Histopathological Changes in Skin-A Tool to Establish Time Since Death in First 24 Hours

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Abstract

Aim: Determination of the time since death is one of the greatest challenge in the practice of Forensic Medicine. There is currently very limited scientific information regarding the process of cutaneous decomposition. This study is aimed at throwing more light towards this field.

Method: 30 skin samples from human cadavers are collected where actual time of death is known, from Yenepoya Medical College, Mangalore. Skin samples (1 x 2 cm) from the anterior chest were collected, parallel to the longitudinal midline incision. Tissues were fixed in 10% formalin, embedded in paraffin wax, and stained with hematoxylin-eosin stain. The skin samples were observed under light microscope for the changes.

Results: Epidermis and Dermis showed marked changes in the histology with increasing PMI and is the most reliable site to look for.

Conclusion: This study will throw a better light to post-mortem skin changes and their role in determination of the time since death.

Key words: *Histology of skin, time since death, postmortem microscopic skin changes.*

Background

Many methods have been attempted to accurately and systematically determine the estimated time of death in autopsy cases. Gross changes of the skin which occur during the post-mortem interval have occasionally been used to estimate the time of death under different conditions; however, the post-mortem gross and histologic changes of the skin have not been systematically analyzed. In this study, an attempt was made to determine the post-mortem skin changes through microscopy and correlate it with the time since death.

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Introduction

An important problem in forensic medicine is the need to fix the probable time of death. It is known that as the interval of time between death and the examination of the body increases, limits of probability also widens out. In most of the investigative procedures, knowing the approximate time is important for the investigating personals to make sure that their investigation is going through the right direction. This places a heavy responsibility on the shoulder of a forensic surgeon who opines regarding the probable time of the period of death.[1]

Structurally, the skin is composed of three major layers which were different physiologically,

histologically and embryologically. The outer layer or epidermis is formed by epithelium and is ectodermal in origin. The dermis, the next layer consists of connective tissue and is of mesodermal origin. Epidermis consists of stratified squamous keratinised epithelium in which five layers can be identified, namely, stratum basale, Stratum spinosum, Stratum Granulosum, Stratum lucidum and Stratum Corneum arranged from deep to superficial.

The basal layer is made up of single layer of columnar cells which lies on basal lamina. It contains the stem cells that undergo mitotic division to form new cells, the keratinocytes. This layer is therefore, also known as stratum germinativum .The renewal of the human epidermis takes about 3 to 4 weeks.

Objectives of the Study

1. To observe various histological changes in skin and its appendages.

2. To study time relation between these histological changes and postmortem interval

Review of Literature

Lovas JGL [2] in 1986 conducted a study, apoptosis in human epidermis by electron microscopy, on skin samples excised from the middle of the medial aspect of the upper arm of m106 consecutive autopsy cases. The time interval between death and biopsy was less than 24 hours in all but three cases. The tissue was fixed overnight in, the tissues were stained Thick sections for light microscopic (LM) survey were stained with toluidine-blue. Thin sections for EM were stained with uranyl acetate and lead citrate, and examined using a Zeiss EM 109 transmission electron microscope. He observed Apoptotic keratinocytes in 95 of the 106 cases. Electron microscopic study of 25 EM blocks of 18cases revealed seven AKs. Apoptotic Keratinocytes was observed in histologically or ultra structurally normal cells. It was not known about any apoptotic activity in epidermis except apoptotic activity in the liver which is induced is experimentally. He observed that when oxygen tension falls below the survival of epidermis is not possible and entire epidermis will undergo necrosis.

Kovarik C, Stewart D, Cockerell C[3] conducted a study in University of Tennessee Anthropology Research Facility in Knoxville, Tennessee, between September 30, 2003, and October 8, 2003 on 3 dead which was donated for research purposes. Bodies were kept outside unclothed in a dry shaded wooded area in a cool to temperate climate (temperature range 38–77°F during the week of analysis), and skin biopsies were systematically taken for analysis. Skin samples were taken from scalp, sole of the foot, dependent area of the trunk. Each specimen was sectioned and stained with hematoxylin and eosin (H&E) for microscopic analysis. They were then analyzed by an experienced forensic pathologist and dermato-pathologist. The general appearance of the skin was normal .From days 1 to 7, except for increased wrinkling in the acral skin. But on histological microscopy he found 3 significant histologic changes were noted, including cleavage of the epidermis from the dermis, eccrine duct necrosis, and dermal degeneration. The post-mortem interval is difficult to determine in autopsy cases in gross examination since many factors influence the rate of decomposition, including the cause of death, condition of the body at the time of death, movement of the body after death, temperature, humidity, and animal activity.

