

Histopathological Evaluation of Burn Injury

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ABSTRACT

Background: Burns always have posed a threat to the sensitive human body. It is a common catastrophe today as burn injury cases are one of the common emergencies admitted to any hospital. In a medico-legal setting, the main difficulty arises when a burnt body found in unknown circumstances is brought for post-mortem examination. In such a case, a forensic expert has to opine whether the burn injuries sustained are antemortem or post-mortem. It is also of paramount importance to determine the age of burn injuries sustained. Aging of burn injuries by the naked eye is sometimes difficult and unreliable. By histopathological examination of burnt tissue, one can predict whether the victim was alive or dead when the fire was going on and determine the age of burn injuries.

Materials and Methods: The present cross-sectional study was carried out in the Department of Forensic Medicine & Toxicology, Pt. B.D.Sharma PGIMS, Rohtak. Total 3033 autopsies were conducted in the mortuary of the Department of Forensic Medicine & Toxicology, PGIMS, Rohtak, from November 5, 2011 to November 5, 2013. During that period, out of 486 cases reported burns, randomly 100 cases were selected for this study.

Results and Conclusions: In the present study, efforts were made to determine burn wound vitality and age by histopathological evaluation. The study revealed that the inflammatory infiltrate consisted mainly of polymorphonuclear leucocytes in initial stages (up to 3rd-day post-burn) with gradual lymphocytes, macrophages, and fibroblasts forming immature granulation tissue. On the 14th-day post-burn, the burn wound revealed repair by regeneration, increased collagen deposition, fibrosis, and granulation tissue formation. It was infiltrated with fibroblasts, macrophages, and lymphocytes. Our study demonstrated that evaluation of various histopathological changes occurring during the distinctive phases of burn wound healing help in the estimation of its vitality and age.

Keywords: Age of burn, Burn, Histopathological evaluation, Medico-legal, Vitality.

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INTRODUCTION

Forensic medicine deals with the application of medical knowledge to aid in the administration of justice. So far as the modes of sustaining burn injuries in India are concerned, the same is everywhere else, i.e., flames, thermal, scalds, and electrical burns. In our country, the most common manner of sustaining flame burns is accidental.¹

The main difficulty arises when a dead body is brought to a forensic expert for opinion in a state of partial skeletonization from burning as well as from advanced putrefaction or the body of a victim who has been killed or murdered by throttling, poisoning or by some other means. Later the body is burnt to conceal the crime and to make it appear like death from burning. As severe burning or charring of the body might produce artifacts of heat ruptures that may resemble lacerated or even incised wounds, differentiation requires differentiation. Therefore, differentiation of antemortem and post-mortem burns need to be affirmatively ascertained. It is also of paramount importance whether a burn injury has a vital reaction, and if so, how long before the burn injury has been sustained.

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Forensic experts can determine how long a victim has been exposed by estimating the age of burn injuries. However, the aging of burn injuries by the naked eye is sometimes difficult and unreliable.² By histopathological examination of burnt tissue, one can surely predict whether the victim was alive or dead when fire was going on and determine the age of burn injuries. Walcher pointed the importance of studying the lesions microscopically in order to distinguish between antemortem and post-mortem injuries. According to him, the leucocytic reaction was the earliest histological sign of

inflammation. The pavement of the inner vessels walls by leucocytes and the beginning of their extravasation may be seen during the first hours after wounding.³

Mallik described histological methods of distinguishing antemortem and post-mortem burns using burns inflicted experimentally on guinea pigs, burns of human skin obtained from autopsy examinations, and burns inflicted experimentally in amputated human tissues. In human burns, the earliest histological change in the antemortem burn was leucocytic infiltration at 6 hours after burning.⁴

The present study was conducted to assess the burn wound vitality and age by histopathological examination of burnt tissue.

MATERIALS AND METHODS

The present cross-sectional study was carried out in the Department of Forensic Medicine & Toxicology, Pt. B.D.Sharma PGIMS, Rohtak. Total 3033 autopsies were conducted in the mortuary of the Department of Forensic Medicine & Toxicology, PGIMS, Rohtak, from November 5, 2011 to November 5, 2013. During that period, out of 486 cases reported of burns, randomly 100 cases were selected for this study. The ethical approval was taken from Institutional Ethical Committee. After obtaining informed expressed consent, the relevant information regarding the case was collected from accompanying relatives, hospital records, and inquest papers. For the purpose of the study, burn injury was defined as a body lesion due to an external cause, either intentional (homicidal or suicidal) or unintentional (accidental) resulting from sudden exposure to thermal energy generated by agent host interaction. Criteria of selection of cases:-

1. All cases of burn injuries that have a definite history of flame burns were taken for the study.
2. The cases with different duration and survival times were studied.
3. Burn injuries due to electrocution, chemical and corrosives were not included.
4. Decomposed bodies and bodies with no specific history were not included.

