DOI: 10.7860/IJARS/2022/55633.2829

Original Article

Anatomy Section

Comparative Cytomorphometric Study of Exfoliated Squamous Buccal Cells among Patients with Iron Deficiency Anaemia and Healthy Individuals in Saurashtra, Gujarat, India

RITIKA M PATEL[1], RIDHDHISH K PATEL[2]

ABSTRACT

Introduction: Cytology is a fast, simple, non invasive, and bloodless procedure. Cytology can be useful in cases where biopsy is contraindicated or not needed, such as in systemically compromised patients, inaccessible areas, recurrent malignancies and mass screening. The most common nutritional disorder in the world is iron deficiency and it is more prevalent in India. Exfoliative cytology is becoming increasingly recognised as a standard technique for screening oral pathologies like anaemia.

Aim: To compare the Nucleus Diameters (ND), the Cytoplasmic Diameters (CD), and the Nucleus/Cytoplasmic ratio (N/C) in cytological buccal smears of iron deficiency anaemic patients and with normal healthy individuals.

Materials and Methods: The case-control study was conducted in Department of Anatomy, G.G hospital connected MP Shah Government Medical College, Jamnagar, Gujarat, India, from August 2013 to February 2016, which included 50 healthy individuals and 50 clinically diagnosed patients of iron deficiency anaemia. Exfoliated buccal smears stained with routine Haematoxylin and Eosin (H&E) staining technique. With the use of ocular micrometer, CD, ND and N/C ratio were calculated. All the parameters were statistically evaluated by using Student t-test.

Results: The mean value of nuclear diameter 11.34±0.58 µm and N/C ratio (0.24±0.02) of buccal mucosa was greater while cytoplasmic diameter 46.57±2.03 µm was lesser in iron deficiency anaemia when compared with healthy individuals. A significant difference was not found when comparing different age groups, as well as male versus female cases and male versus female controls. Comparing cases and controls by gender, however produced significant statistical results.

Conclusion: Statistically significant difference was found between iron deficiency anaemia patients and normal healthy individuals regarding changes in various parameters of buccal squamous cells. In the case of iron deficiency anaemia, oral exfoliative cytological techniques could be used as an alternative non invasive diagnostic tool.

Keywords: Buccal smear, Cytoplasmic diameter, Nuclear cytoplasmic ratio, Nuclear diameter, Screening oral pathologies

INTRODUCTION

Cytology is a quick, easy, bloodless and convenient procedure. The procedure does not replace the surgical biopsy, but rather complements it. It is useful in detecting lesions whose gross appearance does not warrant biopsy [1]. In some cases, cytology may be more valuable than biopsies, such as for systemically compromised patients, hard-to-reach areas, recurrent malignancy, and mass screening [2].

Certain types of systemic diseases such as anaemia, leukaemia, vitamin deficiency, and infectious diseases have a particularly high sensitivity to the oral mucous membrane [3]. As a matter of fact, oral symptoms presage overtly systemic signs and symptoms that are caused by haematological abnormalities [4]. The most common nutritional disorder in the world is iron deficiency. Iron deficiency anaemia is more prevalent in developing countries, including India [5]. The Global anaemia prevalence was 24.8%, affecting 1.62 billion people with about 293 million children of preschool age, 56 million pregnant women, and 468 million non pregnant women estimated to be anaemic. Anaemia is estimated to contribute to more than 115,000 maternal deaths and 5,91,000 perinatal deaths globally per year [6]. The National Family Health Survey-3 (NFHS-3) data suggests that anaemia is widely prevalent among all age groups, and is particularly high among the most vulnerable, nearly 58% among pregnant women, 50% among non pregnant non lactating women, 56% among adolescent girls (15-19 years), 30% among adolescent boys and around 80% among children under 3 years of age [7]. When compared to all other developing countries, India is estimated to have a higher prevalence of anaemia [8].

