Journal of Pakistan Association of Dermatologists. 2021;**31(4)**:637-642.

Original Article

Role of autophagy related Gene 16 L1 in psoriasis pathogenesis

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Abstract

Background Psoriasis is a chronic hyperproliferative inflammatory disease, in which genetic and environmental factors have an important role, but the exact cause is yet unknown . Autophagy is a strictly regulated lysosomal degradation pathway that is crucial for maintaining intracellular homeostasis and normal development. The dysregulation of autophagy-associated genes was recognized to increase the susceptibility to multiple diseases, including inflammation, autoimmune disorders, and cancer.

Objective Our study aimed to detect the expression of autophagy related gene 16 L 1(ATG 16L) in psoriasis patients compared to normal control persons to investigate the possible role of autophagy in pathogenesis of this disease.

Methods This case - control study included thirty psoriasis patients and thirty healthy controls .Four mm punch skin biopsies were obtained from psoriasis lesions and from the controls and they were kept in lysis solution for the stability of the studied parameters and were kept frozen at -80 Celsius till analysis of ATG 16L using real time polymerase chain reaction.

Results The level of autophagy related gene 16 L1 in lesional skin of psoriasis was significantly increased compared to normal control persons ($p<0.001$).

Conclusion Autophagy may play a role in the pathogenesis of psoriasis disease.

Limitation Limited number of patients included in our study (30 patients).

Key words
Psoriasis, Autophagy, Autophagy-related gene 16 L1.

Introduction

Psoriasis is a chronic inflammatory skin disease that affects around 0.5%–1% of children and 2%–3% of the world's population.[1] Psoriasis is believed to be multifactorial with multiple key components such as genetic susceptibility, environmental triggers together with skin barrier disruption and immune dysfunction.[2]

Autophagy is a process that is present in all cells at low levels under normal conditions, but many stimuli like hypoxia or starvation may lead to its upregulation. Cytoplasmic components are broken down into basic components and returned to be reused in the cytosol.[3]

Autophagy is mediated by an organelle called autophagosome. As autophagosomes engulf a part of cytoplasm, the autophagy is generally thought to be a nonselective degradation system

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.Besides mediating survival and homeostasis of the cells, autophagy is important in the antigen processing and presentation and in secretion of proinflammatory cytokines like type I-interferon and tumor necrosis factor-(TNF).[3]

The ATG16L1 molecule, which is encoded by the ATG16L1 gene (2q37), is a key component of a large protein complex essential for autophagy; and polymorphisms in Atg16L1 gene contributes to the risk of psoriasis vulgaris.[4]

The aim of our study was to evaluate role of Autophagy in pathogenesis of psoriasis through the evaluation of autophagy related gene 16 L1 expression in tissue biopsies from normal and psoriasis skin

Methods

Thirty (30) patients with psoriasis and thirty (30) healthy controls were enrolled in the study. All patients and healthy controls recruited from individuals attending the outpatient Clinic of Beni-Suef University Hospitals, Egypt in the period (from June 2019 to March 2020).

Exclusion criteria included the use of any topical or systemic treatment for psoriasis in the last 3 months, Patients with associated systemic, dermatological or other autoimmune disease.

Patient information was collected by single dermatologist (RMN) including age, sex, family history, extent of lesion and duration of the disease.

The aim of our study was explained to each patient, an informed consent was taken from each patient, the protocol of the study conforms to ethical guidelines of the 1975 Declaration of Helsinki as reflected in the *priori* approval by Institution Human Research Committee.

Estimation of ATG 16 L1 in tissue using quantitative reverse transcription polymerase chain reaction (qRT PCR)

RNA extraction

Total RNA has been isolated using Qiagen tissue extraction kit (Qiagen, USA) according to the instructions of manufacture then 30 mg of the human tissue sample was excised and placed directly into a suitably sized vessel for disruption and homogenization**.** The tissue was disrupted, lysed in lysis Buffer RLT and the lysate was homogenized by tissue homogenizer for 40 seconds. The lysate was centrifuged for 3min. at full speed and the supernatant had been removed carefully and transferred into a suitable microcentrifuge tube. One volume (350 µl) of 70% ethanol had been added to cleared lysate. Seven-hundred µl of the sample had been transferred to RNeasy spin column placed actually in a 2 ml collection tube and centrifuged for 15 sec. (at ≥8000) rpm. Five-hundred µl Buffer RPE was then added to RNeasy spin column, and then centrifuged for 15 s at (≥ 8000 rpm) to wash spin column membrane. RNeasy spin column had been placed in the new suitable 1.5 ml collection tube. 30-50µl RNase-free water had been added to spin column membrane, then centrifuged for 1 min at(≥8000 rpm)to elute RNA. The eluate was carefully transferred to the new suitable eppendorf tube and stored at -80 °C for further use. Purity (A260/A280 ratio) and concentration of RNA were then obtained using the spectrophotometry (dual wave length Beckman, Spectrophotometer, USA).

