Research Article

Leptin Receptor Expression in Obesity-Associated Acanthosis Nigricans

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Abstract

Background: Obesity-associated acanthosis nigricans is the most common type of acanthosis nigricans. **Aim of wok:** To detect leptin receptor expression in the skin lesion affected by obesity-associated acanthosis nigricans. **Patients and methods:** Twenty patients suffering from obesity-associated acanthosis nigricans were included in this prospective study. Complete history taking, thorough general examination and calculation of body mass index were done for all patients. Leptin receptor expression was investigated in skin lesion and perilesional skin by immunohistochemistry. **Results:** The mean age of the patients was 19.5 ±7.561 years and the mean duration of the disease was 8.6 ± 6.44 years. 14 patients were females and 6 patients were males. The mean BMI of the examined patients was (31.5 ± 4.4). The expression of leptin receptors was significantly higher in skin lesions than perilesional skin of the patients.

Conclusion: leptin receptor expression was significantly higher in skin lesions affected by obesity-associated AN.

Keywords: Obesity, Acanthosis nigricans , leptin.

Introduction

Acanthosis nigricans (AN) is a mucocutaneous disorder that is characterized by focal or diffuse symmetrically distributed hyperpigmented and hyperkeratotic lesions of the 1skin (Zekiiayi Kutlubay et al.; 2015).

Obesity-associated AN, formerly called pseudo-AN, which is associated with primary obesity, is the most common cause of AN in children and adults worldwide (Sinha S, Schwartz, 2007). Obesity-associated AN is weight dependent, and lesions may completely regress with weight reduction. Insulin resistance is often a primary feature in these patients (Garofalo et al; 2003).

Leptin is a hormone predominantly made by adipose cells that helps to regulate energy balance by inhibiting hunger (Brennan and Mantzoros, 2006).

In obesity, like the resistance of insulin in type 2 diabetes, a decreased sensitivity to leptin occurs, resulting in an inability to detect satiety despite high energy stores (Pan et al., 2014). Leptin receptor is expressed primarily in the hypothalamus but is also expressed in various tissues including peripheral blood mononuclear cells, endothelial cells and fibroblasts (Tartaglia, 1997).

Aim of the study

The aim of this study was to investigate the possible role of leptin in the pathogenesis of Obesity-associated AN.

Patients and Methods

This study included 20 obese patients (BMI ≥ 30) suffering from Obesity-associated AN were included in this prospective study. The selected candidates for this study were patients attending the outpatient clinic of Al-Azhar University hospital (Assiut) between January 2018, and July 2019. The diagnosis of Obesity-associated AN was done clinically and confirmed by histopathology. An informed consent was taken from all patients prior to the study. This study was approved by the Institutional Ethical Committee of the Al-Azhar university.

Exclusion criteria included pregnancy, endo-crine diseases lead to obesity, other causes of

AN, patients received medications interfere with leptin levels as glucocorticoids, antihyperlipidemic agents, and oral contraceptive pills and history of systemic diseases as diabetes mellitus, hypertension or hyperlipidaemia or patients with BMI <30.
BMI was calculated for all patients according to the following formula: BMI = Body weight (in Kilograms) + (Height in Meters)^2 .

After cleaning with antiseptic solution, local anaesthesia was given in the site of biopsy with 2% lignocaine. A 5 mm punch biopsy was taking from the skin lesion and another one was taking from perilesional skin in the same area.

The biopsies were sent to Pathology Department for routine tissue processing with paraffin embedded blocks formation. Two sections were cut from each block, one to be stained by haematoxylin and eosin staining for diagnosis, The second for immunostaining detection of leptin receptor.

The method used for immunostaining was streptavidin-biotin–amplified system. From each block, 4μm thick sections were cut on positive charged slides, which were subjected to subsequent steps of deparaffinization and rehydration. Antigen retrieval was performed by boiling in citrate buffer saline (pH 6) followed by cooling at room temperature. The primary antibody used was rabbit polyclonal antihuman leptin (Cat No. GTX130069 Gene Tex, Inc., North America), diluted (1:200) and incubated overnight at room temperature. Then the secondary antibody (Ultravision detection system anti-polyvalent Horseradish Peroxidase (HRP)/3,3'-Diaminobenzidine (DAB), ready-to-use, Neomarker was applied with DAB as a chromogenic substrate and Mayer's haematoxylin as a counter stain. Replacement of the primary antibody step with a blocking buffer was included in the staining procedure as a negative.

