ORIGINAL RESEARCH

# Cytomorphometric Analysis of Buccal Exfoliated Cells in Anaemic Patients Among Female Population: A Cross-Sectional Study

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## Abstract

**Background:** Cytology is an easy, rapid and a non-invasive procedure that can be used as a screening tool in oral and systemic diseases. Anaemia is the most common encountered disease across the world and is more prevalent in India especially among females.

**Aim:** To evaluate the cytomorphometric changes among anaemic females of different age groups respective to their hemoglobin levels.

**Materials and Methods:** The study group included total number of 60 females of which (n= 45) anaemic group and (n=15) control group. The anaemic group was categorized into 3 groups as young, middle and old age consisting (n=15) samples in each group. Informed consent was obtained. Individuals with < 12mg/dl hemoglobin were considered anaemic. Buccal smears were collected and cytomorphometric analysis was done.

**Results:** The present study showed a noticeable decrease in cellular diameter (Mean - 8393.18), increase in nuclear diameter (Mean - 281.6) and nuclear cytoplasmic ratio (Mean -0.0445894) in anaemic female young adults between 18-30 years. There was a noticeable decrease in cellular diameter (Mean- 6534), nuclear diameter (Mean-227) and nuclear cytoplasmic ratio (Mean- 0.043954) in anaemic females of middle age between 31-45 years. There was a noticeable increase in cellular diameter (Mean- 5479.82), nuclear diameter (Mean-219.07) and nuclear cytoplasmic ratio (Mean-0.0508656) in anaemic females above 45 years.

**Conclusion:** All the parameters such as cellular diameter, nuclear diameter & nuclear cytoplasmic ratio showed alteration among the different female anaemic groups. Further studies have to be performed with larger sample size to obtain promising results.


**Keywords:** Anaemia, Cytomorphology, Anaemic females, Hemoglobin

## Introduction

The oral mucous membrane serves as a window into one's overall health, and certain systemic diseases like anaemia, leukemia, vitamin deficiency, and many infectious diseases can manifest through oral symptoms. These oral symptoms can cause early mucosal alteration. The mucosal changes can be detected by exfoliative cytology being a non-invasive method. Invasive method such as biopsy can be used, however in absence of oral symptoms biopsy is not appropriate and hence exfoliative cytology plays a significant role to evaluate the mucosal changes in the absence of oral symptoms.[1] Cytology is the study of the cells. Cellular morphology reflects biological behaviour of the tissue & the host and genetic & molecular biology of the cells.[24] It appears as one of the best techniques for evaluating the oral mucosa, which is less expensive, non-invasive, particularly for the early detection of mucosal changes unlike biopsy which is expensive and an invasive procedure. There are other various light-based detection systems such as chemiluminescence, self-fluorescence imaging, tissue autofluroscence, optical coherence tomography, nano detection system, artificial intelligence.[1]

The common systemic disease worldwide is anaemia affecting globally half a billion women of 15-49 years of age and in India its prevalence being 52% (WHO). Anemia is defined

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as a reduction of the total circulating red cell mass below normal limits.[4] The most common cause is due to iron deficiency. Various host factors significantly affect the prevalence of iron deficiency such as age, gender, physiological, pathological, environmental, and socioeconomic circumstances. It is widely prevalent across all age groups, and is especially high among females, including approximately 58% of pregnant women, 50% of non-pregnant, 56% of adolescent girls (15–19 years old), 30% of adolescent boys, and roughly 80% of children under the age of three, according to data from the National Family Health Survey-3 (NFHS-3).[2,3,4]

Anaemia causes systemic manifestations such as fatigue, generalised muscular weakness, lethargy, headache, pallor of skin, mucous membrane and conjunctiva, dyspnoea on exertion, nausea, constipation, weight loss, menstrual disturbances such as amenorrhoea and menorrhagia. Anemia for a longer duration causes epithelial tissue changes that occur in nails ((koilonychia or spoon-shaped nails). Oral manifestation associated with anaemia are atrophy of mucosa, atrophic glossitis and angular cheilitis. The common investigations used to detect anaemia are complete blood count, serum iron level, total iron binding capacity (TIBC), and serum ferritin levels.[4]

