



Histopathological changes as tools to discriminate antemortem and post-mortem wounds in rats: Prospective applications in forensic medicine

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Abstract

Wound age estimation is one of the chief exciting subjects in forensic medicine. It is substantial to determine the most likely accurate when wounds happened, whether during antemortem or post-mortem conditions. Moreover, histological change is a method that assists available parameters in determining the antemortem and post-mortem period. This study aims to observe the histopathological changes in induced wounds to determine when the injury occurred and whether the injury occurred during antemortem or post-mortem conditions. Thirty-nine rat wound skin biopsies were studied. All samples were taken in antemortem groups at 30, 60, 180, and 360 min, and in post-mortem groups within 30, 60, 180, and 360 min with control samples (unwounded group). The skin sections were seen by microscope to observe the changes in the following criteria: the ratio and distribution of the neutrophil and macrophage, congestion and dilatation of capillaries, and degree of autolysis. For the antemortem wounds, the number of neutrophils appeared at 30 minutes and sharply increased from 60 minutes after the wound. In addition, there was an increase in macrophages from 180 min after the wound. For the post-mortem wounds in all times examined, the degree of autolysis was the best criterion for knowing the wound timing. These results propose that histopathological changes can be used as a critical criterion for finding the time of wounds and comparing antemortem and post-mortem incisional wounds in forensic medicine.

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Introduction

Determination of wound age and clarifying the consecutive arrangement of actions is a significant part of the effort in forensic medicine. It is essential to tell of the potential accurateness when wounds happen, whether during the antemortem or post-mortem (1,2). The most challenging problem is distinguishing between wounds inflicted shortly before and after death. Forensic specialists are often enquired to comment on the wounds ages in court, and the justification can have crucial medico-legal penalties (3,4). In many countries, people typically use eye inspection to know the age of a wound injury (5). There is apparent strangeness present in the books, suitable to see the difficulty dating wounds and injuries depending on a naked eye (6). Skin

wound healing begins directly after injury and involves three stages: inflammation, proliferation, and remodelling (7). Various biochemical materials and cells are included in the healing route to complete tissue repair (8). As a result of the unsure and inconstant consequences of the gross examination of skin wounds, it is crucial to histopathologically study the time of wound injuries despite the use of up-to-date methods such as immunohistochemistry for dating the wounds. The histopathological changes in wounds are still indispensable (9). Generally, it has been exposed that clear responses do not happen in post-mortem wounds (10). However, in antemortem wounds, an organized order of steps is associated with wound repair (11). These responses make it likely to demonstrate, step by step, and allow the making of a model that might be useful to many

problems in forensic medicine (2). Study of wound age indicators, for example, the inflammatory cells and mediators, are essential for identifying the lesions' vitality and establishing lesion timing, ante- and post-mortem (12). While a traditional method is, even so, a part of concern in current forensic medicine, there is an incessant need for more research and information to be helpful in daily applied work (13). Previously, the histological changes had noted the antemortem or post-mortem skin wound findings alone (14,15). Nevertheless, it has not been sufficient to be beneficial to actual circumstances and used to precisely inform the time of injury between antemortem or post-mortem and compare them. In the current study, skin wound dating was observed using the histopathological changes in induced wound skin in rats for a short time after inducing wound to investigate their possible implication in forensic practice, for example, time of death inference.

Materials and methods

Animal experiments

All the experiments were accepted by the University of Mosul, College of Veterinary Medicine and complied with the ethical approval number (Approval no. UM.VET.2021.011). Thirty-nine rats (weighing 170-200 g) were obtained from the animal house of the College of Veterinary Medicine, University of Mosul, and housed in a well-ventilated area and under pathogen-free conditions. Three rats were housed in each cage with wood bedding, good dry commercial pellets, and water. They were exposed to the environment for two weeks prior to the start of the experiment to ensure their health.

