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EPITHELIAL AND STROMAL CHARACTERISATION WITH POST MORTEM INTERVAL IN ORAL MUCOSA- A HISTOLOGICAL STUDY

Forensic Medicine

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ABSTRACT

Background: Currently, many approaches to PMI estimation focus on observable and measurable changes to the body after death. These gross changes occur because of changes at the cellular level. Degenerative post-mortem changes to the epithelium and connective tissue in the oral mucosa may demonstrate specific patterns that could prove useful to determine PMI. **Aim and Objectives:** The present study is aimed to characterise time-wise histological changes in oral tissues after autopsy. **Materials and Method:** 50 oral mucosal tissue samples each form buccal mucosa were collected during autopsy. Tissues were routinely fixed, processed and stained using H&E stain. PAS and Verhoff's van geison staining were used to evaluated acinar & collagen changes. Histological changes were assessed quantitatively & qualitatively. **Result:** Oral mucosa shows histological & cytonuclear degenerative changes at architectural & cellular level which are correlated with postmortem interval. **Conclusion:** Histological changes indicate that microscopic evaluation of the oral mucosa is effective for PMI estimation.

KEYWORDS

Postmortem Interval, Autopsy, Forensic Pathology, Oral Autopsy

BACKGOURND

Post mortem interval (PMI) estimation focus on observable and measurable changes to the body after death such as autolysis, algor mortis, rigor mortis, livor mortis and the digestive status of the stomach contents. Following death, many physical and chemical changes begin to take place and continue until the body eventually decomposes.[1] After death, a sequence of changes naturally occurs in the human body. These gross changes occur because of changes at the cellular level.[3] Eventually, cellular metabolism comes to an end and the subsequent predominance of lytic enzymes results in autolysis.[4]

Utilising light microscopy, cells are perceived as dead after they have undergone a series of specific morphological changes which occur as a result of two distinct cellular processes: apoptosis and necrosis, a disorderly and unpredictable form of cell death. Necrotic cells show an increased eosinophilia. Nucleus of the epithelial cells may undergo changes like karyolysis, pyknosis and karyorrhexis. [5,6] Autolytic enzymes are released by the oral mucosal cells after death, resulting in abnormal morphology. Therefore, degenerative post-mortem changes to the epithelium and connective tissue in the oral mucosa may demonstrate specific patterns that could prove useful to determine PMI.[1]

Aim & Objective

To characterise time-wise histological changes in oral tissues after autopsy and estimating their potential for PMI prediction.

MATERIALS AND METHOD

The study was undertaken at Shree Bankey Bihari Dental College and Research Centre. 50 Buccal mucosa samples were collected from the bodies of 50 deceased individuals. In each case, information was obtained concerning cause of death, age, gender, time and date of death and time spent outside or in a natural environment since death. The bodies of individuals with a history of communicable disease were excluded, as were those of pregnant females, victims with facial burns and those who had been preserved after death.

The bodies were placed in a dry environment with adequate light and kept at room temperature. Oral tissues were collected under the direct supervision of a forensic medicine and toxicology specialist. The buccal mucosa was accessed using diagnostic instruments including a mouth mirror, a no. 5 interproximal explorer, a pair of tweezers and a pair of toothed forceps. Samples were collected using a no. 15 Bard-Parker® stainless steel blade (Aspen Surgical Corp., Caledonia, Michigan, USA) and a Langenbeck retractor to retract the buccal mucosa. A total of 50 buccal mucosa samples of approximately 6 × 8 × 5 mm in size were collected.

The excised tissues were fixed in 10% buffered formalin. Subsequently, formalin-fixed paraffin embedded (FFPE) blocks were prepared. The FFPE blocks were sectioned into slides of 4 µm in thickness and stained with routine haematoxylin and eosin (H&E) stain. In addition, 50 slides were stained with Verhoeff's van Gieson stain and 50 slides were stained with periodic acid-Schiff (PAS) swastain to determine changes in the intensity of collagen/reticulin fibres and basement membranes/mucous acini, respectively, with time since death.

