Cutaneous Expression of Estrogen, Androgen and Glucocorticoid Receptors in Striae Distensae

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Abstract

Background: Stretch marks or striae distensae (SD) can be considered a common skin disorder, but their physiopathogenic mechanisms have not been totally clarified. Although it is considered an esthetic complaint, it may have serious psychosocial consequences besides the local and systemic alterations of the conjunctive tissue. This study aims at assessing and quantifying the estrogen, androgen and glucocorticoid receptors in skin samples with striae and comparing with normal skin.

Methods: Skin samples for biopsy were obtained from thirty patients with (SD) (10) patients following systemic corticosteroid therapy and (10) patients following pregnancy, and (10) from obese patients and (20) normal controls. Tissue specimens were immediately fixed in neutral buffered formalin (10%) for 24 hours and underwent processing to assess histopathological features by H&E stain for all slides to be studied by ordinary microscopy and to estimate Estrogen, Androgen and Glucocorticoid receptors expressions by immunohistochemical analysis as anti-Estrogen & anti-androgen were performed for all biopsies using Avidine-Biotin complex technique while anti glucocorticoid was performed for all biopsies using (AEC) technique.

Results: When the estrogen receptor in the skin with (SD) was compared with healthy skin, it was significantly higher in healthy skin than skin with (SD), while it was found that androgen and glucocorticoid were higher in skin with (SD) than healthy skin.

Conclusions: These findings indicate that regions undergoing greater mechanical stretching of the skin may have altered hormonal receptor expression and this varied expression may influence the metabolism of the extracellular matrix, causing the formation of (SD). The preliminary results appeared to be relevant and represented an initial step towards an understanding of the pathophysiology of (SD).


Introduction

STRIAEDISTENSAE (SD) or (Stretch Marks) are a common disfiguring skin disorder of significant cosmetic concern. Striae distensae are well defined linear atrophic skin lesionsand secondary to connective tissue abnormalities, changes in weight or muscle mass and skin tension, leading to dermal damage produced by progressive or rapid stretching of the dermis [1-3]. In the early stages, striae may appear pink to red (Striae Rubra) which over time become atrophic and attain white color (Striae Alba) [4]. Striaedistensae are often found on the abdomen, thighs, buttocks, breasts, and sacro-lumbar region, and are often associated with the topical [5] and systemic [6] use of corticosteroids, adolescence, pregnancy, infectious processes such as tuberculosis and typhoid fever, a rapid gain or loss of weight, Marfan and Cushing syndromes, tissue expansion [7] augmentation mammoplasty [8] and tension-requiring skin sutures. Hence, their origin is multifactorial and, despite several studies, their physiopathogenic mechanisms are not entirely understood [9]. The histology of stretch marks is that of a scar, and the development of SD has been likened to that of wound healing or scar formation [10]. Some authors believe that SD result from an initial inflammatory reaction that destroys collagen and elastic fibers, followed by the regeneration of collagen and elastic fibers in the direction imposed by mechanical forces. Lesions appear as a result of the stretching and rupture of rigid cross-linked collagen and “elastic” unlinked collagen, thus permitting a limited degree of stretch and limited intradermal rupture [11]. The inflammatory condition that appears seems to be an unresolved question; however the pathogenesis of (SD) is still unknown, but probably relates to changes in the structures that provide skin with its tensile strength and elasticity. Such structures include components of the extracellular matrix (ECM), including fibrillin, elastin and collagen [9]. Currently, the etiologic mechanisms involved in (SD) are usually classified under the headings of “Genetic Predisposition”, “Biochemical or Hormonal Disorders” and
“Mechanical Disorders”. Hormonal factors as other studies have indicated the interference of estrogen in the mechanism of wound repair and extracellular matrix reorganization \[12\], the participation of estrogens \[13\] and androgens \[14\] in skin homeostasis. It is also known that; under physiologic conditions, glucocorticoids regulate the synthesis of glycosaminoglycans in skin fibroblast culture. Moreover, topical corticosteroids reduce collagen synthesis and induce skin atrophy \[9\].