Wound induction and sampling

Rats were anesthetized with intramuscular doses of xylazine 10 mg/kg and ketamine 90 mg/kg prior to wound induction (16-18). The wound lines were marked with a marker before shaving the dorsal back of the animal with an electric shaver. After that, a 1.5 cm linear wound was produced in the back area, piercing all layers of skin with a sterile medical blade. The wound was induced in antemortem groups, including wounds at 30, 60, 180, and 360 min (3 rats at each time point). In addition, the control samples (unwounded group) were collected from the same area as the wounded groups (3 rats). After the specified period for each group, skin samples were taken, and the animals were euthanized. While in the post-mortem imposed skin wound, the rats in these groups were sacrificed, and post-mortem wounds were imposed within 30, 60, 180, and 360 min after death in the same region as the antemortem groups (3 rats each time point). In addition, the control samples (unwounded group) were collected from the same area as the wounded groups within 30, 60, 180, and 360 min (3 rats each time point). After the specified period for each group, skin samples were taken. The skin wound samples from the

antemortem and post-mortem groups were collected and stored in 10% neutral buffer formalin (pH 7.0) for subsequent histopathological examination.

Histopathological analysis

Wound tissue samples that were formalin-fixed and paraffin-embedded were sliced into 4µm tissue slices. For histological inspection, the sections were stained with hematoxylin and eosin (H&E) (19). Wound tissue samples were assessed based on the following parameters: congestion, edema, hemorrhage, autolysis, and infiltration of inflammatory cells (20). In addition, some parameters were observed for several neutrophils and macrophages using a light microscope in three different fields (21). Neutrophils were well-known as lobulated nuclei with granules in the cytoplasm (22). In the wound area, macrophages were recognized as large round cells with a round nucleus (23).

Results

The histopathological examination of the control skin tissue sections (unwounded rats) revealed normal skin with a normal epidermis lined by all the layers; granular, basal, spinous, cornified layers, and stratified squamous epithelium. The standard dermis layer contained normal blood vessels (Figure 1). The histopathological examination of skin sections at 30min reaction for the antemortem incision exhibited mild hemorrhage, destroyed tissue, and moderate Zenker's necrosis of muscle fibers at the wound edges (Figure 2 a). In addition, the blood vessels in the dermal layer showed congestion and dilatation of capillaries and arterioles (Figure 2 b), with the migration of leukocytes toward the blood vessel walls (Figure 2 c). On the other hand, in the first 30 min of the post-mortem skin examination, the histopathological analysis of the unwounded rats showed no noticeable change (Figure 2 d). However, in the post-mortem imposed wounds within 30 min exhibited mild hemorrhage within the tissue (Figure 2 e), but without cellular reaction, congestion, and dilatation of capillaries (Figure 2 f).

In the 60 min wound reaction time, the histopathological analysis of wound sections for the antemortem incision revealed a severe hemorrhage at the borders of the incision and severe congestion in the dermis (Figure 3 a). Furthermore, there was an increase in inflammatory cells. Generally, neutrophils are marginalized at the inner surface of blood vessel walls, and the amoeboid migration of neutrophils outside the blood vessels across the tissue (Figure 3 b). At the same time, the histopathological picture of post-mortem skin sections in the unwounded rats showed autolysis with epidermal loss related to post-mortem autolysis (Figure 3 c). Nevertheless, the post-mortem skin wounds at 60 min demonstrated severe hemorrhage at the borders of the incision and with post-mortem autolysis of the tissue, but without congestion and cellular reaction (Figure 3 d).

