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Original article

MORPHOHISTOLOGICAL CHARACTERISTICS OF BRUISES WITH DIFFERENT AGE - QUANTITATIVE STUDY

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Abstract

Bruises as common skin lesions are 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 histological characteristics.

The study included 60 human skin samples divided into control and experimental groups A, B, C, D. Group A included skin samples with < 1day old bruises, group B included skin samples with 1-3 days old bruises, group C included skin samples with 3-7 days old bruises, and group D included skin samples with 7-14 days old bruises. Paraffin sections of the skin stained with Hematoxylin-eosin, Giemsa, Perl's Prussian Blue and Masson-Goldner methods were evaluated by light microscopy and photodocumented,using stereological method for cell count and photodocumented.

Histological analysis in group A showed presence of dilated fibrous septa in dermis and hypodermis with extravasated erythrocytes and intense infiltration with neutrophils. In group B there was initial infiltration by macrophages. Group C also showed presence of macrophages, but it was significantly more expressed than in group B ($p<0.001$). Group D showed a significant presence of macrophages when compared to groups B and C ($p<0.001$).

Infiltration with neutrophils occurred shortly after the initiation of the bruise, while after few days of initiation, usually 1-3, there was macrophage infiltration. Presence of macrophages significantly increased as bruises age. These histological characteristics speak in favor of healing the bruises.

Keywords: age of bruises, histological characteristics, microscopic analysis

Introduction

Bruises are the most common injuries that occur since the early childhood. There are numerous studies in the literature that describe bruises seen in different age groups of people - babies, children, adults and the elderly[1].

These injuries are caused by rupture of small blood vessels in the skin, which lead to its discoloration, but the epidermis of 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. Deep bruises may not present on the skin and deep tissue incisions should be made at autopsy to prove them [2]. In the legal proceedings when it comes to cases where a violent death occurred and a forensic medical examination is required, great attention is paid to the description of these injuries because they may correspond to the causes by which injury occurred. Also, attention is paid to distinguishing bruises from livor mortis and a detailed description of bruises is required[3,4]. In forensics, bruises are particularly significant injuries because they can lead to death if they are extensive[5-7]. 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 morphofunctional 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[7].

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 for our analysis, 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 assigned into 5 experimental groups. 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, localized near bruised tissue. Paraffin sections of the skin were stained with Hematoxylin-eosin, Giemsa, Perl's Prussian Blue, Masson-Goldner, Elastica van Gieson methods of staining and application of anti-HO-1 antibody. All paraffin sections were evaluated by light microscopy and photodocumented. For cell count we used stereological method with M42 test system to determine numeric cell`s density [18], on Olympus CX21 microscope with magnification x40.

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 (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

Microscopic analysis of the bruises in this study provided data on the chronological insight on their reparative cell infiltration. Infiltration with different cell types, neutrophils and macrophages into the field of bleeding at different time intervals was noticed (Table 1).

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. Additional staining with anti-HO-1 antibody showed the activity of macrophages in the field of bleeding through the production of the enzyme heme oxygenase 1 (Figure 8).

Table 1. Descriptive statistics – Macrophages and neutrophils density in the bruises in A, B, C, D experimental groups

		Minimum	Maximum	Mean value ($\bar{x}$)	Standard Deviation (Σ)
Macrophages	Group A	0.28	0.35	0.3100	0.01886
	Group B	2.03	2.93	2.6550	0.25596
	Group C	7.02	7.95	7.6320	0.30929
	Group D	13.50	14.10	13.7760	0.22157
Neutrophils	Group A	12.71	13.60	13.0545	0.3176
	Group B	/	/	/	/
	Group C	/	/	/	/
	Group D	/	/	/	/

Table 2. ANOVA - numerical density of macrophages and numerical density of neutrophils

		Sum of Squares	df	Mean Square	F	Sig
Macrophages	Between groups	1066.599	3	355.533	6751.876	<.001
	Within groups	1.896	36	.053		
	Total	1068.495	39			
Neutrophils	Between groups	1338.672	3	446.224	6572.858	<.001
	Within groups	2.444	36	.068		
	Total	1341.116	39			

Table 3. Multiple comparison with Tukey HSD – density of macrophages and density of neutrophils in the experimental groups A, B, C and D

