DISCUSSION

“Burn injury treatment has improved dramatically to increase the probability of survival, but burn survivors still suffer from excessive scarring and skin contractures, which substantially compromise their health and quality of life.”

- Anonymous

Wounds of scar are subject to constant deforming contractile forces because of the underlying anatomy. This results in linear scars and tends to depress the overlying tissue. Moreover linear scars have a greater tendency to bowstring over the surface, thus creating more noticeable scars. This observable fact is more likely to occur over concave surfaces like medial canthus. It may also present over convex surfaces like malar eminence, cheek etc as a well-defined depressed scar (Kotler, 2009).

Skin reconstruction of the face is a challenging task for the plastic surgeon due to multiple aesthetic subunits at different areas on the face (Ali et al., 2014). Surgical manipulation over the face region is a complex procedure requiring intense attention, as the attributes of wound healing and leaving an acceptable scar should be borne in mind. Proper knowledge of various skin tension lines associated with underlying pattern of dermal connective tissue fiber orientation should be considered before performing such procedures.

For over a century, surgeons have been on the lookout for the best direction to follow while making elective incision so as to obtain most aesthetic scar. It is indeed a collective fact that, if an incision follows a certain direction on the skin, an acceptable
scar will be obtained than if it is at right angles to that direction. Several concepts of skin lines have been put forward. But, Langer’s line (often referred as cleavage line) is the most popular amongst the lines employed in surgical approach. The fundamental basis for Langer’s line lies on the pattern of arrangement of collagen fibers in the dermis, thus attributing to its flexibility (Borges, 1984). But, selection of Langer’s line and its expected consequences is being debated among the aesthetic surgeons across the world. It has been popularly thought that understanding the direction of Langer’s lines in the specific area of the skin before surgical incision is important for cosmetic value. However, the exact direction of these lines are unknown, as in some regions of the body there are differences among the individuals and these directional changes are known to occur in the course of a person’s lifetime (David, 2008). Similarly, individual features of Kraissl’s lines also vary in subjects as its principle relies on the differences in underlying muscular development (Borges, 1984). Unlike Langer’s line, which is described in cadavers, Kraissl’s lines are maximum skin tension lines which are demonstrated in living individuals.

Relaxed skin tension lines (RSTL) follow the skin furrows formed when the skin is relaxed. But they are not visible like wrinkle lines. Skin is generally relaxed by joint mobilization, muscle contraction and by pinching. According to Borges and Alexander (1964), former two factors may produce false lines as they depend on the degree and direction of joint mobilization and the muscle undergoing contraction.
Relaxed skin tension lines (RSTL) frequently lie at right angles to the muscle fibers. In cases of multiple muscles with muscle fibers running in different direction, RSTL is perpendicular to the resultant directional pull (perpendicular to the resultant vector force produced by all the muscles lying under the skin) that exists in the relaxed skin. So, according to Langer’s line, skin tension is dependent on the enclosed body contents and on joint movements, which is not true in all the regions. Thus, when a scar crosses these RSTLs, it would have a greater tendency to stretch or become hypertrophic. On the face, keeping the above said in mind, the ideal positions for avoiding such scars are at junctions between anatomical areas such as the nose and the cheek or the cheek and the ear, or the junction between hairy and hairless areas (Norman et al., 2008).

Song (1993), through his histological study on the relationship of dermal collagen and elastic fibers to the Langer’s and Kraissl’s lines on the face reported that, collagen underlying the Langer’s lines were irregular with weaving pattern of arrangement in contrast to parallel and regular pattern underlying Kraissl’s lines. The elastic fibers underlying Langer’s line are arranged in parallel orientations to epidermis where as they are perpendicular to Kraissl’s lines. Due to distinguishable differences in arrangements of both collagen and elastic fibers, it has been suggested to follow Kraissl’s line as it runs along the direction of the greatest skin tension. Consequently, the face region can be divided into 3 territories depending upon the direction of incision as (1) Incision made along the Kraissl’s lines, (2) Incision made along both lines (Kraissl's and Langer's) and (3) Incision not made along both the lines (Song, 1993). However, according to Wilhelmi et al., (1999), lines
of Borges may be considered as the best guideline for elective incisions on the face, while Kraissl’s lines would be suitable for the body.

