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MORPHO-HISTOLOGICAL CHARACTERISTICS OF BRUISES WITH DIFFERENT AGE - qualitative study

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Abstract

Bruises are common skin lesions that occur as the force hits the skin, but integrity of the skin is not impaired. Their significance is important in forensic expert reports in determining the time of death in many cases of domestic violence or child abuse. The aim of this study was to present the differences between bruises with different time of origin by evaluation of their morpho-histological characteristics.

The study included 60 human skin samples divided into control and experimental groups A, B, C, D. The experimental group A included bruised human skin samples with <1day old bruises, experimental group B included bruised human skin samples with 1-3 days old bruises, experimental group C included bruised human skin samples with 3-7 days old bruises and experimental group D included bruised human skin samples with 7-14 days old bruises. Paraffin sections of the skin were stained with Hematoxylin-eosin, Giemsa, Perl's Prussian Blue, Masson-Goldner methods of staining and application of anti-HO-1 antibody. All paraffin sections were evaluated by light microscopy and photodocumented.

Our results have shown changes in color in bruises with different age due to infiltration with different immune cells and metabolism of extracellular hemoglobin. Granulocyte infiltration is seen shortly after the initiation of the bruise, while after few days of initiation, usually 1-3, there is macrophage infiltration in the field of bleeding. Presence of macrophages in the field of bleeding increases as bruises age. These histological characteristics appear in direction of healing the bruise, together with phagocytosis of the erythrocytes and removing the tissue debris.

Keywords: bruises, time of origin, morpho-histological characteristics, microscopic analysis.

Introduction

Bruises are injuries caused by rupture of small blood vessels in the skin, which lead to its discoloration, but the skin has preserved integrity. Due to the damage of small blood vessels, the blood flows into the connective tissue of the skin and macroscopically causes a change in the color of the skin. Bruises can also occur as a result of injury of deeper tissues, such as muscles or broken bones. This often happens in traffic accidents. In these cases, bruises are located deeper, in the soft tissues under the skin. Deep bruises may not present on the skin and deep tissue incisions should be made at autopsy to prove them [1].

In the legal proceedings when it comes to cases where a violent death occurred and a forensic medical examination is required, when interpreting bruises, great attention is paid to the description of these injuries because they may correspond to the reasons by which the injury occurred [2].

When interpreting bruises, sometimes another assessment should be made, after about 24 hours, especially if the assessment is made immediately after the occurrence of the injury,

if bruises are analyzed ante-mortem or in living individuals. Attention is paid to distinguishing bruises from livor mortis and a detailed description of bruises is required [3].

In forensics, bruises are particularly significant injuries because they can lead to death if they are extensive [4,5,6]. There is a difference between bruises that occur during life and changes that occur after death. In bruised tissue, there is edema in the field of hemorrhage, discoloration of the skin and coagulation of extravasated blood in the subcutaneous tissue. These signs are not present if the change occurred post-mortem. The term bruise, as an injury, implies a morpho-functional disorder of the integrity of the tissue. Lifelong injury is characterized by onset of series of vital reactions that do not occur in case of a post-mortem injury. Because vital reactions occur in a certain chronological order and dynamics, they allow the process by which an injury is healed to determine the time at which the injury occurred [6].

Material and methods

Our study included 60 human skin samples with bruises and 60 human skin samples with normal structure. The material for our study were bruises that were subject to forensic treatment in persons who had died of violent death. Tissue samples with dimensions of 2 cm^2, according to the code of ethics for working with human material were taken during routine autopsies at the Institute of Forensic Medicine and Criminology in Skopje. The material was grouped in 4 experimental groups and one control group. Experimental group A included bruised human skin samples with <1day old bruises; experimental group B included bruised human skin samples with 1-3 days old bruises; experimental group C included bruised human skin samples with 3-7 days old bruises; and experimental group D included bruised human skin samples with 7-14 days old bruises. Group E included 60 samples of intact normal skin, located near bruised tissue. Paraffin sections of the skin were stained with Hematoxylin-eosin, Giemsa, Perl's Prussian Blue, Masson-Goldner methods of staining and application of anti-HO-1 antibody. All paraffin sections were evaluated by light microscopy, with photodocumentation of macroscopic and microscopic analyses.

