ORIGINAL ARTICLE

Feasibility of Age Estimation from Exfoliated Buccal Mucosal Cells in Adults: A Cytomorphometric Study

Madhan B,[1] Toi P Ch,[2] Krishnan B.[3]

Professor and Head,[1] Professor.[2-3]

1,3. Department of Dentistry, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry.

2. Department of Pathology, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry.

Abstract:

This study aimed to evaluate the age and gender-related cytomorphometric changes in the buccal mucosal cells of Indian adults and the feasibility of age estimation from them. Exfoliative cytology smears were collected from the clinically normal buccal mucosa of 90 individuals (30 in age groups of 21-30, 31-40, and 41-50 years). They were fixed in 95% alcohol and stained with the Papanicolaou technique. The Cytoplasmic Area (CA) and Nuclear Area (NA) in square micrometers and the Nuclear area: Cytoplasmic area Ratio (NCR) were estimated for each individual by averaging the measurements from 100 clear and unfolded cells. The age and sex-based intergroup comparisons were made with Analysis of Variance and Independent samples T-test (or their non-parametric equivalents), respectively. Pearson and Point Biserial correlations were used to assess the association of NA, CA, and NCR with age and gender, respectively. The cytoplasmic area showed a statistically significant difference (p=.04) between 21-30 years (2572.34 ± 516.54) and 31-40 years (2230.15 ± 516.12). NA and NCR did not differ between age groups. While females (0.030 ± 0.007) showed a higher NCR than males (0.026 ± 0.005, p=.009), no sexual dimorphism was noted for CA and NA. No statistically significant correlations were found between age and CA, NA, or NCR. Though the buccal mucosal cells from adults exhibited some age and gender-related cytomorphometric changes, it is not feasible to predict the chronologic age of an individual from them.

Keywords: Age estimation; Cytomorphometrics; Exfoliative cytology.

Introduction:

Current methods of human age estimation from oral structures are based on assessing the age-related morphological, physiological, radiological, histological, or biochemical changes noted in the teeth.[1–9] The histological and biochemical methods require extracted teeth from the individual and are often not feasible in living subjects.[6,7] The visual techniques based on the number of teeth, eruption pattern, teeth color, attrition, gingival recession, etc., are the easiest for this purpose but are less precise.[1,10-13] In the last few decades, radiographic methods based on stages of tooth development and changes in pulp size have evolved to become the mainstay of age estimation using oral structures in living individuals.[1,4,7,10,11,14,15] While there is little doubt about the utility of radiographic techniques, the need for radiation exposure has ushered in a search for alternatives.

Recent studies on the exfoliated epithelial cells from the normal oral mucosa of living subjects have documented age-related morphologic changes in the cell that can be quantified and analyzed.[16-28] This provides a potential for cytomorphometric evaluation of these cells as a non-invasive method for age estimation in living individuals. Shetty et al. noted a significant negative correlation between the size of exfoliated cells from the normal buccal mucosa and the individual's age.[22] Nallamala et al. found the chronologic age of the individual to be better correlated to the cell size than the pulp-tooth area ratio and hence the better suitability of the former in age estimation.[23] Sex is another variable investigated for effect on the cellular size, but the results are inconclusive.[17,18,27]

Hence, the current study was undertaken to assess the

1. Age and sex-related changes in the dimensions of the normal buccal mucosal cells.
2. Association, if any, between these variables and
3. Feasibility of estimating the chronologic age of an individual by cytomorphometric analysis of exfoliated buccal mucosal cells.

Methodology:

This cross-sectional study was conducted in the Department of Dentistry, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry, after approval from the Institute Ethics Committee (Human Studies) and following the ethical standards in the 1964 Declaration of Helsinki and its later amendments. Participants were recruited from the Outpatient section of the department. All were apparently healthy individuals aged 21- 50 years and willing to participate in the study. Individuals with deleterious habits such as smoking, alcohol, tobacco, and areca nut use (currently or within the past six months) were excluded. Other exclusion criteria were known systemic diseases or use of medications that can affect oral

Corresponding Author
Dr. Balasubramanian Madhan
Email : madhanb@hotmail.com
Mobile No.: +91 94980 66130

Article History
DOR : 02.11.2023; DOA : 31.02.2024

mucosa, clinically evident abnormal alterations in the oral mucosa, multiple grossly decayed teeth, recurrent cheek biting habit, and history of antineoplastic therapy.

