Thanatological Study: Cytomorphometric Analysis of Buccal Cells for the Correlation of Time since Death with Cellular and Nuclear Area

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Abstract- Determination of time since death is one of the important components of post-mortem examination. Molecular death of tissues and cells individually take place usually in 1-2 hours after stoppage of vital functions. Oral mucosa and its cellular content also show morphological and morphometric changes along with other changes in the body after death. Various studies in mention about the morphometric analysis of buccal cells using exfoliative cytological procedures that can determine the cellular alterations in relation to various associated pathology. Furthermore, studies have been done related to establishment of time since death with by determining the onset of rigor mortis and other changes. Studies have been done on the same, with subject to blood; therefore the scope for doing it on buccal cells could enhance the accuracy by using non-invasive method. The present study refers to determining the buccal cell cytomorphometry of an individual after death due to distinct causes of death and certain time interval range. Time since death is correlated with respective parameters such as cellular area, nuclear area and the ratio of both that are utilized for the Cytomorphometric analysis of buccal cells with the help of image analysing software (Image J version 1.51H).

Keywords- Buccal cells, Time since death, Cytological examination, Morphometric analysis, Cellular diameter, Nuclear diameter.

I. INTRODUCTION

Thanatology deals with death in all its aspects. There is a progression from clinical death to cerebral death, biological death then cellular death. Death is defined as irreversible cessation of life. It is classified as somatic and molecular. Estimation of your time since death is one among the foremost important object of post-mortem examination. It continues to be a serious problem for the forensic pathologist and its determination plays a crucial and vital issue in medico legal cases due to the very fact that forensic experts are very often required to answer questions relating to time of death in the courts of law. The main objectives of the study are to establish the possible correlation between the morphological and morphometric changes that occurs in the buccal cells with respect to time since death by cytological examinations. And to find out the correlation between the causes of death and the cellular dimensions in with respect to post mortem interval with microscopic examination.

Though there are plenty of studies done on time since death with establishing the onset of rigor mortis and other changes. Also on the same with blood and therefore there is a scope for doing it in the buccal cells on the similar basis. As mentioned in the past forensic practice, the application of histological techniques has become important part of the armamentarium of tools used in modern forensics. Therefore the interest in the field of oral exfoliative cytology has re-emerged following advancements which serve as a powerful diagnostic as well as forensic tool.

II. MATERIALS AND METHODS

Total 50 Samples have been collected for the study among dead bodies at mortuary of Civil hospital, Gandhinagar and V.S. Hospital, Ahmedabad. Buccal smears have been collected from all the dead bodies that is sent for the autopsy examination (the consent from relatives and autopsy surgeons has been obtained. Only cases satisfying all the following criteria will be included in the study i.e. Dead individual of gender, any age and death occurring in the hospital or anywhere in which case time since death is known. Time since death elapsing 18 hours has not been considered.

2.1 Sample Preparation and Observation-

The glass slides should be cleaned with ethanol and air dried. The smear is to be prepared immediately after the scrapping of the buccal cells from the site of interest. The samples are there after fixed with Cytofix spray fixative at the same time. The fixed slides are stored in the sterilized coupling jar. Not more than 4 slides should be kept in each jar as per the given column in it to avoid the destruction of the smears and then sent it to laboratory. The slides were stained with RAPID-PAP™ Papanicolaou Stain Kit which is a user friendly. Total 9 steps are there in this process, it took 3 minutes, 4 coloured staining techniques which require minimum skill and laboratory aids to achieve best staining result. The prepared sample slides are observed under Zeiss Scope

A1 Axio, Phase contrast microscope with 63X lens and the images of the cells were captured with Canon Power shot G11 SLR camera with 7.0X zoom in. Image analyzing (Image J 1.5oi) software is used to measure the cellular area, nuclear area and the ratio of both and tabulated for statistical analysis.

