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Full Length Research Paper

A histological study on the distribution of dermal collagen and elastic fibres in different regions of the body

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The aim of the study was to assess the unequal distributions of dermal collagen and elastic content in skin sections perpendicular to each other from different regions of the body. We collected 20 skin samples from 10 regions of a cadaver. From each region, 2 samples were collected in 2 directions which were perpendicular to each other. Histological slides were prepared and stained for collagen and elastic fibres'. Photomicrographs taken from special stained sections were analysed using simple and reliable software "tissue quant" method. Various ratios were calculated. The quantitative fraction in terms of percentage area occupied by collagen and elastic fibres were observed to be different in different regions as well as in different directions of sections taken. This unequal distribution of the dermal collagen and elastic fibres in 2 different directions of the sections will possibly have an effect on wound healing process which in turn may have an effect on varied scar appearance and behaviour based on the region and direction of wound.

Key words: Collagen, elastic, tissue quant, image analysis, scar.

INTRODUCTION

The skin performs many vital roles both as a barrier and a regulator between the outside world and the internal environment. The physical toughness of the skin prevents the ingress of harmful chemicals and invading organisms, such as bacteria and viruses. It also provides resistance to shocks for the more sensitive tissues underneath.

Biochemically, the papillary dermis is composed of type III collagen and reticular dermis composed of type I collagen. Elastic fibres appear in dermis much later than the collagen fibres. They undergo significant changes during life. Changes in aging is best studied in non exposed skin, elastoid degeneration is the result of chronic sun exposure. In very old persons, fragmentation and disintegration of some of the elastic fibres may be observed (David, 2009).

Much importance is attached to the appearance of skin, especially in our modern society. Medical conditions affecting the skin can have marked effects, not only on our state of well being but also on the ways we interact with other people and on our suitability for certain occupations.

Appearance of scar after the wound healing is a natural process. It is still a challenging task for the aesthetic surgeons to minimise the scar appearance in the procedures of wound healing. This is because of the fact that, scar tissue is composed of same collagen as in normal skin but with differences in arrangement pattern and composition (Sherratt, 2010). Scars in the skin are less resistant to ultraviolet (UV) radiation and sweat gland, and hair follicles do not grow back in scars.

Despite of the existence of sophisticated treatment and availability of multi drug therapy, the consequences following the wound healing resulting in scar appearance

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and its behaviour is mysterious task for both general and plastic surgeons.

Langer's line is the line of choice in surgical approach which explains the orientation of collagen fibres beneath the skin. However, differential content of connective tissue fibres in different regions of the body as well as differences in the same area between 2 different directions may also be responsible for the alteration in the appearance and behaviour of scar and should be considered.

Aims and objectives

1. To evaluate quantitative fraction of collagen and elastic fibres in skin samples that are cut in perpendicular direction to one another.
2. To analyse the significance of difference in collagen and elastic fibres in terms of percentage area in different regions of the human body.
3. To measure and compare the various ratios of collagen and elastic fibres in the dermis of skin sections perpendicular to one another in different parts of the body.

MATERIALS AND METHODS

Sample collection

Skin samples (20) were collected from 10 different regions of a formalin embalmed cadaver. From each region, two elliptical (1 × 0.5 cm) full thickness skin sections perpendicular to each other were collected. Sample 1 was taken horizontally in all the regions except at joints and was marked as "horizontal". Sample 2 was taken adjacent to sample 1 area and in perpendicular direction to it. It was marked as "vertical". However, in the region of joint, section taken parallel to the joint represents "horizontal" and perpendicular to it as "vertical".

Skin samples were collected from following regions: Scalp (about 3 cm superior to auricular attachment), forehead (in the midline at the middle of the forehead), lateral canthus (immediately lateral to lateral angle of the eye), neck (in the midline at the middle of the front of the neck), shoulder joint (immediately lateral to bony landmark of coracoid process), chest (in the midline, roughly at the middle of the body of the sternum), wrist (on the dorsal surface about 2 cm above the wrist joint), abdomen (in the midline about 2 cm below the umbilicus), groin (at the midpoint region of inguinal ligament), and thigh (on the middle of the medial surface of the thigh).

The aforementioned regions of interest are the areas which are either cosmetically important or important from the point of view of placing scar for achieving more aesthetic scar.

Tissue processing

All the skin samples were processed for histological study. The following staining methods were used:

(1) Haematoxylin and Eosin (H&E) staining: for overall histological appearance of connective tissue fibres in the dermis of the skin.
(2) Combined Verhoeff stain-Van Gieson stain: to demonstrate elastic fibres (by Verhoeff stain) and collagen fibres (by Van Gieson stain).