Skin tissue was taken from the burnt and junctional area of the burnt and unburnt region and preserved in 10% formalin for fixation. The fixed tissues were processed, stained with routine H& E staining and subjected to histopathological examination.

The data was collected and compiled in an excel sheet and analyzed using SPSS (Software Package for Social Studies) version 20, and chi-square test and percentages were applied to find out any significant statistical difference between different groups, and if required, a logistic regression test was applied.

RESULTS

Histopathological examination of normal human skin revealed that it has two layers-outer epidermis and the inner dermis. The epidermis consists of five layers and is separated from the dermis by a basement membrane. The dermis forms the bulk of skin and consists of an upper papillary layer and the lower

reticular layer. It is composed of mature, fibrous connective tissue that contains capillaries, sweat glands, sebaceous glands, hair follicles, and nerve endings. The dermis is attached to underlying loose connective tissue, which consists primarily of adipose tissue (Figure 1).

As shown in Table 1, redline of demarcation was present in 39% of cases and absent in 61% of cases (Figure 2). Redness was present in 40% of cases and absent in 60% of cases. Blisters were present in 30% of cases and absent in 70% of cases. Pus was present in 61% of cases and absent in 39% of cases. Granulation Tissue was present in 51% of cases.

As shown in Table 2, Separation of epithelium and breaking of epithelium were present in 99% of cases, respectively. Vacuolization was present in 71% of cases. Petechial hemorrhages were present in 33% of cases. Epithelial cells were elongated and flattened in 72% of cases, respectively.

As shown in Table 3, Capillary dilatation was present in 59% of cases. Edema and congestion were present in 57 and 45 % of cases, respectively. Margination of leucocytes was present in 67% of cases. Infiltration of leucocytes was present in 95% of cases.

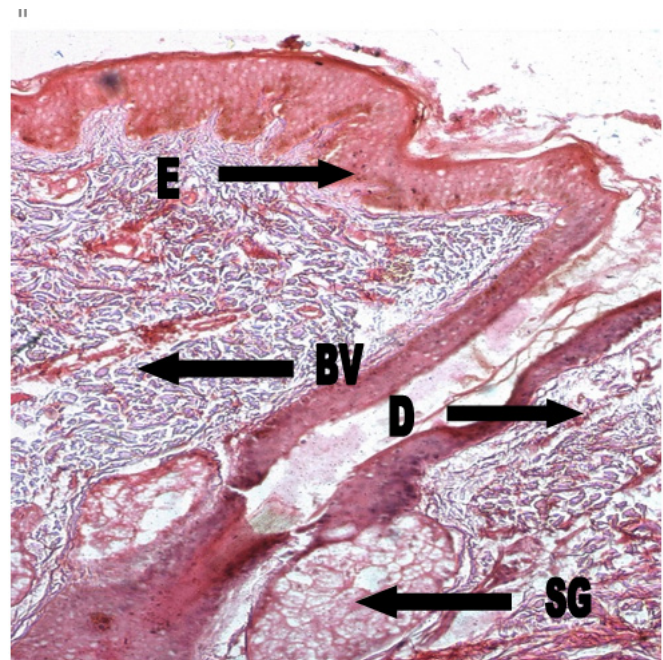


Figure 1: Photomicrograph of histology of normal unburnt skin showing intact epidermis [E], and dermis [D] showing redline of demarcation (H & E; 20x)

Table 1: Incidence and distribution of gross features of burnt skin

Gross features of burnt skin	Present	Absent
	No. (%)	No. (%)
Red line of demarcation	39 (39)	61 (61)
Redness	40 (40)	60 (60)
Blisters	30 (30)	70 (70)
Pus	61 (61)	39 (39)
Granulation Tissue	51 (51)	49 (49)