Assessment of the oral mucosa can be done using a number of methods, including tissue biopsy. As a result of aggressiveness and patient rejection, most biopsy procedures are not applicable, particularly in the absence of clinically visible disease. Exfoliative cytology appears to offer the best method for evaluation of oral mucosa due to its lower cost, less aggressive nature, and lack of damage to oral tissues [9]. There are no visible clinical signs or symptoms associated with cellular alterations in oral mucosa at an early stage [10]. Cytology can be used to detect iron deficiency anaemia even when no symptoms are present.

Results from exfoliative cytology in the study of iron deficiency anaemia have been inconsistent [10-12]. Such a study has not been reported in the Saurashtra population, Gujarat. An early diagnosis of anaemia is essential in order to begin treatment as soon as possible. Therefore, the aim and objective of present study were to determine whether oral exfoliative cytology with morphometric analysis of cells can be useful in detecting iron deficiency anaemia and to compare the ND, the CD, and the N/C ratio in cytological buccal smears of iron deficiency anaemic patients with normal healthy individuals.

MATERIALS AND METHODS

The case-control study was conducted in the Department of Anatomy, from August 2013 to February 2016 on 100 individuals. Total 50 (25 male, 25 female) clinically diagnosed cases of iron deficiency anaemia and age and gender matched 50 healthy individuals were selected as control from Outpatient Department of Medicine, G.G hospital connected MP Shah Government Medical College, Jamnagar, Gujarat, India. The study was conducted, after obtaining ethical clearance from the Institutional Ethical Committee of tertiary care hospital connected medical college. Age group ranged between 18-60 years in both case and control group.

Inclusion criteria: Study (cases) group consisted of 50 (25 male and 25 female, age range between 18-60 years) anaemic patients (haemoglobin ranged from 4-10 gm % in females and 8-13 gm% in males) [13] who had already been diagnosed with iron deficiency anaemia. Control group consisted of 50 healthy subjects (25 male and 25 female), age range between 18-60 years haemoglobin ranged from 12-16 gm% [13], with normal appearing buccal mucosa and normal jaw mobility.

Exclusion criteria: Patients using tobacco in any form or consuming alcohol and smoking were excluded. Those with oral lesions, on radiation or corticosteroid therapy, or medically compromised patients were excluded from the study.

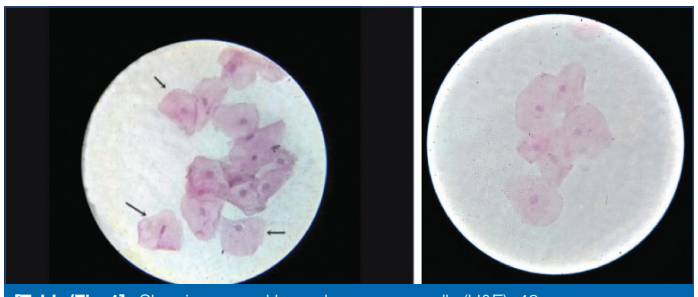
Study Procedure

Details regarding laboratory investigation result such as peripheral blood smear, haemoglobin estimation, total Red Blood Cell (RBC) count, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were obtained in both the control and iron deficiency anaemia groups.

Peripheral blood smear showing hypochromic microcytic pictures were considered under the iron deficiency anaemia group [14]. Both the groups in the study were subjected to oral exfoliative cytological studies.