Selection of the experimental material:

- Inclusion criteria:
  - age of deceased persons 18-65 years
  - persons who had died of violent death.
- Exclusion criteria:
  - persons who had died of natural or violent death for whom there was a history of diseases that caused changes in coagulation and bleeding (in certain diseases of the cardiovascular and digestive systems);
  - persons who had died from burns (due to damage to the epidermis and dermis of the skin by thermal injury);
  - persons who had been dead for more than 48 hours ago (autodigestation and putrefactive processes);
  - persons under 18 years of age (due to the characteristics of the fragility of the skin as the body grows).

Results

Macroscopic analysis

By initial macroscopic analysis of the material, data about dimensions, location on the body, shape and color of the bruises were obtained. The dimensions ranged from a few millimeters to several centimeters, and there were cases of extensive bruises. According to the code of ethics for experimental studies with human material, a small sample of less than 2 cm^2 was taken from the extensive bruises. Most often, an individual had multiple bruises, sometimes diffuse, in different regions of the body. In rare cases, we encountered isolated bruises. In some samples of the examined bruises the shape was oval, while some had an irregular, undefined shape (Table 1). In the experimental group A, fresh bruises had a reddish-purple color. They were vaguely limited to the surrounding healthy tissue and showed a mild edema on the surface of the skin (Fig. 3).

In the central and peripheral part of the bruise, the color was not uniform due to the effect of gravity on the bloodflow. The surface of the skin was intact, with a preserved morphological structure. Bruises in the experimental group B had purple color. Their edges were already clearly defined by the surrounding healthy tissue. In these bruises, a tint of violet discoloration was observed, which corresponded to hemoglobin metabolism in the field of bleeding (Fig. 5).

The surface of the skin was intact, with a preserved morphological structure. The color of the bruises was clearly visible through the epidermis. Bruises in the experimental group C were severely limited by the surrounding healthy tissue. The skin on the surface did not show edema. Its surface had a preserved morphological structure. Subcutaneous discoloration of the underlying connective tissue was seen throughout the epidermis. Bruises 3-7 days old were light purple in color, with a slight gradation of color from the periphery to the center of the bruise due to hemoglobin metabolism (Fig. 7).

Bruises in the experimental group D were strictly limited by the surrounding healthy tissue. Their color was brownish-yellowish, with a color gradation from the periphery to the center of the bruise.

The edema was absorbed and the bruises were at the level of the skin surface. Figure 9 shows two bruises, the lower one, with brown-yellow color refers to this group, while the upper bruise with purple color refers to the previous experimental group, C.

The surface of the skin was intact, the color of the bruise illuminated through it. In the control group E, healthy, intact skin around the bruise had normal morphological structure. Its surface was smooth, without edema, without discoloration, and without macroscopically visible lacerations. Depending on the region in which the analyzed bruises were located, healthy skin was covered with hair or had more pronounced pigmentation.

Microscopic analysis

Microscopic analysis was performed qualitatively. This analysis provided data on the location of bruises in the skin layers as well as chronological insight into their reparative cell infiltration. According to the location, most often bruises were observed in the dermis and hypodermis of the skin (Figs. 1,2).

In one case, location of the bruise was observed in the epidermis itself, with involvement of the dermis, but the surface of the epidermis was intact. Microscopic analysis of fresh bruises, group A, showed that epidermis of the skin had a preserved morphological structure. Figure 12 clearly shows all the layers with their structure. Enlargement of fibrous septa was seen as a result of edema and extravasated erythrocytes during bleeding. The presence of fibrin was also noted in the field of bleeding. In this experimental group infiltration with neutrophil granulocytes was noticed (Fig. 4).

Neutrophil infiltration was seen throughout the field of bleeding. Microscopic analysis in bruises 1-3 days old, experimental group B, showed extravasated erythrocytes in the bleeding field and infiltration with rare macrophages (Fig. 6).

