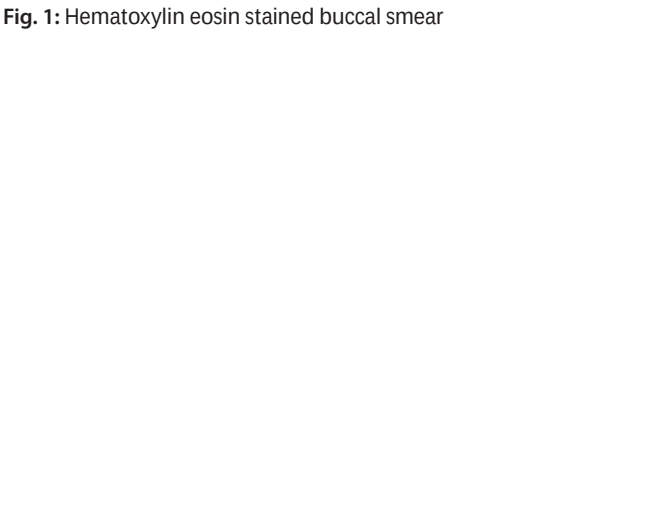

Fig. 1: Hematoxylin eosin stained buccal smear

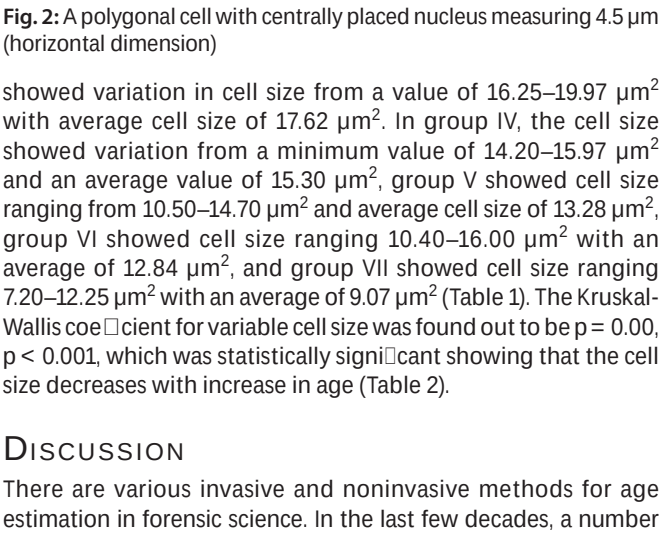

Fig. 2: A polygonal cell with centrally placed nucleus measuring 4.5 µm (horizontal dimension)

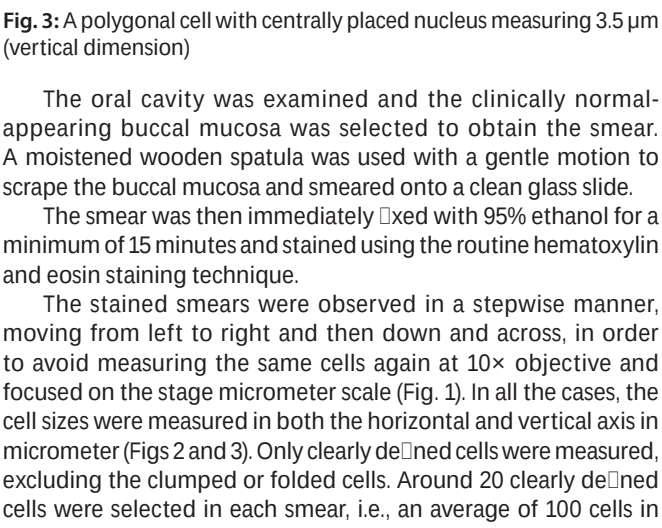

Fig. 3: A polygonal cell with centrally placed nucleus measuring 3.5 µm (vertical dimension)

The oral cavity was examined and the clinically normal-appearing buccal mucosa was selected to obtain the smear. A moistened wooden spatula was used with a gentle motion to scrape the buccal mucosa and smeared onto a clean glass slide.

The smear was then immediately fixed with 95% ethanol for a minimum of 15 minutes and stained using the routine hematoxylin and eosin staining technique.

The stained smears were observed in a stepwise manner, moving from left to right and then down and across, in order to avoid measuring the same cells again at 10× objective and focused on the stage micrometer scale (Fig. 1). In all the cases, the cell sizes were measured in both the horizontal and vertical axis in micrometer (Figs 2 and 3). Only clearly defined cells were measured, excluding the clumped or folded cells. Around 20 clearly defined cells were selected in each smear, i.e., an average of 100 cells in each age group. The average cell size values were obtained for each case and statistically analyzed using Chi-square and Kruskal-Wallis comparison tests.