Chi-square=23.3; p-value=.000



On the 3rd day post-burn, the burn wound revealed separation of the epidermis from dermis and breaking of epithelium, vacuolization of epidermal and dermal cells, petechial hemorrhages, congested and dilated capillaries with areas of necrosis, and formation of immature granulation tissue. The inflammatory infiltrate consisted mainly of polymorphonuclear leucocytes [Figure 3(a,b,c)]. On the 3rd to 7th day post-burn, the burn wound showed the presence of elongated and flattened epithelial cells. The inflammatory infiltrate consisted of lymphocytes and macrophages in the burn wound (Figure 4). On the 7th day post-burn, the burn wound revealed epidermal regeneration, neo-proliferation of small-sized blood vessels, inflammatory cells infiltration consisting primarily

of macrophages, lymphocytes, fibroblasts, and the presence of immature granulation tissue (Figure 5). On the 14th day post-burn, the burn wound revealed repair by regeneration, increased collagen deposition, fibrosis, and granulation tissue formation. In addition, it was infiltrated with fibroblasts, macrophages, and lymphocytes (Figure 6).



Figure 2: Photograph of burnt skin containing blood vessel [BV] & sebaceous gland [SG]

Table 2: Incidence of Histopathological findings of Burnt skin

Burnt Skin	Total number of cases	Percentage (%)
Separation of epithelium	99	99%
Breaking of epithelium	99	99%
Vacuolization	71	71%
Petechial hemorrhages	33	33%
Epithelial cells elongation	72	72%
Epithelial cells Flattening	72	72%

Table 3: Incidence of Histopathological findings of Junctional skin

Junctional Skin	Total number of cases	Percentage (%)
Capillary dilatation	59	59%
Oedema	57	57%
Congestion	45	45%
Margination of Leucocytes	67	67%
Infiltration of Leucocytes	95	95%

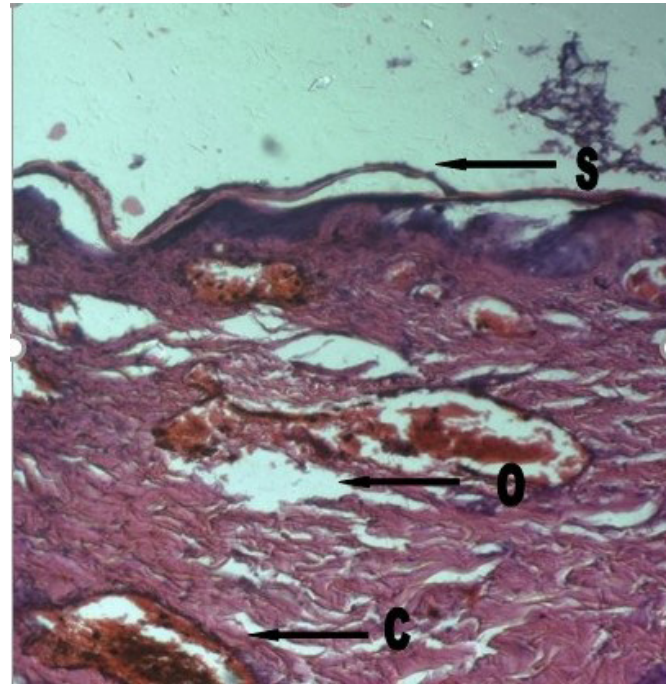


Figure 3a: Photomicrograph of skin, 3 days post-burn showing congestion [C], edema [O] and separation of epidermis from dermis [S] (H&E; 10x)