Stained tissue sections were viewed under a light microscope to evaluate epithelial and connective tissue changes. These changes were categorised according to PMI stage as either early: 18 Deceased individuals (<12.5 hr since death), intermediate: 22 Deceased individuals (12.5–20.5 hours since death) or late: 10 Deceased individuals (>20.5 hours since death). Epithelial changes included cytoplasmic vacuolation, changes to the cellular outline, homogenisation, changes to the nuclear outline, nuclear degeneration, nuclear vacuolation, arc-shaped nuclei, chromatin clumping and epithelial shredding (i.e. separation of layers of the epithelium). Connective tissue changes to the lamina propria included collagen lysis, fibroblast vacuolation, inflammation, homogenisation, clumping of the red blood cells (RBCs) and melanin incontinency. In addition, the buccal submucosa was examined for degeneration of the myofibrils and salivary glands. The interface between the epithelium and connective tissue was also studied. Changes in the intensity of Verhoff's Van Gieson stains in the collagen fibres as well as that of PAS stains in the basement membrane and mucous acini were recorded via consensus.

Data were entered into an Excel spreadsheet, Version 2013 (Microsoft Corp., Redmond, Washington, USA), and analysed using the Statistical Package for the Social Sciences (SPSS). The postmortem samples were compared for the frequency of microscopic changes in the epithelium, lamina propria and submucosa. Early, intermediate and late changes in post-mortem samples were identified depending upon a minimum frequency of >40% of specific microscopic features at each PMI stage. Logistic regression analysis was conducted to determine

the predictive efficiency of these parameters in the estimation of time since death. Changes in staining intensity of the collagen fibres, mucous acini and basement membrane for special stains were evaluated with respect to changes in PMI using analysis of variance.

Ethical approval for this study was obtained from the institutional ethical committee (EC/NEW/INST/2023/3008). Written consent was obtained from the next of kin of the deceased individuals.

RESULTS

A total of 50 buccal mucosa samples were obtained from 50 deceased individuals. The subjects were categorised as per PMI with <12.5 hours, 12.5–20.5 hours and >20.5 hours.

Comparison of histological changes according to PMI groups (Table.1) shows comparison of Homogenisation, Epithelial Shredding and Melanin Incontinency shows increases from group 1 to group 2 and then decreases from group 2 to group3 which is statistically significant. Nuclear Degeneration, Collagen lysis, Myofibril degeneration and RBC clumping is a constant finding. Myofibril degeneration, Arc shaped nuclei, Mucosal degeneration and RBC lysis shows statistically insignificant results.

PMI group comparison with different parameters (Table.2) shows statistically insignificant results with homogenization. Nuclear degeneration, Collagen lysis, Myofibril degeneration and chromatin clumping are constant findings. Chromatin clumping decreases with increases in PMI (From group 1 to group 3) which is statistically significant. Epithelial shredding, melanin incontinency, loss of EP-CT interface, Mucosal degeneration, RBC lysis and cytoplasmic vacuolisation shows increase from group 1 to group 2 and decrease from group2 to group 3 which is statistically significant.

There was a progressive decrease in staining intensity of collagen fibres in Verhoeff Van Gieson stains. The intensity of verhoeff van Gieson stains in collagen fibres was found to be a significant predictor of time since death in both buccal mucosa. While there was no progressive change in PAS staining intensity in the basement membranes of buccal mucosa samples as PMI increased, PAS staining intensity in salivary gland acini increased significantly with PMI.

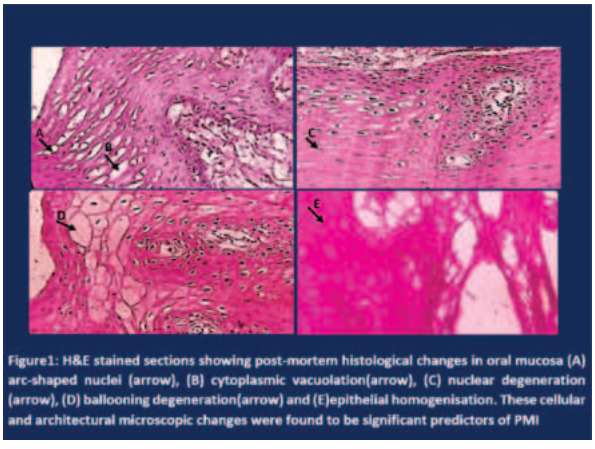

Figure1: H&E stained sections showing post-mortem histological changes in oral mucosa (A) arc-shaped nuclei (arrow), (B) cytoplasmic vacuolation(arrow), (C) nuclear degeneration (arrow), (D) ballooning degeneration(arrow) and (E)epithelial homogenisation. These cellular and architectural microscopic changes were found to be significant predictors of PMI