cDNA synthesis

Total RNA (0.5-2µg) was used for cDNA conversion using the high capacity cDNA reverse transcription kit Fermentas, USA). One µl of the random primers had been added to 10µl of RNA which had been denatured for 5 minutes

at 65°C in the thermal cycler. RNA primer mixture had been cooled to 4°C. the cDNA master mix had been prepared according to the kit instructions and was then added (for each sample) The last mixture had been incubated in programmed thermal cycler one hour at 42°C followed by inactivation of the enzymes at 95°C for 10 minutes, and then cooled at 4°C. RNA was then changed to cDNA. The converted cDNA was then stored at -20 °C.

Real-time quantitative polymerase chain reaction using SYBR Green I

Real-time qPCR amplification were performed by using 10µl amplification mixtures containing power SYBR Green PCR master mix (Applied Biosystem, StepOne Plus™, USA), equivalent to 8ng of reverse –transcribed RNA and 300nM primers,the sequences of PCR primer pairs used for each gene are shown in **(Table 1).** Reactions were then run on an ABI PRISM 7900 HT detection system. The PCR reactions consisting of 95°C for 10 min (1 cycle), 94°C for 15 s and 60°C for 1 min (40 cycles). Data were analyzed with ABI Prism sequence detection system software and quantified using the v1.7 Sequence detection software from PE Biosystems. Relative expression of the studied genes was then calculated using comparative threshold cycle method.

Statistical analysis

Data had been coded and entered using statistical package SPSS (Statistical Package for Social Sciences) version 3.1. Data had been summarized using mean, standard deviation in the quantitative data and using frequency (count) and relative frequency (percentage) for the categorical data. Suitable statistical tests were used (Chi-square (χ^2), one way ANOVA, one sample t-test, Person's and Spearman's correlation) whenever needed, P-values equal to or less than 0.05 were considered statistically significant.

Results

The gender ratio ,age were not substantially different for each variable among patients with psoriasis (20 men,10 women; mean±SD age (35.6±10.9) and healthy controls (22 men, 8 women, mean±SD age 31.1±6.4) . Clinical data of participants are presented in **(Table 2).**

Table 1 The primer sequence of the studied gene.

	Primer sequence	*Gene bank accession number*
ATG 16 L1	Forward primer: 5-CCTCGTGCCCTGGAGATTA3 Reverse primer: 5AGAACCGCATCAAAGAAAGC3	NM_173681

Table 2 Dermographic data, clinical characteristics of the psoriasis patients and controls.

	Patients(n=30)	*control(n=30)*
Gender n(%)		
Males	20 (66.7)	22 (73.3)
Females	10 (33.3)	8 (26.7)
Age (Years), Mean±SD	35.6 ±10.9	31.1±6.4
Duration of psoriasis (years)		
Mean±SD	4.7 ±4.0	-
Extent of psoriasis, Mean±SD	43.3± 23.9-	
Family history of psoriasis n(%)		
Negative	3 (10%)	-
Positive	27 (90%)	-
PASI, Mean±SD	20.8 ±7.3	-

PASI: Psoriasis Areas and Severity Index.
SD: Standard Deviation.

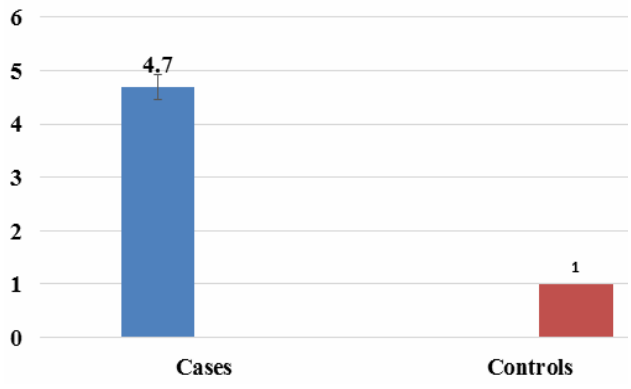

Figure 1 ATG 16 level

The tissue autophagy related gene 16 L1 expression. The expression level of ATG 16 L1 in psoriasis cases ranged from 2.2 to 9.10, with mean value of (4.7±1.8). While in controls, it ranged from 0.97 to 1.2, with mean value of (1±0.04) p value<0.001 **(Figure 1)**.