Sample Collection

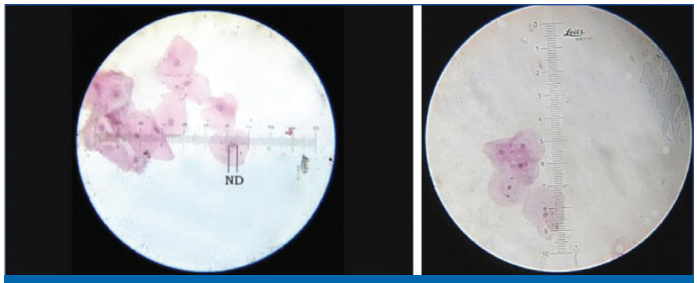
With the consent of the participants, samples were taken with a moistened wooden spatula from the buccal mucosa of the case and control groups and placed on labelled, cleaned, dry glass slides. Slides were immediately fixed with 95% ethyl alcohol. Then slides were stained using the routine Haematoxylin and Eosin staining technique. The cells were examined under 40x microscopes. Unfolded cells with clear outline were only selected for the study [Table/Fig-1]. The sampling was done by moving the microscope stage in "Z" shape to avoid recounting of the same cell. In each smear, cells were observed by using stage micrometer and ocular micrometer. In all cases ND and CD were measured in both horizontal and vertical axis in micrometer. The mean ND and CD value were obtained for each case [Table/Fig-2,3]. The N:C ratio was calculated for each cases.



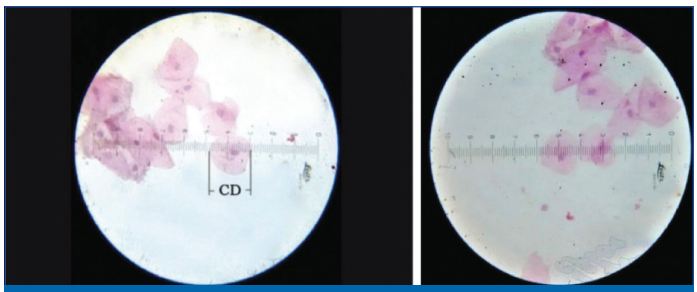
[Table/Fig-1]: Showing normal buccal squamous cells (H&E), 40x.

STATISTICAL ANALYSIS

Student t-test was used and "p-value" was calculated by using MedCalc®V12.5.0 software for comparison of various parameters between males and females within a group and in males and females of different age groups. A p-value <0.05 was considered as statistically significant.



[Table/Fig-2]: Showing measurement of Nuclear Diameter (ND) in squamous buccal cell (H&E), 40x.



[Table/Fig-3]: Showing measurement of cytoplasmic Diameter (CD) in squamous buccal cell (H&E), 40x.

RESULTS

Total 50 iron deficiency anaemia patients, age ranges from 18-60 years were compared with gender matched 50 healthy controls. Out of 50, 25 were males and 25 were females in each group. The mean age of subjects in control group was 31.06±11.59 years and in cases was 35.34±11.91 with p-value >0.01.

There was significant difference observed in various parameters of blood between cases and controls like haemoglobin, total RBC count, MCV, MCH and MCHC [Table/Fig-4].

Parameters (mean±SD)	Cases	Control	Results
Haemoglobin, (g/dL)	10.47±1.56	14.14±1.34	T=13.703, p<0.0001
Total RBC (mill/mm^3)	4.25±0.531	4.90±0.686	T=5.331, p<0.0001
MCV (µm^3)	73.35±8.36	81.35±11.47	T=3.98, p=0.0001
MCH (pg/cell)	25.58±2.05	27.91±2.159	T=5.54, p<0.0001
MCHC (g/dL)	31.69±1.75	34.40±1.842	T=7.546, p<0.0001

[Table/Fig-4]: Comparison of various anaemic parameters to distinguish between cases and controls.
Student's independent t-test were used to calculate the p-value (significant at 5% level of significance)

There was a significant difference (p-value <0.0001) in CD, ND, and N:C ratio in between both groups. The mean value of the cytoplasmic diameter (µm) of the buccal mucosa was less in the iron deficiency anaemia (46.57±2.03) as compared with the healthy individuals (57.11±3.65). The mean value of nuclear diameter (µm) of buccal mucosa was greater in iron deficiency anaemia (11.34±0.58) when compared with healthy individuals (10.13±1.07). Mean value of nuclear cytoplasmic ratio of buccal mucosa was greater in iron deficiency anaemia patients as compared with healthy individuals [Table/Fig-5].