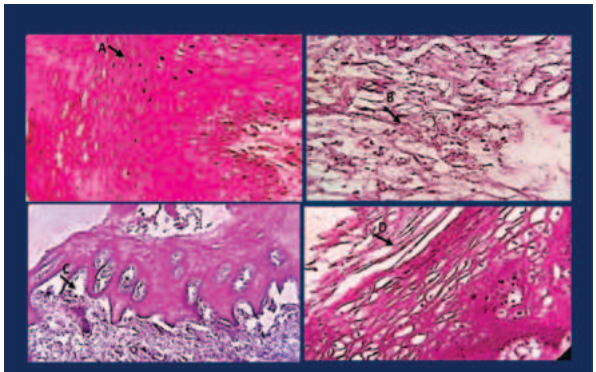

Figure2: H&E stained sections showing post-mortem histological changes in oral mucosa (A) chromatin clumping(arrow), (B) collagen lysis (arrow), (C) epithelial connective tissue separation(arrow), (D) epithelial shredding(arrow)

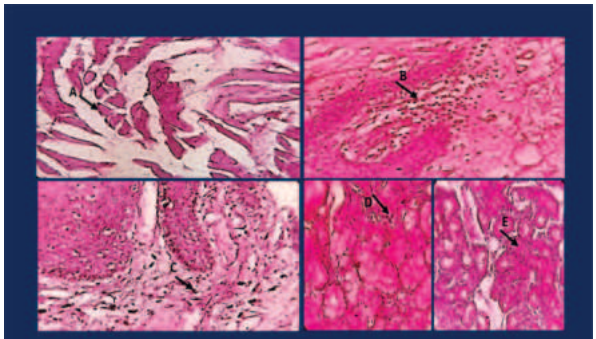

Figure3: H&E stained sections showing post-mortem histological changes in oral mucosa (A) myofibril degeneration (arrow), (B) Infiltration (arrow), (c) melanin incontinency (arrow) and (D) & (E) degeneration of the mucous acini (arrow)

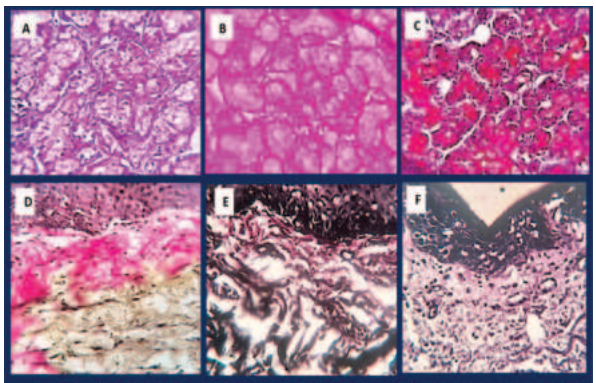

Figure4: Periodic acid-Schiff stains at 40x magnification showing the (A) mild, (B) moderate and (C) severe increase in staining intensity of mucous acini in post-mortem samples of the oral mucosa as the post-mortem interval increased. Verhoeff's van Gieson stains at 40x magnification showing the (D) mild, (E) moderate and (F) severe decrease in staining intensity of collagen fibres in post-mortem samples of the oral mucosa as the post-mortem interval increased.