Material and Methods

Skin biopsies were obtained from 30 patients with (SD) from Dermatology & Andrology outpatient Clinic of Benha University Hospitals during the period from Jun.; 2012 – Dec.; 2014.

Inclusion criteria:

Thirty patients with SD of middle age and of both sexes with striae divided into three groups:

Group (1): Consists of 10 female patients suffering from striae caused by pregnancy.

Group (2): Consists of 10 obese patients suffering from striae.

Group (3): Consists of 10 patients suffering from striae after using local or systemic cortisone therapy.

In addition, 20 healthy volunteers were chosen as control subjects for comparison of the results. Biopsies were taken from normal skin of outpatient's clinics for irrelevant conditions. Considering that both patients and controls were sex and age matched.

Exclusion criteria:

- Patients with history of hemorrhagic disease.
- Diabetic patients as they are more liable for infection and delayed healing at sites of punch biopsies.
- Patients with a history of diseases more liable for koebner phenomenon like psoriasis and vitiligo.
- Patients with history of keloid or hypertrophic scar.

Punch biopsies from striae sized (4mm) after sterilization with alcohol (70%) under local anesthesia with (2cm) carbol using a disposable punch needle of 4ml diameter; tissue specimen were immediately fixed in neutral buffered formalin (10%) for 24 hours, transferred to ascending grades of alcohol, cleared in xylene then impeded in paraffin wax. Sections were cut at (3:5) micron thick by rotatory microtome then dewaxed in xylene and rehydrated in decreasing grades of alcohol & stained by H&E stain to be studied by ordinary microscopy to assess histopathological features. Tissue sections for immunohistochemical stain were cut on adhesive positive charged glass slides and prepared as follows:

Anti-Estrogen & anti-Androgen were performed for all biopsies using Avidine-Biotin complex technique while anti-Glucocorticoid was performed for all biopsies using (AEC) technique. Sections were cut at (4um) & mounted on positively charged slides. Sections were deparaffinized in xylene and rehydrated through decreasing grades of ethyl alcohol.Slides were put in a slide rack & placed in a koplin jar containing solution of citrate monohydrate buffer (PH 6.0) for antigen retrieval. Koplin jar was placed in a water bath to keep a humid atmosphere inside the microwave oven where they undergo 3 treatments for each specimen at (1000, 800 & 600 wave). Endogenous peroxidation was inactivated by immersing the sections in 3 % hydrogen peroxide for (1 min). One to two drops of primary monoclonal antibody (AR & ER) (Neomarker, Labvision, USA) Prediluted ready to use & GR (Santa Cruz Biotechnology, Santa Cruz, CA, USA) diluted (1: 100) were applied to each section. Slides were incubated in humid chamber for 1hr at room temperature. The sections were incubated for (20min) with biotinylated solution and then sections were rinsed with distilled water that is followed by another (20min) of incubation with streptavidin solution. The chromogen reaction was developed with diaminobenzidine (DAB) and all sections were counterstained with Meyer’s haematoxyline. Positivity was considered when there was nuclear staining to (AR & ER). Sections were screened on low power while (AEC) single solution (Invitrogen Cat. No. 00- 1111), (DAB) kit for (GC) were done.

Positive controls: Breast tissue for ER, Prostatic tissue for AR and Adrenal tissue for GR.

Interpretation of results:

ER, AR & GR Status: Either Negative (–) or Positive Nuclear Immunostaining that was Interpreted according to intensities as Mild (+), Moderate (++) and severe ( +++ ) \[15-17\].

Results

The study included 30 patients with SD; 10 males (33.3%) and 20 females (66.7%) and 20 controls; 7 males (35.0%) and 13 females (65.0%). The ages of the patients ranged from 22 to 42 years
with mean values of 31.66 in group (A) and that of the control from 22 to 45 years with main value of 31.40 in group (B). There was no significant difference between age in patient group and control group (p > 0.663), also there was no significant difference between the different groups of the patients due to different causes as regarding age of patients which was divided into 3 groups (striae due to pregnancy, striae due to obesity and striae due to cortisone therapy) (p-value 0.145), also there was no significant difference of expression of oestrogen and androgen due to different cause while there was significant difference in expression of glucocorticoid receptors regarding the cause.