Verhoeff-Van Gieson stained slides are further analysed for tissue quant-image analysis to analyse the percentage area distribution of collagen and elastic fibres which is referred to as quantitative fraction.

Processing and staining procedures were followed according to standard techniques explained by John (2002). Processed tissues were embedded with paraffin wax followed by section cutting using rotary microtome. Sections from each skin sample were stained with routine H&E staining and with combined Verhoeff-Van Gieson stain for elastic and collagen fibres.

Slides were observed under light microscope to assess the general pattern of distribution of collagen and elastic fibres in the dermis before being subjected to image analysis.

Image analysis by tissue quant method

The sections stained by Verhoeff-Van Gieson method, were subjected to image analysis using the tissue-quant software. For the image analysis by tissue quant method, photomicrographs (20× magnifications) were obtained by inverted phase contrast microscope. Images from 3 different fields were taken from each slide and were analysed. The staining property of the collagen and elastic fibres are processed by image analysis using simple and reliable software "tissue quant". The tissue quant is an in-house developed software wherein the collagen and the elastic fibres are segmented out of the image by appropriately adjusting the colour settings. The areas covered by these tissues are then calculated by the software in terms of the number of pixels. All the images were acquired under the same magnification. Analysis from minimum of 3 fields of image from each stained sections were done. Mean percentage of collagen and elastic concentrations were calculated. From the mean values, various ratios of collagen (C1/C2) and elastic fibres (E1/E2) between 2 directions were calculated.

RESULTS

The dermal morphology was studied under light microscope using both H&E and special stained sections for overall pattern of connective tissue fibres. It was found to be normal throughout except for the elastoid degeneration in some regions.

The tissue quant analysis gives the data of quantitative fraction of dermal connective tissue components in terms of percentage area occupied by them based on the image analysis (Figures 1 and 2).

Data of quantitative fraction by dermal collagen and elastic fibers in two directions of skin samples obtained from different regions

Results depicted in the Table 1 and its graphic representation (Figure 3) indicates that, the percentage area occupied by collagen is much higher in groin (49%) region in its horizontal section and lowest in similar section of lateral canthus (16.8%). Similarly, the higher value was recorded for elastic fibres in horizontal section of wrist (22.5%) and lowest in scalp (0.5%) of same section.

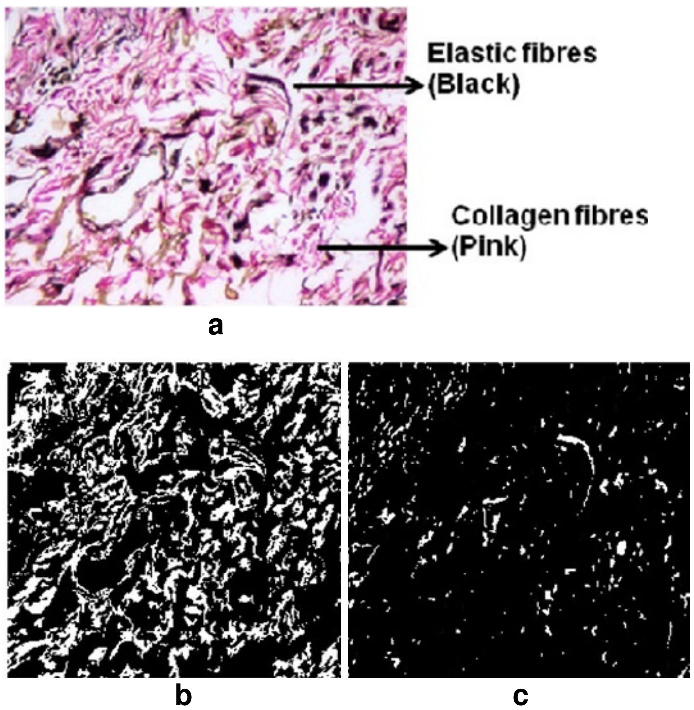

Figure 1. (a) Appearance of collagen and elastic fibres in the dermis of horizontal section of lateral canthus (Verhoeff-Vangieson method 20×). (b and c) Segmentation of collagen and elastic fibres in horizontal section of lateral canthus, respectively by tissue quant image analysis.