Wound healing process following the injury to the reticular layer of dermis is believed to be attributed in the formation of keloids and other varieties of hypertrophic scars. Structurally, the collagen in normal dermis appears to be much relaxed and arranged in a random orientation. However, in the keloids and hypertrophic scars, they are stretched and aligned in accordance to plane of epidermis. And also, they are comparatively thicker and more abundant in keloids (Meenakshi et al., 2005). Subcutaneous or fascial tensile reduction sutures tend to apply negligible tension on the wound and hence, this approach is advisable for reduction in the recurrence of keloids or hypertrophic scars (Ogawa et al., 2011).

Pattern of collagen and elastic fiber content when studied experimentally between photo-damaged facial skin and its radiofrequency rejuvenation revealed marked increase in the type I and III collagen content from the baseline. However, its elastic counterpart showed significant decline from its abnormal increment in the photo-damaged skin (Moetaz et al., 2011). These changes are believed to be due to translocation of elastotic material caused by the photo-damage of the epidermis and subsequent restoration of normal elastic fibers in the dermis of the skin.

Temporary lack of elastic fibers often seen in scars, are morphologically characterized by presence of fragmented and degenerative appearance as observed in hypertrophic scars. Disrupted pathway in the elastin protein synthesis is said to be result of failure of elastogenesis (Hugo and Duane, 1976)
Reduced tensile strength in scar tissue following cutaneous wounding is said to be due to decreased amounts of elastic fibers (Levenson et al., 1965). Role of dermal collagen and elastic fibers studied experimentally with the application of processed native collagen and elastin for the formation of neo-dermis revealed the replacement of newly formed collagenous texture in 3 weeks of post grafting procedure. However, elastic membrane was found to be still present even after 20 weeks (Hafemann et al., 1999). Correspondingly, application of native collagen matrices made of native bovine collagen I and elastin showed the dermal regeneration and remarkably reduced wound contraction (De Vries et al., 1995).

Following the uncertain factors that are accountable for the concept of various lines of choices, subjective principles have been put forward by the researchers about these lines (Song, 1993; Wilhelmi et al., 1999; David, 2008). A step ahead of this can also be explained under the light of pattern of dermal collagen and elastic fiber content in different planes within the dermis. Under this context, quantifying the collagen and elastic fibers at two minimum orientations shall probably furnish the cause for the diverse nature of the scars. The morphometric analysis of dermal collagen and elastic fiber content as measured by image analysis could be considered as confirmatory procedure against immuno-histochemical staining method (Moetaz et al., 2011).

Considering the extensive debate about the interrelation between skin incisions and scar related complications, the unequal distribution of dermal collagen and elastic fiber content between two orientations of skin samples in different regions of the human body was evaluated through this research work. For which, five
representative areas from three major regions (head and neck, trunk and extremity) were chosen.

For each region, the evaluation was discussed under three analyses.

1. **Quantitative fraction evaluation:** In this analysis, the percentage area occupied by the dermal collagen and elastic fibers in two different directions i.e., horizontal and vertical directions of skin samples were measured histomorphometrically. The results of the quantitative fraction analysis were comparable with their functional significance in wound healing consequences. Knowledge of pattern of predominant content in a particular direction renders valuable information to the aesthetic surgeons in placing elective incisions in the direction which maximally utilizes the anatomical factors for better aesthetic result.

2. **Correlation pattern evaluation:** The detailed results of Spearman’s correlation data are analyzed and corresponding scattered plot illustrations are made from SPSS version 15.0 (Figures 21 to 23). The strength of association was categorized into 3 groups (strong, moderate and low) based on the statistical reference chart as shown in table 3 with the consideration of statistically significant correlations. The resulting data are summarized in Tables 6 (for head and neck), 9 (for trunk) and 12 (for extremities). The positive or negative correlations between the variables determine the corresponding changes in their content irrespective of significant difference in their quantitative fraction. Therefore, correlation analysis is an independent observation which provides the trend of correlation among two inter-complimentary variables.
3. **Ratio value evaluation**: Various ratio values of dermal collagen and elastic fibers between two orientations (horizontal and vertical) helps in understanding their relative proportional differences in two directions of skin samples obtained (Tables 13 to 18).

6.1. **Quantitative fraction evaluation in head and neck region** (Tables 4 and 5)

Results of quantitative fraction (QF) assay of the dermal collagen and elastic fibers in the head and neck region can be summarized as follows.