The presence of fibrin was observed in the bleeding field. Fibrous septa in the skin were still dilated as a result of extravasated erythrocyte cells and macrophages (Fig. 8).

Macrophage infiltration was microscopically seen throughout the bleeding field, but it was not particularly pronounced. Neutrophil infiltration in this group was not found. In the experimental group C, epidermis had a preserved morphology. There was moderate infiltration of the macrophage in the field of bleeding, as well as the absence of neutrophil granulocytes. The presence of fibrin was also noted. Infiltration with macrophages using immunostaining with anti-HO-1 antibody was confirmed (Fig. 8).

Macrophage infiltration was observed throughout the bleeding field, whereas neutrophil infiltration was not observed in this group. Fibrous septa were dilated due to profuse erythrocyte extravasation and moderate macrophage infiltration. In the experimental group D, epidermis of the skin had also preserved morphology, with the presence of all structural layers in its structure, which was identical with the other experimental groups. Histological analysis showed intense infiltration in the field of bleeding with macrophages, as well as the presence of hemosiderin granules and tissue debris (Fig. 10).

Macrophages actively produced the enzyme heme oxygenase 1, shown by immunostaining with anti-heme oxygenase 1 antibody, presenting a strong brown signal in these cells (Figs. 6,8,10,11).

Fibrous septa were still dilated due to abundant infiltration by erythrocytes and macrophages in the field of bleeding. Histological analysis of healthy skin, control group E, showed a normal morphological structure.

The epidermis was intact, represented by all the structural layers of cells. There was presence of cutaneous adnexa (hair follicles, sweat and sebaceous glands) and intact small blood vessels in the dermis. In the predominantly adipose tissue of the hypodermis, normal intact vascularization with the presence of larger blood vessels was observed.

Figures 12 and 13 show the structural parts of the skin as well as the blood vessels in it. Figure 13 also shows ruptured blood vessel in the fibrous septa of the skin.

Table 1. Summarized data for macroscopic analysis of all examined groups.

	Group A Bruises not older than 24 hours	Group B Bruises 1-3 days old	Group C Bruises 3-7 days old	Group D Bruises 7-14 days old	Group D Control group
Age of deceased	18-65	18-64	18-68	18-65	18-68
Dimensions of bruises	13-19 mm	10-17 mm	15-19 mm	15-20 mm	/
Shape of bruises	Irregular oval, sharply defined	Irregular oval, well defined	Irregular oval, well defined	Irregular oval, sharply defined	/
Location in the body	Upper limbs torso and neck	Upper and lower limbs and torso	Upper and lower limbs and neck	Upper and lower limbs and neck	Healthy skin in the same area as the bruise
Color of bruises	Reddish-pink	Dark violet	Light purple, shades of purple	Yellow-green	Normal skin color

Microphotographs

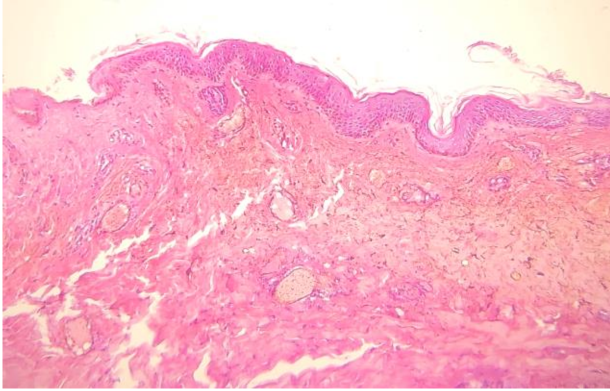

Figure 1. Bruise localized in the dermis of the skin
Hematoxylin-eosin staining, magnification x 4.