**Histopathology:**

SD showed perivascular lymphocytic infiltrate around the venules especially in recent lesions. Collagen bands on the upper third of the reticular dermis were stretched and aligned parallel to the surface of the skin. There was thinning of the epidermis due to flattening of the rete ridges and loss of collagen and elastin especially in later stages. In addition, hair follicles and other appendages were absent. ER, AR and GR were observed in epidermal keratinocytes and dermal fibroblasts.

- **Hormone receptors expressions in SD patients and control groups:**

  According to the statistics illustrated in the Table (1), out of 30 patients with striae; 22 (73.3%) patients showed mild intensity (+) of ER expression, 8 (26.7%) patients showed moderate intensity (++) of ER expression (Fig. 2).

  On the otherhand, out of 20 healthy volunteers; there is 5 (25%) of the healthy control with mild intensity of ER expression, 12 (60%) showed moderate intensity of ER expression and 3 (20%) of patients showed high intensity of ER expression (Fig. 1).

  As we observe here, the moderate and high intensity of ER expression was more in the healthy control group than in the patients group, while most SD patients expressed mild intensity of ER and none of them showed high intensity expression.

  Hence; there was significant difference between ER in patients and control groups as (p-value 0.001).

  According to statistics illustrated in the Table (1), out of 30 patients with striae; 8 (26.7%) patients showed mild intensity (+) of AR expression, 18 (60%) patients expressed moderate intensity (++) of AR and 4 (13.3%) of patients showed high intensity (++++) of AR expression (Figs. 4,5).

  On the otherhand, out of 20 healthy volunteers; 16 (80%) showed mild intensity of AR expression, 4 (20%) showed moderate intensity of AR expression (Fig. 3).

  As we observe here, most patients with SD showed moderate and high intensity of AR expression, while most of healthy control group expressed mild intensity AR and none of them showed high intensity of AR expression.

  So there was significant difference between AR in patients and control groups as (p-value 0.001).

  According to the statistics illustrated in the Table (2), out of 10 patients with striae due to pregnancy; 6 (60%) patients showed mild intensity (+) of GR expression, 12 (40%) patients showed moderate intensity (++) of GR expression and 7 (23.3%) of patients have high intensity (++++) of GR expression (Figs. 7,8).

  On the otherhand, out of 20 healthy volunteers; 15 (75%) showed mild intensity of GR expression, 5 (25%) showed moderate intensity of GR expression and there is no healthy control with high intensity of GR expression (Fig. 6).

  We can observe here that most patients with SD showed moderate and high intensity of GR expression, while most healthy volunteers expressed mild intensity GR expression and none of them expressed high intensity for GR expression.

  So there was significant difference between GR in patients and control groups as (p-value 0.012).

- **Hormone receptors expressions in SD patients regarding cause:**

  According to the statistics illustrated in the Table (2), out of 10 patients with striae due to pregnancy; 6 (60%) patients showed mild intensity (+) of ER expression, 4 (40%) patients showed moderate intensity (++) of ER expression and there was no patients with high intensity (++++) of ER expression.

  While out of 10 patients with SD due to obesity; 7 (70%) patients showed mild intensity of ER expression, 3 (30%) showed moderate intensity of ER expression and there is no patient with high intensity of ER expression.

  Whereas out of 10 patients of striae due to cortisone therapy; 9 (90%) of patients express mild intensity of ER, 1 (10%) expressed moderate intensity of ER and there was no patients with high intensity of ER expression.
Fig. (1): Normal skin (control) stained by immunoperoxidase DAB technique showed marked intensity (+++) of brownish nuclear expression of ER receptors (X 630).

Fig. (2): Striae distensae in obese patient stained by immunoperoxidase DAB technique showed mild (+) nuclear expression of ER receptors (X 400).

Fig. (3): Normal skin (Control) stained by immunoperoxidase DAB technique showed mild nuclear expression (+) of AR receptors (X 630).