The collagen content difference between the horizontal and vertical directions was found to be statistically significant at the scalp ($C_H$=53.63%, $C_V$= 49.7%, $p=0.027$), forehead ($C_H$=54.45%, $C_V$= 50.41%, $p=0.028$) and neck areas ($C_H$=53.53%, $C_V$= 49.53%, $p=0.024$). In all the above areas, the collagen fiber content is significantly higher in horizontal direction than in the vertical directions. The remaining two areas, submandibular area ($C_H$ versus $C_V$; $p=0.238$) and lateral canthal area ($C_H$ versus $C_V$; $p=0.301$) however did not show significant differences in its collagen fiber content between horizontal and vertical directions.

Significantly greater presence of elastic fiber content was observed in horizontal direction of forehead ($E_H$=10.37%, $E_V$= 8.30%, $p=0.036$) when compared to its vertical direction. Whereas, in the scalp ($E_H$=8.68%, $E_V$= 12.14%, $p=0.001$), submandibular area ($E_H$=13.60%, $E_V$= 18.47%, $p< 0.001$) and neck area ($E_H$=10.84%, $E_V$= 14.4%, $p=0.003$) elastic fiber content was significantly higher in vertical direction than the horizontal direction. Lateral canthus, however did not show any significant difference in the elastic fiber content between horizontal and vertical directions ($E_H$=12.08%, $E_V$= 11.01%, $p=0.125$) similar to its collagen fiber content.
This result can be discussed with the profound texture of the skin over the head and neck region. Where the skin is relatively thick and tense and subjected to stretch force during routine movement (as in scalp, forehead and neck area), the difference in QF of collagen between horizontal and vertical orientations is statistically significant. In other areas (lateral canthus and submandibular area) where skin is relatively loose and thin and is not subjected to stretch force during routine movements, the difference was not statistically significant.

Similar to collagen, the difference in the quantitative fraction of elastic fiber content between two directions was statistically significant in regions where the skin is thick and tense (scalp, forehead and neck). Contrary to collagen, the difference in the quantity of elastic fibers across two directions was found to be statistically significant at submandibular area, where the skin is relatively lax.

On the other hand at the lateral canthus; though there is noticeable difference in the content of collagen and elastic fibers between 2 directions, the difference was statistically insignificant. Even though it is not subjected to stretch force, there is a slight muscular force of underlying subcutaneous muscles.
6.2. **Correlation pattern evaluation in Head and Neck region** (Table 19, Figure 21)

Table 19.

*Correlation trend of dermal collagen and elastic fibers in horizontal and vertical directions at Head and Neck region*

<table>
<thead>
<tr>
<th></th>
<th>Between (C_H) and (C_V)</th>
<th>Between (E_H) and (E_V)</th>
<th>Between (C_V) and (E_V)</th>
<th>Between (C_H) and (E_H)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Scalp</strong></td>
<td>Low +ve ((r=0.48))</td>
<td>Moderate +ve ((r=0.60))</td>
<td>Low –ve ((r=-0.46))</td>
<td>-</td>
</tr>
<tr>
<td><strong>Forehead</strong></td>
<td>Moderate +ve ((r=0.63))</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Lateral canthus</strong></td>
<td>Strong +ve ((r=0.79))</td>
<td>Moderate +ve ((r=0.63))</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Sub mandibular</strong></td>
<td>Moderate +ve ((r=0.67))</td>
<td>Strong +ve ((r=0.72))</td>
<td>Strong –ve ((r=-0.73))</td>
<td>-</td>
</tr>
<tr>
<td><strong>Neck</strong></td>
<td>Moderate +ve ((r=0.61))</td>
<td>Low +ve ((r=0.40))</td>
<td>Low –ve ((r=-0.39))</td>
<td>-</td>
</tr>
</tbody>
</table>

\(r\) = Spearman’s correlation coefficient, +ve : Positive correlation, -ve : Negative correlation

\(C_H\) = Collagen fibers in horizontal direction, \(C_V\) = Collagen fibers in vertical direction, \(E_H\) = Elastic fibers in horizontal direction, \(E_V\) = Elastic fibers in vertical direction.