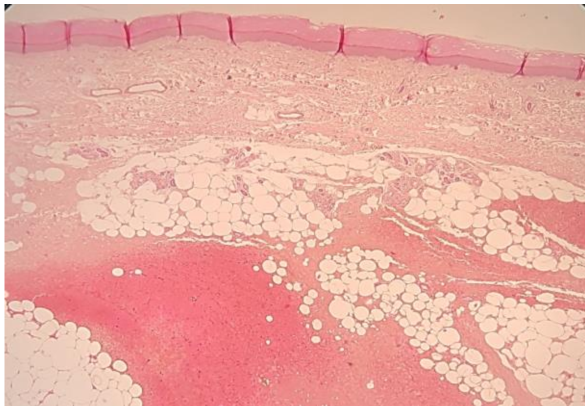

Figure 2. Bruise localized in the hypodermis of the skin
Hematoxylin-eosin staining, magnification x 4.

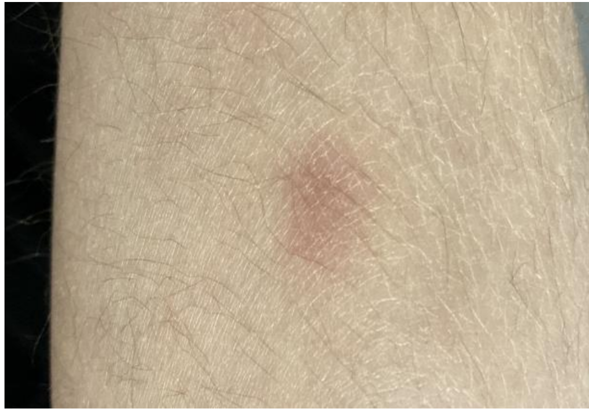

Figure 3 - Macroscopic appearance of a bruise not older than 24 hours
(pink-reddish pigmentation of the bruise).

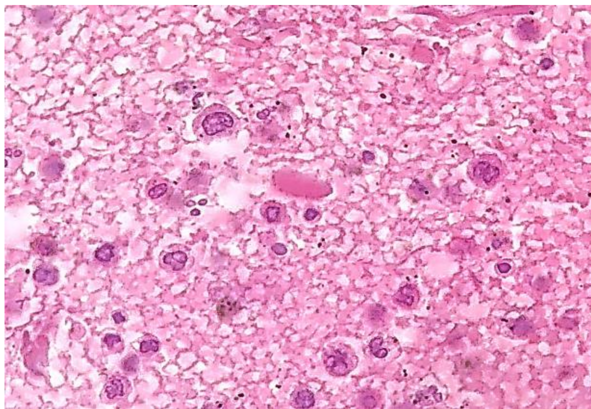

Figure 4. Microscopic findings in bruises not older than 24 hours, late stage of formation,
(presence of a large number of extravasated erythrocytes and neutrophil granulocyte infiltration)
Giemsa staining, magnification x 100.

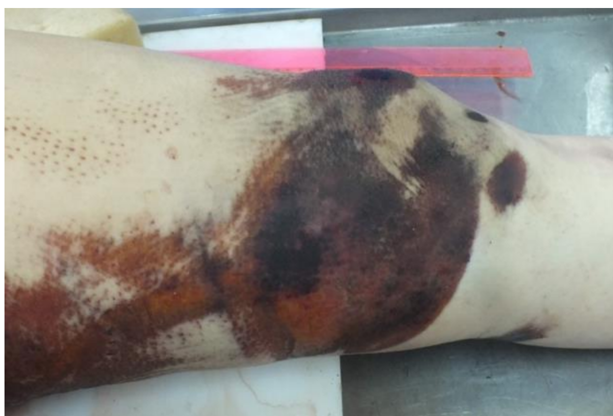

Figure 5. Macroscopic appearance of a bruise 1-3 days old
(intensive violet-black pigmentation of the bruise**).**

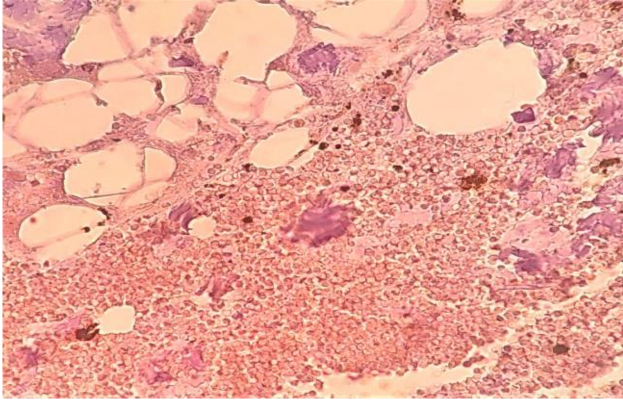

Figure 6. Presence of macrophages in bruise 1-3 days old Anti-HO-1 antibody, magnification x 40.