Fig. (4): Striae distensae caused by cortisone therapy stained by immunoperoxidase DAB technique showed marked intensity (+++) of brownish nuclear expression of AR receptors (X 400).

Fig. (5): Striae distensae in obese patient stained by immunoperoxidase DAB technique showed moderate intensity (+++) of brownish nuclear expression of AR receptors (X 630).

Fig. (6): Normal skin (Control) stained by immunoperoxidase AEC technique showed mild intensity (+) of reddish nuclear expression of GR receptors (X 630).

Fig. (7): Striae distensae caused by cortisone therapy stained by immunoperoxidase AEC technique showed marked intensity (+++) of reddish nuclear expression of GR receptors (X 400).

Fig. (8): Striae distensae caused by cortisone therapy stained by immunoperoxidase DASE AEC technique showed marked intensity (+++) of reddish nuclear expression of GR receptors (X 400).
And so there was insignificant difference in ER intensity regarding the cause of striae ($p$-value 0.303).

According to the statistics illustrated in the Table (2) above, out of 10 patients with striae due to pregnancy; 5 (50%) patients showed mild intensity (+) of AR expression, and 5 (50%) patients showed moderate intensity (++) of receptors expression and there was no patients with high intensity (+++) of AR expression.

While out of 10 patients with SD due to obesity; 2 (20%) patients showed mild intensity of AR expression, 5 (20%) expressed moderate intensity of AR and 3 (30%) patients showed high intensity of AR expression.

And out of 10 patients of striae due to cortisone therapy; one (10%) patient showed mild intensity of AR expression, 8 (80%) expressed moderate intensity of AR and 1 (10%) patient expressed high intensity of AR expression.

And so there was insignificant difference in AR expression regarding the cause of striae ($p$-value 0.101).

According to the statistics illustrated in the Table (2), out of 10 patients with striae due to pregnancy; 6 (60%) patients expressed mild intensity (+) of GR, 4 (40%) patients showed moderate intensity (++) of GR expression and there was no patients with high intensity (+++) of GR expression.

While out of 10 patients with SD due to obesity; 5 (50%) patients showed mild intensity of GR expression and 5 (50%) patients showed moderate intensity of GR expression and there was no patients with high intensity of GR expression.

And out of 10 patients of striae due to cortisone therapy, 3 (30%) patients showed moderate intensity of GR expression and 7 (70%) showed high intensity of GR expression.

And so there was significant difference in Glucocorticoid receptors intensity regarding the cause of striae ($p$-value 0.001).

According to Table (3); there was statistically significant positive correlation between the three receptors (Estrogen, Androgen and Glucocorticoid) in patients of striaedistensa as ($p$-value 0.001).

And finally after statistical analysis of these tables and figures we concluded that patients with-SD express more Androgen and Glucocorticoid receptors than healthy volunteers while healthy volunteers express more ERs than striae patients.

Table (1): Estrogen, androgen and glucocorticoid receptors expressions in all cases.

<table>
<thead>
<tr>
<th>Intensity of expression</th>
<th>Estrogen R</th>
<th>Androgen R</th>
<th>Glucocorticoid R</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients</td>
<td>Control</td>
<td>Total</td>
</tr>
<tr>
<td>Mild</td>
<td>22 (73.3%)</td>
<td>5 (25%)</td>
<td>8 (26.7%)</td>
</tr>
<tr>
<td>Moderate</td>
<td>8 (26.7%)</td>
<td>12 (60%)</td>
<td>18 (60%)</td>
</tr>
<tr>
<td>Marked</td>
<td>3 (15%)</td>
<td>3 (6%)</td>
<td>4 (13.3%)</td>
</tr>
<tr>
<td></td>
<td>30 (100%)</td>
<td>20 (100%)</td>
<td>50 (100%)</td>
</tr>
</tbody>
</table>

Chi-Square $\chi^2$ $p$-value

Table (2): Estrogen, androgen and glucocorticoid receptors expressions in striae patients regarding the cause.

