

Expression of Beclin-1 Gene in Patients with Genital Warts and Its Clinical Significance in Suez Canal University Hospitals

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ABSTRACT

Background: Genital warts are a common infectious sexually transmitted disease. Autophagy plays an essential role in eliminating pathogenic viruses and it is regulated by autophagy-related genes (ATGs). Beclin-1 gene is known to be one of ATGs, mediates autophagosome formation. Many viruses have evolved strategies to interfere with the formation or maturation of autophagosomes. There are lack of studies evaluating serum and tissue expression of Beclin-1 gene in genital HPV (human papilloma virus) infections and their correlation with the disease outcome.

Aim: To measure tissue expression of Beclin-1 gene and its protein level in plasma in patients with genital warts.

Patients and Methods: This cross-sectional study was conducted at Dermatology Outpatient Clinic and Andrology, Infertility and Sexually Transmitted Diseases Outpatient Clinic of the Suez Canal University Hospitals, Ismailia. The study included two groups; 30 patients with genital wart and 30 healthy controls. Beclin-1 gene expression in plasma and in tissue biopsies of 30 patients with genital warts was measured using real time polymerase chain reaction (PCR) and Beclin-1 protein level in plasma of both groups was measured using enzyme-linked immunosorbent assay (ELISA).

Results: Both Beclin-1 gene expression in plasma and Beclin-1 protein level in plasma and their tissue expression were significantly decreased in genital warts patients compared to controls.

Conclusion: Beclin-1 as an essential ATG with its critical role in the formation of autophagosome, which may affect cellular defense and immune response to pathogens, provides a promising therapeutic target for new treatment modalities of infectious diseases.

Keywords: Beclin-1, Genital warts, Autophagy.

INTRODUCTION

The prevalence of genital human papillomavirus (HPV) infections is believed to be 10% to 20%, with clinical symptoms occurring in 1% of cases. Infections with HPV are becoming more common. There are two types of HPV in the human genital tract and external genitalia: high-risk HPV and low-risk HPV groups based on their potential to cause cancer, including cervical intraepithelial neoplasia and cervical cancer ⁽¹⁾.

Through the transport of antigens for MHC-I and -II, the major histocompatibility complex classes I and II, presentation, autophagy is a crucial mechanism for the adaptive immune response to infection. When three things suppress autophagy-methyladenine (3-MA), a PI3K class III inhibitor that prevents the production of autophagosomes, HPV infectivity rises. As a result, these studies point to autophagy as having a crucial function in avoiding HPV infection ⁽²⁾.

Beclin-1, a protein encoded by the Beclin-1 gene and a key component of the Class III PI3K complexes, plays a vital role in regulating autophagy and other processes related to membrane trafficking. Without Beclin-1, the stability of other members of the Class III PI3K complex (such as ATG14 and UVRAG) is compromised. Beclin-1 is crucial for interconnected communication involves protein-protein interactions, phosphorylation, ubiquitination, cleavages, and other biological processes between autophagy, apoptosis, and cell proliferation ⁽³⁾.

Beclin-1 expression dramatically increased in patients with chronic hepatitis who tested positive for the disease as their hepatitis stage advanced and as

cirrhosis developed. positive for the HCV, and it significantly decreased in HCC cases compared to hepatitis or normal tissue ⁽⁴⁾. As HPV induces cervical transformation in infected tissues, Beclin-1 expression was found to decline in samples of dysplastic cervix and even more so in samples of cervical cancer. This suggests a progressive downregulation of the autophagic response ⁽⁵⁾. Infectious disease treatment may take a new turn considering how vital autophagy is to the elimination of infections, including pathogenic bacteria and viruses. The relationship between HPV and autophagy is crucial for the potential development of new anti-viral tactics intended to reduce HPV infectivity ⁽⁶⁾. We did this work to emphasize the potential function of Beclin-1 gene in genital warts pathogenesis in order to improve the management strategies of this prevalent venereal disease because there are not enough studies about the expression of Beclin-1 gene and its biologic role in genital warts. So, this study aim was to improve the management of genital warts infection by studying Beclin-1 gene expression in this common venereal disease.

PATIENTS AND METHODS

Study design

In this cross-sectional study, 30 patients with external genital warts and 30 control participants were enrolled. Patients from the Dermatology Outpatient Clinic were chosen for enrollment during the period from 1/3/2023 to 28/3/2023. The Oncology Diagnostic Unit of the Molecular Laboratory conducted laboratory measurements. Instead of external genital warts or other

conditions impacting the level of Beclin-1, control volunteers complained.

Patients with history of allergic skin disorders, systemic or dermatological inflammatory, autoimmune diseases (e.g., vitiligo, psoriasis, SLE, autoimmune urticaria, inflammatory bowel illness, multiple sclerosis, and type 1 diabetes mellitus or malignancies, with diseases that affect Beclin-1 level (neurodegenerative, heart, infectious diseases and cancers), pregnant or lactating patients or with other viral infections (HCV, HBV, HIV, HSV, etc.) were excluded. Confirmed diagnosis of external genital warts was done by trained well-qualified dermatologists or andrologists.