In the scalp area, low positive correlation between \(C_H\) and \(C_V\) and moderate positive correlation between \(E_H\) and \(E_V\) but low negative correlation between \(C_V\) and \(E_V\) were observed. As a result, when the collagen or elastic fiber content along the horizontal direction of scalp increases, similar amplification in their content is also observable at vertical direction and vice versa. But, mean time, if the collagen in vertical direction increases, the elastic fiber content in the same direction tends to decrease due to negative correlation between them.
In the forehead area, moderate positive correlation has been observed only between \( C_H \) and \( C_V \). As a result, when there is an alteration in the collagen content in horizontal direction, corresponding changes in its vertical direction are also observed. In the lateral canthal area, since there is strong positive correlation between \( C_H \) and \( C_V \) and moderate positive correlation between \( E_H \) and \( E_V \), the changes in the content of collagen or elastic fibers in horizontal direction also result in the corresponding alterations in vertical direction accordingly.

The submandibular area exhibits moderate positive correlation between \( C_H \) and \( C_V \) and strong positive correlation between \( E_H \) and \( E_V \). Therefore, the changes in collagen and elastic fibers content between two different orientations will be in positive manner individually. However there exists strong negative correlation between \( C_V \) and \( E_V \). This implies that the quantitative changes between collagen and elastic fibers in the vertical direction at submandibular area are oppositional. As a result, when the collagen content increases along vertical direction at submandibular area, the elastic fiber content tends to decrease or vice-versa.

Similar to scalp and submandibular area, there is a positive correlation between \( C_H \) and \( C_V \) (moderate positive) and between \( E_H \) and \( E_V \) (low positive) in neck area. Hence, when the collagen or elastic fiber content increases in horizontal direction, its increased content is also observable at vertical direction. However, due to low negative correlation between \( C_V \) and \( E_V \) alternative changes between collagen and elastic fibers could be observed at vertical direction.
Irrespective of significant or non significant differences in QF of collagen and elastic fiber content between the two directions, the association between them still persists in the form of correlation. The knowledge of these associations is clinically imperative in terms of understanding varied nature of scars in certain areas of human body.

Neck being high tension anatomical location, is prone for hypertrophic scar (Elliot et al., 1985; Muir, 1990; From & Assad 1993; Hawkins, 2007; Norman et al., 2008). The cheek and earlobes are prone to keloid formation (Niessen et al., 1999).

The glabella, nose and lateral canthal areas have conflicting RSTLs and lines of minimal tension where repeated muscular pull creates permanent skin creases that override the intrinsic tension lines of the skin. Probably this is the reason, in lateral canthal area, where no significant difference in QF of dermal collagen and elastic fibers across two orientations was observed from our study. In these areas, it is usually best to orient wounds and scars with the skin creases rather than the RSTLs for best cover-up (Tadros, 2007). Surgical incision when placed parallel with the skin cleavage lines over the zygomatic arch are found to result in attractive appearance (Shikimori & Motegi, 1986).

Histomorphometric analysis confirming asymmetrical content of dermal collagen and elastic fibers between two orientations of skin at different areas of head and neck region provide anatomical basis of explanation to earlier experience that, the scar placed in a particular direction in a given region gives better aesthetic result (Naveen et al., 2014a).
6.3. Quantitative fraction evaluation in trunk region (Tables 7 and 8)

Importance of skin lines in aesthetic approach gained much attention even though there is no clear explanation from researchers’ view. This made each researcher to attempt the explanation based on 3 factors of skin tensions; physical, anatomic, functional or experimental (empirical) methods (Borges, 1984).

This actuality is being supported by plastic surgeons, as in the living individuals gaping of the wound may be produced by anatomical (due to underlying dermal elastic content) or functional (as at joints) or physical (closure under tension) reasons. The later factor is dependent on surgeons in the act of closure of skin under tension; the scar is often unacceptable due to physical reason.