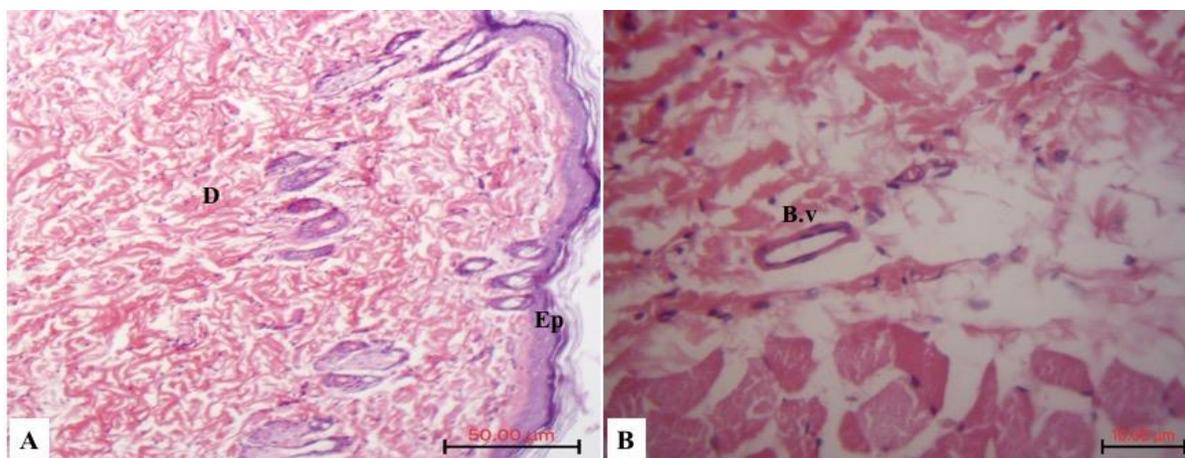


Figure 1: Histological appearance of the skin of the control group (unwounded rats). A. The normal skin showing normal epidermis (Ep) displays all layers (granular, basal, spinous, and stratified squamous epithelium) and normal dermis (D). H&E. 50µm. B. normal blood vessel in the dermis (B.v). H&E. 10µm.