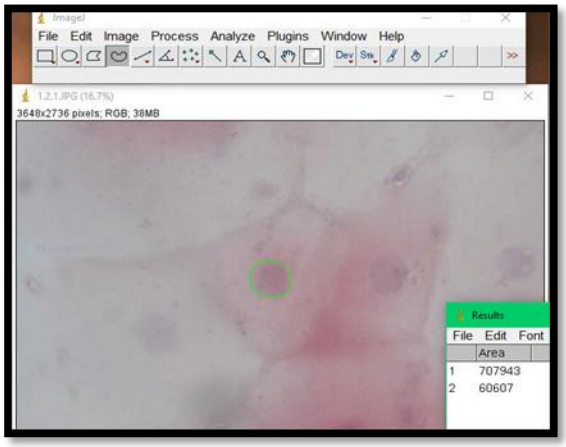

Figure 1. Obtained nuclear area value from Image J software

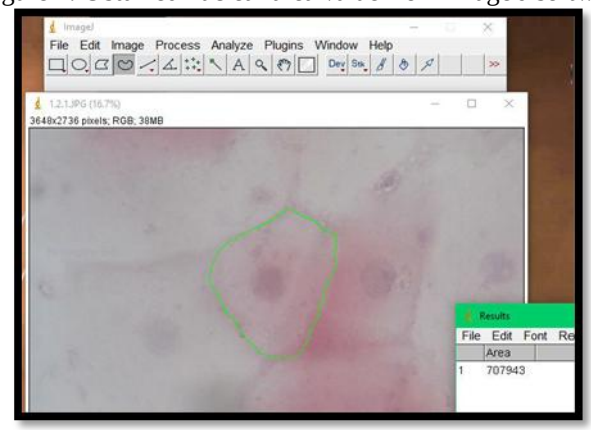

Figure 2. Obtained cellular area value from Image J software

III. EXPERIMENT AND RESULT

The data regarding the state of post mortem lividity and rigor mortis obtained from the post mortem report from the autopsy room of each deceased from where the samples were collected showed that post mortem lividity was fixed in the entire individual grouped under 4-6 hours, 6-12 and more than 12 hours. Similarly rigor mortis was developed in the individual grouped under 4-6 and 6-12 hours and well developed in more than 12 hours.

Time Period	Postmortem Lividity	Rigor Mortis
4-6 hrs	Fixed	Developed
6- 12 hrs	Fixed	Developed
>12 hrs	Fixed	Well Developed

Table -1 State of Post-mortem Lividity and Rigor mortis with respect to time

Mean and Standard Deviation of CA and NA of total 149 samples was calculated using SPSS Statistic software used for statistical analysis.

	Mean	Std. Deviation	N
Cellular Area	954391.8389	390778.68938	149
Nuclear Area	38539.8389	16446.75623	149

Table -2 Mean and Standard Deviation of Cellular Area and Nuclear Area

	Cellular Area	Nuclear Area
Pearson Correlation	1	0.462**
Cellular Area Sig. (2-tailed)		0.000
N	149	149
Pearson Correlation	0.462**	1

Nuclear Area Sig. (2-tailed)	0.000	
N	149	149

** Correlation is significant at the 0.01 level (2-tailed).

Table -3 Correlation test for Cellular Area and Nuclear Area

According to this correlation test, the P value is <0.01 (0.000) and Pearson Correlation value is 0.462. Therefore Cellular Area and Nuclear Area have positive correlation and it is significant at 0.01 levels.

The Correlation test between the cellular and nuclear area resulted into a significant result that states, with an increase with cellular area change there is also a change observed with respect to the nuclear area with a degree of dependence to each other.

	Sum of Squares	Df	Mean	Square F	Sig
Between Groups	739455862635.839	4	184863965658.960	1.218	0.306
CA Within Groups	21861325780428.297	144	151814762364.085		
Total	22600781643064.137	148			
Between Groups	3461688150.486	4	865422037.622	3.408	.011
NA Within Groups	36571688855.648	144	253970061.498		
Total	40033377006.134	148			

Table -4 ANOVA test for Cellular Area and Nuclear Area with respect to age groups Cellular Area is not significant, Nuclear Area is significant.

The null hypothesis is that there is no significant difference in Cellular Area and Nuclear Area between different age groups. This was tested using ANOVA test.

