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ISSN: 0015-5659

e-ISSN: 1644-3284

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DOI: 10.5603/fm.102163

Article type: Original article

Submitted: 2024-08-19

Accepted: 2024-09-11

Published online: 2024-09-13

This article has been peer reviewed and published immediately upon acceptance.
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Articles in "Folia Morphologica" are listed in PubMed.

ORIGINAL ARTICLE
DOI: 10.5603/fm.102163
Snezana Lestarevic et al., Aging, sun exposure and skin mast cells

The effect of aging on mast cell density in human skin: a comparative analysis of photoexposed and photoprotected regions

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ABSTRACT

Background: Mast cells are mononuclear cells originating from bone marrow. They produce various biologically active substances, which allow them to actively participate in immune and inflammatory processes associated with intrinsic and extrinsic skin aging. This research focused on distribution and density of mast cells in healthy skin in different stages of skin aging.

Material and methods: This project included samples of photoexposed and photoprotected skin, obtained from 90 cadavers aged 0–82 years. The samples were classified into five age groups: newborns, young age, middle age, senior age and the oldest age. In order to visualize the mast cells, we have employed several histochemical staining protocols.

Results: The number of mast cells of the photoexposed skin significantly correlated to the individual's age. The number of mast cells of the photoprotected skin was in general statistically significantly lower in younger compared to older groups; however, the correlation of the mast cell density in photoprotected skin and the age did not reach statistical significance. In middle age, senior age and the oldest age groups, a significantly higher number of mast cells was recorded in the skin of the photoexposed compared to photoprotected region.

Conclusions: The increase in mast cell density correlated with age only in photoexposed skin. Age-related higher accumulation of dermal mast cells in photoexposed skin can be an important factor in the photoaging process, as well as the contributing factor in the occurrence of skin cancer.

Keywords: mast cells, photoaging, photoexposed skin, photoprotected skin

INTRODUCTION

A vast number of different cell types are involved in the pathogenesis of skin aging, and among them, mast cells (MC) occupy a significant role [10, 16]. Mast cells are long-lived tissue-resident cells that originate from hematopoietic pluripotent progenitors in the bone marrow, which differentiate and mature in peripheral tissues [20, 40]. While ubiquitous in the body, they are highly concentrated in the regions where the body is directly exposed to the outer environment [33]. This strategic arrangement of mast cells suggests their importance in protecting against infiltration of pathogens and toxins [37] as well as the effects of UV radiation [31].

The skin is abundant with mast cells, and they are most densely distributed in the skin of the arms and legs [38]. Mast cells constitute about 8% of all the cells in the dermis [8, 17, 19, 22]. Dermal MCs are located in the connective tissue of the papillary dermis, in proximity to sensory nerve terminals and blood vessels [14, 27]. Numerous researchers have studied the role of mast cells in the dermis in physiological and pathological conditions [11, 13]. The density of mast cells in the papillary dermis increases with age [29]; their high density in the dermis of older people, and low prevalence in the dermis of the fetus or young people could indicate their in the skin aging process [40]. However, some data indicate that the density of mast cells might even be higher in children compared to adults [7]. Furthermore, while it is suggested that UV radiation has an effect on the stimulation of mast cells, the nature of this effect [5, 13], as well as the interplay between physiological aging and sun exposure in influencing mast cell density is yet to be clarified.

Therefore, the aim of the study was to examine the distribution and density of mast cells in various stages of intrinsic and extrinsic skin aging using different histological techniques.

MATERIALS AND METHODS

Experimental protocol

Skin samples were obtained from autopsy material at the Institute of Forensic Medicine in Nis. The study involved 90 cadavers (50 men and 40 women), aged 0 to 82 years, of white Caucasian race (skin phototype III, IV and V according to Fitzpatrick) who were classified into five different age groups (Table 1). The methodology of this study was approved by the Ethics Committee of the Medical Faculty in Nis and the Ethics Committee of the Medical Faculty in Pristina/Kosovska Mitrovica (decision number 112).

To explore the mast cells distribution, tissue blocks sized 4.0 mm in diameter were taken from the skin of the cadavers using cylindrical (punch) technique. In order to examine the photoexposed skin, the skin samples were obtained from the front of the neck region, while the photoprotected skin samples were obtained from the front of the abdominal region. Two tissue clippings were obtained from each cadaver.

