

Study of YKL-40 expression in chronic plaque psoriasis: a case controlled study

ABSTRACT

Background: Psoriasis is a common inflammatory skin disorder. The typical cutaneous lesions are papules and plaques covered with silvery scales distributed mainly on extensor aspects of extremities. The course and progression of the disease are unpredictable and vary in each patient. YKL-40 is a new inflammatory biomarker which may be involved the pathogenesis of the disease.

Objective: The aim of this study was to study YKL-40 expression in patients with psoriasis to evaluate its possible role in the pathogenesis of psoriasis.

Patients and Methods: This study included 30 patients with chronic plaque psoriasis and 10 healthy individuals of matched age and sex served as a control group. Punch biopsies of four mm were taken from the skin of psoriatic patients as well as from corresponding sites of control subjects. All specimens were examined by both haematoxylin and eosin stain and anti-YKL-40 stain.

Results: The intensity of YKL-40 expression was upregulated in both epidermis and dermis of psoriatic skin in comparison with normal skin of control subjects. YKL-40 expression in psoriatic skin showed significant positive correlation with Psoriasis Area and Severity Index (PASI) score.

Conclusion: YKL-40 involved in the pathogenesis of psoriasis manifested by its up-regulation in skin of psoriatic patients group in comparison to control group.

Keywords: YKL-40 – Psoriasis

Introduction

Psoriasis is a chronic, immune-mediated, inflammatory systemic disease with predominant skin manifestations that affects approximately 2-3% of the population worldwide. It presents clinically as well demarcated dusky red plaques covered with lamellated silvery scales with positive Auspitz sign. Although it can be classified as plaque, guttate, pustular and erythrodermic⁽¹⁾, about 80-90% of patients with Psoriasis have the plaque form of the disease⁽²⁾. The course and progression of the disease are unpredictable and vary in each patient. In the last few years, psoriasis has been recognized as a systemic disorder associated with comorbidities of various grades impacting on patients morbidity and mortality^(3,4). The disease has significant implications on physical, psychological and social wellbeing of the individuals.

YKL-40, also known as chitinase-3-like-1, is a 40-kDa glycoprotein which gene encoding is located on chromosome 1q31-q32⁽⁵⁾. The protein is secreted by numerous tissues with inflammation and cells such as activated macrophages and neutrophils which play important role in psoriasis⁽⁶⁾. Serum levels of YKL-40 were increased in a variety of inflammatory conditions such as rheumatoid arthritis and chron's disease⁽⁷⁾. Moreover, YKL-40 serum level was increased in some inflammatory skin diseases such as Ps, lichen planus and hidradenitis suppurativa⁽⁷⁾.

The aim of this study was to study YKL-40 expression in patients with psoriasis to evaluate its possible role in the pathogenesis of psoriasis.

Patients and Methods

This study was carried out on 30 patients with chronic plaque psoriasis who were collected from the Outpatient Clinic of Dermatology and Venereology Department, Tanta University Hospitals. Ten healthy individuals of matched age and sex served as a control group. Ethical approval was obtained from the Research Ethical committee before the commencement of the study. All subjects included in the study were newly diagnosed patients of psoriasis or who did not receive any systemic treatment for at least 4 weeks or topical treatment for at least 2 months before biopsy taking. Patients with any other dermatological or systemic disease, pregnant and lactating females were excluded from the study. After taking written consent, all participants were subjected

to complete history taking as well as general examination and detailed dermatological examination were done.

Skin biopsy

Four millimeter punch skin biopsies were taken from involved skin of psoriatic patients as well as from corresponding sites of control group. Biopsies were fixed in neutral formalin 10% and processed for two paraffin sections, each 4 micron thick, were cut from each block. One of them was stained by Haematoxline & Eosin to confirm the diagnosis while other section was cut on poly L lysine coated slide for immune-staining by YKL-40 antibody.

Staining procedure:

- 4µm-thick formalin-fixed paraffin-embedded tissue sections on the detection platform Dako Autostainer Link.
- The slides were deparaffinized, rehydrated and subjected to target retrieval in the pretreatment module, PTLINK (Dako, Glostrup, Denmark) at 95°C in Target Retrieval Solution High pH (Dako).
- Then the slides were cooled in TBS with 0.1% Tween (TBS-T).
- IHC reactions were performed using ImmPRESS Reagent Kit, Peroxidase, Anti-Goat IgG (Vector Laboratories, Burlingame, CA, USA).
- During the first stage, the endogenous peroxidase activity was quenched by incubation in Peroxidase-Blocking Reagent for 5 min.
- After rinsing in TBS-T, the slides were incubated with Normal Horse Serum for 20 min and subsequently with anti-YKL-40 (R&D Systems) diluted 1:50 in Antibody Diluent (for 30 min at room temperature).
- The slides were rinsed in TBS-T and incubated with secondary antibodies for 30 min.
- After rinsing in TBS-T, the slides were incubated for 10 min with diaminobenzidine.