Parameters	Cases (Mean±SD)	Control (Mean±SD)	Results
CD µm	46.57±2.03	57.11±3.65	T=17.824, p<0.0001
ND µm	11.34±0.58	10.13±1.07	T=-7.031, p<0.0001
N/C ratio	0.24±0.02	0.18±0.02	T=-17.452, p<0.0001

[Table/Fig-5]: Showing comparison of cases (male+female) and control (male+female) groups with respect to mean CD, ND and N/C ratio.
Student's independent t-test were used to calculate the p-value (Significant at 5% level of significance)

The study showed no statistical significance between CD, ND, and N/C ratio between case and control groups with respect to different

age groups (p>0.05). The study also showed that there were no significant results observed (p>0.05) when compared between male and female cases, neither between male and female control.

When statistical analysis was conducted between male cases and controls, there was a significant difference (p-value <0.0001) found in CD, ND, and N/C ratio. Mean CD (µm) was lesser (47.95±1.56) in male cases as compared with controls (57.83±4.33). Mean ND (µm) and mean N/C ratio (µm) was greater in male cases as compared with controls [Table/Fig-6].

Parameters	Cases (Mean±SD)	Control (Mean±SD)	Results
CD, µm	47.95±1.56	57.83±4.33	T=10.743, p<0.0001
ND, µm	11.29±0.65	10.17±1.03	T=-4.582, p<0.0001
N/C ratio	0.24±0.02	0.18±0.02	T=-12.152, p<0.0001

[Table/Fig-6]: Showing comparison of male cases and male controls with respect to mean CD, ND and N/C ratio.
Student's independent t-test were used to calculate the p-value (p<0.05 was considered significant)

Similar significant results (p<0.0001) were found when comparison was done between female cases and female controls. There were decrease in CD in cases (45.20±1.44) compared to controls (56.39±2.73), respectively. Whereas increase in ND (11.39±0.51) and N/C ratio (0.25±0.02) of female cases compared to ND and N/C ratio of female controls, respectively [Table/Fig-7].

Parameters	Cases	Control	Results
Cytoplasmic diameter µm (CD)	45.20±1.44	56.39±2.73	T=18.152, p<0.0001
Nuclear diameter µm (ND)	11.39±0.51	10.08±1.13	T=-5.269, p<0.0001
N:C ratio	0.25±0.02	0.18±0.02	T=-13.539, p<0.0001

[Table/Fig-7]: Showing comparison of female cases and female control groups with respect to mean cytoplasmic diameter µm CD, ND and N/C ratio.
Student's independent t-test were used to calculate the p-value (p-value <0.5 was considered as significant)

DISCUSSION

Iron deficiency is the most common deficiency in the world. Errors in iron balance lead to iron deficiency anaemia, resulting from faulty iron absorption, chronic blood loss, inadequate iron intake and increased iron requirements during infancy, childhood, adolescence, and pregnancy [4]. There may be burning sensations in the oral mucosa, lingual varicosities, dry mouth, oral lichen planus and atrophic glossitis due to Iron deficiency anaemia [15]. Localised infections and systemic disorders are first manifestations seen in oral mucosa [3].

Oral Cytology is a fast, simple, painless, bloodless non invasive diagnostic test. Quantitative parameters such as nuclear diameter, cytoplasmic diameter, and nuclear: cytoplasmic ratio have been useful in identifying oral lesions associated with iron deficiency anaemia [16].