The samples for the study were divided into three age groups: Group 1 – 21 to 30 years, Group 2 – 31 to 40 years, and Group 3 – 41 to 50 years. The required sample size was estimated in GPower 3.1(©2021 Heinrich-Heine-Universität Düsseldorf),[29] based on previously reported correlation (r=0.692) between buccal mucosal cell size and chronologic age. To detect a difference between the null hypothesis correlation of 0.692 and the alternative hypothesis correlation of 0.5 using a two-sided hypothesis, the alpha error probability of 0.05 and power of 0.8, a sample size of 88 is required. Therefore, to obtain an equal number of samples in all groups, 90 samples were recruited (30 per group).

Written informed consent was obtained from participants just before data collection. The demographic details collected included age in years (cross-verified with medical records) and sex (Male/Female). The exfoliative cytology smears were obtained by scrapping the normal buccal mucosa using a cytobrush. The scrapings were smeared onto the clean and dry glass slides, serially numbered, and stored for the study. The specimens were fixed immediately by submerging them in 95% alcohol and sent for processing. The smears were stained with the Papanicolaou technique.

The cells were examined in an Olympus CX41 compound microscope (Olympus Corporation, Tokyo, Japan) under 10x magnification. The calibrated images of the different fields were obtained with the integrated Optikam microscopy camera and Optika view 7 software (OPTIKA S.r.l., Ponteranica (BG), Italy). To avoid repetitive inclusion of the same cells, the fields were photographed in a non-overlapping, uniform, and stepwise manner, starting from the upper right corner, moving from left to right, and then down to the next row. The estimation of cellular dimensions was performed in FIJI software. The measurements included the cytoplasmic area in square micrometers (CA), the nuclear area in square micrometers (NA), and NA: CA Ratio (NCR). The photographs were imported into the software and calibrated for size in micrometers. The CA and NA were obtained by tracing the outlines digitally and recording the software-generated value for the selected region(Figure 1). A minimum of 100 unfolded non-overlapping cells with clear outlines were assessed for each participant, and the averaged data were used for data analysis. The stepwise procedure, as described above, was used even while selecting the cells for measurement.

To ensure methodological consistency, the same operators did all the critical steps (smear preparation, cytological processing, image acquisition, and analysis). The intra-observer reliability for measuring NA, CA, and NCR was calculated by repeating the measurements for 20 samples after one month and comparing them with their first set.

Statistical Analysis: The normality of NA, CA, and NCR data distribution was analyzed with the Kolmogorov-Smirnov test. They were normally distributed in all groups based on age and sex except for the NCR in Group III (p=.043) and Females (p=.041). The inter-group differences in NA and CA, based on sex and age, were evaluated with an independent sample T-test and One-way Analysis of Variance (followed by Bonferroni post-hoc tests), respectively. The same for NCR was analyzed with Mann Whitney U and Kruskal Wallis Tests, respectively.

Pearson and Point Biserial correlations were used to assess the association of NA, CA, and NCR with age and gender, respectively. If significant correlations were observed, it was planned to evaluate the possibility of age estimation from these variables using multiple linear regressions with age as the dependent variable and CA, NA, NCR, and gender as independent variables. The intra-observer reliability for measuring CA, NA, and NCR was evaluated with the Intraclass correlation coefficient (Two-way random effects, absolute agreement, single rater/measurement). The alpha for all tests was kept at 0.05, and the data analysis was done in IBM SPSS Statistics for Windows, version 25 (IBM Corp., Armonk, NY, USA).

Table 1. Demographic characteristics of the sample.

Age Group (in years)	Male Mean ± SD (n)	Female Mean + SD (n)	Overall Mean + SD (n)
I (21 – 30)	25.25 ± 2.86 (16)	25.50 ± 2.68 (14)	25.36 ± 2.73 (30)
II (31 – 40)	34.41 ± 2.46 (12)	35.16 ± 2.35 (18)	34.86 ± 2.38 (30)
III (41 – 50)	44.70 ± 4.35 (17)	44.30 ± 2.54 (13)	45.20 ± 3.67 (30)
Overall	35.04 ± 9.07 (45)	35.24 ± 8.33 (45)	35.14 ± 8.66 (90)

Table 2. Comparison of Cytoplasmic Area (CA), Nuclear Area (NA) and Nuclear Area: Cytoplasmic Area Ratio (NCR) by sex and age groups.