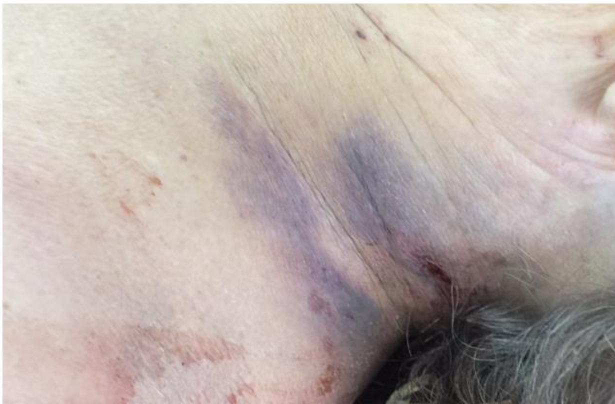

Figure 7. Macroscopic appearance of a bruise 3-7 days old (light to dark violet pigmentation of the bruise).

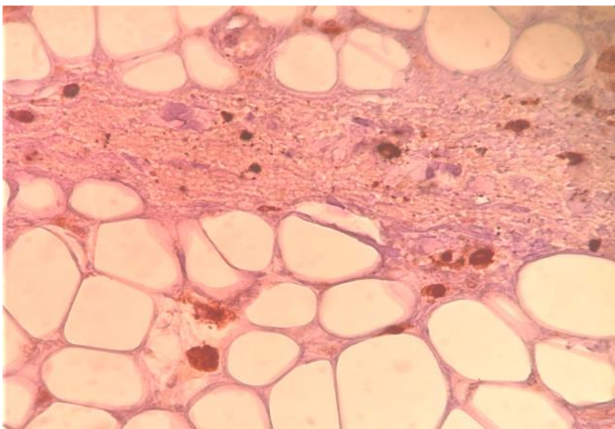

Figure 8. Microscopic findings in bruises 3-7 days old (presence of mononuclear cells in the field of bleeding) Anti-HO-1 antibody, magnification x 100.

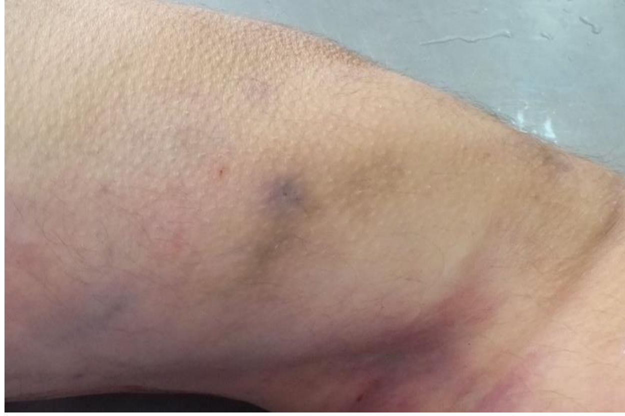

Figure 9. Macroscopic appearance of bruise 7-14 days old
(brownyellow pigmentation of the bruise, with blurred edges towards the surrounding healthy tissue)
NB - also there is a bruise with purple pigmentation, aged 3-7 days.