In this study, mean CD value in iron deficiency anaemia was 46.57 µm. On comparing it with that of the control group, it was seen that the mean CD in iron deficiency anaemia was lesser, which was significant. Result obtained in this study was in agreement with studies by Boddington MM (1959) [17] and Vanishree M et al., (2014) [16] who found smaller mean cytoplasmic diameter in iron deficiency anaemia. Sumanthi J et al., (2012) [11] and Mahdieh RM et al., (2020) [10] reported that there was no significant change in CD in iron deficiency anaemia and Cowpe JG et al., (1991) [18] compared the values obtained by plane metric method with that of image analysis system and found that there was a significant elevation in the measurement of cytoplasmic size. This difference may be due to application of different methods of measurement and difference in the population included in the study. Changes in the cytoplasmic diameter may be due to variety of tissue changes that have been described in patients suffering from iron deficiency anaemia and although it is generally assumed that these result from derangement of intracellular iron metabolism, no satisfactory evidence of this relationship had been established by Jacobs A (1969) [19].

In this study, mean ND value in iron deficiency anaemia was 11.34 µm. On comparing it with that of the control groups, it was found to be greater in the cases with iron deficiency anaemia which was significant. Similar result was found by Gururaj N et al., (2004) [12], Sumanthi J et al., (2012) [11] and Vanishree M et al., (2014) [16], Boddington MM (1959) [17], found increase in mean ND in both the cases of papillated and depapillated tongue of iron deficiency anaemia patients. While it is difficult to determine the exact cause of increased ND, iron may be required for ribonucleotide reductase, which reduces nucleotide sugar groups to their respective deoxy derivatives, the Deoxyribonucliec Acid (DNA) precursors. When this enzyme is decreased, DNA synthesis is impaired, resulting in increased nuclear diameter of exfoliated cells in iron deficiency anaemia [11]. The present study results were contradicted by the study done by Mahdieh RM et al., (2020) [10] in which no significant difference was found in ND between cases and controls.

In this study, mean N/C ratio in iron deficiency anaemia was 0.24 µm on comparing it with that of the control group, there were increase in the mean N:C ratio in iron deficiency anaemia and the result was found to be significant. Similar result were obtained by Boddington MM (1959) [17], Rennie JS et al., (1984) [20], Scott J et al., (1985) [21], Gururaj N et al., (2004) [12], Sumanthi J et al.,(2012) [11], Vanishree M et al., (2014) [26] who found there were statistically significant increase in N:C ratio in iron deficiency anaemia. Mahdieh RM et al., (2020) found no significant difference in N:C ratio between cases and controls [10]. As the activity of the cell increases, two morphological changes occur in the cell with regard to the relationship between the nucleus and the cytoplasm. Because the cytoplasm has less ability to mature into its most mature cell type, there is a greater immaturity to the cytoplasm, which has a high activity level. Moreover, cells make less cytoplasm relative to nucleoplasm, so the N/C ratio increases [22].

Present study showed no statistical significance between CD, ND and N:C ratio in different age groups. This was in correlation with other study done by Macleod RI et al., (1988) [23] and Sumanthi J et al., (2012) [11]. It may be a result of hormones like oestrogen and progesterone that promote anabolism and growth and increase during puberty but decrease over time. Both genders are equally affected by these hormones in relation to cellular diameter and nuclear diameter as they age [12]. In both men and women, cellular senescence can account for age-related variations [24]. Basal cells can divide only for certain numbers of times. As cells age, their ability to renew themselves declines, resulting in the accumulation of senescent cells. In the oral cavity, cells that spend a longer period of time succumb to various local environmental factors [25].

There was very significant difference found in CD, ND and N:C ratio when compared between male cases with male control groups as well as female cases with female control groups. Result obtained in this study was similar to Scott J et al., (1985) [21]. But present study also revealed that there was no significant difference found in CD, ND and N:C ratio when compared between male cases with female cases and male controls with female control. Similar result were found by Scott J et al., (1985) [21], Sumanthi J et al., (2012) [11] and Vanishree M et al., (2014) [16], According to them age and sex did not show any significant influence on buccal cell of study group.