On microscopy he observed separation of the epidermis from the dermis in several skin biopsies. This focal separation likely leads to the gross appearance of bullae on the skin with further decomposition. Another histologic finding he observed was appreciated in many of the skin biopsies was eccrine gland necrosis. The timing of eccrine duct necrosis from the acral skin and scalp was not predictable.

Bardale RV, Tumram NK, Dixit PG, Deshmukh AY [4] conducted a study at the Department of Forensic Medicine, Government Medical College Hospital, Nagpur. They observed normal morphology of the epidermis and the dermis 0-6 hours. Appearance of vacuoles in cytoplasm of basal layer and spinous layer. Rarefaction of the dermis, elastic fibers get prominent with focal fragmentation in 6-9 hours. Distorted basal layer cells with incontinence of melanin pigment, focal epidermo-dermal separation 9-12 hours. Fragmentation of the dermis in 18 hours samples

According to Knight B [5] many methods are followed for determination of the post-mortem interval. Whatever be the method used all the variable factors must be taken into account along with the light of previous

experiences, for giving a final opinion. When time of death is decided upon ,it must be used to construct a "bracket of probability", giving an earliest and latest time between which the doctor feels that death must have occurred instead of quoting a single point of time to the investigator. This time bracket should depend on the uncertainty of the factors.

Materials and Methods

The study was conducted at the Department of Forensic Medicine, Yenepoya Medical College, Mangalore from August 2012 to August 2014. The study consists of 30 human dead bodies comprising of 26 male and 5 female, and their age ranged from 20 to 64 years. The death was due to various causes (head injury n = 12, hanging n = 6, poisoning, n =8 Intracranial hemorrhage n = 12, Natural death n=3, Electrocution n= 2,). The bodies were kept at room temperature in the waiting room of the mortuary. The average ambient temperature during the period varied from 23-C to 37-C, and the average humidity ranged from17% to 87%. At autopsy, skin samples from the anterior chest were collected, parallel to the longitudinal midline incision. The cases were divided into 0-6 hours, 6-12 hours, 12-18 hours, 18-24 hour intervals after death.

Materials Used

Dissection set, metal capsules for sample collection, 10% formalin, tissue processer, microtome, L-blocks, wax, slides, covering slip, H & E stain, PAS stains, microscope, digital camera.

Methods

2 skin samples from each dead body were collected from those bodies where actual time of death was known. Written consent was obtained from the relatives of the deceased. Certain inclusion and exclusion criteria were formulated as described below.

Inclusion criteria: 1. Sample collected from cadavers where actual time of death is known

Exclusion criteria: 1. Cadavers with extensive skin diseases.

2. Cadavers with extensive tattoos over chest region.

3. Those cadavers where actual time of death is not known.

4. Death due to burns

5. Body submerged in water

Skin samples (1 x 2 cm) from the anterior chest were collected, parallel to the longitudinal midline incision.

The skin samples will be carefully taken to avoid traumatic artefact. .The samples were carefully dissected out and immediately transferred to a capsule. Tissues were fixed in 10% formalin, embedded in paraffin wax, and stained with hematoxylin-eosin (H and E). The skin samples were observed under light microscope for the changes in the epidermis and dermis.

Observations

The following observations are seen in the microscopic study of the samples collected. The findings in epidermis and dermis are recorded and separately tabulated in tables- 1-5 respectively in accordance with PMI.

In epidermis, (TABLE:1)up to 0-6 hours. PMI, normal histological features are maintained. Minimal vacuolation appeared from 6th hour onwards. All samples at 6hours PMI showed vacuolations [figure.1] which is showing a tendency to become prominent over time.

Samples taken at 6-12 PMI period, up to 8 hours, normal histology is maintained even though 1 sample at 9 hours also maintained the same. Vacuolation started by 9 hours and is becoming a prominent feature by 12 hours. In 12-18 hours PMI periods, Normal histology is maintained up to 13 hours after which vacuolation started appearing and becomes a prominent feature by 18 hours. No samples showed normal histology after 18 hours and vacuolation was an established feature.

