International Journal of Innovative Research in Medical Science (IJIRMS)
Volume 10, Issue 02, February 2025,
https://doi.org/10.23958/ijirms/vol10-i02/2032

Original Article

Exfoliative Cytology for Age Evaluation: A Comparative Study in Different Age Groups

Dr. Vardendra Manvikar [1], Dr. Pradeep M R [2], Dr. H Aparna Latha *[3], Dr. Shreekanth N G [4], Dr. Vijaya Kumar Velpula [5], Dr. K. M. Parveen Reddy [6]

[1]Associate Professor, Department of Oral and Maxillofacial Pathology and Microbiology, Navodaya Dental College and Hospital Raichur, Karnataka, India.
[2]Associate Professor, Department of Oral and Maxillofacial Pathology, AME's Dental College, Raichur, Karnataka, India.
[3]Assistant Professor, Department of Oral and Maxillofacial Pathology and Microbiology, Navodaya Dental College and Hospital Raichur, Karnataka, India.
[4]Associate Professor, Department of Oral and Maxillofacial Pathology, Srinivas Institute of Dental Sciences, Mukka, Mangalore, India.
[5]Oral Pathologist, Ongole, Andhra Pradesh, India.
[6]Associate Professor, Department of Pediatrics and Preventive Dentistry, Navodaya Dental College and Hospital, Raichur, Karnataka, India.

*Corresponding author: Dr. H Aparna Latha; *aparnalatha1993@gmail.com*

Received 18 January 2025; Accepted 09 February 2025; Published 14 February 2025

Abstract

Introduction: In the context of a criminal investigation or a mass tragedy, determining the age of missing human remains is crucial because details like gender, birthdate and year of death can help investigators narrow down a vast pool of potential matches and identify the right person. Oral exfoliative cytology is a simple, relatively easy procedure that requires little discomfort from the patient to be repeated. ***Objective:*** The purpose of the study was to use image analysis morphometric software to compare the average cell size in buccal smears in order to estimate an individual's age. ***Materials and Methods:*** Buccal smears were collected from 100 apparently healthy individuals. The smears were stained according to the standard Papanicolaou laboratory protocol after being fixed in 95% alcohol. The image analysis program version 4.3 from Dewinter was used to calculate the average cell size. ***Results:*** The average cell size of each individual significantly decreased as age increased, according to the results. The average cell size decreased with age, according to cytomorphometry. A statistically significant reduction in the average cell size was revealed by analysis, using one-way ANOVA and Bonferroni methods ($P < 0.005$). ***Conclusion:*** Buccal smears show age-related changes that can be utilized as an age estimation technique. Compared to other screening modalities, which are typically either invasive or costly, cytomorphometric examination of exfoliated cells of the buccal mucosa offers as a potential alternative non-invasive approach in evaluation and correlation of an individual's age.

Keywords: ***Cytomorphometric Analysis, Cell diameter, Age estimation, Exfoliative cytology, Image analysis, Papanicolau Stain***

Introduction

The identification of human bodies is a challenging issue for the investigators when there are no hints as to the identity from circumstantial facts. The age and gender of the body can be vital information for investigators to narrow down their search for people who might fit missing person lists and, as a result, reduce efforts involving extremely implausible alternatives. Age cannot be easily ascertained via DNA testing, even though gender can now be ascertained [1]. The term "Forensic Age Estimation" (FAE) refers to a forensic medical specialty that attempts to determine, as precisely as possible, the chronological age of an individual whose age is unknown but who is involved in court or legal proceedings [2]. When evaluating a person's development, both mentally and physically, age is an essential instrument. An individual's age is helpful for documentation and authentication in addition to being a source of information. In the realm of forensic sciences, age and gender are two crucial factors for identifying a deceased person when there is no other viable means of identification. The degree of tooth wear, the quantity of secondary dentin production, the tooth-to-pulp ratio, DNA analysis, and the architecture of the skeletal system are some of the techniques utilized in forensic investigations to estimate age. The current age estimation methods are either overly simplistic and inaccurate, or they are too costly and sophisticated. While

radiocarbon dating of teeth is not cost-effective, age estimation methods based on factors such as tooth wear, quantity of secondary dentin development, tooth-to-pulp ratio, etc., are less precise. Moreover, radiological evaluation of skeletal and dental development is less helpful in adults than in children [4]. When estimating age in children and adolescents, morphological techniques such radiologically examining the development of the skeleton and teeth are frequently used [3]. More precise laboratory techniques, such as radiocarbon dating of dental enamel or racemization of aspartic acid in dentin or tooth enamel, are not economical [1]. Exfoliative cytology is a non-invasive method that makes it easy and painless to extract intact cells for microscopic analysis from various layers inside the epithelium [5]. The development of cytomorphometric image analysis tools has greatly simplified the process of determining the size of exfoliated cells. Cytomorphometry has also been used to assess other cellular properties, such as nuclear size and perimeter and cellular perimeter. On the other hand, cell size has shown to be an important factor in determining an individual's age. Results can be obtained in a few hours on the same day. Additionally, the arsenal needed is smaller and more affordable [6]. As part of regular physiological turnover, epithelial cells are constantly renewing themselves. They are exfoliated when they rise from the basal layer to the surface. With a sensitivity of 89% and specificity of 89.5%, oral exfoliative cytology is a quick, easy, non-invasive treatment. The majority of oral epithelium research is conducted when the tissue is diseased. The detection of oral premalignant, possibly malignant, or malignant lesions has been accomplished with the use of oral exfoliative cytological method. However, until the basic findings in normal oral mucosal cells are established, secrets of pathology cannot be studied. There are very few cases where normal buccal mucosal smears are researched since the first investigations of normal epithelial smears by Miller and Montgomery in 1951 [7]. Because of the subjective nature of its interpretations and frequent false-negative outcomes, oral exfoliative cytology was not used as often in the past. Quantitative techniques, like image analysis systems, were introduced to address these constraints, particularly in the evaluation of cytomorphometric cellular alterations [5]. It was first suggested by Donne in 1945 that it would be possible to measure the size of tiny objects. Since then, quantifying cells and their constituent parts has been a cerebral task. Technology for image analysis can replace human vision by being programmed to analyse cells. The image analysis approach for examining leukocytes was first devised in 1960 by Prewitt and Mendelson. Later, Weid and his colleagues adapted it to analyze, cells in cervical smears. When diagnosing malignant and premalignant diseases, as well as Type 2 diabetes mellitus, morphometric analysis can be employed in oral smears. Atrophy, which is defined as a reduction in a part's size and functional activity and can be either localized or generalized, is frequently associated with becoming older. Specific regions may experience simple, degenerative, or numerical changes as a result of local atrophy. Individual cells shrink in size in the simple variety [6,8]. In this study, the exfoliated cells from the buccal mucosa are examined using EC to determine the size of the cells and estimate an individual's age.