Table 1: Comparison Of Histological Changes According To PMI Groups

Parameter	PMI GROUP	Absent	Present	N	VALUE	P VALUE
HOMOGENISATION	1	16	6	22	7.759a	0.021
	2	10	8	18	8.072	
	3	2	8	10	7.239	
EPITHELIAL SHREDDING	1	14	8	22	6.612a	0.037
	2	6	12	18	6.829	
	3	8	2	10	0.071	
MELANININ INCONTINENCY	1	8	14	22	9.158a	0.01
	2	14	4	18	9.382	
	3	8	2	10	7.269	
LOSS OF EP-CT INTERFACE	1	16	6	22	4.147a	0.126
	2	12	6	18	6.412	
	3	10	0	10	1.796	
CYTOPLASMIC VACUOLISATION	1	4	18	22	3.784a	0.151
	2	8	10	18	3.695	

	3	2	8	10	0.309	
NUCLEAR DEGENERATION	1	NIL	22	22	NA	
	2		18	18		
	3		10	10		
CHROMATIN CLUMPING	1	2	20	22	1.419a	0.492
	2	4	14	18	1.486	
	3	2	8	10	0.923	
ARC SHAPE NUCLEI	1	12	10	22	4.310a	0.116
	2	4	14	18	4.455	
	3	4	6	10	1.436	
COLLAGEN LYSIS	1	NIL	22	22	NA	
	2		18	18		
	3		10	10		
MYOFIBRIL DEGENERATION	1	NIL	22	22	NA	
	2		18	18		
	3		10	10		
MUCOUSAL DEGENRATION	1	12	10	22	.725a	0.696
	2	8	10	18	0.727	
	3	6	4	10	0.008	
RBC CLUMPING	1	NIL	22	22	NA	
	2		18	18		
	3		10	10		
RBC LYSIS	1	12	10	22	.725a	0.696
	2	8	10	18	0.727	
	3	6	4	10	0.008	

Table 2: PMI Group Comparison With Different Parameters

PMI	N	Homogenisation			Nuclear Degeneration			Chromatin Clumping			ARC Shape Nuclei		
		Value	Df	p value	Value	Df	p value	Value	Df	p value	Value	Df	p value
1	50	41.883a	14	0	NA			30.159a	14	0.007	36.111a	14	0.001
2		57.503	14					28.691	14		48.573	14	
3		7.629	1					0.795	1		0.044	1	
PMI	N	Epithelialshredding			Collagen Lysis			Melaninin Incontinency			Loss Of EP-CT Interface		
		Value	Df	p value	Value	Df	p value	Value	Df	p value	Value	Df	p value
1	50	31.061a	14	0.005	NA			30.556a	14	0.006	31.725a	14	0.004
2		42.226	14					40.934	14		36.379	14	
3		1.542	1					8.207	1		5.419	1	
PMI	N	Myofibril Degenration			Mucousal Degenration			RBC Clumping			RBC Lysis		
		Value	Df	p value	Value	Df	p value	Value	Df	p value	Value	Df	p value
1	50	NA			32.639a	14	0.003	NA			23.291a	14	0.056
2					44.961	14					31.778	14	
3					0.194	1					0.009	1	
PMI	N	Cytoplasmic Vacuolisation											
		Value	Df	p value									
1	50	26.852a	14	0.02									
2		32.929	14										
3		0.908	1										

DISCUSSION

The microscopic results of the present study revealed significant difference in microscopic finding at different interval of time since death. Parameters including homogenization (Fig1, E), statistically significant in PM1 group 1, 2&3; Epithelial shredding (Fig2, D) shows statistically significant results in PMI group 1&2; Melanin incontinence (Fig3, C) and loss of epithelial- connective tissue interface (Fig2, C) significant in all 3 PMI groups. (Table1) Role of melanin incontinency in estimating PMI has been evidenced in the skin, no previous study had yet explored its potential in the oral mucosa. In a previous study, Mahalakshmi et al.[6] evaluated histological changes in gingival tissue based on an evaluation of unfixed antemortem tissues alone. Gururaj et al.[7] have suggested that the decomposition process begins in the initial 24 hours, while Pradeep et al. concluded that the decomposition process at the cellular level begins within 10 hours of death.