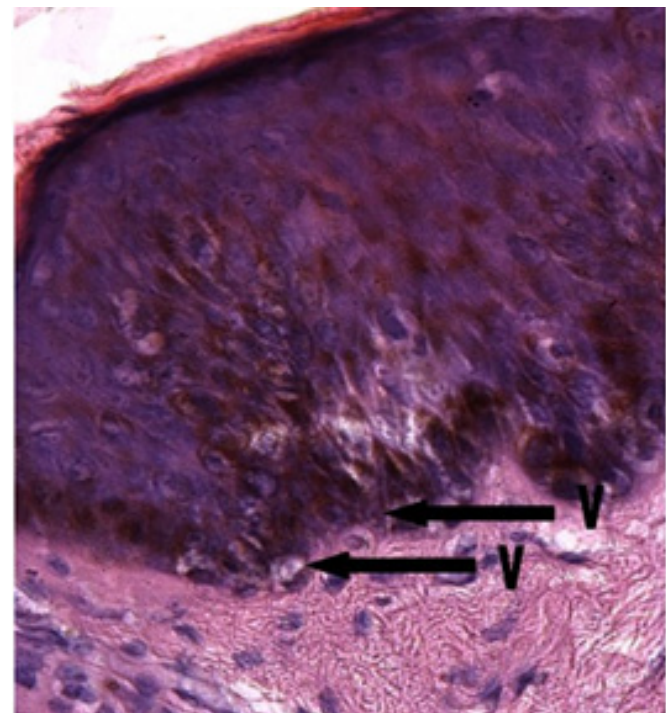


Figure 3b: Photomicrograph of skin, 3 days post-burn showing vacuolization [V] of basal cell layers (H&E; 40x)

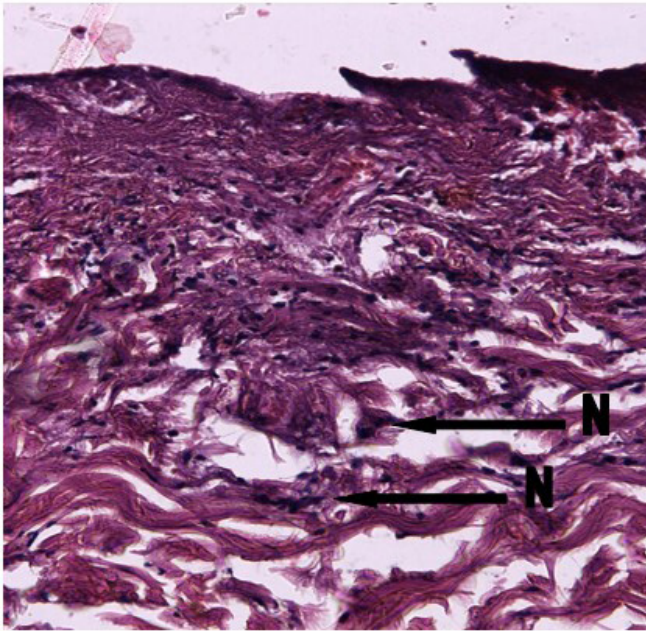


Figure 3c: Photomicrograph of skin, 3 days post-burn showing dermal infiltration by neutrophils [N] (H&E; 20x)

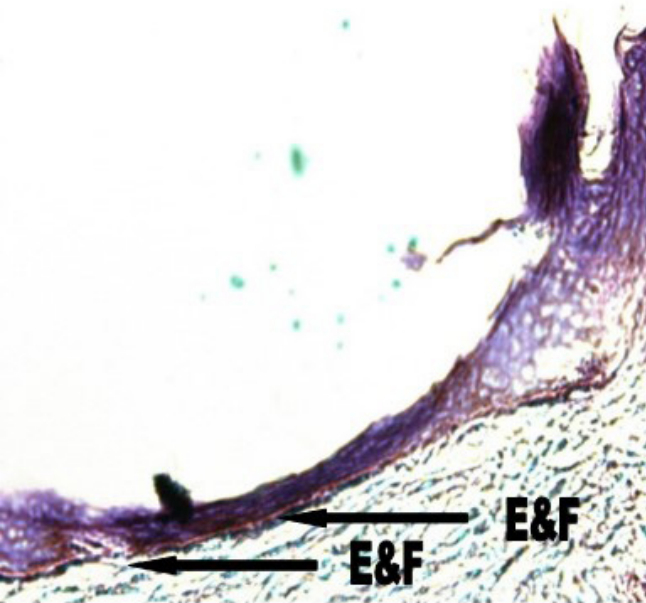


Figure 4: Photomicrograph of skin, 3 to 7 days post-burn showing elongation & flattening [E&F] of basal epithelial cells (H&E; 20x)

DISCUSSION

The histopathological findings of burnt skin are initially due to direct effects of thermal injury and subsequently due to inflammatory healing response.⁵ Therefore, it could be possible to estimate the vitality and age of burns by examining the order and time at which various cellular components of the healing process are present in burn wounds.