Dependent variable	(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound, Upper Bound	
Macrophages	Group A	Group B	-2.34500*	.10262	<.001	-2.6214	-2.0686
		Group C	-7.32200*	.10262	<.001	-7.5984	-7.0456
		Group D	-13.46600*	.10262	<.001	-13.7424	-13.1896
	Group B	Group A	2.34500*	.10262	<.001	2.0686	2.6214
		Group C	-4.97700*	.10262	<.001	-5.2534	-4.7006
		Group D	-11.12100*	.10262	<.001	-11.3974	-10.8446
	Group C	Group A	7.32200*	.10262	<.001	7.0456	7.5984
		Group B	4.97700*	.10262	<.001	4.7006	5.2534
		Group D	-6.14400*	.10262	<.001	-6.4204	-5.8676
	Group D	Group A	13.46600*	.10262	<.001	13.1896	13.7424
		Group B	11.12100*	.10262	<.001	10.8446	11.3974
		Group C	6.14400*	.10262	<.001	5.8676	6.4204
Neutrophils	Group A	Group B	13.36000*	.11652	<.001	13.0462	13.6738
		Group C	13.36000*	.11652	<.001	13.0462	13.6738
		Group D	13.36000*	.11652	<.001	13.0462	13.6738
	Group B	Group A	-13.36000*	.11652	<.001	-13.6738	-13.0462
		Group C	.00000	.11652	1.000	-.3138	.3138
		Group D	.00000	.11652	1.000	-.3138	.3138
	Group C	Group A	-13.36000*	.11652	<.001	-13.6738	-13.0462
		Group B	.00000	.11652	1.000	-.3138	.3138
		Group D	.00000	.11652	1.000	-.3138	.3138
	Group D	Group A	-13.36000*	.11652	<.001	-13.6738	-13.0462
		Group B	.00000	.11652	1.000	-.3138	.3138
		Group C	.00000	.11652	1.000	-.3138	.3138

Infiltration with neutrophil granulocytes was first observed. This finding was characteristic for fresh bruises, not older than 24 hours, group A, $\bar{x}$=13.0545 (Figure 3). In the experimental group B, in bruises aged 1 to 3 days, macrophage infiltration was seen throughout the field of bleeding, but it was not particularly pronounced, $\bar{x}$=2.6550. Neutrophil infiltration in this group was not found (Figure 4). Macrophage density in the field of bleeding progressed significantly in the next experimental group C - bruises aged 3 to 7 days, $\bar{x}$= 7.6320, when compared to group B, $p<0.0001$ (Figures 4,5). The presence of macrophages in group D, bruises aged 7 to 14 days, was significantly increased, with $\bar{x}$=13.7760 and $p<0.0001$, when compared to the presence of macrophages in group B and group C (Figures 4,5,6). In the control group E, normal skin, there was no infiltration with inflammatory cells in all the layers of the skin, which was with normal morphological structure (Figure 7). The statistical analyses demonstrated a strong positive correlation between the age of bruises and macrophage infiltration (Figure 2).

The results of the one-way ANOVA and Tukey test (Tables 2,3) showed a significant difference between the means in all four experimental groups for the parameters numerical density of macrophages ($p <0.001$). Numerical density of neutrophils showed a significant value of p ($p <0.001$) only between groups A and B.

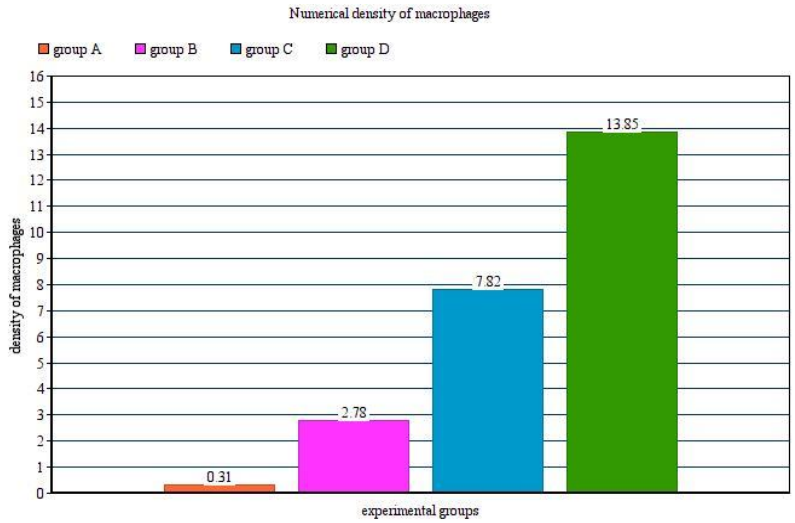

Fig. 1. Numerical density of macrophages in A, B, C, D experimental groups

Figure 1 shows numerical density of macrophages in the experimental groups A, B, C and D. In all four experimental groups, the presence of macrophages was observed. Numerical density of macrophages increases as the age of the bruises progresses, i.e., the lowest numerical density was observed in group A, in bruises not older than 24 hours, while the highest numerical density was observed in group G, in bruises aged 7-14 days. Figure 2 shows the correlation of the age of bruises and their infiltration with macrophages.