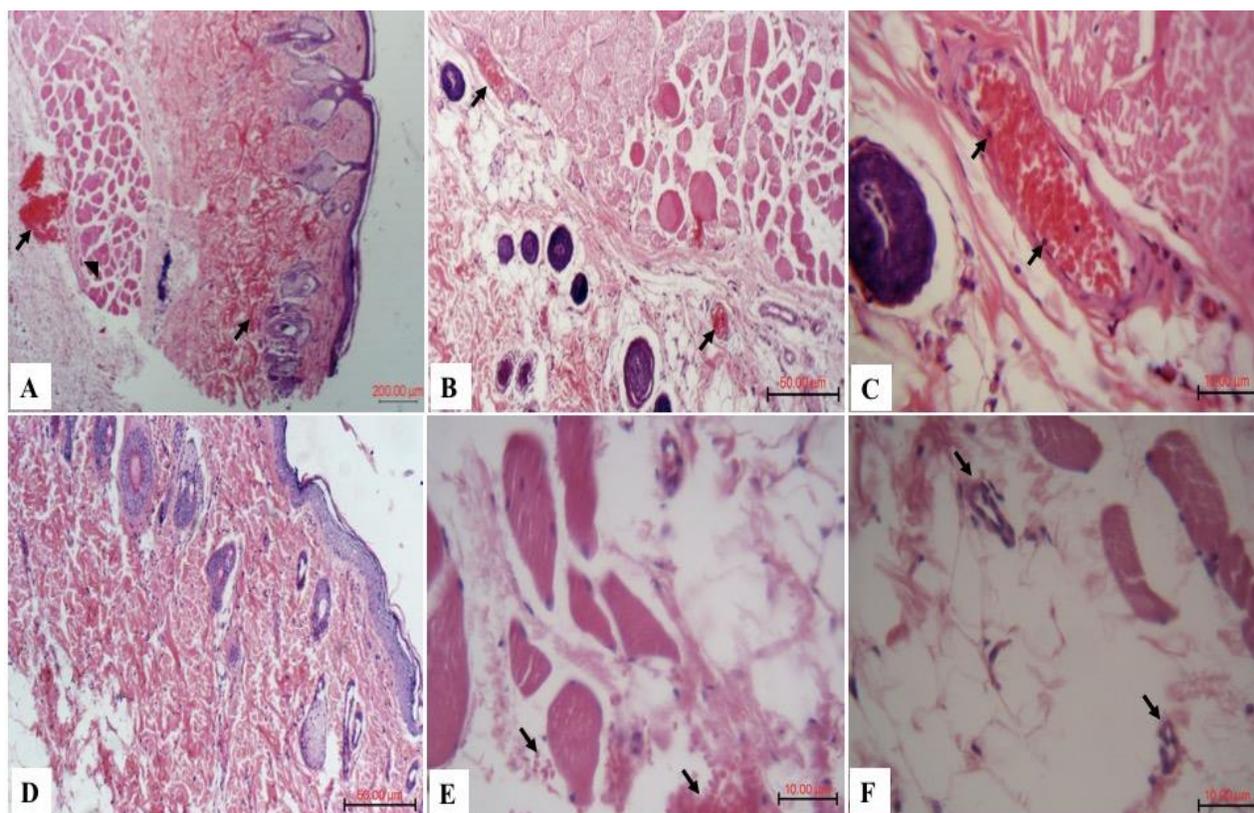


Figure 2: Histopathological changes after 30 min of antemortem and post-mortem skin wound in rats. A. In the group of antemortem skin wounds, the wound edge shows hemorrhage (arrows) and Zenker's necrosis of muscle fibers (arrowhead). H&E. 200µm. B. Dilatation of capillaries and arterioles with congestion in antemortem skin wound (arrows). H&E. 50µm. C. Leukocyte migration toward the endothelium of the blood vessels in an antemortem skin wound. (arrows). H&E. 10µm. D. In the group of post-mortem skin wounds, the histopathological examination of the unwounded rats showed no recognizable change in all layers of skin. H&E. 50µm. E. The post-mortem-induced wounds within 30 min exhibited hemorrhage within the skin layers (arrows). H&E. 10µm. F. No congestion and dilatation of capillaries in a post-mortem skin wound. (arrows). H&E. 10µm.