On the other hand, widely accepted Langer’s line has been established by the observation of gaping (elliptical shaped opening) on the skin by the puncture wound made in a particular direction. The direction which produced less gaping was considered as a Langer’s line of that particular area without any suitable reason (Borges, 1984). And also, one can explain the difference in collagen content in 2 different directions. The collagen content is meant for the strength of the structure or scar. Therefore, collagen content will often be more in one or both directions (horizontal /vertical) depending on the repeated stress due to associated elastic fiber content, physical stretching or functional reason. Accordingly, present findings can be justified as follows (Naveen et al., 2014b).
a. In the upper back area (supra-scapular area)

Anatomical cause was subsided, as there was no significant difference in the elastic fiber content between the two directions ($E_H$ versus $E_V$; $p=0.25$) of the sample taken. Hence it is expected that, anatomical factors may not be significant in determination of quality of scars at the upper back area. It is in accordance with the general experience of surgeons that, at upper back or supra-scapular area, scar in any direction is usually un-aesthetic. But, due to functional reason, as a result of repeated stretching over the back, by the flexion of spines in daily activities might tend to exert more stress in horizontal direction. Therefore, collagen content along the horizontal direction is mandatory to augment the strength of dermis as evident from the current findings, wherein statistically significant higher content of collagen was observed in horizontal direction ($C_{H}=51.90\%$, $C_{V}=49.20\%$, $p=0.03$).

b. Pre-sternal area

As in supra-scapular area, even at pre-sternal area the anatomical factor does not seem to play any role in the formation of unacceptable scar, due to status of elastic fiber content between 2 directions ($E_H$ versus $E_V$; $p=0.18$). Thus, Langer’s line concept for aesthetic scar will not be acceptable in pre-sternal area as is the general experience of surgeons. However, due to functional reason, where the repeated stretching in upward and oblique direction by the shoulder movements, and thus with the thoracic cage, the collagen content was expected to be more in horizontal direction than in vertical directions. This is evident from the result of current study wherein significantly higher collagen fiber content in horizontal direction ($C_{H}=52.30\%$, $C_{V}=47.50\%$, $p=0.01$) could be observed.
c. Lateral chest area

Anatomical cause factor in lateral chest area was also found to be playing an obscure role in the formation of an unacceptable scar, due to its outline of elastic content between two directions ($E_H$ versus $E_V$; $p=0.16$). The functional cause resulting from the expansion of chest during respiration leading to increase in the girth and thence excess stretch will be more along the horizontal direction. This necessitates significantly increased collagen fiber content in horizontal direction than in its vertical direction, which is evident from the resulting data of current study ($C_H=54.18\%, C_V=49.77\%, p=0.02$).

d. Abdomen:

Since, both the collagen and elastic content is significantly higher in horizontal direction in the abdomen region, an anatomical cause factor is mildly subsided. Continuous abdominal movement due to pushing down of abdominal content by the diaphragmatic contraction suits an appropriate functional reason. Increased collagen along the horizontal plane ($C_H=52.16\%, C_V=47.16\%, p=0.009$) explains more stress along the horizontal direction which is not due to anatomical reason as explained by elastic fiber content. The fact that increased elastic content in horizontal direction ($E_H=14.70\%, E_V=12.85\%, p=0.04$) is responsible for scar in vertical direction is unacceptable. Thus, Langer’s line concept holds well over the abdomen.
e. Groin:

Over the groin, the elastic fiber content is significantly higher in vertical direction than in the horizontal direction ($E_H=11.58\%, E_V=13.44\%, p=0.02$). Thus, scar along the crease expected to be under stress, giving rise to un-aesthetic result, which is contrary to routine observation by the surgeons. This may be explained by nullified action of elastic fibers by flexed posture of hip joint during most of the physical activities (functional). The collagen fiber content difference between two directions is insignificant ($C_H$ versus $C_V$; $p=0.25$).

6.4. Correlation pattern evaluation in Trunk region (Table 20 and Figure 22)

Table 20.

<table>
<thead>
<tr>
<th></th>
<th>Between $C_H$ and $C_V$</th>
<th>Between $E_H$ and $E_V$</th>
<th>Between $C_V$ and $E_V$</th>
<th>Between $C_H$ and $E_H$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Upper back</strong></td>
<td>Strong +ve $(r=0.75)$</td>
<td>Strong +ve $(r=0.77)$</td>
<td>Low –ve $(r=-0.37)$</td>
<td>-</td>
</tr>
<tr>
<td><strong>Pre- Sternal</strong></td>
<td>Moderate +ve $(r=0.56)$</td>
<td>Moderate +ve $(r=0.62)$</td>
<td>-</td>
<td>Moderate –ve $(r=-0.55)$</td>
</tr>
<tr>
<td><strong>Lateral chest</strong></td>
<td>Moderate +ve $(r=0.55)$</td>
<td>Low +ve $(r=0.43)$</td>
<td>Low –ve $(r=-0.43)$</td>
<td>-</td>
</tr>
<tr>
<td><strong>Abdomen</strong></td>
<td>Moderate +ve $(r=0.57)$</td>
<td>Moderate +ve $(r=0.69)$</td>
<td>-</td>
<td>Moderate –ve $(r=-0.57)$</td>
</tr>
<tr>
<td><strong>Groin</strong></td>
<td>Strong +ve $(r=0.77)$</td>
<td>Moderate +ve $(r=0.62)$</td>
<td>Low –ve $(r=-0.42)$</td>
<td>-</td>
</tr>
</tbody>
</table>

