MORPHOMETRIC AND DISTRIBUTION OF COLLAGEN FIBERS IN DERMIS OF LOCAL CANINE SKIN IN BASRAH PROVINCE

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ABSTRACT

The present study was carridaed of normal skin of local dogs canins lupus familiaris age of animals was (8 -24 mounths). The range and distribution of collagen fibers was studed. The results showed that the posterior limbs recorded the highest values for distribution and thickness compare with abdomen region. Also the results confirmed that the orientations of collagen fibers were variations between the regions because of stress factors.

INTRODUCTION

The skin consists of two major layers of a variant thickness, where it is thicker in the hairless regions than that of the hairy ones (1). The epidermis is the outermost dermis protective layer which is in a contact with the external environment and it lacks the blood supply. The dermis which comes beyond the epidermis, thicker than it and consists of a connective tissue which contains collagen, elastic and reticular fibres in addition to the accessories like the coetaneous glands, primary and secondary follicles, and supplying blood vessels and nerves (2).

Collagen fibers play a vital role in maintaining structural integrity, and also in determining tissue function, therefore, search to detect , quantify, and analyze collagen are valuable. Skin consists of two- and three-dimensional complex networks of collagen and elastic fibres (1, 2). There are regional differences of the epidermis and dermis in the histology of the skin of the dog and cat related to the thickness, collagen fibers distribution and hair growth (1, 3).

In biological tissues, cell and fiber orientation distributions are important since they define their mechanical properties and function. The amount and the orientation of

fibres are variable and depend on the localization and their specific function in a given organ (4, 5). It is known that mechanical stimuli induce remodelling of collagen fibers, which may include changes in the organization of fibers, fibers thickness, collagen type, collagen synthesis, enzymatic degradation, and crosslinking (6, 7).

A number of studies have used statistical distributions to describe the fiber arrangement in soft tissues in human and animals (8, 9, 10), but few research on collagen fibers distribution in canine dermis was found, for that the present study was aimed at performing a histological investigation of collagen and elastic fibers in different regions of the dermis in dogs with different ages as well as some morphometric parameters and histological features related to the site.

MATERIAL AND METHODS

24 samples were collected from clinically healthy native male dogs from two different body regions (abdomen and posterior limb) at Garmat Ali suburb in Basra governorate at (2011 – 2012). Twelve samples were collected from dogs aged between (8- 12 months) and the other samples collected from the dogs aged (20- 24 months). Fresh tissues from excisional biopsies were provided by the surgical unit of the Department of Veterinary Medicine and Surgery.

The sections were fixed with 10% formalin then treated routinely as the processes of alcohol dehydration, clearing with xylol, embedding with paraffin wax, then cutting with rotating microtome to obtain slides of a thickness 5 microns. Morphologic features were evaluated on haematoxylin and eosin stained sections. Van Giessen's staining Kit (New Delhi, India) was used to stain and differentiate collagen fibres in (red), other tissue (in yellow). The mean area and thickness of collagen fibres were measured on XSZ-N107 Taiwan 400 X microscope using the analysis programme of Soft 40 Imaging System GmbH (11).

The statistical analysis was accomplished by calculating the mean and standard deviation. The comparison of different groups within each group was done using Freidman analysis of variance (ANOVA) test with posthoc multiple two-group comparisons. For comparing categorical data, Chi square ($\chi 2$) test was performed. A $P< 0.05$ was considered statistically significant.

RESULTS

Histological examination of collagen fiber bundle orientation in abdomen skin in relation to epithelium showed that the majority of fiber bundles are parallel to the epidermis (figure -1), while are arranged randomly in relation to epidermis in posterior limb skin (figure -2).