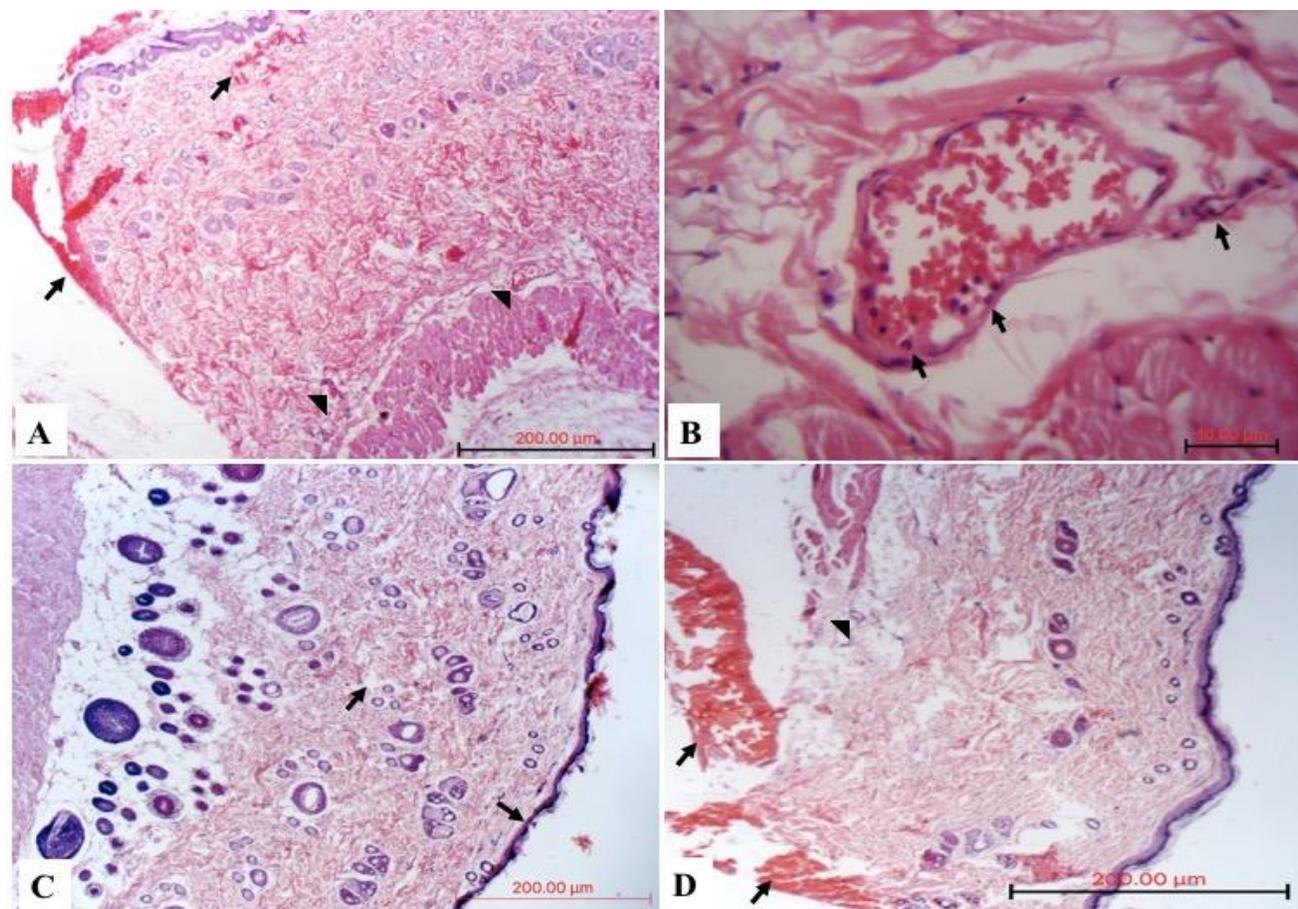


Figure 3: Histopathological changes after 60 min of antemortem and post-mortem skin wound in rats. A. In the group of antemortem skin wounds, the wound edge reveals severe hemorrhage (arrows) and severe dermal congestion (arrowheads). H&E. 200μm. B. Accumulation of neutrophils with marginalized at the inner surface of blood vessel walls, and migration of neutrophils outside the blood vessels (arrows). H&E. 10μm. C. In the post-mortem group, the skin wounds and the histopathological examination of the unwounded rats showed post-mortem autolysis (arrows). H&E. 200μm. D. The post-mortem-induced wounds within 60 min exhibited severe hemorrhage at the wound edge (arrows) but without congestion and cellular reaction (arrowhead). H&E. 200μm.

In the 180 min wound reaction time, the antemortem stab wound reaction showed infiltration of hemorrhage at the wound edge with an increase in the numbers of neutrophils around perivascular tissue and an increase in the movement of these cells to the site of the wound edge (Figure 4 a and b). In addition, at this reaction wound time, a small number of macrophages were observed in the wound region (Figure 4 c). On the other hand, the histopathological photo of post-mortem skin sections at 180 min in the unwounded rats displayed moderate autolysis with loss of the epidermal layer (Figure 4 d). However, the post-mortem skin wounds at 180 min showed hemorrhage and autolysis of the tissue (Figure 4 e). In addition, hemolysis blood filled the blood vessels without infiltrating inflammatory cells (Figure 4 f). In antemortem skin wounds, after 360 min, there were congestion, hemorrhage, and massive neutrophil infiltrations

in all skin layers (Figure 5 a). Moreover, at this wound time, there were increased numbers of macrophages in the wound region (Figure 5 b). Concurrently, the histopathological picture of post-mortem skin sections in the unwounded rats showed extensive autolysis with severe tissue loss (Figure 5 c). Nevertheless, the post-mortem skin wounds at 180 min showed bleeding, edema, and autolysis of the tissue (Figure 5 d). The number of neutrophils and macrophages of each antemortem group in variations over time was represented in figure 6. The number of neutrophils increased gradually after 30 and 60 min and increased very quickly after 180 min with more than 50 cells in each field and reached a maximum value at 360 min with more than 80 cells. Correspondingly, macrophages appeared at 180 min and peaked at 360 min, with more than 25 cells in each field.