Cytological studies have also been conducted on many other diseases showing significant changes. Paraizo JU et al., (2013) revealed that sickle cell anaemia patients had a significantly increased nuclear area compared to controls, but no other morphological differences were found between the groups [26]. According to Pankaj KA et al., (2021) [27] individuals with sickle cell anaemia, regardless of clinically visible oral lesions, show cytological changes in oral mucosal epithelium. Anaemia associated

S. No.	Author's name and year	Place of study	Sample size (cases: IDA)	Parameters compared	Conclusion
1	Boddington M et al., (1959) [17]	Headington, Oxford	10 cases and 10 control	CD, ND, Nuclear abnormalities	In iron deficiency anaemia CD was reduced whereas ND was decreased when compared with healthy controls.
2	Scott J et al., (1985) [21]	Liverpool	12 cases and 161 controls	ND, N:C ratio, MEAN epithelial thickness, thickness of Maturation layer, and thickness of Progenitor layer of tongue	There is no significant changes found in ND but N:C ratio was high in IDA patients.
3	Gururaj N et al., (2004) [12]	Chennai, India	40 cases and 40 controls	ND. CD, N:C ratio	All ratios were increased in cases. Iron deficiency can induce various changes in buccal mucosa at cytological level.
4	Sumanthi J et al., (2012) [11]	Andhra Pradesh, India	40 cases and 40 controls	CD, ND, N:C ration, Red blood cell parameters	Using cytomorphometric quantification by computerised image analysis, the study found encouraging results in estimating CD, ND, and N/C ratios. Screening for iron deficiency anaemia can be done with this prospective, non invasive technique.
5	Vanishree M et al., (2014) [16]	Karnataka, India,	30 Cases and 30 controls	CD, ND and N:C ratio	Iron deficiency can causes significant changes in oral exfoliative cells. Cytomorphometric analysis of smears is useful in detecting the changes in iron deficiency anaemia.
6	Mahdieh RM et al., (2020) (10)	Iran	40 cases and 40 controls	Cellular clumping, CD, ND, cytoplasmic area (CA), nuclear area (NA), nucleus to cytoplasmic area ratio (NA/CA), cellular and nuclear pleomorphism, micronuclei (Mn), binucleation, bacterial colonies, and keratin flakes	The study demonstrated that iron deficiency anaemia causes significant changes to the oral mucosa. Using cytomorphometry and exfoliative cytology, it is possible to assess the mucosal changes in anaemia patients with iron deficiency.
7	Present study	Jamnagar	50 cases and 50 controls	ND, CD and N:C ratio	The decrease in cytoplasmic diameter and increase in nuclear diameter in iron deficiency anaemia suggested that iron deficiency causes significant changes in oral exfoliative cells

[Table/Fig-8]: Comparison of various similar studies: Comparison of the Cytoplasmic diameter, Nuclear diameter and N:C ratio between different studies [10-12,16,17,21].
IDA: Iron deficiency anaemia

with iron deficiency induces significant changes in oral mucosal cytology [10]. According to Aktunc E et al., (2016) Exfoliative cytology and cytomorphometric analysis can be used to detect cytomorphometric changes in buccal cells associated with Behcet's disease, it is a multisystemic inflammatory disease characterised by oral and genital ulcers along with involvement of the cutaneous surface, ocular surface, arthritic process, vascular system, central nervous system, and gastrointestinal tract [28]. Shrilatha T et al., (2021) also conducted cytological study among Smokers alone, as well as those with hypertension and smoking, exhibit significant cytomorphometric changes. There were increase in ND, CD and N:C ratio among study group compared with controls [29]. Suvarna M et al., (2012) conducted cytological study on diabetic patient and found that there was significant changes in ND and N:C ratio in diabetic compared to healthy individual [30].

Thus the methodology of present study may be helpful in diagnosing iron deficiency anaemia in present study population. Although the methodology and population studied was different from various other workers, the results were similar to many workers. Various similar studies have been cited in [Table/Fig-8] [10-12,16,17,21].