Cytoplasmic immunoreactivity in any number of cells was required to assign leptin positivity. Semi-quantitative evaluation was used to Staining intensity was classified according to the following criteria: +1 negative immune-staining weak staining in at least 25% of cells, +2 moderate expression in 25–50% of cells and +3 strong expression in ≥ 50% of the cells.

Statistical analysis

The SPSS program, version 21 (SPSS Inc., Chicago, IL) was used in statistical analysis. The data were expressed as mean ± SD and the differences were evaluated One-Sample t- test. Correlation between values were studied by linear regression test. P<.05 was set as statistically significant.

Results

Table (1): Comparison between percentage of leptin receptors expression in skin lesion versus perilesional skin of the patients (n=20).

leptin receptors expression (%)	Perilesional skin	Skin lesion	P-value
<5%	12.(60%)	0	
5- <25%	8. (40%)	0	
25- <50%	0	8 (40%)	
≥50%	0	12 (60%)	
Mean ± SD	1.40 ± 0.516	3.60 ± 0.516	0.000*

One-Sample t- test was used * Significant P < 0.05.

Table (1) : shows The expression of leptin receptors in normal skin was <5% in 12 (60%) of patients and 5-25% in 8 (40%) of patients. The expression of leptin receptors in lesional skinwas 25-50% in 8 (40%) of patients and >50% in 12 (60%) of patients, as shown in table (3), and figures (6,7).

Table (2): Correlation coefficient between leptin receptors in affected skin of the patients and other variables (n=20) .

	t-test	p- value
Age (Years)	-0.313	0.38
Sex	0.089	0.8
Weight	-0.561	0.09
Height	0.293	0.4
BMI	0.764	0.01*

Linear regression test was used
P. value <0.05 is significant

Table (2): A statistically significant positive correlation was seen between leptin receptor expression and BMI ,while no significant correlation between leptin receptor expression and patients age, sex or duration.