Aim and Objective

The study was done to estimate the age and gender of an unknown individual using smear of exfoliative cells of buccal mucosa stained with hematoxylin and eosin by comparing the average cell size using a stage micrometer-microscopic grid.

Materials and Methods

A total of one hundred seemingly healthy subjects, twenty in each age group (10 males and 10 females), were chosen at random from the Navodaya Dental College and Hospital's outpatient department. Group I consisted of respondents aged 10-20, Group II of subjects aged 21-30, Group III of subjects aged 31-40, Group IV of subjects aged 41-50, and Group V of patients aged >50. The study comprised clinically normal adults without a history of systemic disease or use of therapeutic drugs. Excluded those who had a history of alcohol consumption, tobacco use, or systemic illness. Individuals from every age group had buccal mucosa samples taken for oral smears. Using a moistened wooden spatula, the smear samples were gently scraped off normal, healthy-looking buccal mucosa and quickly smeared onto a clear glass slide. After that, it was fixed for 15 minutes with 95% ethyl alcohol before the Papanicolaou staining method was used. For image analysis, the stained smear samples were examined under a microscope. Photos were focused on a stage micrometre scale with a 40x magnification. A mean of twenty distinct cells was analysed and quantified in micrometres on both the horizontal and vertical axes. Cells that were folded or clumped were excluded from the measurement. Using the Lawrence and Mayo LM-52-3001 microscope, pictures were taken and projected onto the computer for image processing. The cells were then manually labelled using Dewinter's image analysis software.

Statistical Analysis

The various parameters were used to analyse the CP, NP and NP:CP ratio of each cell in relation to age and sex of apparently healthy subjects. One-way ANOVA test was used for analysis of CA, NA, and N:C ratio in relation of age and sex. The average cell size values were obtained for each case and statistically analysed using one-way ANOVA, Bonferroni comparison tests. Student's t test was used to evaluate the difference in CA, NA, and N:C ratio in male and female in various age groups. The level of significance was set at $P<0.05$. Data was reported as mean and standard deviations. Ethical clearance was obtained from the Institutional Ethics Committee.

Results

Buccal smears were collected from 100 subjects with 5 age groups (Group I 10-20 years, Group II 21-30 years, Group III 31-40 years, Group IV 41-50 years, Group V > 50 years) with 20 subjects in each age group (10 Males & 10 Females) as shown in [**Table 1**] and CP, NP, and CP:NP ratio was measured. The mean values of CP in Group I-232.25, Group II-272.56, Group III-278.68, Group IV-178.98 and Group V-129.26. The mean values of NP in Group I-77.06, Group II-77.23, Group III-75.42, Group IV-70.40 and GroupV-59.29. The mean values of CP:NP in Group I-34.44, Group II-32.39, Group III-36.88, Group IV-28.19 and Group V-27.16 as shown in [**Table 2, 3, 4, 5 & Graph 1**]. CP, NP & CP:NP was decreased from Group I to Group V and also showed statistically significant difference. With respect to gender, CP was increased in females when compared to males. Hence, comparison of CP with respect to gender showed statistically significant difference ($P= 0.000 < 0.001$), but NP & CP:NP did not show ant statistically significant difference as shown in [**Table 6 & Graph 2**]. Comparison of CP & NP in Group I with respect to gender showed statistical significance ($P=0.000 < 0.001$, $P=0.002$) respectively, but CP:NP showed statistically significant difference as shown in [**Table 7, Graph 3**]. In Group II only CP showed statistically significance ($P=0.000 < 0.001$) with respect to gender as shown in [**Table 8, Graph 4**]. In Group III both CP & NP showed statistical significance ($P=0.002$, $P=0.027$) with respect to gender as shown in [**Table 9 & Graph 5**]. CP:NP in Group IV with respect to gender showed statistically significant difference as

shown in [**Table 10 & Graph 6**]. CP & NP in Group V with respect to gender showed statistically significance (P=0.019, P=0.010] as shown in [**Table 11, Graph 7**]. There was a difference in terms of CP, NP & CP:NP with respect to age and gender.

Table 1: Division of study subjects based on their age and gender

		Sex		Total
		Male	Female	
AGE in Years	10 - 20 Years	10	10	20
	21 - 30 Years	10	10	20
	31 - 40 Years	10	10	20
	41 - 50 Years	10	10	20
	> 50 Years	10	10	20
Total		50	50	100

Table 2: Comparison of CP, NP & NP:CP within age groups

Parameters	Age Group	N	Mean	Std. Deviation	F Value	P Value
Cell Perimeter	10 - 20 Years	20	232.25	58.381	35.216	**0.000 < 0.001**
	21 - 30 Years	20	272.56	63.719		
	31 - 40 Years	20	278.68	56.397		
	41 - 50 Years	20	178.98	21.126		
	> 50 Years	20	129.26	21.133		
Nuclear Perimeter	10 - 20 Years	20	77.06	9.164	25.362	**0.000 < 0.001**
	21 - 30 Years	20	77.23	5.394		
	31 - 40 Years	20	75.42	6.593		
	41 - 50 Years	20	70.40	5.017		
	> 50 Years	20	59.29	6.610		
Nuclear: Cytoplasm Ratio	10 - 20 Years	20	34.44	6.069	11.77	**0.000 < 0.001**
	21 - 30 Years	20	32.39	6.221		
	31 - 40 Years	20	36.88	5.416		
	41 - 50 Years	20	28.19	4.995		
	> 50 Years	20	27.16	3.735		