Dermis showed a normal histology up to 5 hours in s samples 0-6 hours. PMI samples, however, 2 samples at 6 hour PMI also showed a normal histology. (TABLE:2) Features like vacuolation, dermo-epidermal separation [figure.3] and dermal fragmentation[figure.2] are identified by 6 hours over I sample in this time interval. At 6-12 PMI set of samples, normal features are seen up to 12 hours, Dermo epidermal separation and dermal fragmentation is seen in samples at 9 hours PMI,

however dermal fragmentation showed its appearance since 7 hours PMI. Of the samples collected at 12-18 PMI, normal histology is shown up to 16 hours, however from 13 hours onwards, dermo-epidermal separation is a feature and dermal fragmentation is seen at 14 hours PMI sample. One sample at 18 hours showed normal histology whereas dermal fragmentation was an established feature in samples at 23 hours PMI. Sample of 23 hour PMI showed dermal degeneration [Figure.4] along with 22.2% of samples with 14-15 hours PMI(TABLE:3)

Results

The results of the present study show that the skin undergoes progressive morphological changes in the post-mortem period. These changes can be observed at the cellular level by light microscopy.

In epidermis, normal histology is maintained up to 13 hours in 2 cases. Vacuolation started appearing by 6 hours and is getting established by 14-16 hours and is a predominant feature of samples over 18 hours in most of the cases.

Dermis exhibited normal histology up to 19 hours in one case. Vacuolation started appearing by 6 hours even though it is not an established feature in dermis. Dermo-epidermal separation getting initiated at 6 hours along with dermal fragmentation is getting established through time. Dermal fragmentation is a distinguishable feature after 14 hours PMI.

Discussion

A limited amount of research has been conducted in the forensic field to determine an accurate estimate of the post-mortem interval. One of the major reasons behind it is that a controlled environment is difficult to obtain in most studies, and the instability of environmental factors has prevented the development of a single reliable predictor. There are no studies that compare post-mortem skin changes of bodies that have been allowed to decompose under the same environmental conditions.

Kushwaha V, Yadav M, Srivastava AK, Agarwal A[6], conducted a study in the Department of Forensic Medicine in collaboration with Department of Pathology, G.S.V.M. Medical College, Kanpur, U.P. A total of 45 cases are taken belonging to both sexes 36 males and 9 females were studied. These are of different age groups. On histological study of these kidney samples he observed changes which are follows: In first 12 hours, all 5 cases show mild degenerative changes. In 13-18 hours, with increasing temperature he observed moderate & severe changes are seen. Only 2 out of 13 cases show severe changes. In 19-24 hours with increasing temperature of up to, severity increases.

Considering the present study, we have sequentially studied the histologic appearance of the skin, sweat glands glands, and hair follicles in same environmental condition with average ambient temperature varied from 23-C to 37-C and average humidity ranged from 17% to 87%.

The major findings of the present study are compared with those of past literatures in Table.4.(TABLE:4)

When the results of the present study were compared with past literatures, the histologic findings are more or less similar, but the histologic changes seems to appear earlier than the other studies, the reason for which may be the environmental difference as extreme environmental factors may speed up decomposition, mainly the cutaneous decomposition.

Summary

-Epidermis and Dermis showed marked changes in the histology with increasing PMI and is the most reliable site to look for. -All these changes had to be correlated collectively taking into account the external as well as internal environment and all the micro factors playing in and around for a better prediction of PMI.

Limitations

This study is limited by availability of inadequate literatures, the small number of samples as Study included cases within 24 hours of death with time of death known and cases of found dead with time of death unknown were not included in the study, and conduction of the study in a single environment; however, useful information can be deduced from the results. The variability of the findings reinforces the difficulty in estimating the exact time since death.

Conclusion

If multicentric studies with similar conditions are carried out, it may establish concrete periodicity of death

based on the changes. This study is a potential tool in determination of time since death. There is currently very limited scientific information regarding the process of cutaneous decomposition. Studies with a larger number of cases being evaluated in different environments will throw a better light to post-mortem skin changes and their role in determination of the time since death.