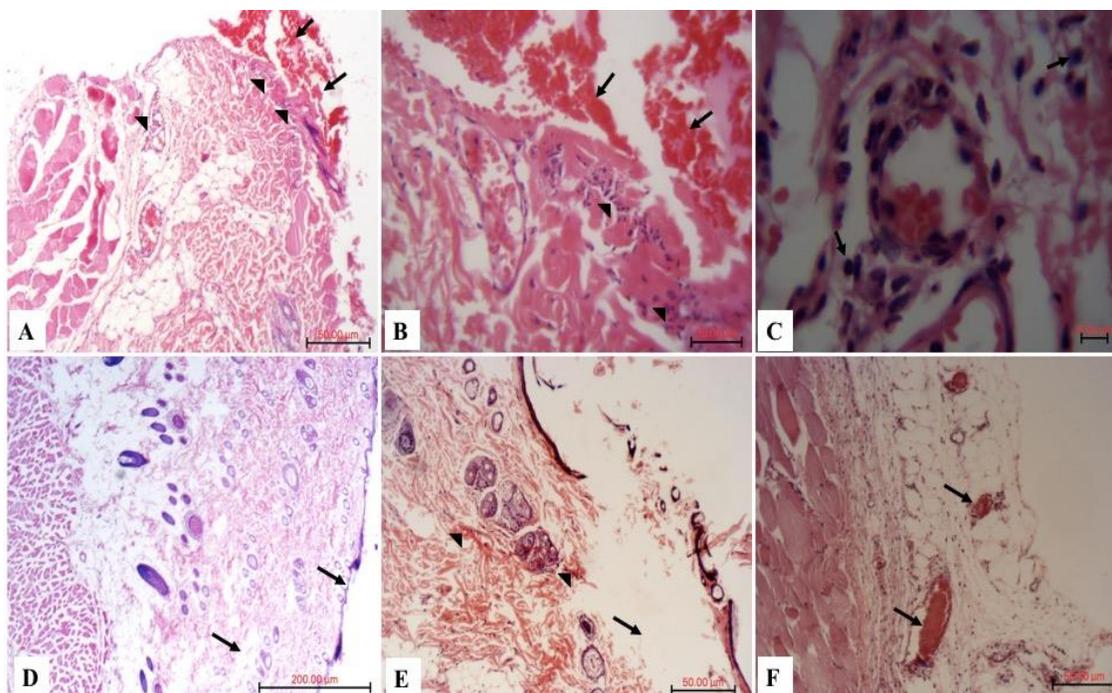
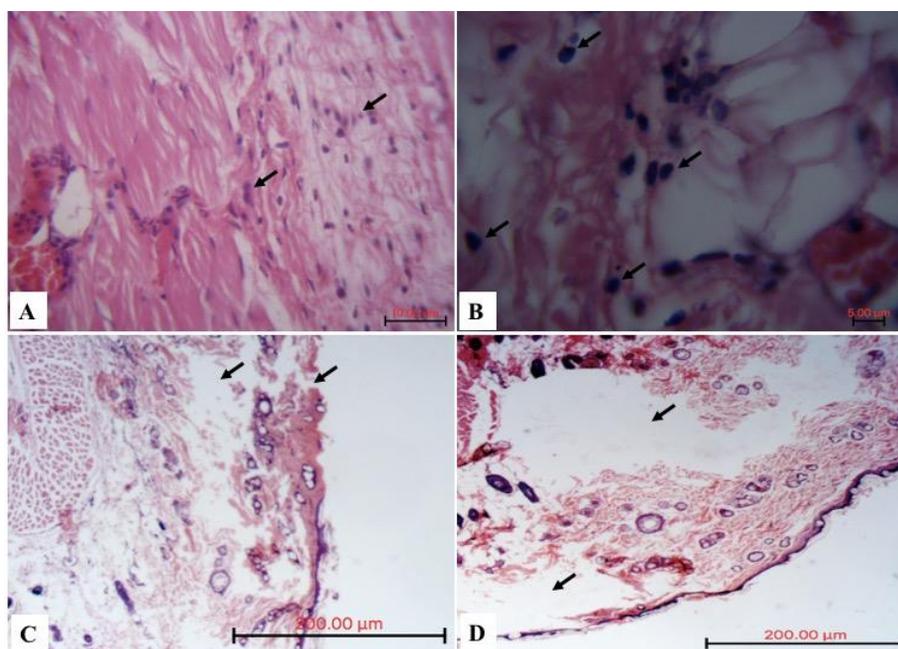


Figure 4: Histopathological changes after 180 min of antemortem and post-mortem skin wound in rats. A. In the group of antemortem skin wounds, the wound edge showed infiltration of hemorrhage (arrows) and increased the numbers of neutrophils around the wound edge (arrowheads). H&E 50µm. B. Hemorrhage at the wound edge (arrows) with the increasing numbers of neutrophils around perivascular tissue and wound margin (arrowheads) in the antemortem skin wound. H&E. 10µm. C. a small number of macrophages were observed around the blood vessels (arrows) in the antemortem skin wound. H&E. 5µm. D. In the group of post-mortem skin wounds, the histopathological examination of the unwounded rats showed moderate post-mortem autolysis with loss of the epidermal layer (arrows). H&E. 200µm. E. The post-mortem induced wounds within 180 min exhibited hemorrhage (arrowheads) and autolysis of the tissue (arrow). H&E. 200µm. F. the blood vessels were filled with hemolysis blood (arrows). H&E. 50µm.