Cell surface loss occurs often in a typical epithelium, while epithelium thickness remains constant. Under normal circumstances, epithelial cells are firmly attached in situ. The cells lose their cohesive force as they exfoliate and the epithelial thickness decreases when there is any underlying disease. Hormones like estrogen and progesterone which promote anabolism and growth, increase at the time of puberty, and decrease as age advances. These hormones have a direct effect on the increase and decrease of cellular diameter and nuclear diameter in relation to age in both genders equally.[6] Since the cell's integrity is lost, exfoliated cells can be gathered and studied under a microscope. One way to observe the consequences of aging is in the oral cavity where the oral epithelium is always changing due to periodic turnover, there should be detectable aging indicators in this tissue. Since exfoliative cytology is rapid and non-invasive, the buccal mucosa in particular is a prospective site for smear collection. Thus, variations in the functional activity of buccal epithelial cells primarily represent the state of local and systemic homeostasis within the body, or its deterioration with age. Age-related alterations are always present in tissues, regardless of gender, as age advances and their regeneration ability decreases.[5]

Iron is needed for ribonucleotide reductase that reduces the sugar group of nucleotides to corresponding deoxy derivatives, the precursors of DNA. If this enzyme is decreased, DNA synthesis will be impaired with resultant alterations leading to the increase in nuclear diameter of exfoliated cells in iron deficiency anaemia.[6] Previous literature discussed on the cytological changes observed in the exfoliated buccal cells in iron deficiency anaemia and stated that age and sex did not show any influence on the cytological parameters however a positive correlation between the red cell parameters and the cytological changes were evident.[3] Sumanthi et al, stated that positive correlation was found between the Hb%, RBC, MCV, MCH, MCHC, Ferritin levels, and CD and ND values, suggesting that the changes in these indices and serum ferritin levels may be related to the changes in the Cellular Diameter and Nuclear Diameter.[6]

This study aims to evaluate and compare the cytological changes of buccal exfoliated cells among the different female age groups of young adults, middle age, and old age.

## Materials and Methods

This cross-sectional study was conducted in Chettinad Dental College and Research Institute, Chennai, Tamil Nadu, after procuring "ethical approval from the institution review board (IHEC-CDCRI/2024/STU-0007) on 14 May 2024."

**Study Population:** The study participants included in this study were the patients reported to the outpatient department of our institution. The sample size was calculated using the G*Power software, $N=[(Z\alpha/2+Z\beta)2.(SD12\ /N1+SD22\ /N2)]/\delta 2$. A total of 60 individuals were included in this study. Three categories of female age groups were included: young adults (18-30), middle age (31-45) and old age above 45 years as group I, II and III respectively. Group III subjects will be in the age of 45 years and above for whom menopausal and premenopausal factors will be considered. These factors include body mass index, physical activity, high consumption of fat, smoking, alcohol consumption, family history, and hysterectomy. These factors were considered because of change in hormonal influences (progesterone and estrogen) which influences the cytomorphology of cells and nucleus. The study group included

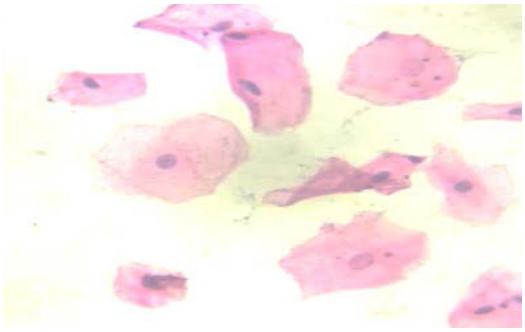

**Fig. 1:** H and E-stained buccal smears at 40X objective

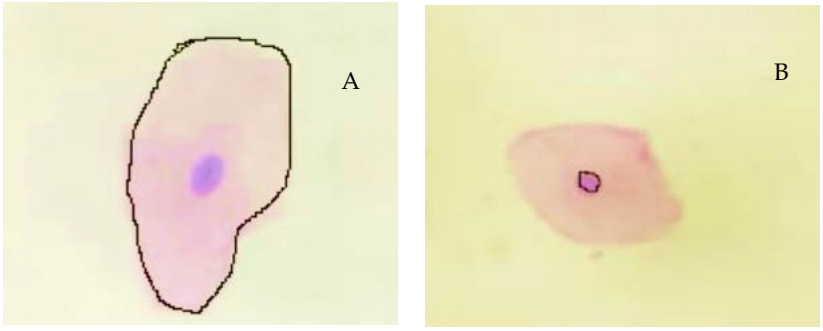


**Fig. 2:** (A and B) Cytomorphometric analysis of cellular and nuclear diameter

45 anaemic females of which (n=15) was taken from each age category and the remaining 15 as control group of which (n=5) was taken from each age group. Selection of the participants was done based on collecting the detailed history including the medical, dental, habit history, family history, history of surgery.