We found no relation between patient age, sex, disease duration, family history, pasi score with autophagy related gene 16 L1 expression both in lesional and nonlesional tissue biopsies.

Discussion

Psoriasis, a chronic skin disease is known to be the most prevalent autoimmune disease in humans. The precise aetiology of psoriasis remains poorly understood, but psoriasis is thought to result from a complex interplay between genetics, environment, skin barrier disruption, and immune dysfunction.[2]

Psoriasis shows traits of an autoimmune disease on an (auto) inflammatory background, with both mechanisms overlapping and even potentiating one another. This because an activation of the innate immune system driven by endogenous danger signals and cytokines characteristically coexists with an auto-inflammatory perpetuation in some patients, and T cell-driven autoimmune reactions in others. The pathogenesis of psoriasis can be conceptualized into an initiation phase possibly triggered by trauma (Koebner phenomenon), infection, or drugs and a maintenance phase characterized by a chronic clinical progress.[5]

Psoriasis shows clear autoimmune-related pathomechanisms include abnormal interactions among innate immunity, T cells, keratinocytes, etc. Disturbances in the innate and adaptive cutaneous immune responses are responsible for the development and sustainment of psoriatic inflammation.[6]

Immune cells in the patients release excess proinflammatory factors, leading to uncontrollable activation of congenital and acquired immune system, such as nuclear factor-κB (NF-kB) signaling pathway and differentiation of T helper (Th) cells toward Th1 and/or Th17 cells. The complex pathogenesis results in tissue and organ damage over time, manifested by hyperproliferation, inflammation, and other clinical syndromes at the lesion sites.[7]

Autophagy is a regulated lysosomal degradation process which is essential for maintaining the intracellular homeostasis and also normal development.[3] Autophagy is included in multiple innate and adaptive immune processes, like pathogen recognition and destruction, antigen processing for major histocompatibility complex (MHC) presentation, regulation of lymphocyte development, function and inflammation. After being stimulated by T cell receptor activation upon antigen recognition, autophagy can be induced in T cells and is required for T cell proliferation, differentiation, survival and death. Autophagy is important trafficking event in mediating T cell response and regulating T cell immunity.[3]

Defects in autophagy-related genes and recruitment of autophagy proteins are important

for autophagic dysfunction, the defect in regulation of autophagy-related genes was recognized to increase susceptibility to different diseases,such as inflammation, autoimmune skin diseases such as vitiligo and cancer.[6] Recently, studies have illustrated that autophagy is intricately related to skin diseases.[8]

There are studies that have shown that autophagy deficiency leads to inflammatory cytokine production and cell proliferation in KCs.[9]

The ATG16L1 gene provides instructions for making a protein that is required for a process called autophagy. Cells use this process to recycle worn-out cell parts and break down certain proteins when they are no longer needed. Atg16L1 deficiency affects the autophagy machinery on signaling pathways that regulate cytokine production and result in accumulation of damaged proteins and organelles that are toxic, leading to cell death, tissue damage, and chronic inflammation.[4]

All the above mentioned prompted us to investigate the possible role of autophagy in psoriasis and in order to do so, we estimated autophagy related gene 16 L1 level in tissue.

We reported the level of ATG 16 in cases of psoriasis ranged from 2.2 to 9.10, with mean value of (4.7±1.8). While in controls, it ranged from 0.97 to 1.2, with mean value of (1±0.04). ATG 16 L1 gene expression had no significant relation to patients' age, sex, disease course, family history, disease duration, lesion extension and PASI score

Conclusion

In conclusion, autophagy may play an important role in the pathogenesis of psoriasis, particularly through autophagy related gene 16 L1**,** it can be used as a biomarker to evaluate its progression and effect of therapeutic interventions.

Study limitation

Limited number of patients included in our study (30 patients) so additional studies on large number of cases is needed to determine its exact role in pathogenesis of psoriasis.

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