Figure 5: Histopathological changes after 360 min of antemortem and post-mortem skin wound in rats. A. All skin layers show massive neutrophil infiltrations (arrows) in the group of antemortem skin wounds. H&E. 10µm. B. Accumulation of macrophages into the wound region (arrows). H&E. 5µm. C. In the group of post-mortem skin wounds, the histopathological examination of the unwounded rats showed severe autolysis (arrows). H&E. 200µm. D. The post-mortem-induced wounds showed bleeding, edema, and autolysis of the tissue (arrows). H&E. 200µm.



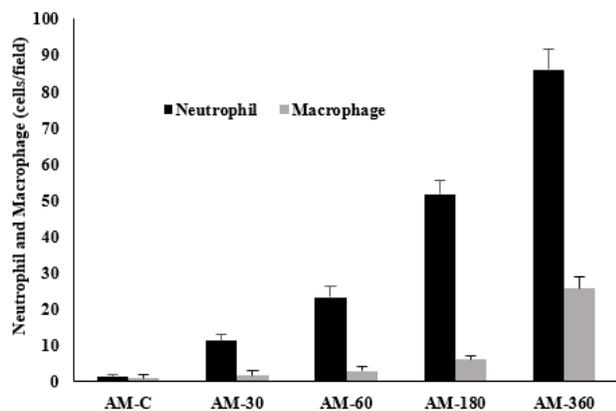


Figure 6: The number of neutrophils and macrophages over the time course of wound age. The data were collected from 3 fields/slides (n=3). The values are represented as mean \pm SD.

Discussion

Determination of wound age and vitality is one of forensic pathologists' central and crucial parts. Pathologists are continuously needed to establish how far before death a skin wound was continued. In addition, forensic pathologists are wanted to differentiate antemortem from post-mortem wounds to appropriately assess the association between wounds and death (2,24). In previous studies, it has been said that histopathological analysis cannot ascertain whether the wound was imposed during the antemortem or post-mortem, especially during the first hours (25). Our study plans to assess the histopathological changes in the experimental incisional wounds along the different periods related to antemortem or post-mortem times during the first minutes and hours. We used histopathological analysis because it gives results in a short time at a low cost and uses daily forensic practice to determine wound dates.

It has been confirmed that skin wound reactions start directly after the injury and comprise a well-organized series of actions (7,26,27). This study detected a difference in the timing of the progressive periods of wounds between antemortem or post-mortem wounds. In 30 min after inducing wounds, the vital responses were noticeable in all antemortem sections but not in any of those from the post-mortem sections. Cellular reactions, congestion, and dilatation of capillaries were present at the edge of the antemortem skin wound. This presence indicates that it is feasible to distinguish between lesions performed in the antemortem from those performed in the post-mortem, even in this short time. It has been stated that cellular reactions are an essential reaction in wound age determination (28,29). In this study, the histopathological technique aided in detecting the migration of leukocytes toward the blood vessel walls in the antemortem group, which can appear within 30 minutes

after injury and would be an easy method to compare wound age between antemortem and post-mortem.

Many studies have informed neutrophils infiltrate the wound site within the inflammatory phase (30-32). In this work, after 60 min of inducing the wound, we detected an increase in the number of neutrophils with marginalized at the inner surface of blood vessels and cells migration outside the blood vessels across the tissue in the antemortem wound. However, no infiltration of neutrophils in the post-mortem wound was observed. Nevertheless, at this time of post-mortem wound, autolysis of the tissue began to start, while in 30 min post-mortem wound showed a regular aspect of tissue.

Our results in the antemortem group with infliction times in 180 minutes showed that the number of neutrophils suddenly increased. In contrast, a small number of macrophages were observed in the wound region. The proportion among neutrophils and macrophages in the wound area can be used to determine wound age (33,34), and consistent with Barrington (35), macrophages appear after 10-12 hours of survival time. However, we detected the first macrophages earliest in an antemortem wound aged three h. The correlation between the number of neutrophils and macrophages may allow the differentiation of antemortem wounds during the early few hours. At the same time, in the post-mortem group, degeneration and autolysis of dermal extensions it was not likely to identify them in the previous group.