A complete history was taken of the patients, covering the course of the condition, the age at which the disease first appeared, any history of atopy, any family history of autoimmune diseases, warts, or atopy, as well as a general and local examination with particular attention to the site (external genitalia, vaginal in females and perianal), size, type, and number of warts. The assessment of the affection of other sites of anogenital area: in male patients: examination of the urethral meatus, in female patients: perianal inspection was provided and speculum examination was offered during the initial assessment if cervical or vaginal lesions were suspected, such as if lesions were identified at the introitus.

Beclin-1 gene expression assessment

A tissue samples from the lesion were done by scrapping of the surface of the wart or by taking small pieces of the wart using scalpel size 16 and putting them in preservative RNA Later solution. This maneuver was done by using xylocaine intradermal infiltration anesthesia then we did electrocauterization to the base of the wart, and all the patients were asked to follow up in the clinic until complete wound healing and the non-lesional tissue samples were obtained by scrapping the normal skin tissue away from the site of lesion in the same patients. The RNeasy Mini Kit (cat no. 74104, Qiagen, Germany) was used to extract RNA was quantified by Nano drop 1000 (Thermo Scientific, USA) and reverse transcription was done using QuantiTect Reverse Transcription Kit (cat no205311, Qiagen, Germany). Beclin-1 gene expression level and the housekeeping gene (GAPDH) was measured using QuantiTect SYBR Green PCR master mix (cat no.204141, Qiagen, Germany) and the specific primers as mentioned in table (1). The reaction was completed the amplification programme comprised two stages: a first denaturation at 95C Taq activation stage for 10 min, followed by 45 cycles of 95C denaturation for 5 s and 60C anneal for 35 s on a step one real time PCR equipment (Applied Biosystems, USA). Following amplification, fluorescence data were gathered to conduct a melting curve study. As an internal control, GAPDH was employed, and the outcomes were examined utilising the Δ CT method of analysis, which is detailed by Wang *et al.* (7).

Table 1: Primers used for real-time polymerase chain reaction Gene Sequence Beclin-1.

Gene	Sequence
Beclin-1 gene	Fwd-5 -GGCTGAGAGACTGGATCAGG-3
	Rev-5 -CTGCGTCTGGGCATAACG-3
GAPDH gene	Fwd-5 - GGA CTGACCTGCCGTCTAG-3
	Rev-5 -TAGCCCAGG ATGCCCTTGAG-3.

*GAPDH: Glyceraldehyde 3-Phosphate Dehydrogenase

Beclin-1 protein level assessment

From each participant's peripheral vein, a 3 ml blood sample was taken in EDTA tubes (patients and controls). EDTA blood samples from patients and controls were centrifuged at 3,000 rpm at 4°C for 20 min to separate plasma that was stored at -80°C until measuring the protein level of Beclin-1 using specific ELISA kits (PEL-BECLIN1-S234-T-2).

Ethical consideration

Participants had the right to refuse to participate in the study. Participants' data were confidential, and any data manipulation or transfer had been done using codes. Participants provided informed consent. Approval from the Faculty of Medicine, Suez Canal University Research Ethics Committee had been obtained before starting the fieldwork with number (4459). The Helsinki Declaration was followed throughout the study's conduct.

Statistical analysis: Software known as the Statistical Package for the Social Sciences was used to analyse the data. Numbers and percentages were used to describe the qualitative data. The distribution's normality was confirmed using the Kolmogorov-Smirnov test. The terms range, mean, standard deviation, median, and interquartile range were used to characterise quantitative data. For categorical variables, the chi-square test was employed to compare various groups. Results were deemed significant at a 5% confidence level (P value < 0.05).

RESULTS

Demographic and clinical characteristics of the participants

The mean age of the recruited patients was 38.27 (\pm 7.53) years, while that of the controls was 35.17 (\pm 6.67). The majority of the patients were females (63.3%), while most of the controls were males (53.3%). None of these characteristics differed significantly between both groups. Most of the cases were married (80%), and the most affected site was the vulva (56.7%), while the least affected sites were the vagina, perineum, and the clitoris. The majority of the patients suffered from a newly occurring disease (66.7%), while 33.33% had a recurrent disease. Furthermore, the mean duration of disease was 17.67 (\pm 30.16) months. Most of the patients suffered from papules (80%), while equal percent of patients had condylomas and plaques (10%).

Beclin-1 gene expression

The mean Beclin-1 gene expression in lesional tissue was statistically significantly lower than non-lesional tissue among the patients (Table 2 and figure 1).

Table 2: Comparison between Beclin-1 gene expression in non-lesional (normal) tissue and lesional-tissue (diseased) in patients.