Results

The cell size in group I ranged from 21.20 to 27.42 µm^2 with an overall average of 24.32 µm^2. In group II, the cell size varied from 17.15 to 25.27 µm^2 with average of 20.56 µm^2. Group III showed variation in cell size from a value of 16.25–19.97 µm^2 with average cell size of 17.62 µm^2. In group IV, the cell size showed variation from a minimum value of 14.20–15.97 µm^2 and an average value of 15.30 µm^2, group V showed cell size ranging from 10.50–14.70 µm^2 and average cell size of 13.28 µm^2, group VI showed cell size ranging 10.40–16.00 µm^2 with an average of 12.84 µm^2, and group VII showed cell size ranging 7.20–12.25 µm^2 with an average of 9.07 µm^2 (Table 1). The Kruskal-Wallis coefficient for variable cell size was found out to be $p = 0.00$, $p < 0.001$, which was statistically significant showing that the cell size decreases with increase in age (Table 2).

Discussion

There are various invasive and noninvasive methods for age estimation in forensic science. In the last few decades, a number of methods have been developed for age estimation among which age estimation in teenagers and adolescents claims relatively accurate estimates.[5]

The EC is the study of exfoliated superficial cells from the mucous membrane of oral cavity, esophagus, and genital mucosa. The normal EC of the oral epithelium was in detail studied by Paul W Montgomery in 1951 after which there are only few studies on normal buccal mucosal smears.[6]

The studies on oral epithelium are largely done in the pathological state. The oral exfoliative cytological technique has been used for the detection of oral premalignant, potentially malignant, or malignant lesions.[7] But secrets of pathology can be explored only when the fundamental observations in normal oral mucosal cells are established.[8,9] The normal oral epithelium is a stratified squamous type, and these cells, as a part of normal physiologic turnover, undergo continuous renewal by migrating from the basal layer to the surface after which they get exfoliate.[10] Exfoliative cytology uses direct scrapings of the surface oral epithelium, which will remove all the layers including the basal cells. Various parameters such as nuclear and cellular size, nuclear and cellular pleomorphism, and the nuclear–cytoplasmic ratio can be analyzed using EC.[4] The cellular activity, cellular organelles, and the epithelial turnover rate decrease as age advances, which could be the reason for the decrease in cell size.[11]

Cowpe et al. piloted a study on smears obtained from different sites of the oral cavity. Their results showed a significant variation

Table 1: Mean and standard deviation of the cell sizes in various age groups

Groups	N	Mean (m^2)	SD (m^2)	Median (m^2)	Minimum (m^2)	Maximum (m^2)	F value	p value
I (21–25)	5	24.32	2.73	24.25	21.20	27.42	29.56	0.000*
II (26–30)	5	20.56	3.12	20.42	17.15	25.27		
III (31–35)	5	17.62	1.73	16.50	16.25	19.97		
IV (36–40)	5	15.30	1.68	15.62	14.20	15.97		
V (41–45)	5	13.28	1.66	14.00	10.50	14.70		
VI (46–50)	5	12.84	2.01	12.48	10.40	16.00		
VII (>51)	5	9.07	1.92	8.40	7.20	12.25		

*Signi□cant $p < 0.001$

Table 2: Signi□cant value of 0.000 using the Kruskal–Wallis test

Test statistics[a,b]	
	Measurement
Chi-square	31.014
Df	6
Asymp. sig.	0.000

[a]Kruskal–Wallis test
[b]Grouping variable: group

in the nuclear diameter with age, but there was no variation in the cell diameter.[11] This is in divergence to the present study where there is decrease in cell size with increasing age. Patel et al. did a cytomorphometric study in normal exfoliated gingival cells. Their results revealed an age-related signi□cant variation in nuclear area, cytoplasmic area, and nuclear–cytoplasmic ratio, irrespective of gender,[12] which is on par with the present study where the cell size showed variation with increasing age. But in our study, gender was not taken as a variable, so the cell size changes could not be related to the gender.

Shetty et al. conducted a study in the normal buccal mucosal smears, which resulted in a signi□cant decrease in the average cell size of an individual with increasing age, which is in accordance with the present study.[1]

Eid et al. conducted a study on age changes in the oral mucosa and concluded with a wide range of cellular morphometric features. Their □ndings suggest that epithelial cells become larger with age as measured by cell area, perimeter, Feret's diameter, and breadth, which is in contrary to our study where cell size decreases as age increases.[13]

Nallamala et al. conducted a study on age estimation using buccal smears and the pulp tooth ratio of the canine tooth and concluded that the age estimated using cell size from buccal mucosal smears is more accurate as compared to that of the pulp–tooth area ratio that can be an additional value in our study as we have used buccal smears. Also in their study there a signi□cant decrease in the cell size with increasing age, which is the same as in our study.[14]

In the present study, age estimated using cell size is comparable to that of chronological age. The plot between the chronological age and cell size showed a signi□cant correlation with only a small variant in the age group of 46–50 years. This suggests that age estimated by cell size is comparable to that of chronological age and that the cell size decreases as the age of the individual increases.

Strengthand Limitations

We used the very simple exfoliative cytological procedure with routine hematoxylin and eosin stain, which is cost-e□ective and a standardized stain. The procedure was less technique-sensitive and also can be used as a reliable method.

Proper scraping of the mucosa to obtain the exfoliated cells and smear preparation were the two crucial factors to be considered in our study.

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