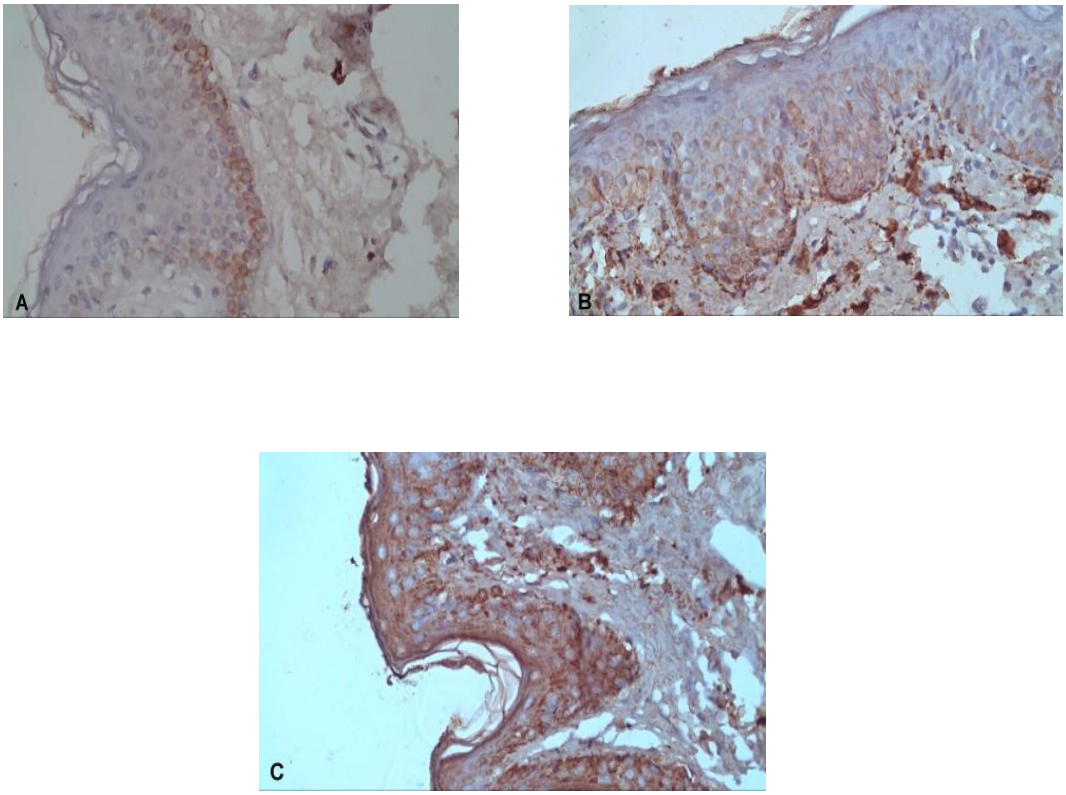

Fig. (1): Immunohistochemical staining of leptin receptor (x400). Leptin expression in perilesional normal epidermis mainly in basal layer with scattered immunoreactivity in suprabasal layers (A). Moderate and marked cytoplasmic expression of leptin in acanthosis nigricans (B&C) respectively.

Discussion

Obesity associated AN is a hyperpigmented skin disease associated with obesity and insulin resistance (IR). Hyperinsulinemia may inhibit IGFBP-1 synthesis and increase the synthesis of IGF-1 in the liver. The increase in IGF-1 circulating concentrations and IGF-1 receptor

activation may trigger dermal fibroblast and epidermal keratinocyte proliferation (Schwartz, 1994; Sinha and Schwartz, 2007; Phiske, 2014; González-Saldivar et al.; 2017).
As AN is linked to obesity and insulin resistance, major adipokines such as leptin, adiponectin, and resisting which are known to be dysregulated in obesity are considered key players in the pathogenesis of AN (Atwa et al., 2014).

In this study we aimed to investigate the expression of Leptin receptors in skin lesions of Obesity-associated AN.
In the present study, AN was more prevalent in adolescents. Previous reports support the notion that AN was frequently detected in adolescents, often begins in childhood (Kluczynik et al., 2012; Ng, 2016 and Ng et al., 2014).

We observed in the present study that 70% of patients were female. In contrast other studies reported that no sex predilection was observed in AN (Abraham & Rozmus, 2012; Brown et al., 2010; Kutlubay et al., 2015). This can be explained by environmental variation and different nutritional and physical habits between countries. In Egypt, obesity which is commonly associated with AN is more prevalent in females. The prevalence of obesity is almost double among females as compared to males (Alebshehy et al., 2016).

Leptin, a polypeptide hormone that is predominantly synthesized in adipocytes, including subcutaneous adipocytes (Klein et al; 2007). Leptin and leptin receptor expression in the human skin was validated and confirmed by real-time quantitative PCR (Johnston et al.; 2008).

Acanthosis nigricans is associated with disorders associated with insulin resistance, including obesity, type 2 diabetes, and the polycystic ovary syndrome (Cruz, Hud,1992). Hyperinsulinemia may also facilitate the development of AN indirectly by increasing the levels of free IGF-1 in the circulation (Nam et al; 1997). IGF-1 is expressed within the stratum granulosum and by dermal fibroblasts and an increase in bioactive IGF-1 facilitating the development of hyperkeratosis and papillo-matosis observed in acanthosis nigricans [Rudman et al.; 1997].

In the present study, the expression of leptin receptors was significantly higher in skin lesions than in nearby normal skin in obesity associated AN patient.
To our knowledge, after reviewing the published data through a detailed PubMed search, found no reports evaluating the expression of leptin receptors in obesity associated AN patient. The mechanism and role of leptin in pathogenesis of AN which depends on keratinocyte hyperproliferation. Leptin, in addition to its role in obesity, a strong mitogenic effect of leptin was observed in vitro on primary epidermal keratinocytes, which was dose dependent (Frank et al., 2000).

Also, in the present study there is a statistically significant positive correlation between leptin receptor expression in skin and BMI. Similar results were reported in an Omanian study (Al Maskari & Alnaqdy, 2006). In conclusion, the expression of leptin receptors is higher in affected than in normal skin of AN patients.

Conclusion

High leptin receptor expression is a good indicator in AN. AN is commonly associated with obesity and higher leptin receptor expression.

Recommendation

This study recommend to use anti-leptin in treatment of obesity- associatd AN. Also decreasing weight can helps in treatment of obesity-associated AN.

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