Table 3: Comparison of cell perimeter among age groups

Multiple Comparisons						
Bonferroni	**Cell Perimeter**					
(I) AGE in Years		**Mean Difference (I-J)**	**Std. Error**	**Sig.**	**95% Confidence Interval**	
					Lower Bound	**Upper Bound**
10 - 20 Years	21 - 30 Years	-40.307	15.193	0.094	-83.974	3.360
	31 - 40 Years	-46.432	15.193	**0.029**	-90.099	-2.765
	41 - 50 Years	53.272	15.193	**0.007**	9.604	96.939
	> 50 Years	102.990	15.193	**0.000**	59.323	146.657
21 - 30 Years	31 - 40 Years	-6.125	15.193	1.000	-49.792	37.542
	41 - 50 Years	93.579	15.193	**0.000**	49.911	137.246
	> 50 Years	143.297	15.193	**0.000**	99.630	186.964
31 - 40 Years	41 - 50 Years	99.703	15.193	**0.000**	56.036	143.371
	> 50 Years	149.422	15.193	**0.000**	105.755	193.089
41 - 50 Years	> 50 Years	49.719	15.193	**0.015**	6.052	93.386

**. The mean difference is significant at the 0.05 level.*

Table 4: Comparison of nuclear perimeter among age groups

Multiple Comparisons						
Bonferroni	**Nuclear Perimeter**					
(I) AGE in Years		**Mean Difference (I-J)**	**Std. Error**	**Sig.**	**95% Confidence Interval**	
					Lower Bound	**Upper Bound**
10 - 20 Years	21 - 30 Years	-0.168	2.123	1.000	-6.270	5.934
	31 - 40 Years	1.642	2.123	1.000	-4.461	7.744
	41 - 50 Years	6.659	2.123	0.023	0.557	12.762
	> 50 Years	17.771	2.123	0.000	11.668	23.873
21 - 30 Years	31 - 40 Years	1.809	2.123	1.000	-4.293	7.912
	41 - 50 Years	6.828	2.123	0.018	0.725	12.930
	> 50 Years	17.939	2.123	0.000	11.836	24.041
31 - 40 Years	41 - 50 Years	5.018	2.123	0.201	-1.084	11.120

	> 50 Years	16.129	2.123	0.000	10.027	22.231
41 - 50 Years	> 50 Years	11.111	2.123	0.000	5.009	17.213
*. The mean difference is significant at the 0.05 level.						

Table 5: Comparison of NP:CP among age groups

Multiple Comparisons						
Bonferroni	**Nuclear: Cytoplasm Ratio**					
(I) AGE in Years		**Mean Difference (I-J)**	**Std. Error**	**Sig.**	**95% Confidence Interval**	
					Lower Bound	**Upper Bound**
10 - 20 Years	21 - 30 Years	2.052	1.696	1.000	-2.822	6.925
	31 - 40 Years	-2.441	1.696	1.000	-7.314	2.433
	41 - 50 Years	6.252	1.696	**0.004**	1.379	11.126
	> 50 Years	7.277	1.696	**0.000**	2.404	12.151
21 - 30 Years	31 - 40 Years	-4.492	1.696	0.094	-9.366	0.381
	41 - 50 Years	4.201	1.696	0.150	-0.673	9.074
	> 50 Years	5.226	1.696	**0.027**	0.352	10.099
31 - 40 Years	41 - 50 Years	8.693	1.696	**0.000**	3.820	13.566
	> 50 Years	9.718	1.696	**0.000**	4.844	14.591
41 - 50 Years	> 50 Years	1.025	1.696	1.000	-3.849	5.898
*. The mean difference is significant at the 0.05 level.						

Table 6: Comparison of CP, NP & NP:CP with respect to gender

		N	**Mean**	**Std. Deviation**	**" t " Value**	**P Value**
Cell Perimeter	Male	50	189.63	43.348	4.183	**0.000 < 0.001**
	Female	50	247.07	86.886		
Nuclear Perimeter	Male	50	71.55	7.298	0.349	0.728
	Female	50	72.21	11.280		
Nuclear: Cytoplasm Ratio	Male	50	32.99	6.496	0.692	0.066
	Female	50	30.63	6.191		

Table 7: Comparison of CP, NP & NP:CP in group I with respect to gender

10 - 20 Years		**N**	**Mean**	**Std. Deviation**	**" t " Value**	**P Value**
Cell Perimeter	Male	10	179.23	12.977	10.892	**0.000 < 0.001**
	Female	10	285.27	27.918		
Nuclear Perimeter	Male	10	71.17	4.414	3.725	**0.002**
	Female	10	82.95	8.979		
Nuclear: Cytoplasm Ratio	Male	10	35.78	6.016	0.986	0.337
	Female	10	33.10	6.131		

Table 8: Comparison of CP, NP & NP:CP in group II with respect to gender

21 - 30 Years		**N**	**Mean**	**Std. Deviation**	**" t " Value**	**P Value**
Cell Perimeter	Male	10	215.02	27.298	10.448	**0.000 < 0.001**
	Female	10	330.10	21.634		
Nuclear Perimeter	Male	10	75.35	4.775	1.628	0.121
	Female	10	79.11	5.545		
Nuclear: Cytoplasm Ratio	Male	10	34.58	6.098	1.643	0.118
	Female	10	30.20	5.819		