$r = $ Spearman’s correlation coefficient, +ve : Positive correlation, -ve : Negative correlation
($C_H$ - Collagen fibers in horizontal direction, $C_V$ - Collagen fibers in vertical direction, $E_H$ - Elastic fibers in horizontal direction, $E_V$ - Elastic fibers in vertical direction)
Among the areas of trunk region, upper back area shows strong positive correlation between $C_H$ and $C_V$ and also between $E_H$ and $E_V$. Lateral chest shows moderate positive correlation between $C_H$ and $C_V$ and low positive correlation between $E_H$ and $E_V$. Groin shows strong positive correlation between $C_H$ and $C_V$ and moderate positive correlation between $E_H$ and $E_V$ contents. But, all the above 3 areas exhibit low negative correlation between $C_V$ and $E_V$.

This indicates that, the content of collagen and elastic fibers in these areas changes in accordance with their positive correlation across horizontal and vertical directions. But when the collagen content in vertical direction increases, the elastic fiber content will decreases due to negative correlation between collagen and elastic fiber content in vertical direction.

In remaining two areas ie, pre-sternal and abdomen, moderate positive correlation was observed between $C_H$ and $C_V$, and between $E_H$ and $E_V$. But in both areas, moderate negative correlation was observed between $C_H$ and $E_H$.

Therefore, like wise afore mentioned 3 areas, changes in collagen and elastic fiber content between horizontal and vertical directions remain progressive. But along the horizontal directions of these areas, when the collagen content increases, elastic content decreases or vice-versa due to negative correlation between $C_H$ and $E_H$.

Hypertrophic scars usually affect the young individuals. Females are more prone for hypertrophic scars when compared to males for unknown etiology. The anterior chest, pre sternum, shoulders, deltoid regions are predisposed to
hypertrophic scars (Elliot et al., 1985; Muir, 1990; From & Assad 1993; Hawkins, 2007 and Norman et al., 2008). Anterior chest area is vulnerable for keloid formation (Niessen et al., 1999). Never the less, scar stretching occurs most frequently in the lower third of the scar overlying the xiphisternum and it may extend onto the abdomen (Eliot et al., 1985).

6.5. Quantitative fraction evaluation in Extremity region (Tables 10 and 11)

The quantitative fraction evaluation of dermal collagen and elastic fibers in the extremities of the body is highly intricate and subjective due to involvement of joints. The present study involved 3 joint areas which are under constant stretching (shoulder joint, wrist and ankle) and two other non stretchable areas (forearm and thigh). For the joint areas, the QF evaluation was made under two factors involving burst force exerted over the stretched skin of flexed/adducted joint (shoulder joint) and stretch force applied during movement at joints that are not acutely bent in rest position (wrist and ankle joint) (Naveen et al., 2014c).

a. Shoulder joint area

During normal adducted position at rest, the maximum stretch on the shoulder skin i.e. over the deltoid region is liable to cause burst force with the tendency to create wound in vertical direction. To counteract this force in nature, strength is required along horizontal direction. Thus, the collagen deposition (that provides strength) predominates along the horizontal direction. This is apparent from our result data in which significant higher collagen fiber content was observable in horizontal direction compared to the vertical direction ($C_H = 53.91\%$ versus $C_V = 49.03\%$, $p=$
0.005). To compensate between excess stretching and laxity on one or other surface of joint during movement, elastic fibers necessitate in increased concentration in vertical direction that is perpendicular to the joint line. This was confirmed with our findings in which elastic fiber content in vertical direction ($E_V=13.07\%$) was significantly higher than horizontal $E_H (10.34\%)$ direction ($p=0.011$).