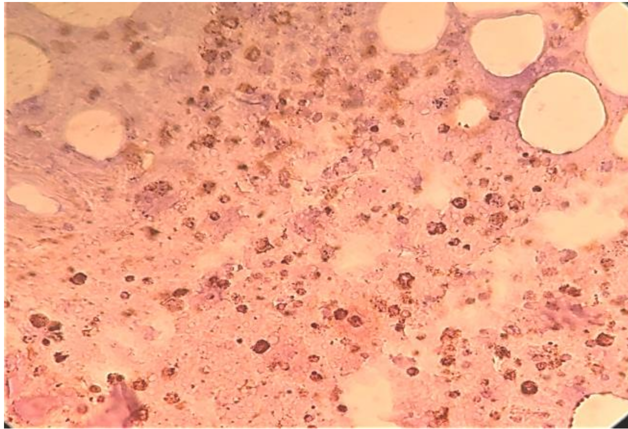

Figure 10. Microscopic findings in bruises aged 7-
14 days (remarkable presence of macrophages in the field of bleeding)
Anti-HO-1 antibody, magnification x 40.

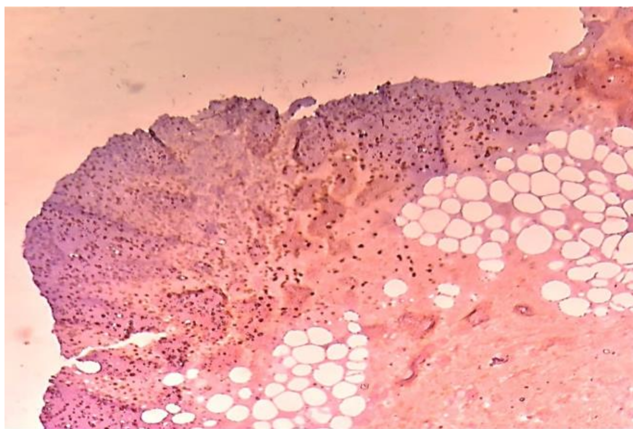

Figure 11. Bruise localized in the epidermis and dermis of the skin
Anti-HO-1 antibody, magnification x 4.

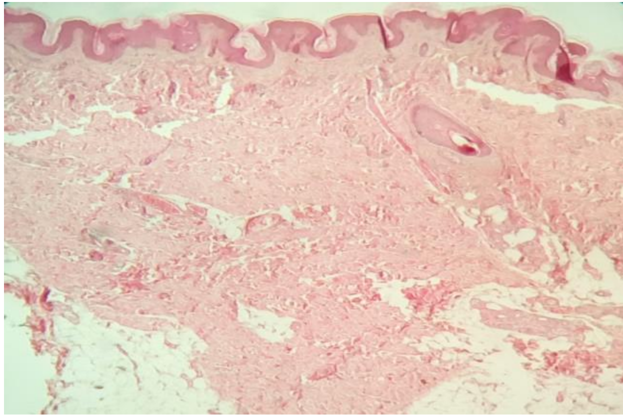

Figure 12. Microscopic appearance of healthy skin
Hematoxylin-eosin staining, magnification x 4.

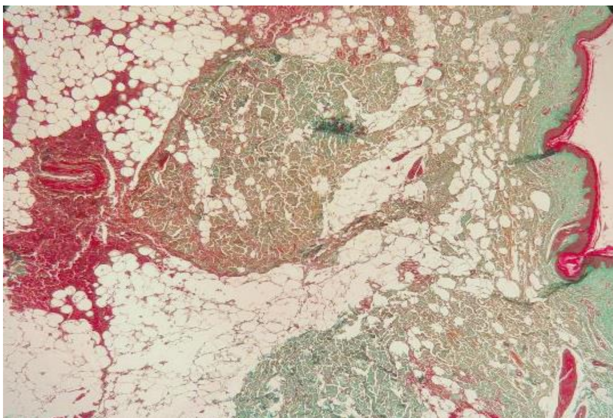

Figure 13. Intact epidermis of the skin in the control group (right), ruptured blood vessel in the adipose tissue of the skin (left) Masson trichrome green staining, magnification x 20.

Discussion

Bruise formed in the tissue changes skin color. Macroscopic assessment of bruises demonstrates the presence of hemoglobin near the surface of the skin shown in red color, but if the hemoglobin is deeper in the tissue, it will be shown in blue. This effect is related to the Rayleigh's bursting phenomenon, the skin absorption coefficient, and the visual interpretation system [7, 8, 9,10].