Patients approaching to the dental hospital for various complaints. Control group included healthy individuals with hemoglobin levels >12mg/dl. The study group consisted of individuals having hemoglobin levels <12mg/dl. Deleterious habits, history of systemic diseases, medications or iron supplements, multivitamins, benign or malignant mucosal oral lesions and pregnant women were excluded from the study.

Informed consent was obtained from study participants. Initially hemoglobin levels were checked by a finger prick and evaluated using a hemoglobinometer. Patients were asked to rinse their mouth with water to remove any debris prior to smear collection. Two smears were collected from the buccal mucosa of the same site. Smears were collected by gently scraping the buccal mucosa using a moist wooden spatula and immediately smeared over a glass slide. The smears were fixed using 95% ethyl alcohol for 30 minutes. The slides were then stained using hematoxylin and eosin (H&E) stain, and microscopic evaluation of the cytosmears were done.

All the results were observed by single observer due to limitation in time and nature of the study. Photographs were taken at 40X magnification and were transferred to a computer (Figure 1). The software used for image analysis was ImageJ software version 1.53 (National Institutes of Health, Bethesda, MD). For image analysis, an average of 5 cells with defined outlines was selected per subject. Overlapping cells were excluded.

The cells were measured for their cell diameter (CD), nuclear diameter (ND), and nuclear-cytoplasmic (N/C) ratio (Figure 2). The cellular size and nuclear size were determined after the cellular and nuclear outlines were outlined using a digitalized cursor and a computer-based measurement tool in the software. The cytoplasmic size was calculated as the sum of the differences between cellular size and nuclear size. The formula NC=nuclear size/cytoplasmic size was used to determine the nuclear-cytoplasmic ratio. The collected data was analyzed using descriptive statistics to generate mean of all the cell parameters in the study groups and control groups.

## Results

The total number of patients included were 60 (45 study groups and 15 control groups). The age groups were divided between young adults 18-30, middle age 31-45 and old age above 45 years. There was a significant difference between the cell parameters of all anaemic age groups.

Results of our study showed decrease in the CD (8393.18) and increase in the ND (281.6) and N/C ratio (0.04) of young anaemic female age groups when compared with the control groups which showed increase in CD (9070.76) and decrease in the ND (278.24) and N/C ratio (0.03) (Table 1).

Among the anaemic middle age group the results showed a significant decrease in all the cell parameters CD (6534), ND (227), N/C ratio (0.04) when compared with the control group which showed an increase in all the cell parameters CD (8165), ND (286) and N/C ratio (0.06) (Table 2).

Among the anaemic age group above 45 years the results showed a significant increase in all the cell parameters CD (5479.82), ND (219) and N/C ratio (0.05) when compared with their control group which showed a decrease in all the cell parameters CD (5133.88), ND (180) and N/C ratio (0.04) (Table 3).

Among the study groups of I, II & III, a significant increase in the CD of group I (8393.18) was noted followed by group II (6534) & group III (5479.82). There was an increase in the ND of group I (281.6) followed by group II (227) & III (219). Similar results were noted in the N/C ratio in group I (0.04) & II (0.04)

**Table 1:** Comparison of cell parameters between cases and controls of Group I (18-31 years)

| Cell Parameters | Study group (Mean) | Control group (Mean) |
|---|---|---|
| Cell Diameter | 8393.18 | 9070.76 |
| Nuclear Diameter | 281.6 | 278.24 |
| Nuclear cytoplasmic ratio | 0.04 | 0.03 |

**Table 2:** Comparison of cell parameters between cases and controls of Group II (31-45 years)

| Cell Parameters | Study group (Mean) | Control group (Mean) |
|---|---|---|
| Cell Diameter | 6534 | 8165 |
| Nuclear Diameter | 227 | 286 |
| Nuclear cytoplasmic ratio | 0.04 | 0.06 |

**Table 3:** Comparison of cell parameters between cases and controls of Group III (Above 45 years)

| Cell Parameters | Study group (Mean) | Control group (Mean) |
|---|---|---|
| Cell Diameter | 5479.82 | 5133.88 |
| Nuclear Diameter | 219 | 180 |
| Nuclear cytoplasmic ratio | 0.05 | 0.04 |

**Table 4:** Comparison of cell parameters between the study groups of I, II and III

| Cell Parameters | Group I (Mean) | Group II (Mean) | Group III (Mean) |
|---|---|---|---|
| Cell Diameter | 8393.18 | 6534 | 5479.82 |
| Nuclear Diameter | 281.6 | 227 | 219 |
| Nuclear cytoplasmic ratio | 0.04 | 0.04 | 0.05 |

with slightly increase in group III (0.05) (Table 4).