Thus, when the scar is placed in vertical direction over deltoid region, the burst force during adducted position tends to exhibit wide and/ or hypertrophic scar in due course of time. On the other hand, when the scar is placed along horizontal direction, the elastic content in vertical direction is divided and exerts pull on the wound edge producing stretched and/ or hypertrophied scar (gaping). Also, the effect of gravity aggravates the force on the horizontal wound edge exerted by divided elastic fibers. When a surgical incision is made along the horizontal direction, the tension produced by elastic fibers on wound/scar edge is probably weaker than burst force due to adducted position (rest position); resulting scar will be better if incision is horizontally placed rather than in vertical direction.

b. Wrist and Ankle area

Similar to shoulder joint area, the functional correlation of elastic fibers in vertical direction is comparable with that of wrist and ankle area, as the quantitative fraction of elastic fiber content was significantly increased in vertical direction at the wrist ($E_H = 6.36\%$ and $E_V = 8.20\%$ with $p=0.014$) and at the ankle ($E_H = 5.06\%$ and $E_V= 8.14\%$ with $p=0.001$) parts.
But, in terms of collagen content, both wrist and ankle parts exhibit higher content of collagen in vertical direction which is contrary to shoulder joint area. However, the difference was observed to be statistically significant at wrist \((C_H \text{ versus } C_V, p=0.016)\) but insignificant at ankle \((C_H \text{ versus } C_V, p = 0.640)\). This diverged fact in collagen content may be attributed to anatomical (rest) position of the joint which in turn is comparable to the fact of burst force versus stretch force as shown in Table 21.

Table 21.

Various force effects applicable at joint areas in anatomical position and during various movements

<table>
<thead>
<tr>
<th>Force applicable</th>
<th>Shoulder joint</th>
<th>Wrist joint</th>
<th>Ankle joint</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burst force due to position</td>
<td>Maximum</td>
<td>Minimum</td>
<td>Moderate</td>
</tr>
<tr>
<td>Stretch force due to movement</td>
<td>Minimum</td>
<td>Maximum</td>
<td>Moderate</td>
</tr>
</tbody>
</table>

c. Forearm and Thigh area

Since there is a minimal effect of movement and gravity over the thigh and forearm skin, no significant differences in their collagen and elastic fiber content between horizontal and vertical directions were to be expected. The results of current study partially support this hypothesis in terms of elastic fiber content except that there was a significant difference at the thigh area \((E_H \text{ versus } E_V, p = 0.028)\). This becomes obligatory due to a possible stretch force produced by the slow circumferential tissue expansion of bulky thigh with growth of the body that necessitates deposition of more elastic fibers along the horizontal direction than
compared to vertical \((E_H > E_V, \ p = 0.028)\) direction. Contrary to this, the circumferential growth of forearm is less as compared to that of thigh region; the above observation as seen in the thigh is not seen at the forearm. As a result, there is no significant difference in elastic fiber content in forearm between the two directions \((E_H= 14.38\%, \ E_V= 14.28\%, \ p=0.892)\).

6.6. Correlation pattern evaluation in Extremity region (Table 22, Figure 23).

Table 22.

*Correlation trend of dermal collagen and elastic fibers between horizontal and vertical directions at extremities*

<table>
<thead>
<tr>
<th></th>
<th>Between (C_H) and (C_V)</th>
<th>Between (E_H) and (E_V)</th>
<th>Between (C_V) and (E_V)</th>
<th>Between (C_H) and (E_H)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shoulder joint</td>
<td>Moderate +ve ((r= 0.66))</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Wrist</td>
<td>Strong +ve ((r= 0.75))</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ankle</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Forearm</td>
<td>Strong +ve ((r= 0.75))</td>
<td>Moderate +ve ((r= 0.65))</td>
<td>Moderate –ve ((r= -0.51))</td>
<td>-</td>
</tr>
<tr>
<td>Thigh</td>
<td>Strong +ve ((r= 0.80))</td>
<td>Low +ve ((r= 0.42))</td>
<td>-</td>
<td>Moderate –ve ((r= 0.65))</td>
</tr>
</tbody>
</table>

\(r = \) Spearman’s correlation coefficient, +ve : Positive correlation, -ve : Negative correlation

\((C_H – \) Collagen fibers in horizontal direction, \(C_V – \) Collagen fibers in vertical direction, \(E_H – \) Elastic fibers in horizontal direction, \(E_V – \) Elastic fibers in vertical direction)
Among the joint areas, the positive correlation between \( C_H \) and \( C_V \) was observed at shoulder joint (moderate) and wrist areas (strong). No other significant correlation pattern was observed. The ankle did not show any significant correlations among the variables tested.