Limitation(s)

The present study was not conducted according to the severity of anaemia, which could have influenced the results. A large sample size research study including the above deficiencies would enlighten aspiring medical practitioners. Another limitation was that, there was no baseline for normal cytomorphometry of buccal cells based on age and gender.

CONCLUSION(S)

The present study showed statistically significant difference between cases and control regarding changes in various parameters of buccal squamous cells in patients of iron deficiency anaemia. Also, the difference was irrespective of gender and age groups. The decrease in cytoplasmic diameter and increase in nuclear diameter in iron deficiency anaemia suggested that iron deficiency causes significant changes in oral exfoliative cells. The analysis of cytomorphometric smears is useful for detecting changes in iron-deficiency anaemia. Thus, cytology could be a non invasive and prospective screening tool for clinicians to identify patients who suffer from iron deficiency anaemia.

REFERENCES

[1] Rajendran R, Sivapathasundharam B. Shafer's textbook of oral pathology. Ch 14 Healing of oral wounds. 7th ed. Reed Elsevier India Pvt Ltd. 2012: pp. 596-97.
[2] Lester R Cahn, Kurt H Thomas. Studies in diagnosis in oral surgery and oral medicine. Oral Surg Oral Med Oral Pathol. 1960:13:994-09.
[3] Lain ES. Lesions of the oral cavity caused by physical and physicochemical factors. Arch Derm Syphilol. 1940:41:295-307.
[4] Rajendran R, Sivapathasundharam B. Shafer's Textbook of Oral Pathology. Ch 18 Diseases of the blood and blood forming organs. 7th ed. Reed Elsevier India Pvt Ltd. 2012: pp. 761-800.
[5] Kumar V, Abbas AK, Fausto N, Aster JC, Robbins, Cotran. Pathological basis of diseases. Ch. 14 Red cells and bleeding disorders. 8th ed. Reed Elsevier India Pvt Ltd; 2013: pp. 639-62.
[6] World Health Organization. World-Wide Prevalence of Anaemia. 1993 to 2005. World Health Organization. Geneva, Switzerland; 2008.
[7] Government of India. National family health survey 4- key indicators: 2015-2016. National Family Health Survey. 2016;4:01-08.
[8] Kalaivani K, Ramachandran P. Time trends in prevalence of anaemia in pregnancy. Indian J Med Res. 2018;147(3):268-77.
[9] Kohli M, Ahuja P, Mehendiratta M, Sharma M, Dutta J. Micronucleus assay: An early diagnostic tool to assess genotoxic changes in patients with tobacco use, oral leukoplakia and oral submucous fibrosis. J Clinic Diagn Res. 2017;11:ZC28.
[10] Mahdieh RM, Hamid A, Maryam M, Hemmat G, Shima N. Cytomorphometric analysis of buccal exfoliated cells in patients with iron deficiency anemia. Acta Medica Iranica. 2020;58:424-29.
[11] Sumanthi J, Reddy GS, Anuradha C, Sekhar PC, Prasad LK, Reddy BR. A study on cytomorphometric analysis of exfoliative buccal cells in iron deficiency anemic patients. Contemp Clin Dent. 2012;3:S156-59.
[12] Gururaj N, Sivapathasundharam B, Sumathy N. Cytological findings in iron deficiency anemia. Indian J Dent Res. 2004;15:126-28.
[13] Ministry of Health & Family Welfare. Guidelines for control of iron deficiency anaemia. National Iron plus Initiative. New Delhi: MoHFW, Government of India; 2013. [accessed on September 23, 2018].
[14] Kumar V, Abbas AK, Fausto N, Aster JC, Robbins, Cotran. Pathological basis of diseases. Ch. 8 Environmental & Nutritional disease. 1st South Asia Ed. Reed Elsevier India Pvt Ltd; 2018: pp. 299-339.
[15] Wu YC, Wang YP, Chang YF, Cheng SJ, Chen HM, Sun A. oral manifestation and blood profile in patient with iron deficiency anaemia. J Formos Med Assoc. 2014;113:83-87.
[16] Vanishree M, Kale A, Hallikerimath S, Thakur B. Quantitative cytology of squames obtained from iron deficiency anaemia and healthy individuals. J Adv Clin Res Insights. 2014;1:10-13.
[17] Boddington MM. changes in buccal cells in anemia. J Clin Pathol. 1959;12:222-27.
[18] Cowpe JG, Green MW, Ogden GR. Quantitative cytology of oral smears. A comparison of two methods of measurement. Anal Quant Cytol Histol. 1991;13(1):11-15.
[19] Jacobs A. Iron deficiency and the oral mucous membrane. Br J Dermatol. 1969;81:861-62.
[20] Rennie JS, MacDonald DG, Dagg JH. Iron and the oral epithelium a review. JR Soc Med. 1984;77:602-07.
[21] Scott J, Valentine JA, St Hill CA, West CR. Morphometric analysis of atrophic changes in human lingual epithelium in iron deficiency anaemia. J Clin Pathol. 1985;38:1025-29.