Table 9: Comparison of CP, NP & NP:CP in age group III with respect to gender

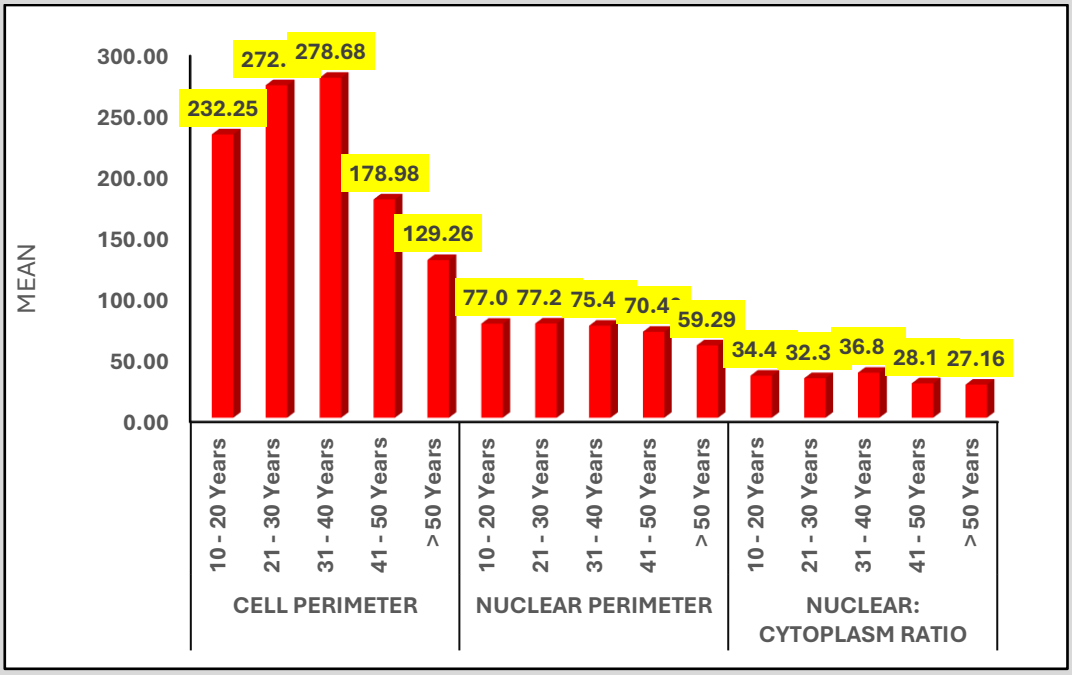
31 - 40 Years		**N**	**Mean**	**Std. Deviation**	**" t " Value**	**P Value**
Cell Perimeter	Male	10	242.99	35.018	3.623	**0.002**
	Female	10	314.38	51.545		
Nuclear Perimeter	Male	10	78.60	4.864	2.412	**0.027**
	Female	10	72.24	6.761		
Nuclear: Cytoplasm Ratio	Male	10	37.92	5.503	0.85	0.406
	Female	10	35.84	5.408		

Table 10: Comparison of CP, NP & NP:CP in age group IV with respect to gender

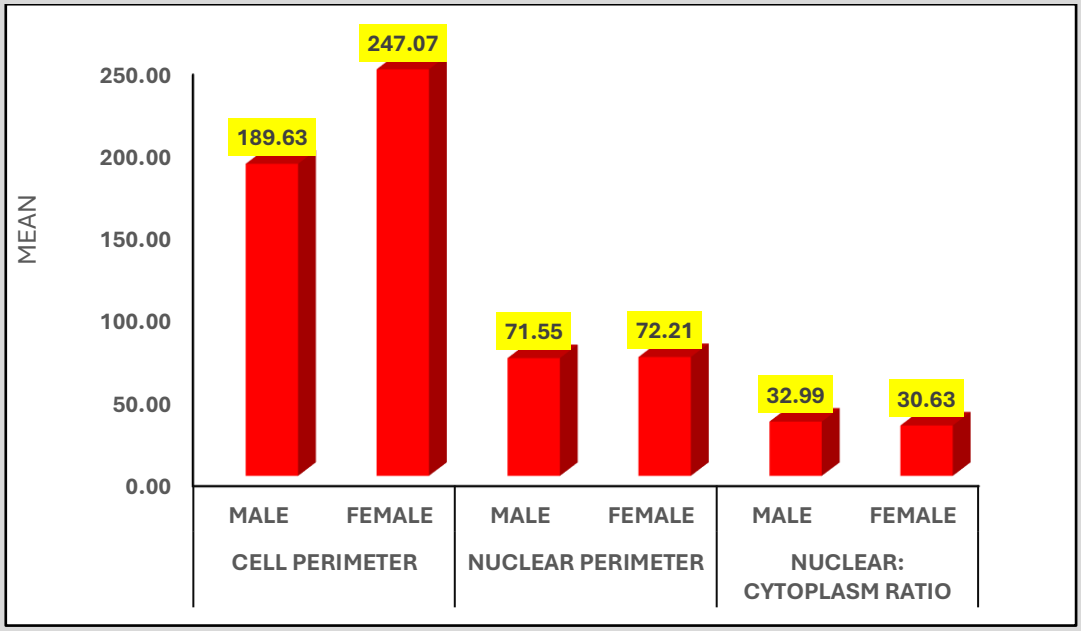
41 – 50		N	Mean	Std. Deviation	" t " Value	P Value
Cell Perimeter	Male	10	170.95	20.554	1.796	0.089
	Female	10	187.01	19.407		
Nuclear Perimeter	Male	10	69.74	5.442	0.577	0.571
	Female	10	71.06	4.750		
Nuclear: Cytoplasm Ratio	Male	10	30.51	3.942	2.304	**0.033**
	Female	10	25.87	5.013		