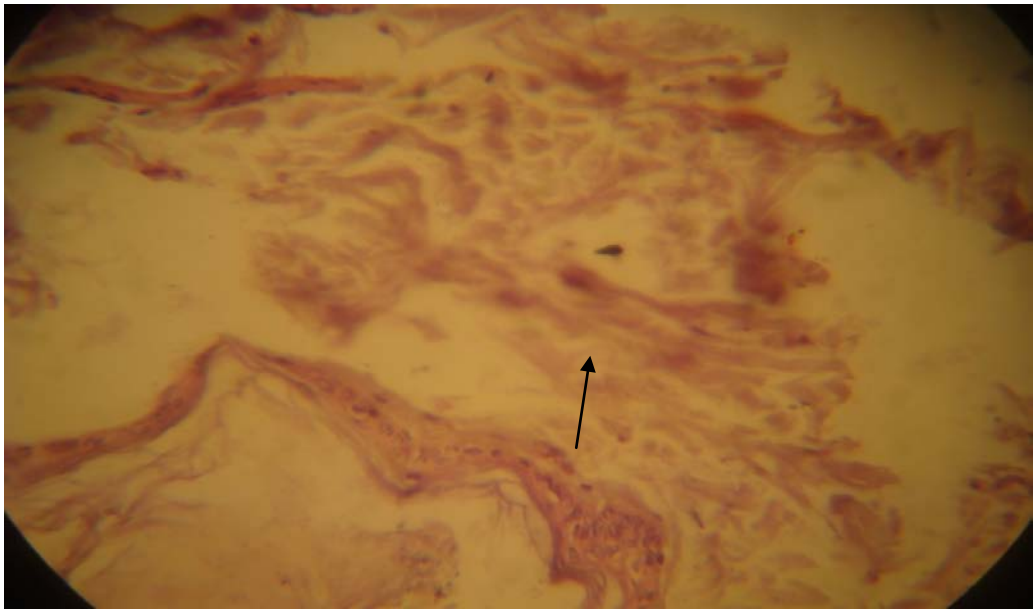


Figure (1) Section stain with hematoxylin and eosin show the collagen fibers parallel arranged in relation to the epidermis of dog (12 months) in abdomen region 40 x.

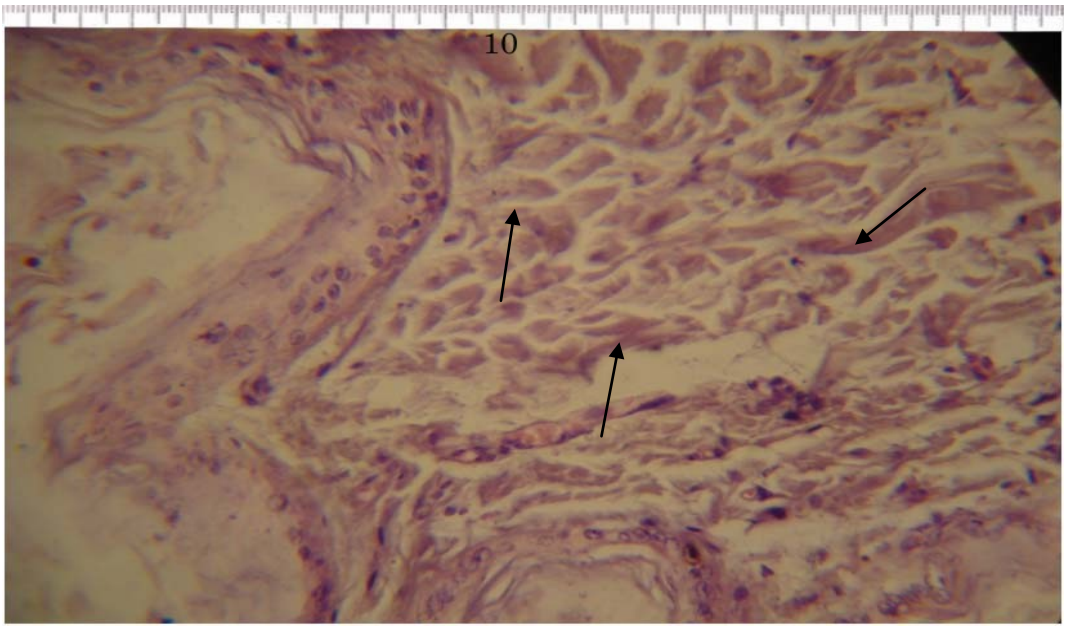
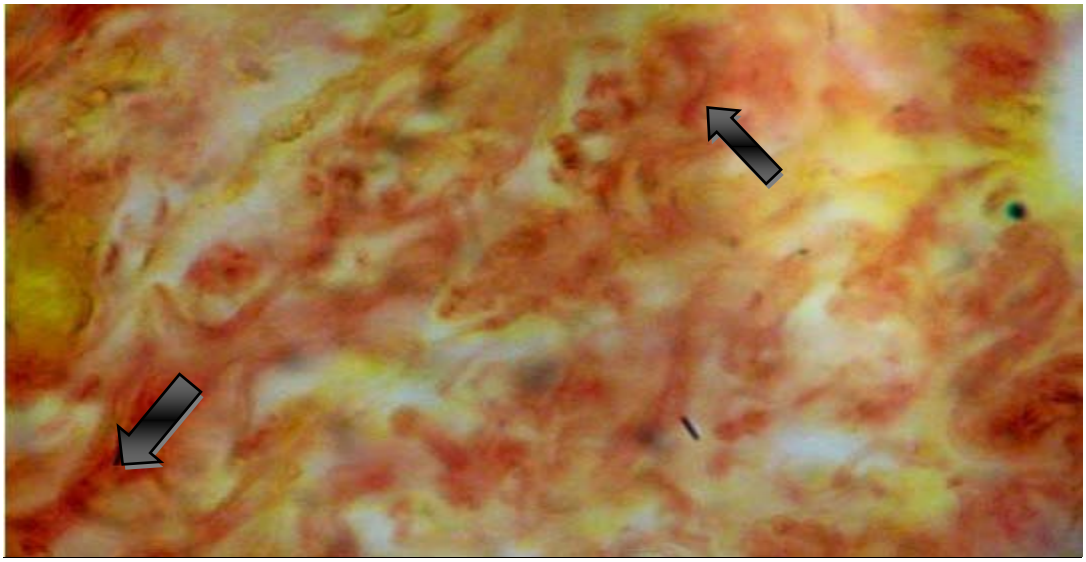


Figure (2) Section stain with hematoxylin and eosin show the collagen fibers randomly arranged in relation to the epidermis of dog (24 months) in posterior limb40x.