Preparation of tissue samples for optical microscory

Fixation of tissue samples was performed using 10% formaldehyde solution in photosphate buffered saline (PBS) 150 mM, pH 7.5, during 24 ± 1 hours. Following the dehydration performed using a series of ethanol solutions, each with a progressively higher concentration, and clearing in butanol, the representative fragments of the skin were embedded in paraffin and sectioned semiserially at 4–5 μm using Reichert sliding microtome.

Histochemical staining protocols for the visualization of the mast cells

Three different types of staining techniques were used to identify mast cells: Toluidine Blue Staining, Aldehyde fuchsin and Giemsa Staining [12, 35].

The preparations were analyzed using a microscope of the Leica brand and photographed by Leica MC190 HD digital microscope camera at 10×, 40× and 100× magnification. To perform the morphometric analysis, we have used the mash (with the surface area of 0.0145 mm^2 at 400 × microscope magnification) which was previously calibrated. For each measurement, the mash was put over the entire tissue sample, whose surface required a certain number of measurements *per* sample. The average number of cells *per* mesh surface was determined for each sample, and by dividing this number by 0.0145, the number of cells *per* mm^2 of derm was obtained. We have also performed the histomorphometric analysis of images, using the image analysis software ImageJ 1.45. available at the Internet address: http://rsb.info.nih.gov/ij. Following the calibration, on the micrographs (the size of digital image was 640 × 480 pixels or 453.33 ×

340.00 μm and 2088 × 1552 pixels or 1353.56 × 1006.10 μm), a mash test system was placed over the examined field with the help of the "grid" option, and the image was analyzed using the cell count tool.

Statistical analysis

The experimental data were recorded and analyzed using SPSS software, version 20 (SPSS Inc., Chicago, IL, USA). Quantitative data are presented as the mean ± standard deviation (mean ± SD). The significance of differences between groups was assessed using one-way ANOVA, followed by Tukey-Kramer post hoc tests for multiple comparisons. A p-value of less than 0.05 was considered statistically significant. Pearson's correlation coefficient was calculated to examine correlations.

RESULTS

Histological analysis of photoexposed and photoprotected skin samples

The skin of newborns (group I) contained a moderate number of mast cells, distributed throughout the dermis, with a slightly higher density in the papillary layer. Mast cells of the skin of newborns were mostly distributed in proximity to the capillaries. Morphologically, they were predominantly round and oval cells, completely filled with granules. In the skin samples of the young group (group II), we observed a large number of mast cells, round, oval or oblong in shape, most often near the capillaries, distributed throughout the dermis. While some proportion of cells were partially degranulated, the cytoplasm of most of the cells was entirely filled with granules. Mast cells are more frequently found in the reticular compared to the papillary layer of the dermis; they are most frequently found at the boundary between the two layers. We observed no significant differences between photoexposed and photoprotected skin. In the samples of the photoprotected skin, in the group of middle (group III) and senior age (group IV), we observed an increased number of mast cells in the reticular dermis layer, with a tendency to cluster around arterial and venous blood vessels. The mast cells, usually oblong in shape, were also rarely present between collagen bundles. The density of mast cells in the dermal papilla was moderate. We did not observe mast cells inside the epithelium in any of the samples. In photoexposed skin samples of the middle (III group) and senior age group (IV group), we observed a significantly higher number of mast cells and a larger proportion of degranulated mast cells compared to photoprotected skin samples. The density of mast cells was increased in proximity of the elastic fiber clusters, as well within the inflammatory infiltrates, if present. Mast cells, most frequently of elongated shape, could also be observed between collagen bundles. Most of them were found precisely in the regions of the dermis containing elastic material, as well as around larger blood

vessels. Although located near the elastomically altered material, we have gained an impression that they are primarily located in those places where collagen fibers are subject to the process of collagenolysis. In these places we observed the clusters of large, round cells abundantly filled with metachromatic granules. In the oldest age group (V group), both in the samples of photoexposed and photoprotected skin, we observed a lower density of mast cells. Morphologically, the mast cells were most frequently degranulated cells, elongated, spindle-shaped (Fig. 1, 2).

Statistical analysis of photoexposed and photoprotected skin samples

The comparison of mast cells density in photoexposed skin is indicated in Table 2. We have observed statistically significant differences in each comparison with the exception of the young age group compared to the oldest age group. Table 3 indicates the comparison of mast cells density in photoprotected skin of different age groups. We have observed a statistically significant positive correlation between the age of the individual and mast cell count in the skin of the neck region ($r = 0.278$, $p = 0.008$); however, there was no statistically significant correlation between the age of the individual and the mast cell count in the skin of the abdominal region ($r = 0.086$, $p = 0.418$). Table 4 indicates the comparisons between the mean mast cell count in photoexposed and photoprotected skin within each age group. We have observed a statistically significantly higher density of mast cells in the photoexposed compared to the photoprotected skin in the middle, senior and the oldest age groups.