<table>
<thead>
<tr>
<th>Intensity of expression</th>
<th>Estrogen R</th>
<th>Androgen R</th>
<th>Glucocorticoid R</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pregnancy</td>
<td>Obesity</td>
<td>Cortisone</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>Total</td>
<td>Total</td>
</tr>
<tr>
<td>Mild</td>
<td>6 (60%)</td>
<td>7 (70%)</td>
<td>9 (90%)</td>
</tr>
<tr>
<td>Moderate</td>
<td>4 (40%)</td>
<td>3 (30%)</td>
<td>1 (10%)</td>
</tr>
<tr>
<td>Marked</td>
<td>3 (30%)</td>
<td>1 (10%)</td>
<td>2 (20%)</td>
</tr>
<tr>
<td></td>
<td>30 (100%)</td>
<td>10 (100%)</td>
<td>10 (100%)</td>
</tr>
</tbody>
</table>

Chi-Square $\chi^2$ $p$-value
Table (3): Comparison between estrogen, androgen and glucocorticoid receptors expressions in striae patients.

<table>
<thead>
<tr>
<th>Intensity of expression</th>
<th>Estrogen R</th>
<th>Androgen R</th>
<th>Glucocorticoid R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild N (%)</td>
<td>22 (73%)</td>
<td>8 (26.7%)</td>
<td>11 (36.7%)</td>
</tr>
<tr>
<td>Moderate N (%)</td>
<td>8 (26.7%)</td>
<td>18 (60%)</td>
<td>12 (40%)</td>
</tr>
<tr>
<td>Marked N (%)</td>
<td>4 (13.3%)</td>
<td>7 (23.3%)</td>
<td>7 (23.3%)</td>
</tr>
<tr>
<td>Total N (%)</td>
<td>30 (100%)</td>
<td>30 (100%)</td>
<td>30 (100%)</td>
</tr>
<tr>
<td>Chi-Square X²</td>
<td></td>
<td>18.678</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Discussion

Striae distensae or stretch marks were described as a clinical entity hundreds of years ago; they are a common disfiguring cutaneous condition, characterized by linear smooth bands of atrophic appearing skin which occurs in areas of dermal damage produced by stretching. The exact etiology still remains controversial and this is partly due to the variability in the clinical situations in which striae arise. The pathogenesis of striae is unknown but probably relates to changes in the components of extracellular matrix, including fibrillin, elastin and collagen [18].

The present study aimed to study the expression of estrogen, androgen and glucocorticoid receptors in normal skin and SD to explore the proposed role of hormonal factors in this disease and if there was a difference in the expression of each hormone in striae patient and normal volunteers (<i>p</i> < 0.05), as we found that androgen and glucocorticoid receptors were more expressed in patients with striae than normal volunteers while estrogen receptor was more expressed in healthy volunteers than in the patients with striae.

There was insignificant difference in estrogen and androgen receptor expression regarding the cause but there was significant difference with glucocorticoid receptor, as we found that glucocorticoid receptors were more expressed in patients with SD caused by glucocorticoid therapy than caused by pregnancy or obesity.

In agreement with us Gilliver et al., [14] reported that similar to scarring; for SD to occur, there must be a reorganization and restructuring of ECM, dependent on the secretion of proteins capable of initiating the degradation of ECM macromolecules, coordinated by hormonal stimulation and explained the occurrence of SD due to shift in the balance between serum estrogen and androgen levels.

Also in support for our results; Strudwick et al., [20] reported that male genotype was a strong positive risk factor for impaired healing in the elderly and castration of male mice results in improved cutaneous wound healing associated with dampened inflammatory response and increased matrix deposition. The presence of ARs in chronic wound healing also suggests that AR may bind to androgen response elements and regulate the expression of various target genes, including KGF, and the presence of ER in normal skin and wound fibroblasts suggests that local oestrogen levels may influence cutaneous physiology, including the wound healing process.

Also Sator et al., [21] observed improved skin moisture, elasticity, and thickness in postmenopausal subjects receiving 6 months of estrogen replacement therapy.