Table 11: Comparison of CP, NP & NP:CP in age group V with respect to gender

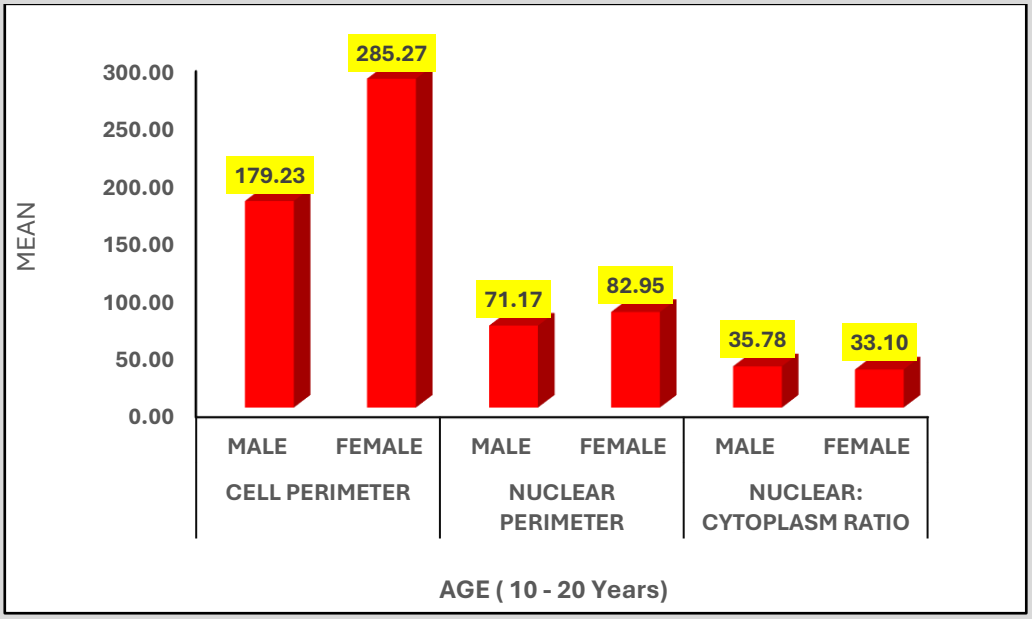
> 50 Years		N	Mean	Std. Deviation	" t " Value	P Value
Cell Perimeter	Male	10	139.94	23.185	2.573	**0.019**
	Female	10	118.58	12.323		
Nuclear Perimeter	Male	10	62.89	6.018	2.862	**0.010**
	Female	10	55.69	5.213		
Nuclear: Cytoplasm Ratio	Male	10	26.18	3.698	1.197	0.247
	Female	10	28.15	3.687		



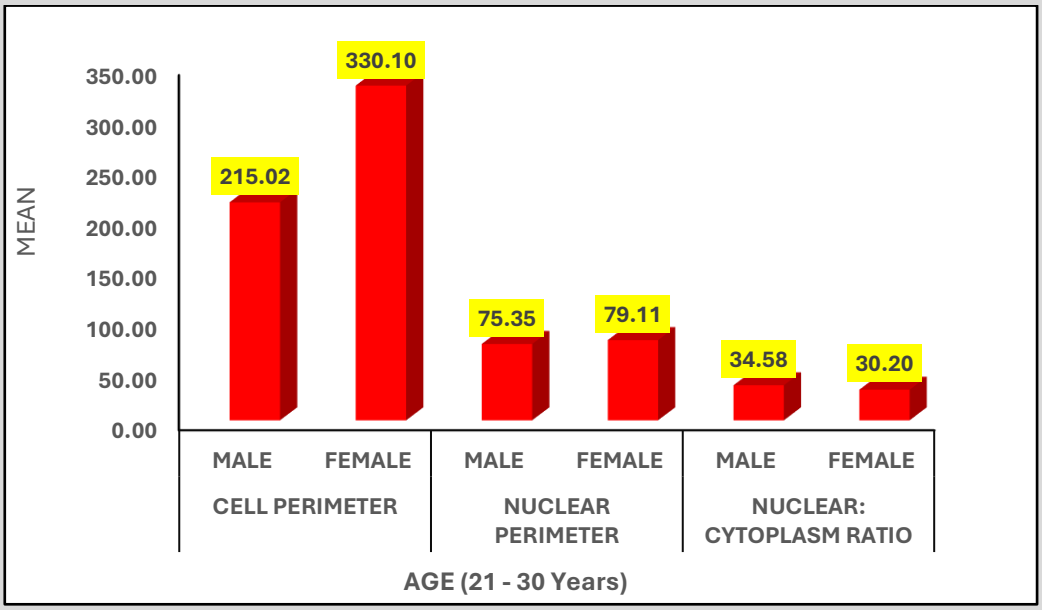
Graph 1: comparison of CP, NP & NP:CP within age groups



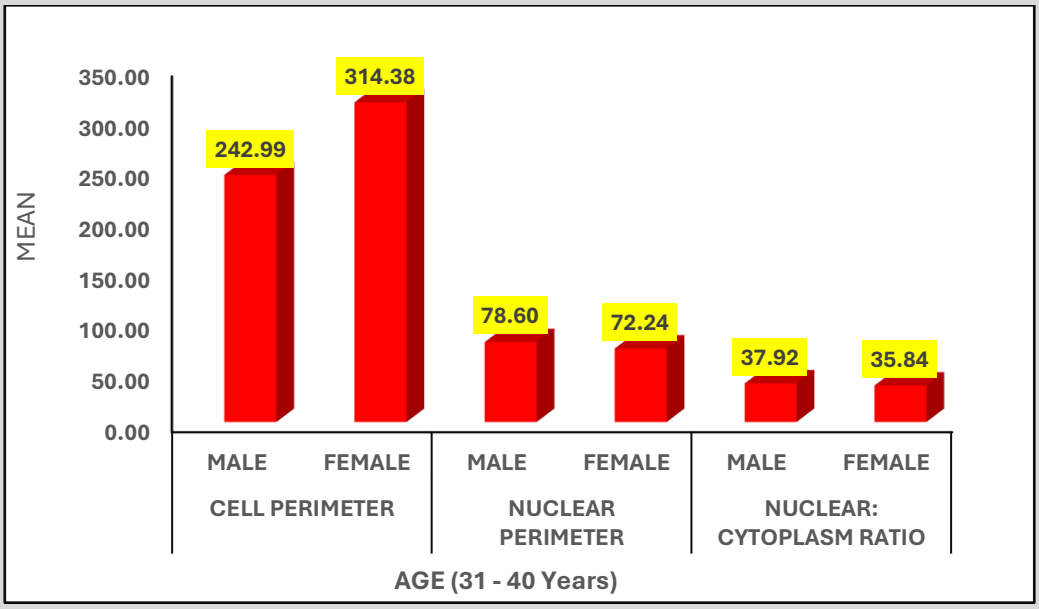
Graph 2: Comparison of CP, NP & NP:CP among genders