DISCUSSION

In this study, we aimed to explore the differences in skin mast cell density related to aging and sun exposure, through examining the distribution and density of mast cells in the neck region, exposed to the constant action of UV rays and constant injury, and abdominal region, covered and protected from sun exposure, of different age groups. We observed the presence of mast cells in the samples of all age groups. The density of mast cells was shown to increase in relation to the age in photoexposed regions; this correlation was not observed in photoprotected regions. The density of mast cells was shown to be significantly higher in photoexposed compared to photoprotected skin in middle age, senior age and the oldest age group.

In our research, the detection of mast cells was carried out using several histochemical staining methods; we argue that the best visual distinction of the mast cells was achieved using Giemsa staining method, which is supported by previous research [28, 34]. Our histological analysis indicated the presence of various shapes of mast cells (spindle and oval-shaped), readily stained and arranged within the dermis. At higher magnifications, we were able to observe the

granulated mast cells characterized by the presence of colored blue/purple metachromatic granules that obscure the central positioned nucleus, or to see “emptied” degranulated cells. Mast cells were distributed throughout the dermis, most frequently as individual cells and less frequently within the small groups in proximity to the epithelium or the blood vessels.

While our research involved determining the mean value of mast cell count within different age groups, we argue that the comparison of mean counts of mast cells throughout different studies is of limited value, primarily due to the substantial methodological heterogeneity; among other things, different ways of counting (manual and automatic), different age groups classifications, different anatomical mast cell localizations, as well as different sample sizes. While Fede et al. presented mast cell density of 20.4 ± 9.4 cells/mm^2 as a normal range of skin mast cells in adults under the age of 40 [9], in our study, the density of mast cells close to that value was registered in the photoexposed and photoprotected skin of the newborns and the oldest age group; however, in the groups of early, middle and senior age group, the density was twofold that value.

Our morphometric analysis indicated that with increasing age, the number of mast cells increases in the photoexposed skin. This positive correlation was previously observed by a number of other researchers [1, 15, 18]. The increase in the number of mast cells in relation to age may be due to previous inflammations, since it has been shown that the cell population of mast cells grows during and following inflammation caused by UV radiation, as well as in various inflammatory skin diseases [36]. The number of mast cells on the photoprotected skin had also shown a growing trend in relation to age, without reaching statistical significance, although we noted a statistically significantly higher density of mast cells in the groups of early, middle and senior age group compared to the newborns. This is somewhat in line with the results of the recent histological and morphometric study of intrinsic skin aging, which, among other things, explored changes in mast cell density by analyzing 25 autopsy skin samples taken from the periumbilical area of four age groups (0–12 years; 13–25 years; 26–54 years; ≥ 55 years). The authors reported that no significant changes in the number of mast cells associated with aging were observed [3].

Despite observing a growing trend of skin mast cell density with aging (which even reached statistical significance in photoexposed region), it is noteworthy to mention that we have detected a reduced number of mast cells on the skin of both the neck and the abdominal region in the oldest age group. A result similar to ours was presented by Kim et al. [21] in a study exploring changes in mast cell counts in chronological aging and photoaging, as well as the influence of UV and infrared radiation on mast cell prevalence and tryptase expression in human skin. They concluded that mast cell counts decrease with age on both photoexposed and

photoprotected skin. Namely, the dermis thins with age and shows reduced production of the extracellular matrix, as well as increased fragmentation and disorganization of matrix components, especially in photoaging, and changes such as collagen and elastin density can affect the distribution and activity of all cells in the dermis, including mast cells [26]. Inflamm-aging is a term that is increasingly used, and the basis of inflammation can be mitochondrial dysfunction, autophagy, the ubiquitin-proteasome system activation and DNA damage, as well as several signaling pathways involved in the regulation of inflammation [2, 24]. The key role of increased levels of pro-inflammatory cytokines and the importance of maintaining proper balance between them are also recognized [25]. Some authors hypothesize that mast cells exhibit reduced synthetic capacity and increased functional activity during skin aging, thus contributing to the aging, and therefore may serve as a potential biomarkers for age-related changes [32].