On the other hand, both forearm and thigh areas showed strong positive correlation between \( C_H \) and \( C_V \), and positive correlations between \( E_H \) and \( E_V \) for forearm (medium positive) and thigh (low positive). While, forearm exhibited moderate negative correlation between \( C_V \) and \( E_V \), the thigh area showed moderate negative correlation between \( C_H \) and \( E_H \).

Therefore, collagen and elastic fiber content will have negative correlation with each other in the horizontal direction of thigh area, whereas, similar pattern of correlation could be seen in vertical direction at forearm.

An interventional study performed on excision wounds of the trunk and extremities when treated with bi-layered closure procedure involving deep dermis revealed to having an overall better appearance of scar than sub-cuticular epidermal closure procedure of the same (Alum et al., 2006).

Hypertrophic scar is common in knees and ankles as they have a constant tension (Elliot et al., 1985; Muir, 1990; From & Assad 1993; Hawkins, 2007; Norman et al., 2008) whereas, shoulders, upper arms are said to be common sites for keloids (Niessen et al., 1999). A study on evaluating pattern of skin cleavage lines of cadaveric feet, suggested that incision should be along or parallel to skin cleavage
lines. If this is not completely possible due to operative requirements, then at least a large part of the incision should follow these lines (Andermahr et al., 2007).

Research studies performed on animal model reported an incremental mean area per unit of collagen fibers in their posterior limb than in abdominal area. This is because limb is exposed to continuous tensions during movements than other parts in animals (Sawsan, 2013).

Changes in the pattern of arrangement of collagen fibers also observed in the animal models with the inference of direction of extracellular matrix changes according to the type of stress and their movements (Houlbrooke et al., 1990; Zanna et al., 2012). This supports the anatomical arrangement of the collagen fibers that lie more or less parallel to epidermis in abdomen area when compared to perpendicular arrangement to the epidermis in posterior limb of animals (Sawsan, 2013)
6.7. Ratio value evaluation of dermal collagen and elastic fiber content in Head and Neck, Trunk and Extremity region

6.7.1 Collagen ratio value evaluation

- In areas with low significant ($C_v/C_h<1$) ratio values (Table 13), there is less distracting force on the horizontal wound edges during movements as compared to the burst force on vertical wound edge during rest period. Clinical experience suggests that lower is the value, better is the long term result of horizontally placed scar.

- In areas with high significance of ($C_v/C_h >1$) ratios (Table 14), the force exerted due to circumferential growth in the areas where body growth is less than the force produced in horizontal direction during movements (for example in lateral bending at lateral chest or during extension at wrist). Hence to provide maximum strength to the skin that has damaged due to maximum stretch during movement, collagen content in vertical direction should be more. In other words, more collagen deposition is expected in vertically placed wound with more distracting force on wound edge due to its orientation.

- In the areas with no significant ratios, (Table 15) various forces produced in different directions on skin due to movement of pull by underlying muscles and/ or growth were not much different. In this group, areas with lower ratio value (0.96 or less) clinically shows better acceptable scar, if wound is horizontally placed (probably due to relaxation produced by the underlying muscles). Areas with relatively higher ratio values (0.99-1.04) show better scar in vertical direction. Areas with very high ratio values (1.01; CV>CH), more
collagen content probably signifies more pull on the horizontally placed scar by underlying muscle (as the role of platysma in submandibular area) and moderate effect of both burst force and stretch force (as in ankle area).

6.7.2. Elastic ratio value evaluation (Tables 16 to 18)

- In areas with low significant ratio values, frequent movement (if any) in vertical direction or rotation and slow expansion causes maximum stretch force in horizontal direction.

- Whereas, the constant stretching in vertical directions as we see in the areas where in high significant ratio value could be appreciated, is probably due to movement or stretching in vertical direction by embryologic growth pattern and gradual growth after birth.

- In the areas, where no stretching or minimal stretching in both directions is observed, no significant difference is expected.