The initial appearance of bruises is associated with the appearance of extravasated blood in the skin. Bruise becomes visible within 15 to 20 minutes of injury at the earliest. According to published data, the initial color of the bruise depends on the location of the extravasated blood in the skin [9].

Red, blue, purple, dark purple, or green can be seen, but these colors are not reliable indicators of the age of the bruise. The change in color may be due to a change in the position of the blood relative to the surface of the skin and may be enhanced by the hemoglobin released during the conversion of oxyhemoglobin to deoxyhemoglobin [11].

In the experimental group A, where fresh bruises were analyzed, not older than 24 hours, macroscopically red discoloration was observed. In group B, when bruises aged 1 to 3 days were assessed, skin discoloration ranged from dark red to purple.

In the experimental group C, the analysis of bruises aged 3 to 7 days, showed bruises colored violet-purple, while in the last observed group D, bruises aged 7 to 14 days color of bruises showed shades of brown to yellow.

According to the hemoglobin metabolism and the change in the color of the bruise, it has been concluded that only the appearance of yellow color in the bruise can give information about its age, when observation is used as a simple method for determining the age of bruises [12].

Stephenson in his paper presents 5 schemes by different authors to interpret the age of bruises, according to their color [13].

According to him, initial color of bruises is almost always red, unless the bruise is deep in the skin and then it is purple or blue. Yellow color cannot be seen until 18 hours after the formation of bruise. Usually after one week, the yellow color is noticed which disappears at the end of the second week. When interpreting the age of bruises, the anamnestic data are especially important, i.e., the time of occurrence of the injury, which is different from the time of the appearance of the bruises [14].

Extravasated blood into the connective tissue of the skin causes an inflammatory reaction [15] and this reaction may be exacerbated by tissue damage from blunt injury [16,17].

As a result of the activation of the inflammatory response, a process of cell infiltration begins in the field of bleeding, in addition to numerous biochemical processes. Polymorphonuclear cells, neutrophils, are the first cells to infiltrate the field of bleeding, but they are not able to metabolize hemoglobin [16].

This is followed by infiltration with other cells of the inflammatory response, macrophages, mononuclear cells that can phagocytose erythrocytes because they contain the enzyme heme oxygenase (HO), which enables the first step in hemoglobin metabolism.

This enzyme converts hemoglobin to biliverdin. Biliverdin is a green pigment and is soon converted to bilirubin, a yellow pigment mediated by the enzyme biliverdin reductase (BVR). BVR is an enzyme found in all tissues under normal conditions, but it is particularly found in reticulocytes and macrophages of the liver and spleen [18,19].

In the experimental group A, where fresh bruises were analyzed, not older than 24 hours, histological analysis showed presence of neutrophils in the field of bleeding. In the experimental group B, when bruises aged 1 to 3 days were assessed, the field of bleeding was infiltrated with rare macrophages, while in the next two experimental groups C and D, with bruises aged 3-7 and 7-14 days respectively, infiltration with macrophages was more pronounced. Infiltration with macrophages in the field of bleeding was most pronounced in the experimental group D.

The production of bilirubin and hemosiderin at the site of the bruise, which gives its color, takes time for macrophage influx, HO enzyme induction, and hemoglobin metabolism [20].

The development of yellow color in the field of bleeding is due to the local production of bilirubin which can be demonstrated in elevated serum concentrations [21].

There is a significant difference in the average time of onset of yellow color in people under 65 years of age [22].

Literature data from experimental animal studies indicate that bruises heal faster in younger individuals and that macrophage function is impaired in older individuals [23,24].

Conclusion

The formation of a bruise causes an inflammatory reaction in the tissue and initiates a process of reparation. Skin changes its color because of the hemoglobin metabolism when it is extracellular found.

Microscopic analysis showed the presence of neutrophils in fresh bruises, while in br uises older than 3 days there were macrophages in the field of bleeding, the presence of which was more pronounced in older bruises, 7-14 days.

All these findings make it possible to determine the approximate age of the bruises. F or more accurate determination of their age, quantitative histological analysis of the cell infiltr ate in the field of bleeding is required.

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