Our histological analysis of the skin of the oldest group indicated the relative abundance of the mast cells in the zone of the papillary dermis. In the skin of the abdominal region, we observed a large number of degranulated mast cells. Furthermore, we observed a large number of degranulated mast cells on the skin of the neck region in all age groups except I and II. In contrast to our results, the study by Pilkington et al. suggested a lower number of degranulated mast cells in older skin [29]. In accordance to our observations, Kowalszky and Weller indicated an increased activity of mast cell tryptase as an effective driver of dermal matrix degradation, while an Egyptian study recognized the presence of active and enlarged degranulated mast cells in the dermis of aging skin [1, 23]. The tryptase can activate MMP, the main enzymes in the process of ECM degradation that leads to direct damage to the ECM proteins, especially collagen types I and III, which are usually seen in aging skin [15]. Although in our study we did not measure the activity of tryptase, in the skin samples of the oldest age group we observed almost devastated dermis with fragmented collagen and elastic fibers, which indirectly indicated the occurrence of the ECM degradation, as well as a depletion of almost all connective tissue cells constitutively located in the dermis. One of the histologically common characteristics of chronological aging and photoaging is the loss or change in the structural components of connective tissue, caused by an imbalance between the synthesis and degradation of the extracellular matrix [6]. Mast cells are known to interact closely with fibroblasts [30], and UV radiation triggers fibroblasts to produce more pro-inflammatory cytokines such as IL-1 and TNF-α and mast cells to produce inflammatory mediators such as prostaglandins and leukotrienes. In such condition the senescent fibroblasts can develop the senescence-associated secretory phenotypes (SASPs), and hence contribute to skin aging through promoting chronic inflammation and degradation of the extracellular matrix, which is also evident in photoaging [4, 39].

In the middle age and senior age groups, as well as in the oldest group, we observed significantly higher mast cell density in the photoexposed compared to the photoprotected skin; this is in line with the results of other authors who showed that exposure to UV radiation increases the density of mast cells in the dermis and that the skin of people, as well and the skin of mice, chronically exposed to the sun, contains significantly higher mast cell density compared to photoprotected skin [5, 13].

Our results, however, should be viewed in the context of several limitations. Firstly, our sample were heterogenous both in terms of age (relatively large age range within age groups) and sex (unequal representation of members of each sex in each of the age groups and within total sample). We attempted to overcome the age heterogeneity by examining the correlation of age as a continuous variable and the number of mast cells within photoexposed and photoprotected skin. In addition, in understanding the role of mast cells in aging, other factors might be of importance, such as the presence of various disorders and the habits of each individual (smoking, diet, sun-related preferences etc.). Unfortunately, these data were not available to us and therefore were not taken into account. Finally, the techniques we used to visualize mast cells are basic, and there are more sophisticated methods available. However, we argue that traditional visualization methods absolutely provide the possibility of accurately estimating the number of mast cells. Bearing in mind the material circumstances of research institutions in developing countries, our goal was, among other things, to increase the contribution of researchers from developing countries to this area of research and to demonstrate that methodologically sound and precisely conducted research is not limited by material limitations. Therefore, we argue that our results provide a very significant, albeit modest, contribution to this area of knowledge.

CONCLUSIONS

Our study indicates that the density of mast cells in photoexposed skin correlates positively with age. The abundance of degranulated mast cells in the skin of older people indirectly shows that the secretory activity of dermal mast cells is increased in older people and that these cells probably play an important role in the dermal remodeling of ECM in older skin. Given the inflammatory mediators and cytokines produced by mast cells, the greater accumulation of mast cells observed in photoexposed skin could increase the risk of cancer. Therefore, further research should be directed towards finding ways to block the immunosuppression caused by mast cell activity. Also, the potential to influence the number and function of mast cells could be important in designing novel anti-aging procedures. Future

research on mast cells is expected to lead to significant improvements, both in the prophylactic and therapeutic area of skin aging.

ARTICLE INFORMATION AND DECLARATIONS

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics statement

This study was approved by the Ethics Committee of the institution in which it was performed (approval 112). Prior to participation in the study, parents/caretakers signed the informed consent form.

Author contributions

SL, SS and MD contributed to the research design. SL, PM, and DM wrote the main manuscript text. MM and MF contributed to data acquisition and interpretation. IBR i TJ performed statistical analysis. All authors reviewed the manuscript.

Funding

The authors declare that no funding was received for this project.

Acknowlegdments

We sincerely thank the donors of cadaveric tissue for their invaluable contribution to this research.

Conflict of interest

The authors declare that they have no competing interests.