Beclin-1	Non-lesional tissue (n = 30)	Lesional tissue (n = 30)	t	P
Expression level of Beclin-1 gene in tissue (FC).				
Mean ± SD.	1.0 ± 0.0	0.47 ± 0.32	9.108*	<0.001*

SD: Standard deviation

t: Student t-test

p: p value for comparing between the studied groups. *: Statistically significant at p ≤ 0.05

The expression level of the Beclin-1 gene in the plasma of patients was lower than controls. Also, the Beclin-1 protein level in plasma was lower among the patients and these differences were statistically significant between both groups (Table 3 and figure 2).

Table 3: Comparison between the two studied groups, controls and patients according to Beclin-1 gene expression in plasma and Beclin-1 protein level in plasma.

Beclin-1	Controls (n = 30)	Patients (n = 30)	t	P
Expression level of Beclin-1 gene in plasma (FC).				
Mean ± SD.	1.0 ± 0.0	0.41 ± 0.26	12.341*	<0.001*
Beclin-1 protein level in plasma (pg/l)				
Mean ± SD.	1.49 ± 0.35	0.32 ± 0.17	16.361*	<0.001*

SD: Standard deviation

t: Student t-test

p: p value for comparing between the studied groups. *: Statistically significant at p ≤ 0.05

DISCUSSION

Research has been done on the function of Beclin-1 in the pathophysiology of several viruses, where **Chawla et al.** ⁽⁸⁾ discovered that in order to evade cellular defences, herpes simplex virus 1 targets Beclin-1 to have an inhibitory influence on autophagosome production. In a similar vein, **Bębnowska and Niedźwiedzka-Rystwej** ⁽⁹⁾ revealed that in order to help infected cells adapt to nutrient-deficient environments, the hepatitis B virus X protein upregulates Beclin-1 expression, which in turn induces autophagy.

A strength in our study is that this is the first study to investigate the plasma level of Beclin-1 protein and the Beclin-1 gene's expression level in genital wart patients' tissue and plasma. According to the results of our investigation, the mean levels of Beclin-1 protein and gene expression in plasma in plasma of patients were lower than controls. Similar readings were found regarding the mean Beclin-1 gene expression level in

lesional tissue compared to the patient's non-lesional tissue mean expression level. There was a statistically significant difference (P value = <0.001).

In support to our results, **Jiang et al.** ⁽¹⁰⁾ reported that Beclin-1 gene expression was markedly downregulated in HPV-infected individuals. They said that there was no discernible difference between the patients who had a mixed HPV infection and those who were not, where the Beclin-1 gene was downregulated in both groups of cases.

Additionally, our findings and those of **Jiang et al.** ⁽¹⁰⁾ were strengthened by the previous findings stated by **Wang et al.** ⁽¹¹⁾ and **Hu et al.** ⁽¹²⁾ who discovered that there was a strong correlation between the downregulation of autophagy and the downregulation of Beclin-1 gene expression. Then **Xie et al.** ⁽¹³⁾ illustrated that the Beclin-1 proteins are also downregulated as a result of the downregulation of the Beclin-1 gene, which

eventually results in the downregulation of the autophagy.

Nevertheless, Wang *et al.* ⁽¹⁴⁾ previously related the reduced Beclin-1 gene expression in cervical cancer tissues relative to healthy cervical tissue. However, they disclaimed that HPV-16 infection was associated with the affection of Beclin-1 gene expression. Nonetheless, the discrepancy between their findings and ours might be because HPV strains 6 and 11 are responsible for 90% of genital warts ⁽¹⁵⁾.

It is worth mentioning that in our investigation we found no discernible changes in the Beclin-1 gene or protein expression by the difference characteristics of our study population, except for the number of lesions, where a moderate positive correlation existed between the gene expression in serum and the existing number of lesions (p value = 0.019). Finally, our study examined the presence of a correlation between the Beclin-1 protein in plasma and the Beclin-1 gene expression level in tissue and plasma and found no statistically significant association between both.

Xie *et al.* ⁽¹³⁾ supported our findings as they explained that there exist many Beclin-1-binding proteins that affect the development and nucleation of autophagy. The expression level of these proteins decreases with the decrease in the Beclin-1 gene expression. However, the contradiction between our results and the explaining of Xie *et al.* ⁽¹³⁾ regarding the correlation between the protein level and the gene expression in tissue and plasma could be attributed to the presence of a large number of Beclin-1 binding proteins, and that multiple proteins could be engaged in the downregulation of the autophagy process as a result of the downregulation of the Beclin-1 gene.

CONCLUSION

Patients with genital warts had significantly lower levels of Beclin-1 protein in plasma and Beclin-1 gene expression in lesional tissue as compared to controls and non-lesional tissue respectively. Taking into account Beclin-1's critical function in the control of cellular processes such as endocytosis, apoptosis and phagocytosis as well as its critical role in autophagy, targeting such critical molecule may help in the development of different therapeutic modalities in the future.

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