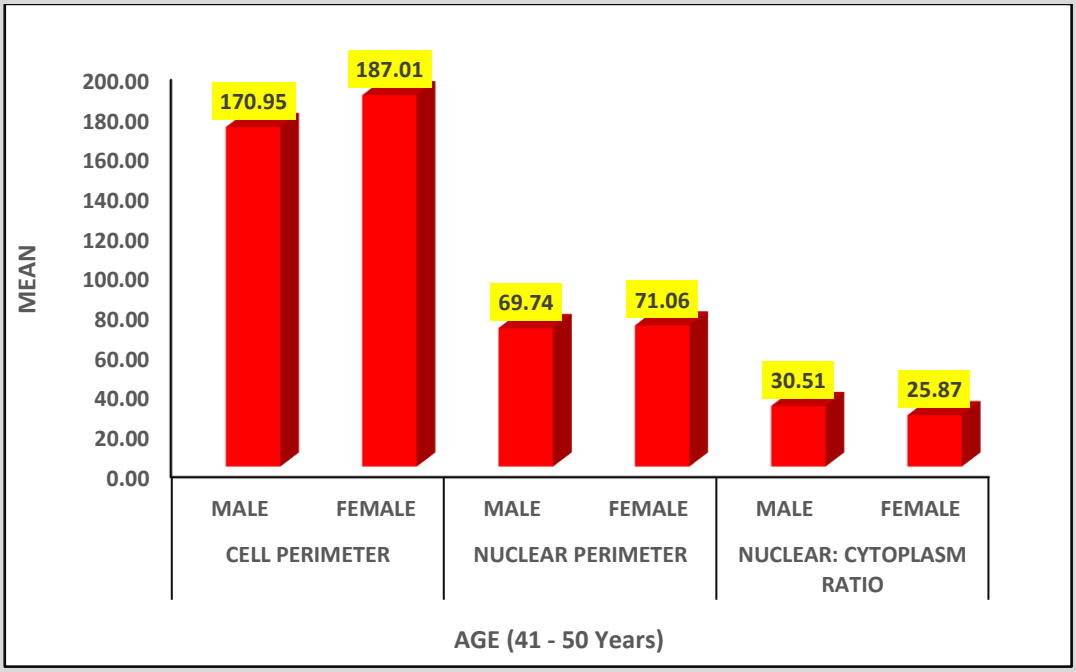
Graph 3: Comparison of CP, NP & NP:CP in group I with respect to gender



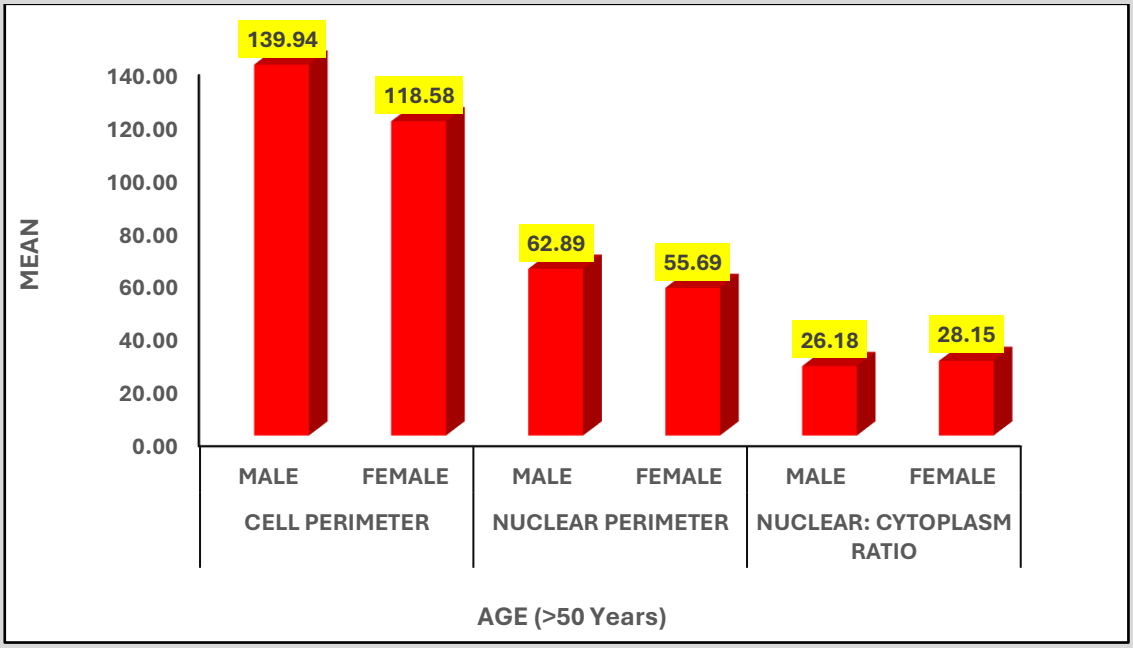
Graph 4: Comparison of CP, NP & NP:CP in group II with respect to gender



Graph 5: Comparison of CP, NP & NP:CP in group III with respect to gender



Graph 6: Comparison of CP, NP & NP:CP in group IV with respect to gender



Graph 7: Comparison of CP, NP & NP:CP in group V with respect to gender

Discussion

The area of dentistry known as forensic odontology is concerned with the appropriate management of dental evidence as well as the appropriate assessment and communication of dental conclusions in the service of justice. When it comes to avoiding clinically perceived lesion misdiagnoses, oral cytology is crucial. A straightforward chairside examination yields a prognostic prognosis. Monitoring the exfoliated cells, or cells that flake off the mucosa, whether by natural or artificial means, is the foundation of exfoliative cytology. Nuclear DNA content, immunohistochemistry, and molecular analysis have recently been performed on cytological specimens [4]. Miller and Montgomery have conducted extensive research on the oral epithelium's typical exfoliative cytology. When the epithelial cell ages, it becomes thinner and more compressed, resembling a wafer. Additionally, the nuclear chromatin condenses into a dense structure with less mass, known as pyknosis, and the nucleus's physiological activity begins to decline. Nucleus completely disappears as the cell continues to cornify, leaving thin squamous epithelium in its place. There are several illnesses that can detected by morphometric analysis, including type 2 diabetes, cancer, and premalignant disorders. The planimetric methods that were used in the past for cytomorphometric analysis have gradually been superseded by computer-assisted image analysis approaches, which are more accurate, faster, and more repeatable [9].