After 360 min of inducing the wound in the antemortem group, the neutrophils were seen in all the layers (dermis, subcutis, and muscle tissue). These findings follow the previous experiments in pigs and mice (36,37). Furthermore, at this time, there were increased numbers of macrophages in the wound region. Previously, the macrophages were noticed at 7 hours of the wound injures (38,39). Simultaneously, the histopathological picture of post-mortem skin sections showed severe degeneration and autolysis of all the tissue. This is in agreement with the results reported by others (40,41).

Wound age determination is an exciting field in forensic medicine and can give to the renovation of crime views. Therefore, the histopathological analysis became a motivating method to evaluate the wound age by giving results in a short period at a low cost. Ultimately, while morphological analysis of the wounds offers a poor assessment of their time, the histopathological analysis will offer a precise timing of their incidence, consequently supporting the direction of justice.

Conclusion

Overall, histopathological studies are the significant importance for determining the age and vitality of wounds. Wound age evaluation should be based on the ratio and distribution of neutrophils and macrophages and the

presence of congestion and degree of autolysis. In the antemortem wound, the number of neutrophils and macrophages around the blood vessels and in the tissue is a vital sign of the timing of the vital wound. Moreover, this work verified that the histopathology analysis of the post-mortem wound could be used to assess the time of wounds by testing the degree of autolysis.

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Declaration of conflicting interests

The author(s) declared no potential conflicts of interest concerning this article's research, authorship, and/or publication.

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التغيرات النسيجية المرضية كأدوات للتمييز بين الجروح قبل الموت وبعد الموت في الجرذان: التطبيقات المحتملة في الطب العدلي

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الخلاصة

بعد تقدير عمر الجرح أحد الموضوعات الرئيسية المثيرة في الطب العدلي. من المهم تحديد الدقة الأكثر احتمالية عند حدوث الجروح وما إذا كان في الفترة ما قبل الموت أو بعد الموت. يمكن أن تكون التغيرات النسيجية طريقة للمساعدة كمعامل متاح وسهل لتحديد فترة ما قبل الموت وبعد الموت. كان الهدف من هذه الدراسة هو ملاحظة التغيرات النسيجية المرضية في الجرح المستحدث والقدرة على تحديد الوقت الدقيق الذي حدثت فيه الجروح وما إذا كان قبل الموت أو بعد الموت. تمت دراسة ٣٩ خزعة جلدية من جلد الجرذان. تم أخذ جميع العينات في مجموعات ما قبل الموت في أعمار ٣٠ و ٦٠ و ١٨٠ و ٣٦٠ دقيقة وفي مجموعات ما بعد الموت في غضون ٣٠ و ٦٠ و ١٨٠ و ٣٦٠ دقيقة مع عينات سيطرة (مجموعة غير مجروحة). تم فحص أقسام الجلد بواسطة المجهر لملاحظة التغيرات في المعايير التالية: نسبة وتوزيع العدلات والخلايا البلعمية، الاحتقان وتوسع الشعيرات الدموية، درجة التحلل الذاتي. بالنسبة للجروح قبل الموت في جميع الفترات الزمنية التي تم فحصها، تم عرض عدد العدلات والخلايا البلعمية في الجروح. كان أول ظهور للعدلات عند ٣٠ دقيقة وزاد بشكل حاد بعد ٦٠ دقيقة بعد الجرح. بالإضافة إلى ذلك، كانت هناك زيادة في الخلايا البلعمية من ١٨٠ دقيقة بعد الجرح. بالنسبة لجروح ما بعد الموت في جميع الأوقات التي تم فحصها، كانت درجة الانحلال الذاتي هي أفضل المعايير لمعرفة توقيت الجروح. تقترح هذه النتائج أنه يمكن استخدام التغيرات النسيجية المرضية كمعايير مهمة لمعرفة توقيت الجروح والمقارنة بين الجروح ما قبل الموت وبعد الموت في الطب العدلي.