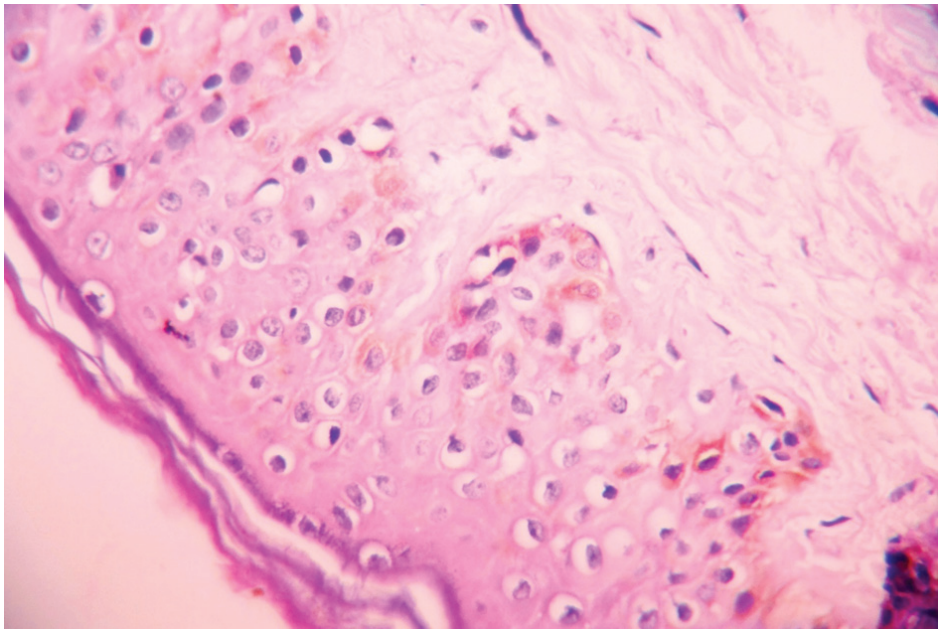

Figure.1: Microphotograph showing vacuolation in epidermis, H&E, 400x

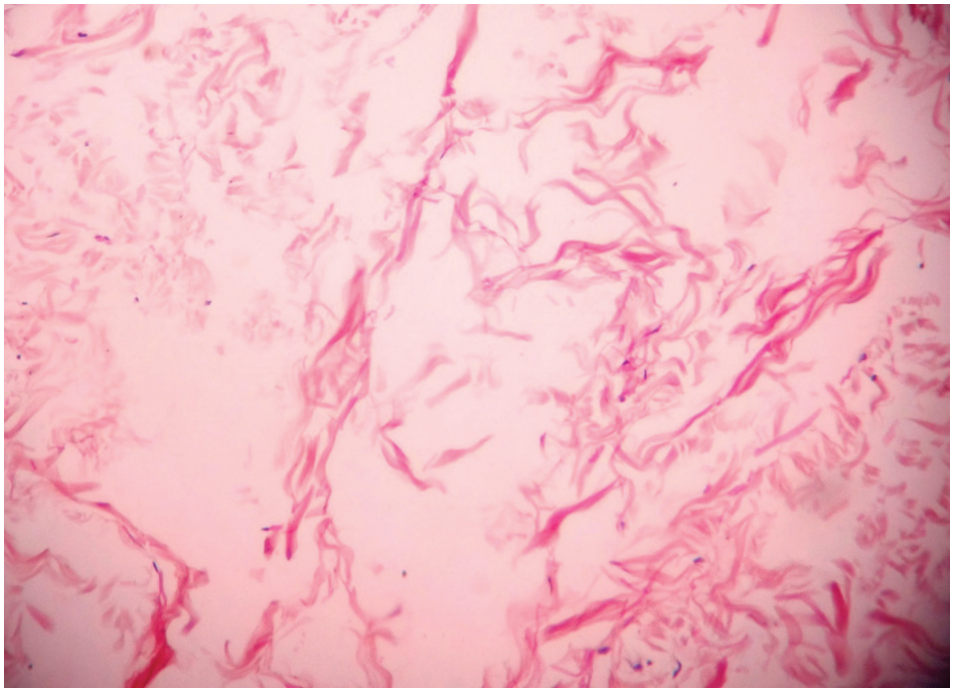

Figure.2: Microphotograph showing dermal disintegration,H&E,100x

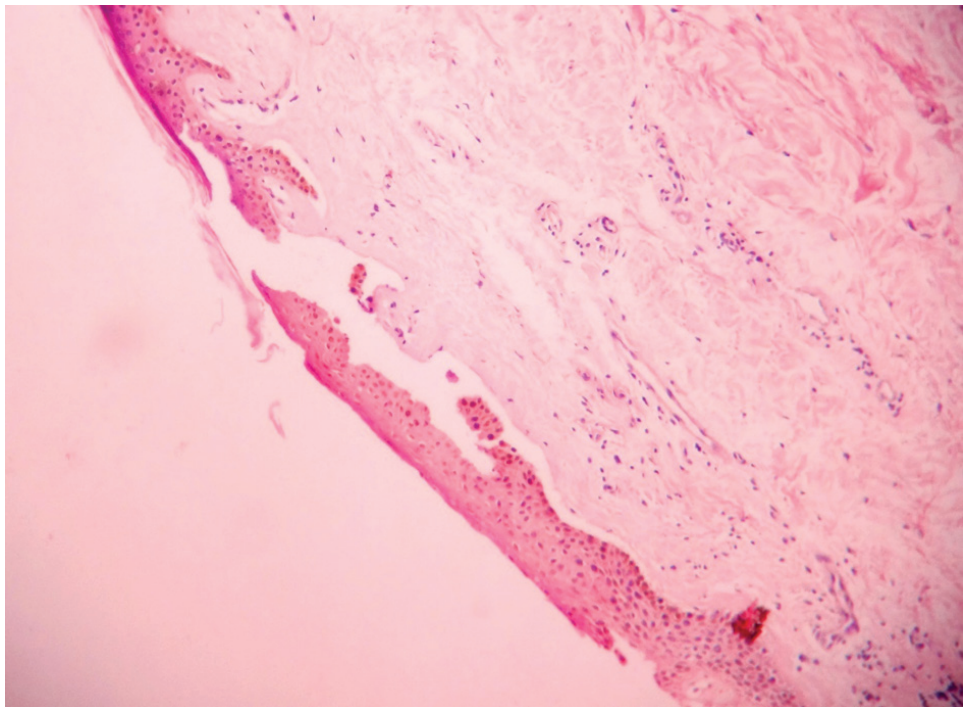

Figure.3: Microphotograph showing dermo-epidermal separation,H&E,100x