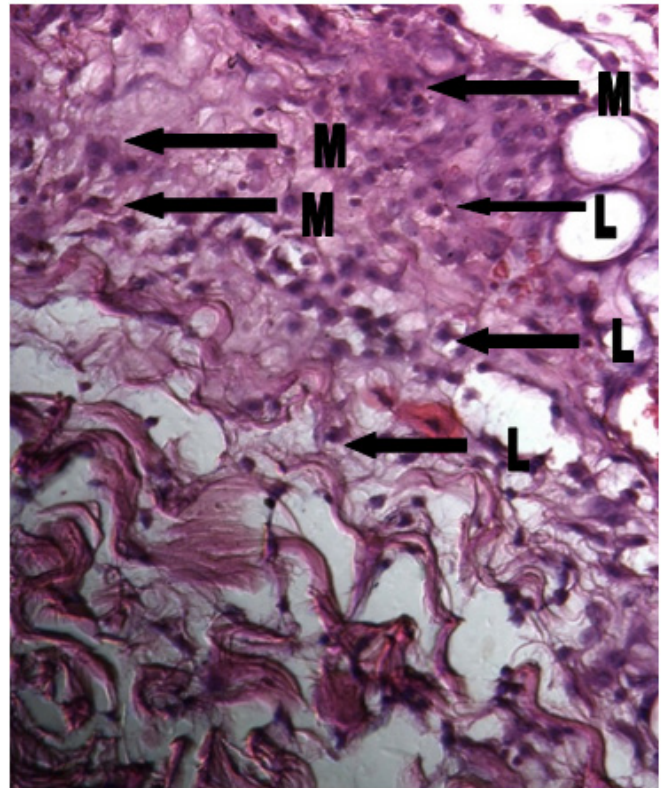


Figure 5: Photomicrograph of skin, 7 days post-burn showing dermal infiltration by lymphocytes [L] & macrophages [M] (H&E; 20x)

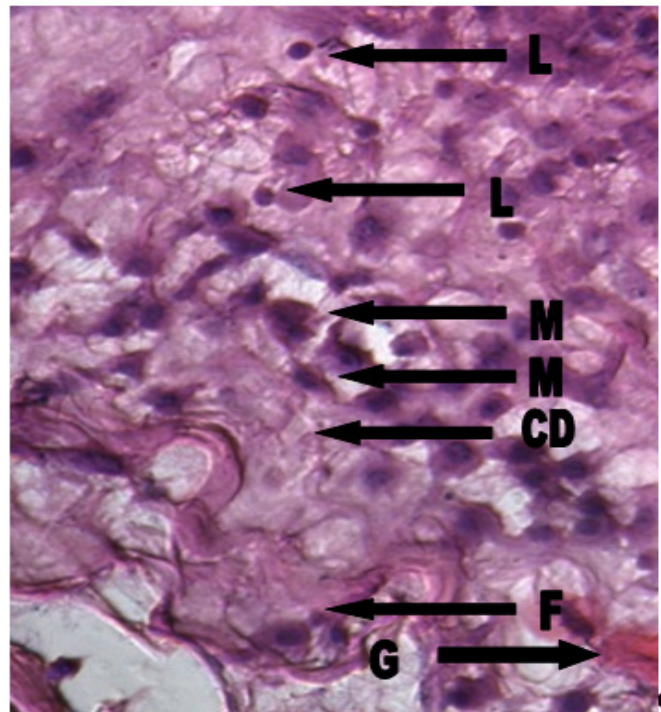


Figure 6: Photomicrograph of skin, 14 days post-burn showing Collagen deposition [CD], fibrosis [F], granulation tissue [G], and dermal infiltration by macrophages & lymphocytes (H&E; 20x)

Histopathological examination of the edge of a burn, including both the burnt area and adjacent grossly unburnt skin, may reveal vital reaction microscopically, consisting of acute inflammation, hemorrhage, edema, necrosis, and vertical streaming epidermal nuclei and homogenization of the dermal collagen. Skin shows petechial hemorrhages in deeper layers, epithelial cells are elongated, flattened and stained deeply with hematoxylin and eosin and vacuolization of epidermal and dermal layers is prominently seen. These findings help in differentiating antemortem burn from post-mortem burn.⁶⁻⁸

In the present study, histopathological examination of burnt and junctional skin, three days post-burn, revealed separation of the epidermis from dermis and breaking of epithelium, vacuolization of epidermal and dermal cells, petechial hemorrhages, congested and dilated capillaries with areas of necrosis, and formation of immature granulation tissue. The inflammatory infiltrate consisted mainly of polymorphonuclear leucocytes in initial stages with gradual involvement of lymphocytes and macrophages.