Variable	Groups by Sex			Groups by age (in years)		
		Mean ± SD in µm2	p		Mean ± SD in µm2	p
CA	Male Female	2480.20 ± 521.40 2310.44 ± 557.19	NS[a]	I (21-30) II (31-40) III (41-50)	2572.34 ± 516.54 2383.47 ± 558.50 2230.15 ± 516.12	I vs. II-NS[c] I vs. III -*[c] II vs. III-NS[c]
NA	Male Female	58.91 ± 11.06 59.94 ± 08.83	NS[a]	I (21-30) II (31-40) III (41-50)	61.56 ± 11.48 59.14 ± 09.21 57.56 ± 08.93	I vs. II-NS[c] I vs. III-NS[c] II vs. III-NS[c]
NCR	Male Female	0.026 ± 0.005 0.030 ± 0.007	**[b]	I (21-30) II (31-40) III (41-50)	0.027 ± 0.004 0.028 ± 0.007 0.029 ± 0.008	I vs. II-NS[d] I vs. III-NS[d] II vs. III-NS[d]

NS – Nonsignificant, *p<0.05, **p<.01, a Independent samples T test, b Mann Whitney U test, c One-way ANOVA with Bonferroni post-hoc tests, d Kruskal Wallis test.

Table 3. Mean, standard deviation, and correlations for Cytoplasmic Area (CA), Nuclear Area (NA), Nuclear area: Cytoplasmic area Ratio (NCR), age and sex.

	Mean	SD	CA	NA	NCR	Age	Sex
CA (in µm2)	2395.32	543.30	-	.38**	-.71**	-.19	-.15
NA (in µm2)	59.42	9.96		-	.20	-.12	.05
(NCR)	0.020	0.006			-	.13	.25*
Age (in years)	35.25	8.95				-	-
Sex (1=Male, 2=Female)	-	-					-

* p<0.05, ** p<0.01.

Results:

The demographic characteristics of the sample are presented in Table 1. Comparisons of CA, NA, and NCR by sex (Table 2) revealed statistically significant differences for NCR, with females (0.030 ± 0.007) showing a higher ratio than males (0.026 ± 0.005) (p=.009). No statistically significant differences were

 DOI : 10.48165/jiafm.2024.46.2(Suppl).15

noted for CA [t(88)=1.65,p=.10] or NA [t(88)=-0.41,p=.68] . One-way ANOVA showed a statistically significant difference in intergroup comparisons based on age for CA [F(2,87)=4.38,p=.015]. The cytoplasmic area in Group I was higher than in Group III (2572.34 vs. 2230.15, p=.04). The intergroup differences for NA [F(2,87)=1.10,p=.33] and NCR (p=.30) were not statistically significant (Table 2).

The Pearson's correlations among the study variables are presented in Table 3, and the scatter plots are shown in Figure 2. No statistically significant correlations were found between age and CA, NA, or NCR. Given the lack of correlation, the proposal to predict the individual's age from these variables using multiple linear regression analysis was deemed inappropriate and abandoned.

The single measures Intraclass Correlation Coefficient indicated that the reliability of CA (.93, 95% CI= .85 - .97) and NCR (.89, 95% CI= .76 - .95) was good to excellent while that of NA (.83, 95% CI=.63 - .93) was moderate to excellent.

Discussion:

In our study, the cytoplasmic area gradually decreased with age, with statistically significant differences noted between age groups 21-30 and 41-30. The decrease in cytoplasmic area/ cellular size with advancing age is consistent with many previous studies.[22–26,28] In contrast, Eid et al.[21] observed that the epithelial cells tended to become larger and flatter with age, and Cowpe et al.[17] observed no change. Conflicting results have been reported for NA too. Our study did not find any variation in NA with age, but both increased[17,28] and decreased[24] nucleus size have been reported. Similar to Ilayaraja et al.,[24] the NCR did not differ by age group in our study. However, Cowpe et al.[17] found significant variations in NCR with advancing age. Reddy et al.[28] noted an increase in NCR with age and attributed the same to a decrease in Cytoplasmic Diameter (CD) rather than the increase in ND with age. Though inconsistent and conflicting, these dimensional alterations in cytomorphometric variables are often ascribed to senescence, resulting in decreased cellular activity and epithelial turnover.[17,18,21,22-26,28]