The present study was undertaken for age estimation in various age groups using exfoliative cytology. The results of this study showed a significant difference in the CP, NP and NP:CP in various age groups. This may be because, as the age advances, the cellular activity and epithelial turnover rate decreases. Decrease in cellular organelles can be a possible reason for decrease in cell size.

Our study samples involved clinically normal individuals, with no systemic illness or habits. The present study was in accordance with the study of Shetty *et al.*[4] who reported the

distribution of cell size with variable group of different ages has a significant difference, showing variation in cell size to be significant in different age groups.

However, Eid *et al.*[21] conducted a study on age changes in the oral mucosa and concluded with a wide range of cellular morphometric features. In addition, their findings suggest that epithelial cells become larger with age as measured by cell area, perimeter, Feret's diameter, and breadth, which is not in relation with our study where cell size decreases as the age increases.

Cowpe *et al.* piloted a study on smears obtained from different sites of the oral cavity in which they observed significant variation in nuclear and cytoplasmic areas among different sites. But their results showed a significant variation in the nuclear diameter with age, but there was no variation in the cell diameter [10]. This is in divergence to the present study where there is decrease in cell size with increasing age.

On comparing our results with previous studies, showed a similar result as shown by Anuradha and Sivapathasundharam *et al.* where similar age related and sex-related alterations are observed in gingival smears [7]. Similarly, Ramaesh et al, showed that smears from normal buccal mucosa of healthy subjects displayed a wide variation in the size of the cells and nuclei [16].

Moreover, our study findings of NP:CP are in accordance with results of Scott *et al.* as they showed a reduction in N:C ratio with advancing age at a similar rate in both males and females [17]. Similarly, Cowpe et al also showed a significant variation in ND with age but there was no variation in CD, whereas the ND and CD varied significantly in various regions in the oral cavity [10]. In the current study, we found statistically significant CP and NP values for age group of 10--20years followed by 21-30, 31-40, 41-50 and >51 years age group.

The study findings of Nayar and Sivapathasundharam in which the ND was reported to increase with age and CD to decrease with age [18]. Also, the study reports of Zitwack et al, where animals treated with estrogen showed larger and more active cells in the vagina with similar changes in gingiva and buccal mucosa [19]. Our study findings are in contrast to the above studies in which CP and NP were decreased with increase of age, females showed more values when compared to males, but in group V (>50 years) particularly was less in females than in males. Our results showed stastically significant differences in CP, NP and NP:CP which are in contrast with the results of Lee et al where no significant variations in ND and CD with age has been reported [20].

Moreover, Reddy *et al.* showed increase in N:C with age as there was a significant elevation in mean nuclear area and significant reduction in mean cytoplasmic area which is in contrast with our study [5]. Finally, our study findings showed that there was a proportionate decrease with increase in age of cell perimeter followed by nuclear perimeter and then NP:CP (CP>NP>NP:CP). When compared to age groups (Group III>GII>GI>GIV>GV) and showed stastically significant difference. When compared to gender females showed more propionate mean values than males (F>M).

Patel *et al.* did a cytomorphometric study in normal exfoliated gingival cells. Their results revealed an age-related significant variation in nuclear area, cytoplasmic area and nuclear-cytoplasmic ratio, irrespective of gender [11] which is in accordance with the present study where the cell and nuclear perimeters, NP:CP showed variation with increasing age. But in our study, gender was taken as a variable in which females of Group I, II, III and IV showed increased perimeters when compared to males. Our results suggested that there was a significant difference in CP, NP and N:C ratio in different age group of females. This may be attributed to the variations in the hormonal levels in female throughout the life time. Both estrogen and progesterone promote protein anabolism and growth of the organ system, including oral cavity. Estrogen influences the cytodifferentiation of stratified squamous epithelium as well as the synthesis and maintenance of fibrous collagen [15]. The variations noticed in Group I are due to hormonal changes occurring at puberty. Data suggest that at puberty there is a significant increase in sex hormone in both male and female life. Changes in hormone levels have been related to an increased prevalence of gingivitis followed by remission. Increase in gingival inflammation is one of the factors that can increase NA and lead to a poorly preserved cytoplasm [12]. The observed variations in Groups II, III might be attributed to the hormonal imbalances occurring during 21-40 years of age. Mostly women in this age group are on oral contraceptives and often experience hormonal changes associated with pregnancy [13]. The variations observed in Group IV could be ascribed to hormonal changes occurring during or after menopause in women's life. The time frame between regular menstrual cycles and the cessation of menstrual periods, called perimenopausal transition, is 2-7years. During this period, the concentration of circulating estrogen decreases, while follicle-stimulating hormone and luteinizing hormone concentrations increase. Consequently, the effects of estrogen on gingival tissue are reduced, therefore compromising the anti-inflammatory effect of this hormone on the gingival tissue and ultimately resulting in cytological variation in the gingival tissues [14]. Hormonal influence happens to be the differentiating factor for the cytomorphometric trends of males and females. Testosterone has been frequently associated with metabolism and maintenance of bone and connective tissue matrix unlike the female sex hormones, which have their effect on the epithelium.