Correlation of the hemoglobin levels with the cell parameters among study groups was evaluated (Table 5). Group I hb levels ranged from 7- 9.5 mg/dl showed a significant increase in cell diameter, nuclear diameter. Group II hb levels ranged from 8-10.5 mg/dl and group III hb levels ranged from 8.5-11.5mg/dl showed a decrease in cell and nuclear diameter. No significant difference was seen in the nuclear cytoplasmic ratio of group I & group II, however a slight increase was noted in group III.

## Discussion

Early oral mucosal cellular changes take place without obvious clinical symptoms or indicators. Oral keratinocytes exfoliate as they proliferate and differentiate along the layers from stratum basale to stratum corneum. Keratinocytes differentiate from spinous layer to granular layer, nuclear size increases, and as they pass from granular layer to the surface layer, the nuclear size decreases. The thickness of the epithelial layers decreases with epithelial atrophy. Moreover, keratinocytes are exfoliated prior to the nucleus contracting and do not show signs of a typical maturation phase. This would also support a theory that keratinocytes of the oral mucosal epithelium of people with IDA have larger nuclei.[21]

The most convenient method for collecting the oral epithelial cells is exfoliative cytology which is a non-invasive, quick, easy, painless, and bloodless diagnostic procedure.

Quantitative factors such as cellular diameter, nuclear diameter, and nuclear cytoplasmic ratio help to determine the changes in the oral mucosa at cellular level. The ability to measure different cell parameters has improved with the use of computer assisted morphometric analysis of exfoliative cytology.[5] Previously used methods for cytomorphometric analysis were planimetric methods but with time, planimetric methods have been replaced by computer-assisted image analysis techniques, which are faster, more accurate, and more reproducible.[18]

There are various systemic and local factors that influence the normal cells. Factors such as smoking, tobacco chewing, anaemia, and diabetes mellitus show cytomorphometric changes in exfoliated cells of oral cavity. It is also useful in early detection of premalignant and malignant conditions of oral mucosa. Fluctuations in hormonal levels also have an influence over the buccal cell morphology resulting in alterations.[17]

**Table 5:** Correlation of Hb range with cell parameters among study groups of I, II and III

| | Hb levels | Cell Diameter (Mean) | Nuclear Diameter (Mean) | Nuclear cytoplasmic ratio (Mean) |
|---|---|---|---|---|
| Group I | 7-9.5 | 8393.18 | 281.6 | 0.04 |
| Group II | 8-10.5 | 6534 | 227 | 0.04 |
| Group III | 8.5-11.5 | 5479.82 | 219 | 0.05 |

In our study we measured the quantitative cell parameters of anaemic females of different age group category and compared with that of their control age group category. Additionally, we contrasted the anaemic female's findings across the three age categories. Our study results showed that there was a significant decrease in the cell diameter, increase in the nuclear diameter and nuclear cytoplasmic ratio of young anaemic females (18-30) compared to their control groups which showed increase in cell diameter, decrease in nuclear diameter and nuclear cytoplasmic ratio.

The results of anaemic young females in this study was in concordant with the previous study by Rithika et al[2] which showed a significant decrease in the cell diameter, increase in the nuclear diameter and nuclear cytoplasmic ratio. Results obtained in young anaemic females was in agreement to those obtained by Boddington[8] and Monto et al[9] who found decrease in cell size and an increase in nuclear size in iron deficiency anaemia. The results were also in agreement with the previous study by Vanishree et al.[3]

In middle age groups (31-45), the study results showed a significant decrease in all three cell parameters compared to their control groups. The study results of anaemic middle age females (31-45) showed decrease in all the three cell parameters which was contradicted to the previous study by Swati et al[5] showing an increase in all parameters. Our study results were contradictory to others because of hormonal influences by stress and lifestyle habits which might have altered the cytomorphology.

In old age groups above 45 years, the study results showed an increase in all the three cell parameters compared to their control groups. Our study results in anaemic females above 45 years was concordant with the previous study results by Swati et al[5] & Gururaj et al[7] showing increase in all the cell parameters. Similar results were observed in a study by Sumathi et al[6] showing a significant increase in the nuclear diameter and nuclear cytoplasmic ratio but the mean cell diameter being normal.