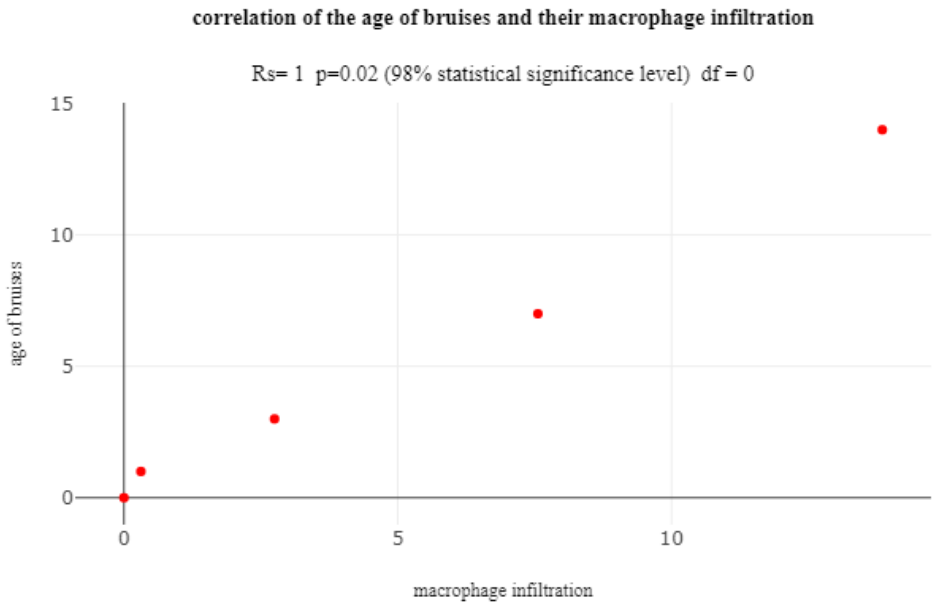

Fig. 2. Correlation of age of bruises and macrophage infiltration

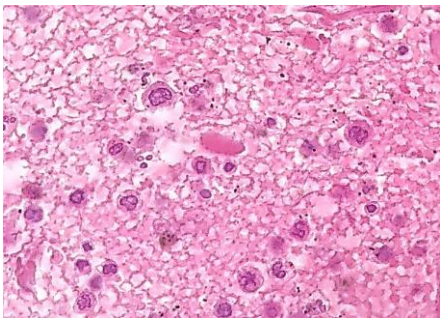

Fig. 3. Microscopic findings in bruises not older than 24 hours (presence of a large number of extravasated erythrocytes and neutrophil granulocyte infiltration); Giemsa staining, magnification x 100

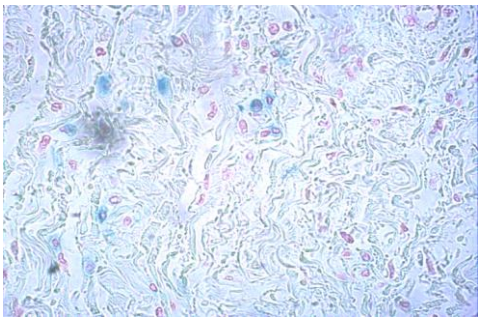

Fig. 4. Microscopic finding in a bruise 1-3 days old (presence of blue-colored macrophages in the field of bleeding); Perl's Prussian Blue staining, magnification x 40

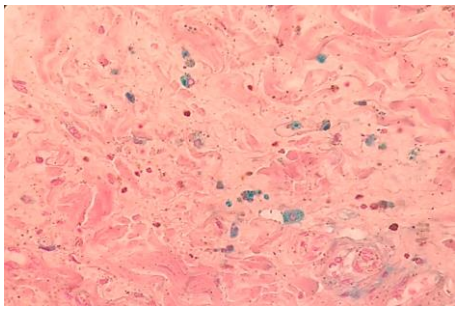

Fig. 5. Microscopic findings in bruises aged 3 to 7 days (moderate presence of mononuclear cells in the field of bleeding); Perl's Prussian Blue staining, magnification x 40