According to PMI group comparison homogenization, Melanin incontinence and Loss of epithelial- connective tissue interface shows statistically significant results in all three PMI groups; Chromatin clumping (Fig2, A), Arc shaped nuclei (Fig1, A), epithelial shredding, Mucous acini degeneration (Fig3, D&E) and RBC lysis are statistically significant in PMI group 1 & 2; Cytoplasmic vacuolization (Fig1, B) shows statistically significant finding in PMI group 2. (Table2) These findings support those of previous research conducted by Jagganath Patro et al.[1] In addition, the present study also focused on additional features such as arc-shaped nuclei (Fig1, A) as an intermediate change in the buccal mucosa. The identification of epithelial shredding as a late microscopic change in the buccal mucosa is in agreement with results reported by Yadav et al.[8] The latter finding supports previous research by Yadav et al.[9] indicating that collagen disruption becomes increasingly apparent over time. However, they reported no abnormalities in the RBCs, a finding which contradicts that of the present study.

Our findings also reveal increase in staining intensity of mucous acini with PAS stain (Fig3, A, B&C) and decrease in staining intensity of collagen fibers with Verhoff's Van Gieson (Fig3, D, E&F) as the PMI progress. Which is in concordance with Jagganath Patro et al.[1] This progressive decrease in stain intensity can be attributed to collagen lysis and late PMI stages; alternatively, this may be attributed to a decrease in the thickness of the submucosa or lamina propria.[9,10]

CONCLUSION

Based on these findings, we suggest that histopathology is a useful method to determine post mortem interval. In addition, advanced histological techniques, such as immunohistochemistry and electron microscopy, should also be investigated as other potential methods of determining post-mortem changes in the oral tissues which can unfolds many mysteries behind death.

REFERENCES

1. Patro J, Panda S, Mohanty N, Mishra US. The Potential of Light Microscopic Features of the Oral Mucosa in Predicting Post-mortem Interval. Sultan Qaboos University Medical Journal. 2021 Feb;21(1):e34.

2. Rajkumari S, Mensudar R, Naveen N, Thayumanavan B, Thammaiah S. Estimation of postmortem death interval from autopsied tongue tissue: A cross-sectional study. J Oral Maxillofac Pathol 2020;24:568-71.
3. Muthukrishnan S, Narasimhan M, Paranthaman SK, Hari T, Viswanathan P, Rajan ST. Estimation of time since death based on light microscopic, electron microscopic, and electrolyte analysis in the gingival tissue. J Forensic Dent Sci 2018;10:34-9.
4. Rajkumari S, Mensudar R, Naveen N, Thayumanavan B, Thammaiah S. Estimation of postmortem death interval from autopsied tongue tissue: A cross-sectional study. J Oral Maxillofac Pathol 2020;24:568-71.
5. Azevedo-Alanis LR, Tolentino ED, ASSIS GF, Cestari TM, Lara VS, Damante JH. Acinar autolysis and mucous extravasation in human sublingual glands: a microscopic postmortem study. Journal of Applied Oral Science. 2015 Sep;23:459-66.
6. Mahalakshmi V, Gururaj N, Sathya R, Sabarinath TR, Sivapathasundharam B, Kalaiselvan S. Assessment of histological changes in antemortem gingival tissues fixed at various time intervals: A method of estimation of postmortem interval. J Forensic Dent Sci 2016;8:114-5.
7. Gururaj NS, Sivapathasundharam B. Post mortem findings in the gingiva assessed by histology and exfoliative cytology. J Oral Maxillofac Pathol 2004; 8:18–21.
8. Yadav AB, Angadi PV, Kale AD, Yadav SK. Histological assessment of cellular changes in postmortem gingival specimens for estimation of time since death. The Journal of forensic odonto-stomatology. 2015 Jul;33(1):19.
9. Yadav A, Angadi PV, Hallikerimath S, Kale A, Shetty A. Applicability of histologic post-mortem changes of labial mucosa in estimation of time of death–A preliminary study. Australian Journal of Forensic Sciences. 2012 Dec 1;44(4):343-52.
10. Nahar N, Ara S, Zohora F, Siddika D, Yesmin T. Histological Study on Thickness of Different Layers of the Gallbladder-A Postmortem Study. Bangladesh Journal of Anatomy. 2011;9(1):41-4.