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Table 1. Participant groups by age.

Group	Age (range, $M^{age} \pm SD$)	n
I	0	10

II	1–20 years, M^{age}9.95 ± 5.92 years	20
III	21–40 years, M^{age} 33.1 ± 4.95 years	20
IV	41–60 years, M^{age} 53.50 ± 4.17 years	20
V	61–82 years, M^{age} 74.90 ± 4.36 years	20
Total		90

Table 2. Post-hoc Tukey HSD analysis of the mast cell density in photoexposed skin between age groups (p values).

Age groups	Newborns (group I)	Young age (group II)	Middle age (group III)	Senior age (group IV)	The oldest age (group V)
Newborns (group I)					
Young age (group II)	**0.002***				
Middle age (group III)	**0.000***	**0.012***			
Senior age (group IV)	**0.000***	**0.000***	**0.010***		
The oldest age (group V)	**0.011***	0.959	**0.001***	**0.000***	

*Statistically significant differences

Table 3. Post-hoc Tukey HSD analysis of the mast cell density in photoprotected skin between age groups (p values).

Age groups	Newborns (group I)	Young age (group II)	Middle age (group III)	Senior age (group IV)	The oldest age (group V)
Newborns (group I)					
Young age (group II)	**0.002***				
Middle age (group III)	**0.000***	0.845			
Senior age (group IV)	**0.000***	**0.030***	0.295		
The oldest age (group V)	0.381	0.113	**0.007***	**0.000***	

*Statistically significant differences.

Table 4. Comparison of the number of mast cells on the skin of the neck and abdominal skin within age groups (M ± SD, t and p values).

Age group	Mast cell count – neck region	Mast cell count – abdominal region	t	p
I	28.7410 ± 12.92219	26.3070 ± 12.64392	0.633	0.543
II	62.3365 ± 13.13861	60.4795 ± 12.01596	0.598	0.557
III	85.6125 ± 24.12470	67.9835 ± 27.49482	2.281	**0.034***
IV	109.3580 ± 27.52138	82.3830 ± 28.78016	3.790	**0.001***
V	57.4955 ± 25.26300	42.5010 ± 24.73973	2.121	**0.047***

*Statistically significant differences.