[22] Bibbo M. Comprehensive Cytopathology. 2nd ed. Philadelphia. WB. Saunders Company. 1977:845.
[23] Macleod RI, Hamilton PJ, Soames JV. Quantitative exfoliative oral cytology in iron-deficiency and megaloblastic anemia. Anal Quant Cytol Histol. 1988;10:176-80.
[24] Wynford Thomas D. Cellular senescence and cancer. J Pathol. 1999;187:100-111.
[25] Coates PJ. Markers of senescence? J Pathol. 2002;196:371-73.
[26] Paraizo J, Rech I, Azevedo-Alanis L, Pianovski M, De Lima A, Machado M. Cytomorphometric and cytomorphologic analysis of oral mucosa in children with sickle cell anemia. J Cytol. 2013;30:104-08.
[27] Pankaj KA, Sonam A, Shivkumar BN, Mosby SP, Kiran KC, Anjali S. Evaluation and comparison of micronuclei in exfoliative cytology of buccal mucosa in normal individuals and sickle cell anemia patients of durg city of Chhattishgarh State. European Journal of Molecular and Clinical Medicine, 2021;7(10):3555-63.
[28] Aktunc E, Oz ZS, Bektas S, Altinyazar C, Koca R, Bostan S. Cytomorphometric characteristics of buccal mucosal cells in Behçet's disease patients. Anal Cell Pathol (Amst). Epub. 2016:5.
[29] Srilatha T, Manthapuri S, Shylaja S, Ramanand OV, Reddy ES, Vamshi VR. Cytomorphometric analysis of exfoliated buccal mucosal cells in smokers and patients with hypertension: A quantitative analysis. J Int Oral Health 2021;13:53-59.
[30] Suvarna M, Anuradha C, Kumar K Kiran, Sekhar P Chandra, Lalith Prakash Chandra K, Ramana Reddy BV. Cytomorphometric analysis of exfoliative buccal cells in type II diabetic patients. Journal of Dr. NTR University of Health Sciences. 2012;1:33-37.

PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Anatomy, Dr. Kiran C Patel Medical College and Research Institute, Bharuch, Gujarat, India.
2. Assistant Professor, Department of Anatomy, Dr. Kiran C Patel Medical College and Research Institute, Bharuch, Gujarat, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Ritika M Patel,
Assistant Professor, Department of Anatomy, Dr. Kiran C Patel, Medical College and Research Institute, Civil Hospital, Bharuch, Gujarat, India.
E-mail: doc.ritika@yahoo.in

PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Feb 17, 2022
- Manual Googling: Jun 17, 2022
- iThenticate Software: Jul 20, 2022 (18%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval Obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. No

Date of Submission: **Feb 15, 2022**
Date of Peer Review: **Mar 11, 2022**
Date of Acceptance: **Jun 18, 2022**
Date of Publishing: **Oct 01, 2022**