Haggag OG et al. in their study observed that on the 3rd-day post-burn, upper layers of dermis showed ulceration, and deeper layers of dermis showed the formation of granulation tissue, diffuse congested capillaries, tissue edema with areas of necrosis. Few tiny perivascular hemorrhages were present in the deep dermis and collagen was destructed. Infiltration of neutrophils was present in burn wounds of moderately-burned patients, but a few neutrophils were present in severely burned patients.⁹

Tarran et al. in their study concluded that the neutrophils were the first inflammatory cells to infiltrate the wound.¹⁰ Peng et al. observed that infiltrative reaction of leucocytes started at 3rd-day post-burn in victims who sustained moderate burns, but in severe burns, there was no infiltration until 1-week after-burn. The infiltration of neutrophils is due to a local increase in a neutrophil chemo-attractant. The delayed neutrophil infiltration in more severe burn wounds was related to the impaired production of local chemokines.¹¹

RJ Brennen, in his study, observed the presence of gross edema, separation of collagen fibers and separation of epidermis almost entirely from the underlying dermis. The capillaries of the dermis were dilated, and congestion was present. In addition, he observed increased numbers of round cells and polymorphonuclear leucocytes in the perivascular tissue. The deeper layers of the dermis showed granulation tissue containing numerous thrombosed capillaries surrounded by areas of microscopic debridement and loss of structure.¹²

Xu et al., on day three post-burn, observed vacuolization of epidermal cells, hyaline degeneration in collagen dermis, and infiltration of the dermis by inflammatory cells consisting mainly of neutrophils.¹³

In the present study, 3 to 7 days' post-burn biopsies showed elongated and flattened epithelial cells. The inflammatory infiltrate consisted of lymphocytes and macrophages in the burn wound. RJ Brennen, in his study, observed fragmentation of the thermally coagulated tissues, microscopic evidence of granulation, and infiltration of the burn wound with

lymphocytes and macrophages. Xu et al. on day 5 post-burn, observed superficial tissues in the dermis were liquefactive necrotized, and exfoliated. The dermis was infiltrated with inflammatory cells consisting mainly of neutrophils and occasional macrophages. A similar result was observed by Haggag OG et al in their study.

In the present study, 7 days' post-burn biopsies showed epidermal regeneration, congested blood vessels, inflammatory cells infiltration consisting primarily of macrophages, lymphocytes and fibroblasts, and the presence of immature granulation tissue. Haggag OG et al. in their study observed that on 7th day post-burn, the epidermis showed regeneration from the basal layer. Congested blood vessels, collagen deposition and edema, were detected in the dermis with the appearance of fibroblasts in clusters. In addition, the presence of neutrophils, lymphocytes, plasma cells, and a few mononuclear cells was noticed in the burn wound. Peng et al found a large number of leucocytes in burn wounds on the 7th-day post-burn. The cellular components consisted of polymorphonuclear leucocytes in the majority and a few lymphocytes and macrophages. Tarran et al. observed that when macrophages are abundant, the wound is probably a few days to weeks old.

In the present study, 2 weeks' post-burn biopsies revealed repair by regeneration, granulation tissue formation, fibroblasts, macrophages, and lymphocytes in burn wounds. Haggag OG et al., in their study on 14th day post-burn observed the same. They observed that there was epidermal growth, collagen fibers of the dermis were denser than normal, and numerous scattered fibroblasts. Mononuclear cells were the main cellular components in the burn wound. Tarran et al. and Peng et al. also observed the same. They found a formation of frank granulation tissues, neo-proliferation of blood vessels, and predominantly mononuclear cells in the burn wound. RJ Brennen, in his study, observed that between the 12th day to 18th day, the morphological structure of the upper layer of the dermis was entirely lost. Dissection of the collagen fibers and vacuolization were still evident. In some areas, there were still present some nests of white cells. Xu et al., on day 15 post-burn, observed that actively proliferated fibroblasts and neo-formative capillaries were visible in the dermis, accompanied by the infiltration of lymphocytes.

CONCLUSION

The present study concluded that its histopathological evaluation can fairly estimate the burn wound vitality and age. Such estimation is of paramount importance, particularly in criminal cases, when the victim is killed by some other means. Later, the body is burnt to conceal the crime and make it appear like death from burning.

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