The dermis was mainly composed of collagen fibers intersecting under various angles. In Van Giessen's staining showed a moderate and well expressed reactivity of collagen and elastic fibers in the dermis (fig. 3).



Figure(3). Seetion staio with van giesson show collagen fibers in dermes are staining with red color 40x.

The percentage of posterior limbs skin collagen mean area of biopsies in both groups . The mean area of collagen in abdomen and posterior limb skin of (8 – 12) month age and (20 – 24) month age of dogs are studied. (8 – 12 &20 – 24) month age was statistically higher than the percentage of collagen mean area per unit of abdomen skin biopsies ($P < 0.05$). Although the percentage of collagen mean area in both skin biopsies (posterior limbs and abdomen) was higher in (20 – 24) month of age than the (8 – 12) month of age dogs group yet, it was no significant ($P > 0.05$) (Table 1).

Table (1): The means area of collagen fiber in skin biopsies (posterior limb and abdomen) of dogs in both age groups.

Age/ months	Abdomen biopsy	posterior limb biopsy	P-value
8 – 12	10.34±5.05	17.12±9.75	0.05
20 - 24	14.21±6.15	21.33±7.25	0.05

P-value	0.126	0.291	-

Table -2 shows that thicker collagen fibers were located in the dermis of posterior limbs 10.06±0.37 µm than in the abdomen 7.07 ±0.44 µm.

Table(2): Collagen fibres thickness (µm) in the dermis of the dog.

Age/ months	Abdomen Mean±SEM (range)	posterior limb Mean±SEM (range)	P-value
8 – 12	7.07±0.44 (5.38–9.56)	10.06±0.37 (6.93–11.88)	*0.05*
20 - 24	8.21±0.15 (7.09–10.36)	12.33±0.25 (10.03–14.88)	*0.05*

*** P<0.05 of dermis in posterior limb vs. abdomen**

DISCUSSION

In biological tissues, cell and fiber orientation distributions are important since they define their mechanical properties and function. According to normal histophysiologies of the skin, collagen fibre predominates in the dermis have tensile strength and provide the skin with strength and toughness. Considering the effect that the mean orientation of collagen fibers has on the mechanical response of skin, the histological method for establishing the orientation and thickness measurements of the fibers is of the utmost importance. Due to the difference in tensil location between the abdomen and posterior limb, the amount, thickness and orientation of collagen fibers is differs (11).

The present study shows an increase in the mean area per unit and thickness of collagen fibers in posterior limb which exposed to continuous tensions during movements of the animal than that in abdominal area (table 1, 2), these result is agreed with the finding of (11, 12, 13, 14).

On the other hand the parallel arrangement of collagen fibers to the epidermis of the abdominal region due to response to the direction of the stress while the perpendicular

arrangement of the most collagen fibers to the epidermis in the posterior limb (figure 1, 2), these finding was corresponding with many authors conclusion that the direction of the extracellular matrix was changed according to the type of stress and their movements (13, 14). The different site of tensions on the posterior limb of skin dermis creat the collagen fibers reorient toward the loading direction increasing its global stiffness.

No significant differences between the mean area of collagen fibers in the two different areas among (8 – 12) and (20 – 24) month of age and that may due to increase mean area of collagen fibers in both during this time.

According to our findings, we conclude that the differences in the means area per unit and direction of stresses that exposed to the dermis on these two regions (the posterior limbs dermis and the abdomen) leads to the variation in the histological features in the distribution, thickness of collagen fibers of canine dermis

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دراسة شكلية قياسية لتوزيع الالياف الغراوية في ادمة جلد الكلاب المحلية في محافظة البصرة

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الخلاصه

تمت دراسة مقاطع من الجلد الطبيعي باخذ 24 عينة من مناطق (البطن والاطراف الخلفية)لذكور الكلاب المحلية من نوع canin lupus family avis وكانت اعمارها تتراوح (8 -24 شهر) تضمنت الدراسة النسجية قياس معدل الالياف الفردية وتوزعها اضافة الى قياس سمكها .اشارت النتائج ان نسب توزيع الالياف الفراوية وسمكها في منطقه الاطراف الخلفيه كانت اعلى من المنطقه البطنيه 0.002(pc 0.05)

بالاضافة الى ذلك بنيت النتائج ان اتجاه الالياف الفراوية كانت متبايه بين المنطقتين المدررسة pvalue= .
بسسب عوامل الاجهاد .

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