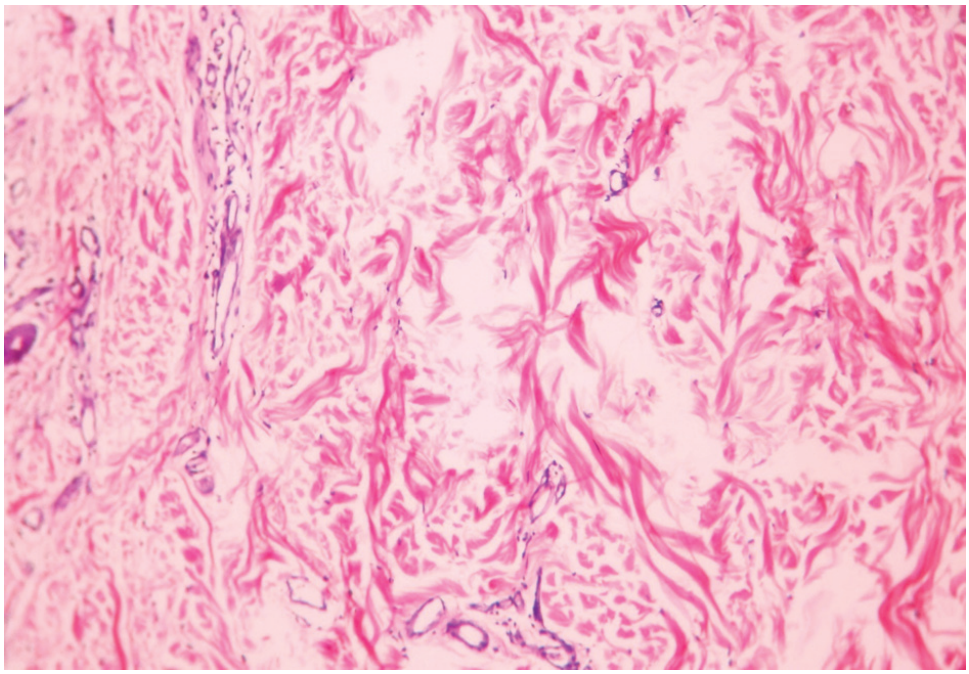

Figure.4: Microphotograph showing dermal fragmentation, H&E, 100x

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Source of Funding- Self

Conflict of Interest - Nil

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