- The slides were rinsed once again in TBS-T. Then the slides were counterstained with hematoxylin, rinsed in distilled water and dehydrated in graded ethanol alcohols and xylene.

Interpretation of YKL-40 expression: ⁽⁸⁾

YKL-40 expression assessment done as follows;

0= negative

1= mild

3= moderate

4= strong

Statistical analysis of the data

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp) Qualitative data were described using number and percent. The Kolmogorov-Smirnov test was used to verify the normality of distribution Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. Significance of the obtained results was judged at the 5% level.

Results

This study comprised 30 patients with Psoriasis vulgaris, 11 males and 19 females. The patient's demographics were shown in (**Table 1**).

Histopathological results

A. Results Haematoxylin and eosin staining

On studying the biopsies of the involved skin of Ps patients, there were parakeratosis, Munro's microabscesses, absent granular cell layer, test tube acanthosis with clubbing of the

rete ridges, thinning of the suprapapillary epidermis, elongation and tortuosity of blood vessels in the papillary dermis and superficial perivascular inflammatory infiltrate.

B. Results of YKL-40 expression were summarized in (Table 2)

Normal control skin showed a negative YKL-40 expression in 100% of specimens.

However, psoriasis specimens showed a positive YKL-40 expression in epidermis and dermis of all specimens with various degrees of intensity. It was mild in 43.3% of specimens, moderate in 50.0% of specimens, and strong in 6.7% of specimens. Hence, there was statistically significant upregulation of YKL-40 expression in both epidermis and dermis of psoriasis in comparison to control with p .value < 0.001 .

DISCUSSION

Serum levels of YKL-40 were increased in a variety of inflammatory conditions such as rheumatoid arthritis and chron's disease ⁽⁷⁾. Moreover, YKL-40 serum level was increased in some inflammatory skin diseases such as Ps, lichen planus and hidradenitis suppurativa. Previous studies have supported that YKL-40 has a role in the pathogenesis of psoriasis manifested by its up-regulation in serum of psoriatic patients ⁽⁹⁻¹²⁾. They concluded that, the increased YKL-40 may point out psoriatic patients with a higher level of systemic inflammation ⁽¹³⁾, and can be used as a new marker for evaluation of disease severity and progression in psoriasis ⁽¹⁴⁾.

This study evaluated the expression of anti-YKL-40 antibody in patients with psoriasis in comparison with normal control skin in order to through a light on its possible role in the pathogenesis of the psoriasis. It included 30 patients presented with psoriasis, in addition to 10 apparently healthy individuals of matched age and sex served as a control group.

In this study, the incidence of psoriasis was more in females (63.3%) than in males (36.7%). This was in the agreement with the result of **Parisi et al., 2013** ⁽¹⁵⁾ who reported slightly higher prevalence of psoriasis in females than males. However, most studies have found that psoriasis is

equally distributed in both sexes⁽¹⁶⁻¹⁸⁾. Women may experience earlier onset of disease but no differences exist in quality of life or in the morphology between male and female^(18,19).

In this study, the age of the psoriasis patients varied from 25 to 65 years with the mean age of 50.10 ± 10.30 and duration of the disease ranged from 0.50 – 40.0 with the mean duration of 9.33 ± 11.22 . **Johnson and Armstrong, 2013**⁽¹⁹⁾ reported that there is a bimodal age distribution for psoriasis presentation. They are typically presented between 20 and 30 years and between 50 and 60 years of age and approximately 75% of patients with psoriasis are presented prior to the age of 40. They also reported that disease onset at a younger age may predict more extensive disease with greater numbers of exacerbations in life. In comparison, patients presenting with psoriasis at an older age usually have a milder and more stable course. However, determination of the age of onset of psoriasis may be inaccurate as it depends on the patient recall and determining onset based on first visit to a physician could underestimate time of occurrence of the disease, as minimal disease may present for years before the consultation.

Regarding family history of the disease, it was positive in 26.7% of the studied patients, which was relatively in agreement with **Mohd Affandi et al.**,⁽²⁰⁾ who found that family history was positive in 23% of their patients. Others reported a big variation of positive family history from 2% to 91%^(21,22). **Basko-Plluska and Petronic-Rosic, 2012**⁽²³⁾ reported that patients with early disease onset tend to have positive family history of psoriasis while those with onset after the age of 40 usually have negative family history.