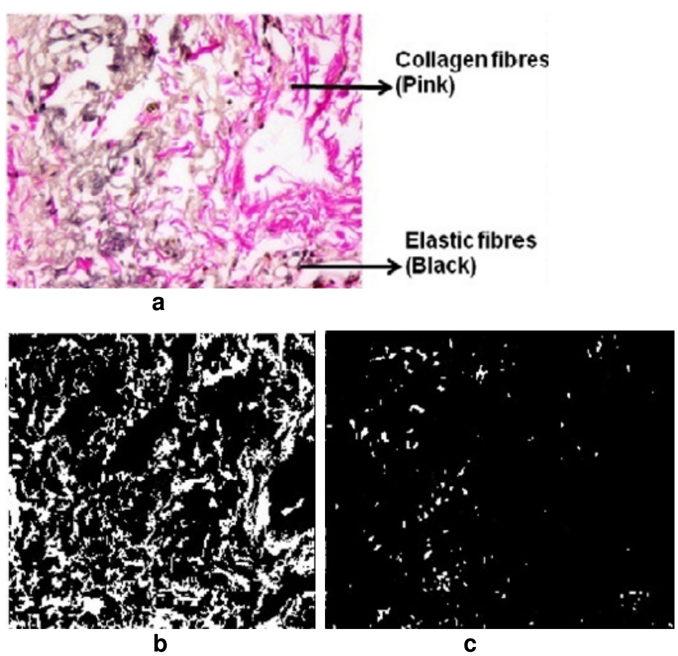

Figure 2. (a) Appearance of collagen and elastic fibres dermis in vertical section of lateral canthus (Verhoeff-Vangieson method 20×). (b and c) Segmentation of collagen and elastic fibres of in vertical section of lateral canthus respectively by tissue quant image analysis.

Comparison of quantitative fraction values of collagen and elastic fibres between horizontal and vertical sections

In this study, the regions such as lateral canthus, chest, forehead, shoulder joint and wrist show that the percentage area occupied by collagen fibre was higher in vertical (C2) sections than in horizontal (C1) sections (H<V), whereas, the percentage occupied by elastic fibres was more in horizontal (E1) sections than vertical (E2) sections of all the regions studied except in scalp (Figure 4).

Ratio analysis

The C1/C2 ratio is calculated by dividing C2 value by C1. Similar calculation has been applied for E1/E2, C1/E1 and C2/E2 ratios.

Various ratio values in the Table 2 are summarised as follows. The ratio value is depicted in the bracket. The quantitative fraction of dermal connective tissue fibres of the horizontal section in both cases, that is, collagen and elastic fibres were kept constant as 1, so that the values denoted here is the proportionate value of its vertical counterpart. Hence, it is very clear that, the percentage area occupied by collagen was higher in the sections taken in vertical direction than horizontal direction in regions such as lateral canthus (1.50 times more than horizontal counterpart), chest (1.36), scalp (1.02), forehead (1.25), shoulder joint (1.35), wrist (1.08), and thigh (1.32), whereas, it was the reverse in the case of abdomen and groin (0.79) and neck (0.76). In these regions, collagen content is more in horizontal direction of the sections than its vertical counterpart.

Similarly, the percentage area occupied by elastic fibres was higher in vertical sections than horizontal observed in all regions studied except in scalp and groin regions. This shows that the quantitative fraction of collagen and elastic fibres in different regions as well as in different sections is not proportionately distributed. This imbalanced distribution of collagen and elastic fibres may play a significant role in the formation and the behaviour of scars during wound healing process

DISCUSSION

The major problem identified in the clinical setup during the process of wound healing is the variant appearance and behaviour of scars. The scars may be hypertrophic or stretched. Scar formation is the natural part of healing process which results from biologic process of wound repair. Hypertrophic scars are due to collagen content and stretching is dependent on elastic content. Appearance of the scar varies from region to region, for example, scar over the back, shoulder joint often stretches and develops hypertrophy, and it also depends