The samples (individuals with known cause of death) were grouped according to the cause of death of the individual and the mean of the Cellular Area CA, Nuclear Area NA and Cellular Area:Nuclear Area CA:NA were tabulated as below:

Causes of Death	Cellular Area (CA)	Nuclear Area(NA)	Ratio Of CA &NA (CA:NA)
Poisoning	914183.1	47738.13	19.875
Accident	1137180	40128.83	30.7
Clinical	839379.4	38318.33	23.7
Burns	864224.5	36971.39	27.7
Drowning	1106060	48508	24.7
Natural	940585.5	40192.94	25.4

Table -5 Values of CA and NA with respect to different causes of death Obtained mean values of CA, NA and CA: NA with respect to cause of death.(irrespective of age and gender)

The null hypothesis is that there is no significant difference in Cellular Area and Nuclear Area between different groups of causes of death. This was tested using ANOVA test.

3.1 Discussion-

In the present study, the obtained record of Post-mortem Lividity and Rigor mortis were compared with the same in relation to changes in body. According to literature, time since death between >1 hour and 2-3 hours, some patches post mortem staining were seen on back and rigor mortis seen on face. Therefore according to this present study during this period of 4-6 hours the value of cellular area of the cells is in the range of 8,00,000-10,00,000 (pixels) and nuclear area is 36,000 (pixels) and the ratio of both ranges between 26-26.50 (pixels) with fixed post-mortem lividity and slightly developed rigor mortis. Time since death between 6-8 hours till 12 hours pertain fixed post-mortem staining and developed rigor mortis on upper part of body, similarly in this study it was observed that post-mortem lividity was fixed and developed Rigor mortis. The value of Cellular area of the cells is in the range of 8, 00,000-10, 00,000 (pixels) and Nuclear area comes around 36,000 (pixels) and the ratio of both is around 27.50 (pixels) in the period of 6-12 hours. Within 12-24 hours, rigor mortis is present all over the body and post-mortem staining is fixed similarly, according to this study the post-mortem lividity was fixed with well developed rigor mortis. The value of Cellular area of the cells is in the range of 10, 00,000-12, 00,000 (pixels) and Nuclear area comes around 42,000- 43,000 (pixels) and the ratio of both is around 28-28.50 (pixels) in this particular time of period. Based on the findings it is possible to mention that there is correlation between time since death and CA and NA of the buccal cells. From 4-6 hours, 6-12 hours and >12 hours irrespective of the time since death the variation is significantly noted with increase in CA and NA. Though the difference between 4-6 and 6-12 hours was found to be less still there is significant, rather a drastic increase in Cellular Area as well as Nuclear Area was noted. The current study, as far as the cause of the death is concerned there is a significant difference between the groups (between different causes of death) there is a high significance noted with cellular area CA. This implies that CA is more dynamic and subject to variation are to causes of death.

3.2 Shortcomings of the study-

The sample is very small, for better results the sample size should have been increased. Specification of gender consideration should have been included. Non uniformity in the age groups should be resolved for accurate results. The method used in the evaluation of Cellular Area and Nuclear Area is sensitive technique due to human observational error that may be replaced by more accurate method for evaluation.

IV. CONCLUSION

Whilst traditionally established techniques are commonly used to estimate the time of death during routine medico legal autopsies there is a trend toward the development and introduction of newer techniques. It is established that the reliance on a single technique can produce erroneous outcomes.

There are very few studies done in the interest of oral soft tissues specifically buccal cells in the post mortem cases. This study is a new effort carried for estimation of time since death from buccal cells inclusive of cytological examination and morphometric analysis by noting the significant difference in the CA, NA and ratio of both. From the results obtained, it can be stated that Cellular Area and Nuclear Area overall have a significant correlation with each other with a degree of dependence with each other irrespective of factors like age, sex, cause of death. There is a significant difference among the Cellular Area and Nuclear Area with respect to time since death was found to be marginally different and it is applicable to time since death up to 8 hours. In more than 12 hours highly significant difference is noted among them. There is significant NA when age groups were taken into consideration. The variation of NA remains same within the age group. CA has a significant correlation with the causes of death. Further studies have to be carried out in future in the same prospect to fulfil the loop holes in the present study to refine the short comings and redefine the accuracy of the result.

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