While the NCR ratio was higher in females than males in our

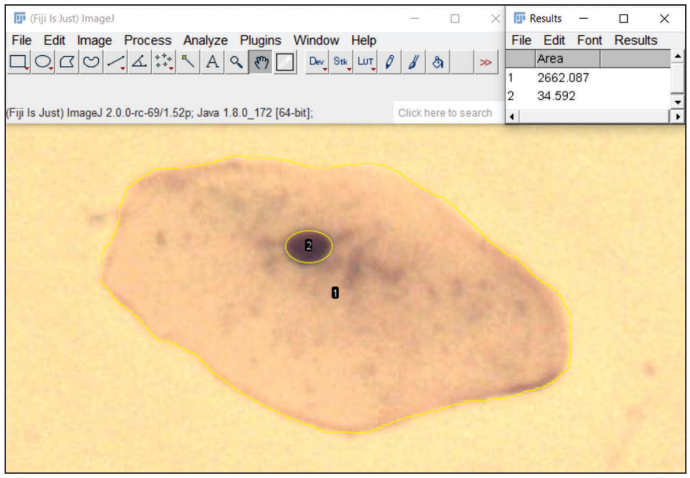

Figure 1. Measurement of the cytoplasmic and the nuclear areas of the buccal mucosal cell in FIJI software.

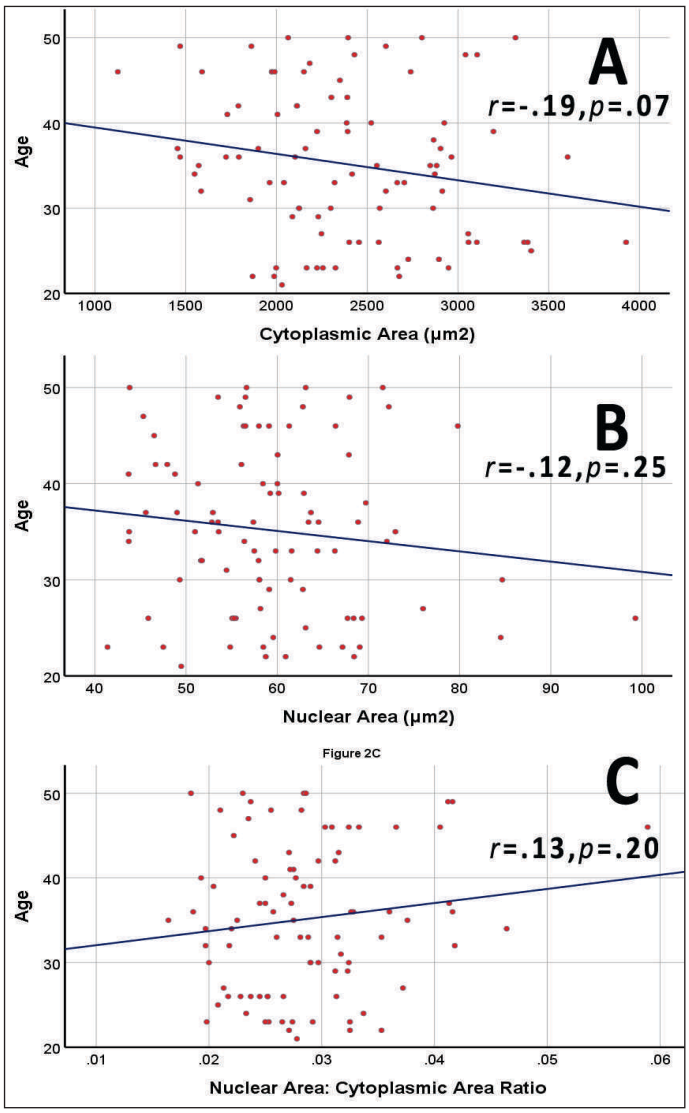

Figure 2. Scatter plots of cytoplasmic area (a), nuclear area (b), and nuclear area: cytoplasmic area ratio (c) against age.

study, no sexual dimorphism was noted for CA or NA. Cowpe et al.[17] found no significant variation between males and females for all three variables. Nayar and Sundharam[18] observed statistically significant sex-related variations only in the 40 – 60 years age group, with males showing comparatively higher values for both. These differences were considered to result from hormonal imbalances occurring in females during this age.