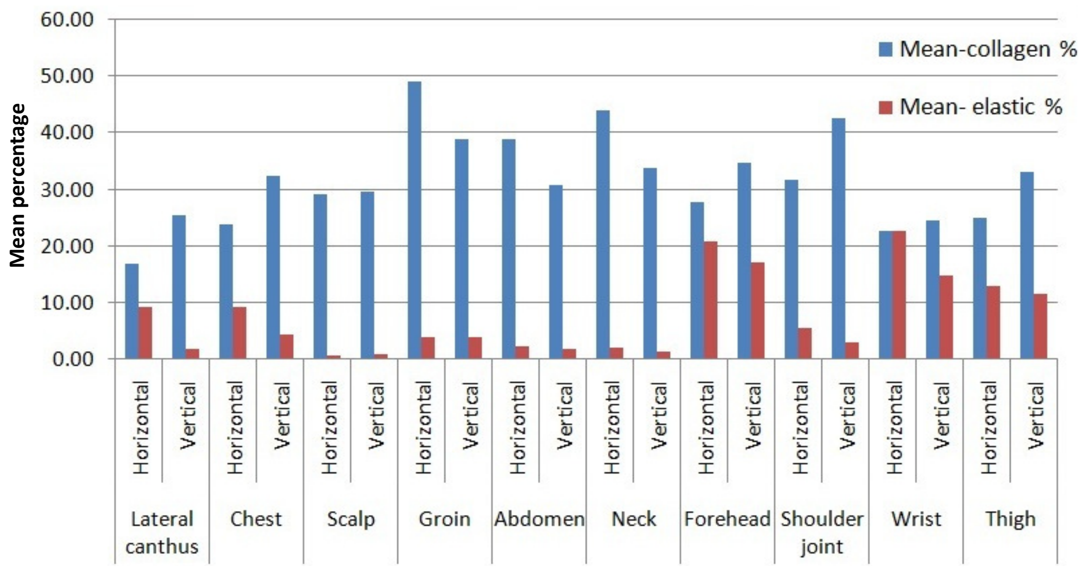

Figure 3. Graphic representation of mean quantitative fraction of collagen and elastic fibres in horizontal and vertical skin samples of various regions.

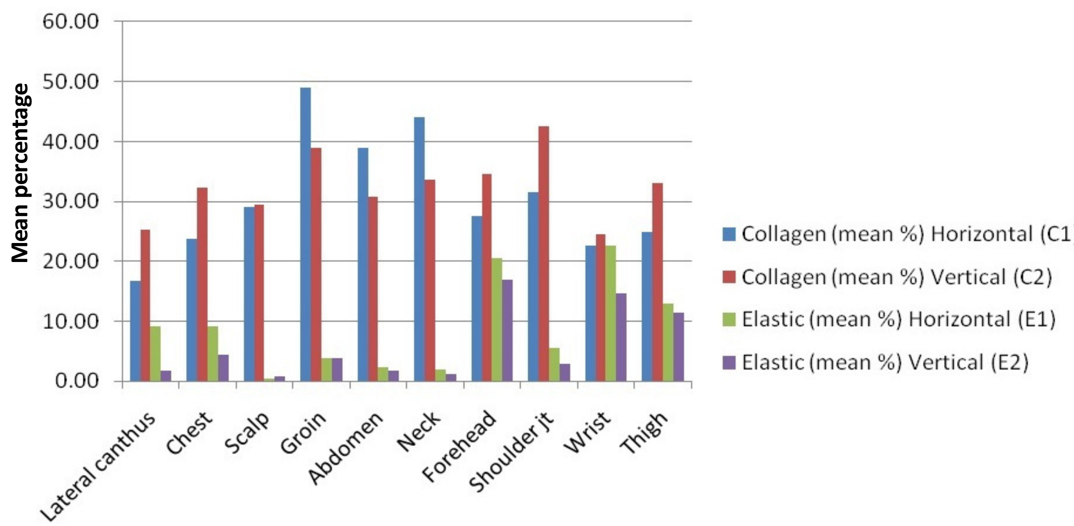

Figure 4. Graphic representation of quantitative fraction values of collagen and elastic fibres between horizontal and vertical sections.

on wounds of one direction to another. It is well evident in clinical setup as wound of horizontal direction on forehead results in an acceptable scar, while in the same region with vertical direction it becomes more obvious scar.

Hence, clinically, the behaviour of scar depends on the site and direction of the wound on the skin. It indicates that scar appearance and behaviour is also dependent on the pattern of quantity and arrangement of underlying anatomy of the dermis. Our study findings indicate that, the collagen and elastic fibre content differs in different direction, not only from region to region, but also in the same region.

The wounds that are under constant deforming contractile forces usually result in unaesthetic scars because of underlying anatomy (Kotler, 2009). The factor responsible for this is the pattern of distribution of elastic fibres in the dermis, which differs from one direction to another direction in a same region. Vitellaro-Zuccarello et al. (1994) reported that the fractional volume of collagen fibres is always higher in females than in males except for the 2nd and 3rd decades of life.