According to Monto et al[9] due to iron shortage, exfoliated tongue epithelial cells revealed a noticeable deficiency in the cornified and keratinized population. A disordered nuclear pattern, an increase in nucleoli, the existence of double nuclei, and karyorrhexis were indicative of abnormal cellular maturation. The cytoplasmic width was decreased with paradoxical enlargement of the nucleus.[9] Alterations in the oral mucosa have also been linked to nutritional deficits. Iron deficiency causes a delay in the production of DNA, the building block of cell nuclei, which might change the size of the cytoplasm and nucleus.[16]

Age-related declines in cellular activity and epithelial turnover rate are well-known, and this may be the cause of the cytoplasmic diameter drop.[22] Senescence can be attributed to age changes where renewal capacity of the cell declines as age advances and the cellular activity and the epithelial turnover decreases resulting in reduction of the cellular organelles which could be the reason for the decrease in cell size.[18] Accumulation of senescent cells, has direct effect of various environmental

factors resulting in increased ND.[21]

The morphometric examination of atrophic alterations in human lingual epithelium in IDA was investigated by Scott et al. They discovered that a higher nuclear diameter led to an enhanced nuclear-cytoplasmic ratio. A trophic impact resulting from the decreased epithelium thickness could be one reason for the higher N/C ratio linked to IDA in this study. Lower concentrations of the inhibitory cycle may occur from the smaller and fewer cells in the maturation compartment of the epithelium in IDA, which would cause a proliferative response in the progenitor layer. Frost stated in actively proliferating cells, there is an increase in the nuclear size.[21]

Morrison et al. (1949) emphasized that understanding the typical cytological appearance is crucial before examining diseased squames.[20] Thus age-related variations cause alteration in the epithelial cell morphology with the influence of various factors. Furthermore, cytoplasm is less able to mature during pathological processes, which results in greater immaturity and a lower amount of cytoplasm relative to nucleoplasm, increasing the nuclear-cytoplasmic ratio.[23] Too much iron in the body can be harmful. One well-known classical histochemical reaction that is frequently used in the field of hematology is the Perl's Prussian blue reaction. Given that the exfoliated cells may reflect alterations in the underlying parent tissue, this technique was applied to the exfoliated buccal mucosal cells.[15]

It is well recognized that a woman's hormone levels fluctuate cyclically from childhood through menstruation and menopause. Various menopausal and premenopausal factors also play an important role in RBC parameters influencing hemoglobin level and also indirectly affects the cytomorphology of the cells. The saturation of mucosa has been demonstrated to be influenced by abnormal sex hormone levels. The cytodifferentiation of stratified squamous epithelium is influenced by estrogen. Increase in the ND and CD can be attributed to the rise in estrogen and progesterone levels in the blood.[19]

Estrogen and progesterone which promote anabolism and growth, increases at the time of puberty, and decrease as age advances. These hormones have a direct effect on the increase and decrease of cellular diameter and nuclear diameter in relation to age in both genders equally. Age-related variation of ND, CD, and N/C ratio irrespective of gender can be ascribed to cellular senescence. The renewal capacity of the basal cells declines as age advances resulting in the accumulation of senescent cells, which has the effect of various environmental factors resulting in increased ND and N/C ratio.

Future research is needed to determine whether lifestyle behaviour has a major effect on the physiological cellular growth pattern and whether there are any changes in the cytomorphometric of buccal smears.

## Limitations

The study was conducted in a single hospital setting with limited resources. Access to a larger sample size would have enlighten the results superiorly. Single blinded study itself has limitation of result interpretation by single observer which affects the sensitivity and specificity of the study. Other red cell parameters were not evaluated in our study. Menopausal and premenopausal factors would also have influenced the results, i.e. levels of hemoglobin and cytomorphological changes in group III females. This shall be considered one of the limitations.

## Conclusion

This study compares the cytomorphological parameters of anaemic females among different age groups. This study indicates that cytological alterations in the oral mucosal epithelium are present in anaemic patients even in the absence of clinically evident oral diseases and gives the possible causes for the variations in the cell parameters among different age groups. Understanding the quantitative changes in anaemic patient's oral epithelial cells is crucial since these changes resemble those observed in precancerous lesions. Hence the study concludes that age influences the cytomorphology of the cell parameters resulting in variations among each age groups.

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