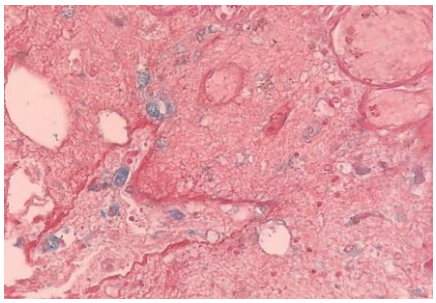

Fig. 6. Microscopic findings in bruises aged 7-14 days (remarkable presence of macrophages in the field of bleeding); Perl's Prussian Blue staining, magnification x 100

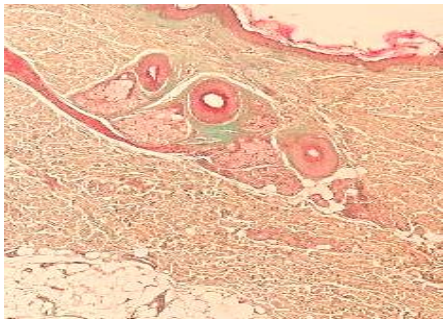

Fig. 7. Microscopic structure of healthy skin; Masson-Goldner, magnification x 4

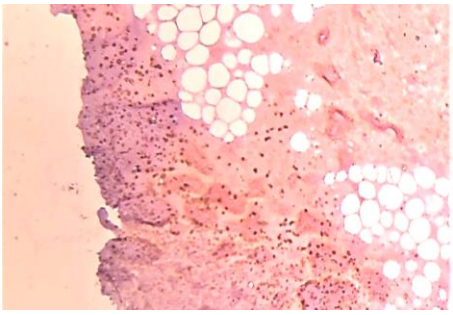

Fig. 8. Microscopic structure of bruised skin; Anti-HO-1, magnification x 4

Discussion

In our study we analyzed 60 bruised tissue samples with different age, divided into 4 experimental groups.

When bruise is formed, extravasated blood into the connective tissue of the skin causes an inflammatory reaction and this reaction may be stressed by tissue damage from blunt injury[8-10]. As a result of the activation of inflammatory response, a process of cell infiltration begins in the field of bleeding, in addition to numerous biochemical processes. Polymorphonuclear cells, neutrophils, are first cells to infiltrate the field of bleeding, but soon they will be replaced with other cells because they are not able to metabolize hemoglobin from extravasated red blood cells[11,12]. This is followed by infiltration with other cells of the inflammatory response, macrophages, mononuclear cells that can phagocyte erythrocytes and metabolize hemoglobin because they contain the enzyme heme oxygenase (HO), which enables the first step in hemoglobin metabolism [13]. Macrophages are essential for wound healing[14]. They replace neutrophils as dominant cells in injury no later than two days after injury. In our study, we concluded that first-line cells that infiltrated the bruise were neutrophils, found in fresh bruises not older than 24 hours, experimental group A. In experimental group B, bruises aged 1-3 days, initial influx of macrophages in the bruised tissue was noticed. The presence of macrophages was more pronounced as the age of bruises increased, in experimental group C, 3-7 days old bruises and experimental group D, 7-14 days old bruises, i.e., we found statistically significant values for numerical density of macrophages in the experimental groups B, C and D, when compared (Table 2).

Heme oxigenase (HO) enzyme has a potential role in modulating the immune response[15]. The form present in macrophages is HO-1 and is usually found in small amounts. The amount of HO-1 enzyme in macrophages is increased by erythrocyte phagocytosis [13,15,16] or by exposure of macrophages to hemoglobin alone[11]. In HO-1-positive cells, which correspond to the macrophage in the field of bleeding, enzyme activity can be detected as early as 3 hours after bruise formation, but peak expression is after 3 days [10]. Nakajima T *et al*. in their paper on subcutaneous hemorrhages analyzed HO-1 enzyme expression and macrophage infiltration. They concluded that determining HO-1 expression could provide useful information in determining the age of bruises [17]. We obtained similar findings in our study. In the second examined group, B, in bruises aged 1 to 3 days, analyzed macrophages showed enzyme production and gave solid brown signal during staining. The same brown signal in macrophages was noted in groups C and D, in bruises 3 to 7 days old and 7 to 14 days old, respectively.

Conclusion

The formation of a bruise causes an inflammatory reaction in the tissue and initiates a process of reparation. According to the cells that infiltrate the bruise, their numerous density and enzyme activity, we can determine their age. Microscopic analysis showed presence of neutrophils in fresh bruises, while in bruises older than 3 days there were macrophages in the field of bleeding and their numerous density was more expressed in older bruises.

Microscopic analysis along with an observational method of analysis can give credible results for determination of the age of bruises.

Conflict of interest statement. None declared.

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