The current work revealed a statistically highly significant increase of YKL-40 expression in the skin of psoriatic patients in comparison to normal skin of control. These results agreed with a previous study done by **Imai et al., 2011**⁽²⁴⁾, who reported that YKL-40 might be a useful biomarker for psoriasis, and it can reflect the severity of skin lesions in psoriatic patients. **Salomom et al., 2017**⁽¹³⁾ reported that the serum level of YKL-40 in psoriatic patients is considerably elevated, and this protein may be involved in the pathomechanisms of psoriasis. **Abu EL-Hamd et al., 2019**⁽²⁵⁾ and **Baran et al., 2018**⁽¹¹⁾ found that there were higher levels of serum YKL-40 in patients with psoriasis

vulgaris compared with healthy control subjects. Likewise, **Jensen et al., 2013 and Ahmed et al., 2015** ^(12,26) reported that serum YKL-40 was higher in psoriatic patients than controls and in psoriatic arthritis patients than in psoriasis patients. On the other hand, **Ataseven and Kesli, 2016** ⁽²⁷⁾ observed no significant difference in the serum concentration of YKL-40 between psoriatic patients and the control group ⁽²⁷⁾.

Conclusion

In conclusion, the current study suggested a possible role of YKL-40 in the pathogenesis of psoriasis. It can be used as a new marker for evaluation of disease severity and progression in psoriasis. In addition it may open the way for a further therapeutic approaches in the treatment of psoriasis.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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Table 1: Distribution of the studied groups according to demographic data:

	patients (n=30)		Control (n=10)		Test of sig.	P
	No.	%	No.	%		
Sex						
Male	11	36.7	4	40.0	$\chi^2=$ 0.036	^{FE} p= 1.000
Female	19	63.3	6	60.0		
Age (years)						
Min. – Max.	25.0 – 65.0		32.0 – 59.0		t=1.485	0.146
Mean ± SD.	50.10 ±10.30		44.60 ±9.63			
Median (IQR)	50.0(45.0–60.0)		43.50(45.0–60.0)			

Family history	No	%
Negative	22	73.3
Positive	8	26.7

Duration since (years)	No.	%
<10	22	73.3
≥10	8	26.7

Table (2): Comparison between the two studied groups according to immunohistochemical expression of YKL-40 in the studied specimens:

YKL-40 expression	patients (n=30)		Control (n=10)		P
	No.	%	No.	%	
Negative	0	0.0	10	100.0	^{MC} _p <0.001*
Mild	13	43.3	0	0.0	
Moderate	15	50.0	0	0.0	
Strong	2	6.7	0	0.0	

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Figure 1: A 50-year old male patient presented with chronic plaque psoriasis on both lower extremities

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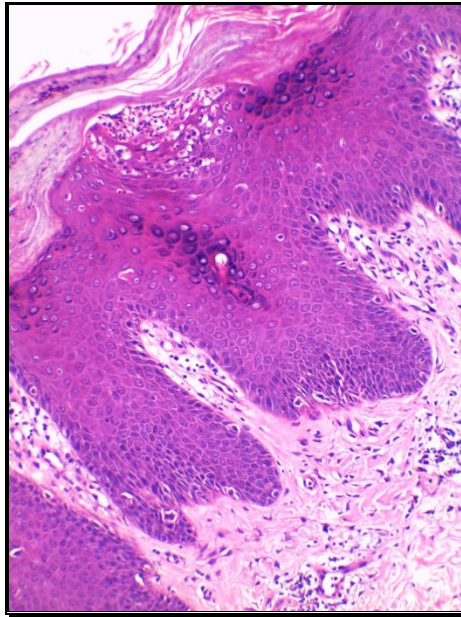


Figure 2: Psoriasis vulgaris shows moderate parakeratosis, Kogoj abscess, Munro's microabscess, absent granular cell layer and moderate acanthosis of the epidermis. The dermis shows moderate perivascular inflammatory infiltrate and moderate angiogenesis [H&Ex200].

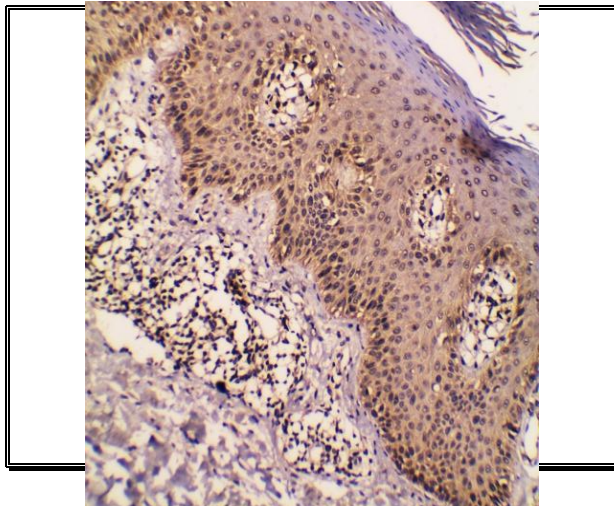


Figure 3: Chronic plaque psoriasis specimen showing moderate (++) expression of YKL-40 in the epidermis and dermis [IHCx200].

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