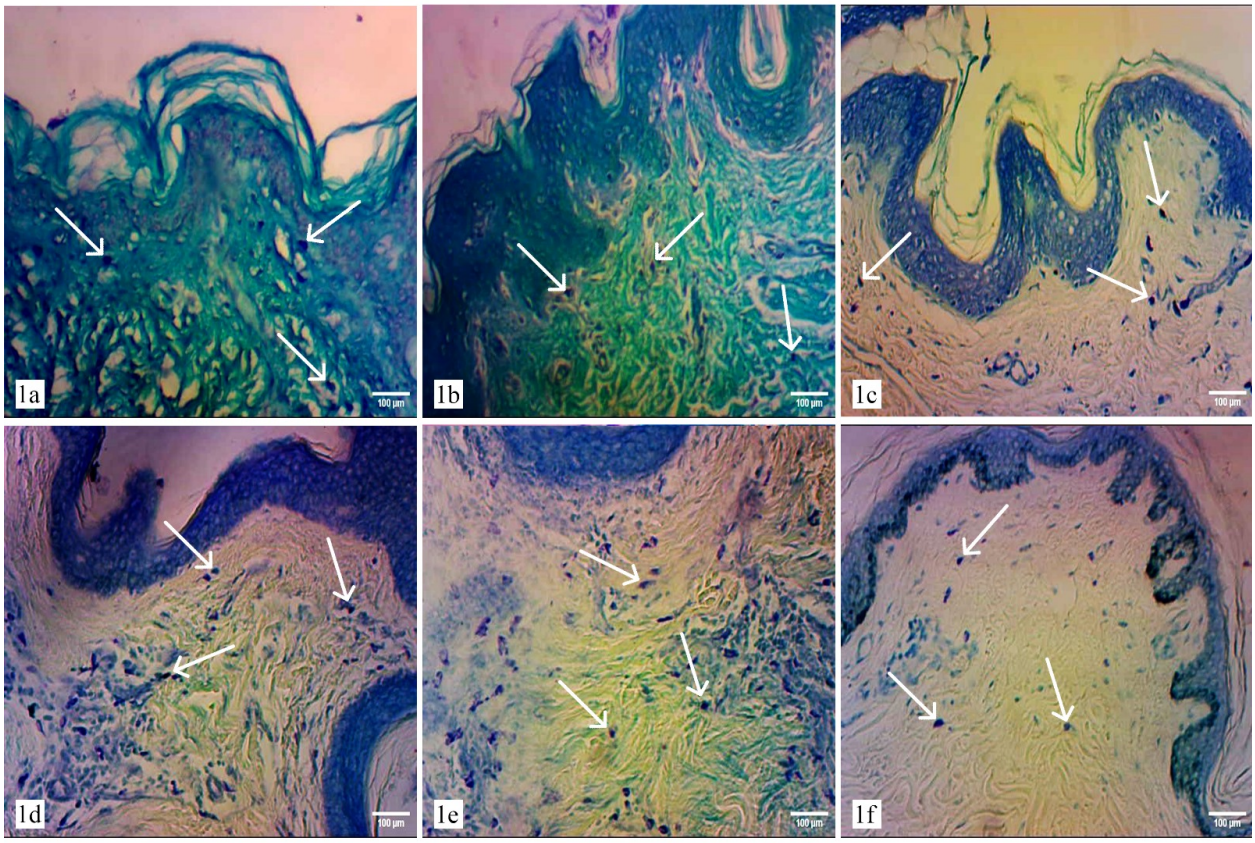

Figure 1. Top row — acidic aldehyde fuchsin staining. **1a)** Skin of the neck region of the newborn. Round-shaped mast cells in the papillary dermis of the skin. **1b)** Boy, 3 years old, skin of the neck. Numerous mast cells at the very border with the epidermis and around the blood vessels. **1c)** Giemsa staining Young man, 16 years old, skin of the neck. In the papillary and reticular dermis, as well as in perivascular spaces and between the bundles of collagen, a large

number of oval and oblong-shaped mast cells is observed. Bottom row — giemsa staining. **1d)** 23-year-old male, skin of the abdominal region. Mast cells of oblong and spindle shape on the border of the papillary and reticular dermis, mainly around the blood vessels. **1e)** 43-year-old male, skin of the neck region. Increased number of diffuse distributed mast cells. **1f)** Woman, 67 years old, skin of the abdomen region. Granulated and degranulated mast cells adjacent to thinned blood vessels in the dermis.

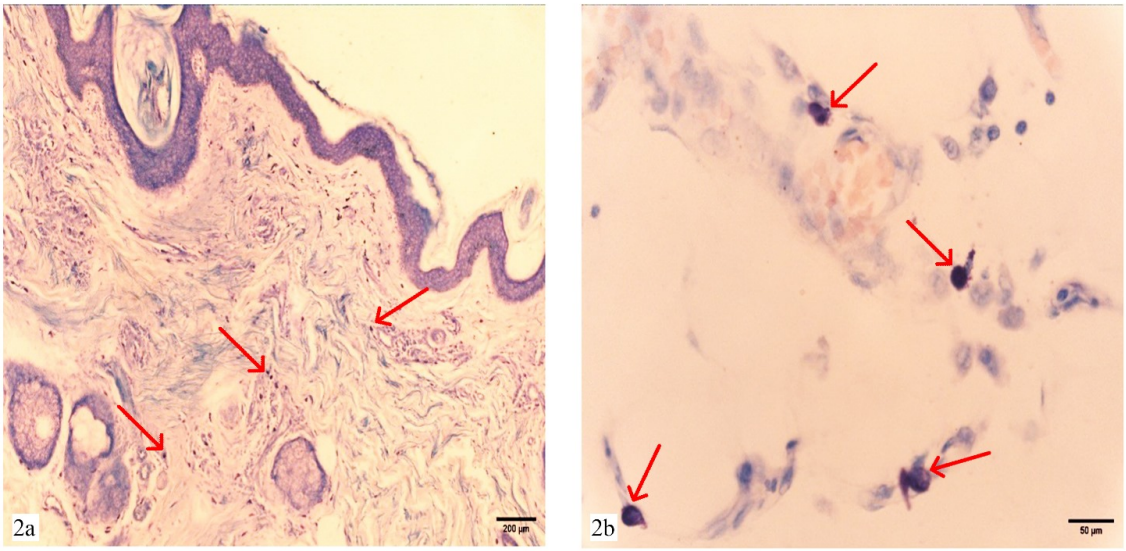

Figure 2. Toluidin blue staining. Woman, 46 years old, skin of the neck region. **2a)** Increased number of diffuse distributed mast cells. **2b)** Several round mast cells next to a small blood vessel.