Gregory et al., [22] explained that the effective reduction in estrogen following menopause has been proposed as a cause of accelerated aging of human skin as collagen content as well as the ratio of type III to type I collagen in the skin showed a strong inverse correlation with years postmenopausal, this suggested that the accelerated collagen decrease was related to estrogen loss, rather than due to aging per se.

Shah and Maibach, [23] also found that epidermal thinning was associated with aging, and topical estradiol has been shown to reduce epidermal thinning in aging skin and maintain skin thickness.

Azzi et al., [24] have confirmed that administration of estradiol to gonadectomised mice increases epidermal thickness in both sexes.

Susan and Julie, [25] stated that an increase in the mitotic activity of epidermal keratinocytes occurred both in women and in vitro in response to estrogens and also ER in human skin may provide protection for the skin continuously exposed to oxidative damage due to UVB radiation.

Gilmore et al., [26] stated that reduced expression of fibronectin and of types I & III procollagen was found to be significantly reduced in fibroblasts from striae in comparison with normal fibroblast, suggesting that there was fundamental aberrations of fibroblast metabolism in striae distensae.

Son et al., [27] found increase in collagen and elastic fibers content in buttock skin from elderly males and females treated with topical estradiol
and type I procollagen, tropoelastin and fibrillin RNAs and proteins were significantly increased in both; yet more in females than in males. Similarly, immunohistochemical expression for type I procollagen protein was increased following estradiol treatment.

Brincat [28] explained that many women reported a sudden onset of skin aging several months after menopausal symptoms began as the menopause causes hypoestrogenism, accelerating age-related deterioration.

Sator et al., [21] found that hormone replacement therapy (HRT) has been shown to increase epidermal hydration, skin elasticity, and skin thickness and also reduces skin wrinkles.

Varila et al., [29] demonstrated an increase in collagen content following topical estrogen administration. They also demonstrated an increase in collagen synthesis as shown by increased type I & type III procollagen levels.

Stephen et al., [30] found that castrated rats heal wounds more rapidly and with reduced inflammation compared with controls. Furthermore, they have shown that blocking the conversion of testosterone to DHT accelerates healing and reduces wound IL-6 levels, suggesting that the negative effects of testosterone are through its metabolism to DHT. These findings may have important implications for male athletes who misuse DHT or other steroids that are metabolized to DHT.

In agreement with us; Raquel et al., [9] found that AR & GR in the SD skin had increased and they stated that they do so by inducing profound alterations on several parameters of ECM homeostasis, such as a decrease in collagen synthesis and altered expression of matrix metalloproteinases (MMPs) and their inhibitors. But this study disagrees with our results regarding ER as when they compared the estrogen receptor expression in the skin with SD with healthy skin and it was observed to have increased twice as much and this difference may be explained as the biopsies were taken from recent striae and it was analyzed by densitometric analysis of western blots.

On the contrary Rotsztejn et al., [31] studied an unusual case of large SD that appeared nearly all over the body although the patients receiving typical doses of steroids and the level of ACTH and cortisol were normal and they explained that due to a genetic factor and not due to hormonal factor which considered a very rare condition.

In contrast to our results; Irene et al., [32] found that long-term exposure to high GC concentrations in vivo was not associated with persisting adverse changes in skin fibroblasts and/or acceleration of the aging process of these cells. Also they found that collagen synthesis was comparable with normal following the removal of skin fibroblasts from the high GC milieu of Cushing’s syndrome patients. Moreover, skin fibroblasts from these patients exhibited an increased proliferative capacity in culture, a higher final cell culture density and an increased contractile ability. As these anabolic responses were in clear contrast to the catabolic effects of GCs which proved in our study this may be due patients with GC administration were usually exposed to a different pattern of GC excess or these anabolic responses which is in contrast to the catabolic effects of GCs can possibly represent a typical “rebound” reaction.

Contradicting our results, Filipe et al., [33] found that SD may be a common but under reported complication after breast augmentation and they owe this to exposure to higher estrogen levels and the important role of this hormone in facilitating the formation of SD. This difference may be explained by mechanical stretching due to augmentation and not due to oestrogen therapy.

**References**


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