameter

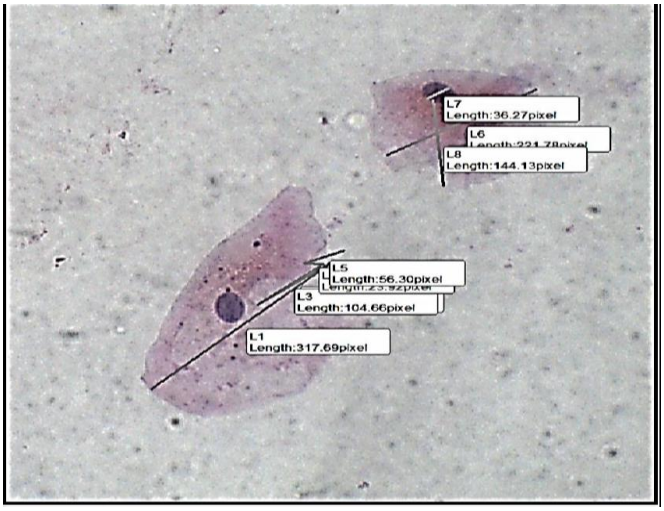

Figure 5 OPTIKA Software which used to calculate the cells ND, CD, and N/D distance in microns (μm).

Discussion

Oral cytology offers a simple, fairly painless, non-invasive diagnostic test that is readily acceptable to both patients and clinicians, and hence it is necessary to enhance sensitivity in the detection of oral diseases. Throughout the past, oral cytology relied largely on subjective interpretation, which showed a great many false-negative results (Mehrotra et al., 2006).

In this research, we attempted to transform a quantitative approach which would represent oral mucosal cytological changes. Despite advances in quantitative oral exfoliated cytology, interest in oral cytology in the diagnosis of oral lesions has once again arisen.

Computer-assisted morphometric analysis of exfoliated cytology samples has increased the capability to calculate specific cell parameters accurately (Beard, 2001; Lozoff et al., 2000). In our study, we used the most advanced OPTIKA software to accurately and precisely measure the CD, ND, and NC ratio.

In this study, we found that a very significant difference between the CD, ND, and N/C ratio in males and females when comparing the mean of anemic patient outcomes with control. Also, we detected very significant differences when comparing the same parameters with the two age groups used in the research, our study is inconsistent with the previous study, Anuradha and Sivapathasundharam, 2007, proposed that age-related variability of ND, CD, and N/C may be due to cellular senescence, regardless of gender. Basal cells' regeneration ability declines as age progress resulting in the accumulation of senescent cells, which has the effect of numerous environmental factors resulting in an increased ND and N / C ratio. Hormones such as estrogen and progesterone promote anabolism and development, increase during puberty, and decrease as age progresses. Such hormones have a direct effect on cellular diameter and nuclear diameter rise and decrease in relation to age in both genders equally (Anuradha and Sivapathasundharam, 2007).

Repairing ad growth of tissue relies on increased cell proliferation rates. For the synthesis of thymidine and pyrimidine bases, folic acid is needed, while zinc contributes to the DNA and RNA polymerase catalytic sites. Since folate, zinc, and iron play critical roles in regulating DNA synthesis, these nutrients are considered a growth-limiting factor (Sumanthi et al., 2012).

Serum ferritin has a positive correlation with CD and is a direct correlation in the sense that the lower the serum ferritin concentration the less CD, whereas the correlation between the ND and NC ratio with the ferritin serum is a negative one. This means that less serum ferritin concentration increases the ND and N/C ratio, although the exact cause of increased nuclear diameter and nuclear/cytoplasmic ratio is difficult to understand. It may be linked to iron required for ribonucleotide reductase which decreases the nucleotide sugar group to the corresponding deoxy derivatives, the DNA precursors. When this enzyme is diminished, the synthesis of DNA will be disrupted with subsequent alterations leading to an increase in the nuclear diameter of exfoliated cells in anemia with iron deficiency. Arrays of critically important iron-containing enzymes are modulated by cofactors containing iron. Those include aconitase, catalase, cytochrome C, cytochrome C reductase, cytochrome oxidase, succinic dehydrogenase, formimonotransferase, peroxidase, xanthine, and tryptophan pyrrolase (Gururaj et al., 2004).

In this study , it was found that there are significant differences when comparing the hematological parameters in patient and control samples with morphometric parameters, and the justification for this is the iron concentration (serum ferritin). Iron plays a key role in the production of red blood cells and the synthesis of hemoglobin and is also involved in the development and maturation of the nucleus and the cell as a whole (cytoplasm), This finding was agreed with Vanishree et al. , 2014, who conducted the study that a mild negative correlation between serum ferritin and nuclear diameter was observed in iron deficiency anemia. (Vanishree et al., 2014).

Iron is an essential cofactor for a wide range of important cellular processes, including oxygen delivery, respiration, the tricarboxylic acid cycle, lipid metabolism, gene regulation, and DNA synthesis. To perform these multiple tasks, iron is integrated into the hemoglobin, myoglobin, and cytochromes group, or is associated with nonheme moieties (e.g., in ribonucleotide reductase) or Fe-S motifs (functionally versatile prosthetic groups associated with proteins that sustain fundamental life processes). Iron can, therefore, be seen as an essential nutrient for nearly all species (Cair et al., 2006). This might be recommended that the use of computerized morphometric analysis software in the quantitative measurement of cytological cells would be very useful in increasing diagnostic precision and accuracy.

Conclusion

The significant decrease in CD and rise of the ND and N / C ratio in iron deficiency anemia was correlated with a decrease in serum ferritin levels suggested that iron deficiency caused significant changes in oral exfoliated cells. Cytomorphometric smear analysis helps to identify changes in iron deficiency anemia.

Compliance with Ethical Standards
Disclosure of potential conflicts of interest

The author declares that they do not have a conflict of interest

Research involving human participants and/or animals

Was approved by the ethical committee of National University

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Conflict of interest

The author declared that there is no conflict of interest in this research.

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