Table 1. Mean values of quantitative fraction of collagen and elastic fibres in horizontal and vertical skin samples of various regions.

Skin site	Direction of section	Collagen (%)	Elastic (%)
Lateral canthus	Horizontal	16.83	9.19
	Vertical	25.29	1.82
Chest	Horizontal	23.85	9.10
	Vertical	32.36	4.33
Scalp	Horizontal	28.99	0.50
	Vertical	29.46	0.73
Groin	Horizontal	49.05	3.92
	Vertical	38.92	3.90
Abdomen	Horizontal	38.86	2.29
	Vertical	30.75	1.75
Neck	Horizontal	44.00	2.05
	Vertical	33.63	1.22
Forehead	Horizontal	27.64	20.62
	Vertical	34.62	16.91
Shoulder joint	Horizontal	31.55	5.54
	Vertical	42.50	2.91
Wrist	Horizontal	22.63	22.54
	Vertical	24.47	14.61
Thigh	Horizontal	24.93	12.92
	Vertical	33.02	11.38

Table 2. Ratio analysis derived from the results of tissue-quant image analysis.

Skit site	C1/C2 ratio	E1/E2 ratio	C1/E1 ratio	C2/E2 ratio
Lateral canthus	1.50	0.20	0.55	0.07
Chest	1.36	0.48	0.38	0.13
Scalp	1.02	1.45	0.02	0.02
Groin	0.79	1.00	0.08	0.10
Abdomen	0.79	0.76	0.06	0.06
Neck	0.76	0.59	0.05	0.04
Forehead	1.25	0.82	0.75	0.49
Shoulder joint	1.35	0.53	0.18	0.07
Wrist	1.08	0.65	1.00	0.60
Thigh	1.32	0.88	0.52	0.34

C1, Quantitative fraction of collagen in horizontal section; C2, quantitative fraction of collagen in vertical section; E1, quantitative fraction of elastic in horizontal section; E2, quantitative fraction of elastic in vertical section.

Collagen fibres density increases with age in both sexes up to 30 to 40 years. In reticular dermis of both sexes, there is an increment of elastic fibres density in the 1st decade of life, followed by a drop particularly marked in males. This clinical observation reveals that scar problem is more in children and young age group patients. This may have some relation with the aforementioned reported values of collagen and elastic fibres.

Clinical research about collagen and elastic content of abdominal skin after surgical weight loss showed undamaged elastic fibre content and moderate increase in epigastrium. Preoperative obesity had a negative correlation with hypogastric collagen content (Simone et al., 2010). This shows that skin stretching due to obesity (fat deposition under the skin) is opposed by elastic fibres. After losing the fat from subcutaneous tissue, the skin becomes loose. Hence, it may be indirectly inferred that the elastic tissue content of skin exerts the stretching force on the scar and thus scar behaviour and appearance is altered in proportion to the force which may vary depending upon the elastic tissue content and inherent property of elastic tissue.

In a study conducted by Gogly et al. (1997), diameters of elastic fibres increased regularly with age in the skin between each age group. The area fraction occupied by skin elastic fibres increased significantly within age group. Collagen diameter in mid-dermis also increased strongly with age group. But the area fraction occupied by the collagen bundles significantly decreased from the age of 50 to 75 years. Since the clinical observation shows that the scar in children and young age group does not settle well and majority of time unacceptable, the aforementioned findings of this study indicates a strong correlation between scar appearance and behaviour with collagen and elastic content of the dermis. Excessive laying down of collagen and stretching of scar is more in more elastic skin (e.g. in children and young subjects) (Berman et al., 2008*).*

Although, the significance of cleavage lines of the skin is well known in plastic surgery, it is considered that there is still uncertainty about their exact location in certain parts such as in mammary region (Zanon and Harp 1993). According to Wilhelmi et al. (1999), many surgeons prefer Langer's lines. However, Kraissl preferred lines oriented perpendicular to the action of the underlying muscles. Later, Borges described relaxed skin tension lines, which follow furrows formed when the skin is relaxed. They also opine that these are only guidelines as there are many factors contributing in the formation and behavior of scars, including wrinkle and contour lines. Borges's and Kraissl's lines (not Langer's) may be the best guides for elective incisions of the face and body, respectively.

Conclusion

This study suggests that the distribution of dermal collagen and elastic fibres varies not only in different regions, but also in two different orientations of the sections taken in the same region. Hence, it may provide an anatomical basis for explanation to earlier experience that the scar placed in a particular direction in a given region gives better aesthetic result.

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