There was a significant variation of CP and NP with respect to gender and irrespective of age. The result showed that CP and NP were significantly greater in females than in males of all age groups except in >50 age group. However, the difference in N:C ratio between males and females was not statistically significant. The variation of CP and NP with respect to gender can be ascribed to sexual dimorphism observed in human and hormonal difference in individuals. Hormonal influence happens to be the differentiating factor for the cytomorphometric trends of males and females. Testosterone has been frequently associated with metabolism and maintenance of bone and connective tissue matrix unlike the female sex hormones, which have their effect on the epithelium [15].

Our research aims to establish a standard for pathological smears made from oral mucosa. If pathology samples are collected and handled similarly in the future as this study has done, the data we have presented may have greater diagnostic utility. Since every study is unique, there are differences in the number of cells counted per slide, the type of fixatives employed, the staining time after smear preparation, the specimen collection time, and the cytomorphometric analysis time. The study's findings indicate that there is a notable gender- and age-related difference in cell size, which is likely due to cellular senescence. The oral epithelium's cells only divide for a set amount of time before stopping. Senescent cells accumulate as a result of a decline in the body's capacity to produce new cells as age increasess. A decrease in cell size may be caused by a decrease in the rate of turnover and cellular activity as a result of a reduction in cellular organelles. As a result, it's imperative to standardize both the cytological and image analysis processes and to provide guidelines for cytological technique. When submitted to cytomorphometric examination, the size of the oral exfoliated cells significantly decreases with age. As a result, it's imperative to standardize both the cytological and image analysis processes and to provide guidelines for cytological technique.

Conclusion

This study provides a baseline of quantitative cyto-morphometric data with which pathological smears can be compared. The CP, NP & CP:NP ratio is found to fluctuate in different age groups with no specific pattern and is showed statistically significant difference with respect to gender. It does this by demonstrating age and sex-related changes of CP, NP, and NP:CP in normal exfoliated gingival cells. Thus, improving the oral exfoliative cytological technique's capacity for screening and diagnosis tests. The aging process is the cause of the reduction in cell size. An individual's age might be used to help with their personal identification. Age can be ascertained by comparing the cell sizes from buccal mucosa smears. Furthermore, the information offered in this study can be used in a more objective way to distinguish between premalignant and malignant gingival lesions. To produce a statistically meaningful association and a regression model for estimating age and gender, more research with a bigger sample size is advised.

Declarations

Ethical Approval and Consent to participate

Not applicable

Funding Statement

None

Conflicts of Interest

None

References

[1] Sakuma A, Ohtani S, Saitoh H, Iwase H. Comparative analysis of aspartic acid racemization methods using whole tooth and dentin samples. Forensic Sci Int 2012; 216:88-91.

[2] Prieto JL, Landa MI. Forensic age estimation in unaccompanied minors and young living adults forensic medicine. From Old Problems to New Challenges 2011. p. 78-121.

[3] Willems G. A review of most commonly used dental age estimation techniques. J Forensic Odontostomatol 2001; 19:9-17.

[4] Shetty DC, Wadhwan V, Khanna KS, Jain A, Gupta A. Exfoliative cytology: a possible tool in age estimation in forensic odontology. J Forensic Dent Sci 2015; 7:63-6.

[5] Reddy SV, Kumar SG, Vezhavendhan N, Priya S. Cytomorphometric analysis of normal exfoliative cells from buccal mucosa in different age groups. Int J Clin Dent Sci 2011; 2:53 66.

[6] Chaudhary R, Sahni P, Shylaja M.D., Patel A. Age estimation by exfoliative cytology: New era of noninvasive forensic science. Int J Forensic Odontol 2018; 3:40-3.

[7] Anuradha A, Sivapathasundharam B. Image analysis of normal exfoliated gingival cells. Indian J Dent Res 2007; 18:63-6.

[8] Mc Connell G. A Manual of Pathology. 3rd ed. Philadelphia: W. B. Saunders Company; 1915. p. 56.

[9] Suvarna M, Anuradha C, Kiran Kumar K, Chandra Sekhar P, Lalith Prakash Chandra K, Ramana Reddy BV. Cytomorphometric analysis of exfoliative buccal cells in type II diabetic patients. J NTR Univ Health Sci 2012; 1:33-7.

[10] Cowpe JG, Longmore RB, Green MW. Quantitative exfoliative cytology of normal oral squames: An age, site and sex-related survey. J R Soc Med 1985; 78:995-1004.

[11] Patel PV, Kumar S, Kumar V, Vidya G. Quantitative cytomorphometric analysis of exfoliated normal gingival cells. J Cytol 2011;28(2):66-72. DOI: 10.4103/0970-9371.80745.

[12] Massler M, Schour I, Chopra B. Occurrence of gingivitis in suburban Chicago school children. J Periodontol; 21:146-64.

[13] Mariotti A. Sex steroid hormones and cell dynamics in the periodontium. Crit Rev Oral Biol Med 1994; 5:27-53.

[14] Treloar AE, Boynton RE, Behn BG, Brown BW. Variation of the human menstrual cycle through reproductive life. Int J Fertil 1967; 12:77-126.

[15] Mascarenhas P, Gapski R, Al-Shammari K, Wang HL. Influence of sex hormones on the periodontium. J Clin Periodontol 2003; 30:671-81.

[16] 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.

[17] Scott J, Valentine JA, St Hill CA, Balasooriya BA. A quantitative histological analysis of the effects of age and sex on human lingual epithelium. J Biol Buccale 1983; 114:303-15.

[18] Nayar AK, Sundharam BS. Cytomorphometric analysis of exfoliated normal buccal mucosa cells. Indian J Dent Res 2003; 14:87-93.

[19] Jenkins Gn. The physiology and biochemistry of the mouth. 4th ed. Philadelphia: Lippincott; 1978.

[20] Lee LH, Pappelis AJ, Pappelis GA, Kaplan HM. Cellular and nuclear dry mass and area changes during human oral mucosal cell. Acta Cytol 1973; 17:214-9.

[21] Eid RA, Sawair F, Landini G, Saku T. Age and the architecture of oral mucosa. Age (Dordr) 2012;34(3):651-658. DOI: 10.1007/s11357-011 9261-1.