No statistically significant correlation was found between age and the cytomorphometric variables (CA, NA, and NCR) evaluated in this study. Hence, arriving at a regression model to predict the individual's age from these variables was not possible. Shetty et al.[22] found a significant correlation (r=-.692) between the cell size (cytoplasmic area) and the individual's age and suggested its use for age estimation. Nallamala et al.[22,23] observed an excellent correlation between the chronological age and the cell size (r=-.934) and arrived at a linear regression model to predict the age [Estimated age = -0.0516 (cell size) + 57.363.]. Further, the chronological age correlated better with the cell size

(r=-.934) than the pulp-tooth area ratio (r=-.76) in mandibular canines. Therefore, they concluded that age assessment from exfoliative cytology was more accurate than radiographic evaluation of pulp-tooth area ratio.

The inconsistency in the literature regarding age and gender-related changes in the cytomorphometric variables could have stemmed from methodological variations. The sample size, the age range of participants, the differences in the grouping of individuals by age, the number of males and females in the total sample, and agewise subgroups are a few factors related to the sample used in these studies. Factors associated with the method of smear preparation and cytomorphometrics include the number of smears per patient, the instrument and method of obtaining the scrappings, the cytological processing, methods of image acquisition and analysis, the use of linear measurements (diameter) vs. area, number of cells counted per individual and the number of observers.

There is no consensus on the minimum number of cells to be counted in each individual to get an accurate and precise estimate of the cytomorphometric variables used in the study. Numbers as low as 20 have been used earlier.[22-26] We included a minimum of 100 cells per individual, assuming it would yield reliable data. Though the intraclass correlation coefficients noted in our study favor this, the veracity of the assumption needs further testing. Another significant limitation of the study needs mention. The participants in the study were considered healthy based on thorough case history and clinical examination. However, no laboratory or radiological investigations were undertaken to ascertain the status. Hence, the possibility of including individuals with subclinical or asymptomatic disease/disorder cannot be ruled out.

Conclusion:

Cytomorphometric analysis of the exfoliated buccal mucosal cells showed a gradual reduction in the cytoplasmic area from 20 to 50 years and no statistically significant change in the nuclear area or the nuclear area: cytoplasmic area ratio. Females showed a higher nuclear area: cytoplasmic area ratio than males. However, given the lack of significant correlation between these variables and age, it is not feasible to estimate an individual's chronological age by cytomorphometric analysis of the exfoliated buccal mucosal cells.

Declarations:

Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors

Conflict of Interest: None.

Ethical approval: The study was approved by the JIPMER Institutional Ethics Committee (Human Studies) Institutional Ethics Committee- Human Studies (No. JIP/IEC/2015/16/606). All procedures performed on the study participants were following the ethical standards of the Institute Ethics Committee and the 1964 Helsinki Declaration and its later amendments.

Consent to participate: Written informed consent was obtained from all participants included in the study.

References:

1. Willems G. A review of the most commonly used dental age estimation techniques. J Forensic Odontostomatol. 2001; 19:9–17.
2. Jeon HM, Jang SM, Kim KH, Heo JY, Ok SM, Jeong SH, et al. Dental Age Estimation in Adults: A Review of the Commonly Used Radiological Methods. J Oral Med Pain. 2014; 39:119–26.
3. Chaudhary R, Doggalli N. Commonly used different dental age estimation methods in children and adolescents. Int J Forensic Odontol. 2018;3:50–4.
4. Verma M, Verma N, Sharma R, Sharma A. Dental age estimation methods in adult dentitions: An overview. J Forensic Dent Sci. 2019;11:57–63.
5. Priyadarshini C, Puranik MP, Uma SR. Dental Age Estimation Methods: A Review. Int J Adv Health Sci. 1:19–25.
6. Gopika M, Manipal U, Dinesh T, Rajmohan M, Naveen, Prabu D, et al. Digital Measurement of Dentinal Translucency in Correlation with Age Maturity - a Fact or Fiction? J Indian Acad Forensic Med. 2017;39:266–70.
7. Jambunath U, Balaji P, Poornima G, Vasan V, Gupta A. Dental Age Estimation by Radiographic Evaluation of Pulp/Tooth Ratio in Mandibular Canines and Premolars. J Indian Acad Forensic Med. 2016;38:416–9.
8. Anju R, Khanna S, Mittal A, Harpreet G, Behera C. Age Estimation by Clinico-Radiological Examination of Third Molar Teeth: A Study from Delhi. J Indian Acad Forensic Med. 2014;36:349–54.
9. Singh A, Gorea R, Singla U. Age estimation from the physiological changes of teeth. J Indian Acad Forensic Med. 2004;26:94–6.
10. Rösing FW, Kvaal SI. Dental Age in Adults — A Review of Estimation Methods. In: Alt KW, Rösing FW, Teschler-Nicola M, editors. Dental Anthropology: Fundamentals, Limits and Prospects [Internet]. Vienna: Springer; 1998 [cited 2021 Jun 15]. p. 443–68. Available from: https://doi.org/10.1007/978-3-7091-7496-8_22
11. Lewis JM, Senn DR. Forensic Dental Age Estimation: An Overview. CDA J. 43:315–9.
12. Baylis S, Bassed R. Precision and accuracy of commonly used dental age estimation charts for the New Zealand population. Forensic Sci Int. 2017;277:223–8.
13. Quaremba G, Buccelli C, Graziano V, Laino A, Laino L, Paternoster M, et al. Some inconsistencies in Demirjian's method. Forensic Sci Int. 2018;283:190–9.
14. Ritz-Timme S, Cattaneo C, Collins MJ, Waite ER, Schütz HW, Kaatsch HJ, et al. Age estimation: the state of the art in relation to the specific demands of forensic practise. Int J Legal Med. 2000;113:129–36.
15. Panchbhai AS. Dental radiographic indicators, a key to age estimation. Dento Maxillo Facial Radiol. 2011;40:199–212.

16. Cowpe JG. Quantitative exfoliative cytology of normal and abnormal oral mucosal squames: preliminary communication. J R Soc Med. 1984;77:928–31.

17. 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.

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

19. Anuradha A, Sivapathasundharam B. Image analysis of normal exfoliated gingival cells. Indian J Dent Res. 2007;18:63–6.

20. Patel PV, Kumar S, Kumar V, Vidya G. Quantitative cytomorphometric analysis of exfoliated normal gingival cells. J Cytol. 2011;28:66–72.

21. Abu Eid R, Sawair F, Landini G, Saku T. Age and the architecture of oral mucosa. Age Dordr Neth. 2012;34:651–8.

22. 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.

23. Nallamala S, Guttikonda VR, Manchikatla PK, Taneeru S. Age estimation using exfoliative cytology and radiovisiography: A comparative study. J Forensic Dent Sci. 2017;9:144–8.

24. Ilayaraja V, Priyadharshini T, Ganapathy N, Yamunadevi A, Dineshshankar J, Maheswaran T. Exfoliative cytology for age estimation: A correlative study in different age groups. J Indian Acad Dent Spec Res. 2018;5:25.

25. Radhika T, Hussain S, Adithyan S, Jeddy N, Lakshmi S. Cytomorphometric Evaluation of Oral Exfoliated Cells − Its Correlation With Age of an Individual. J Orofac Sci. 2019;11:84–8.

26. Radhakrishnan S, Venkatapathy R, Balamurali PD, Babu P, Prasad KS, Thawfeek M. Exfoliative Cytology in Age Estimation. J Sci Dent. 2019;9:33–5.

27. Donald PM, George R, Sriram G, Kavitha B, Sivapathasundharam B. Hormonal changes in exfoliated normal buccal mucosal cells. J Cytol. 2013;30:252–6.

28. Reddy V, Kumar G, Vezhavendhan N, Pruya S. Cytomorphometric Analysis of Normal Exfoliative Cells from Buccal Mucosa in Different Age Groups. Int J Clin Dent Sci. 2011;2:53–6.

29. Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. Behav Res Methods. 2009;41:1149–60.