Received: 11 July 2021 | Revised: 13 August 2021 | Accepted: 20 August 2021

DOI: 10.1111/jocd.14430

JCD Journal of Cosmetic Dermatology WILEY

ORIGINAL ARTICLE

Galectin-3 and Interleukin-17: A potential role in the pathogenesis of human papilloma virus infection

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Abstract

Background: In many cases, human papillomavirus (HPV) infection is self-limiting, as innate and adaptive cell-mediated immunity is needed for infection elimination; however, the inability of the immune system in some patients to clear the infection is a matter of debate. The interleukin-17 (IL-17) family cytokines play an important protective role in host immune response to infections, through maintaining immunity against specific pathogens, induction of antimicrobial proteins, and recruitment of neutrophils to sites of invasion. Galectin-3 (Gal-3) may be considered as a marker of viral infection as many studies reported its increased serum level in viral infections.

Aims: To evaluate levels of serum Gal-3 and IL-17 in patients with verrucas and to explore the potential role of these markers in the pathogenesis of the disease.

Methods: Fifty patients suffering from HPV Infection, and fifty healthy controls were included in this study. Serum levels of Gal-3 and IL-17 were assessed using enzyme-linked immunosorbent assay.

Results: The patients' serum Gal-3 was significantly higher, while IL-17 was significantly lower than that of the healthy controls (p-value < 0.001). Moreover, a statistically significant positive correlation was found between Gal-3 serum level and disease duration and number of warts. Significant negative correlation exists between IL-17 and Gal-3 levels.

Conclusion: Our results indicate a potential role of both IL-17 and Gal-3 in the pathogenesis of warts and open a new opportunity in targeting these markers in the future in treating warts.

KEYWORDS
Galectin-3, interleukin-17, warts

1 | INTRODUCTION

The different clinical presentations and course of HPV may be attributed to many factors. These factors include the immune response of the patient, the genotype of HPV, and the infected epithelial cell phenotype. Moreover, these factors are influenced with patients' environment and lifestyle.[1]The inability of the immune system in many cases to recognize and deal with HPV is still a matter that is not completely understood. However, one of the explanations of this failure is the fact that the infectious life cycle of HPV is non-cytolytic or necrolytic, so there is no inflammation, no inflammatory cytokines release, and no activation of antigen presenting-cells (APCs).[2]The protective role that interleukin-17 family cytokines play against infections is through the recruitment of neutrophils to sites of infection and also through stimulation of antimicrobial peptides in order to maintain immunity-specific pathogens. The protective

role of IL-17 is mediated by the release of pro-inflammatory molecules, which help in controlling these pathogens. During viral infection, IL-17 either potentiate early neutrophil infiltration at the site of infection or inhibit natural killer cell-mediated host immune response.[3]Galectin-3 is considered to be a unique member of a lectin family named galectins. Members of this family have the ability to bind to β-galactosides through conserved sequence elements of carbohydrate-recognition domain.[4]

Galectin-3 regulates many functions at the cellular level such as cell attachment, migration, and proliferation, as well as cell signaling and apoptosis, and plays various roles in the pathogenesis of many infections. Regarding its distribution, it is expressed in various leukocytes including eosinophils, neutrophils, and macrophages, its role in T lymphocytes activation, growth, and apoptosis and in the modulation of the inflammatory response has been suggested.[5]Increased levels of serum Gal-3 in many viral infections pointed out its role as a possible marker of viral infection. The Gal-3 regulatory function is either extracellularly through its ability to bind to glycosylated proteins on the viral envelope, and also by interacting with immune cells ligands or receptors, or intracellularly by interacting with cellular components in the cytoplasm.[6]

Galectin 3 plays an important role in the regulation of Th1 and Th2 cytokines production by differentiated T cells[7] and also has a role in the regulation of IL-23/IL-17A—axis responses to infection.[8]This study aims were to measure serum levels of Gal-3 and IL-17 in patients with viral warts, and also to explore the possible role of these markers in the pathogenesis of the disease.

2 | PATIENTS AND METHODS

This case-control study was performed on 50 patients with any type of warts and 50 apparently healthy age- and sex-matched control subjects without a history of warts attending the Outpatient Clinic of Dermatology and Andrology Department of Benha University Hospitals from January 2020 to July 2020. Participants gave their informed written consents before enrolment, and approval of the local ethics committee of research of Benha Faculty of Medicine was obtained before conducting the study.

All cases enrolled in the study had clinically confirmed HPV infection with any number and any site and aged more than 18 years old. Patients suffering from any systemic disease (tumors, infections, and autoimmunity), patients with any skin disease other than HPV infection, and pregnant women were excluded from the study. None of the patients were on systemic or topical treatment for warts for one month before the study or taking any therapy that may affect serum levels of Gal-3 or IL-17.

Detailed history was taken from all patients, including personal history (age, sex, and occupation), HPV infection (duration, course, site, number, and pervious treatments), and the history of other skin or systemic diseases or drug intake. Complete examination was done to exclude any systemic or other skin diseases and to evaluate site, type, and number of warts.

2.1 | Laboratory investigations

From each patient and from controls, six-ml venous blood was withdrawn by a disposable plastic syringe; the collected blood was placed on plain tube without anticoagulant for 30 min at room temperature till coagulation occurs; after this, centrifugation of tubes was done for 20 min at 1000 *g*. The serum was separated into two aliquots and placed at −20 degrees Celsius for further analysis. Serum Gal-3 was measured using human Galectin-3 ELISA kits supplied by SunRed, China, catalogue number (201-12-1952), while serum IL-17 was measured using human Interleukin-17 ELISA kits supplied by SunRed, China, catalogue number (201-12-0134).

2.2 | Statistical analysis

The SPSS (Statistical Package for Social Sciences) version 22 for Windows® (IBM SPSS Inc.) was used for processing and analyzing the collected data. Frequencies and relative percentages were used to express the qualitative data, while quantitative data were expressed as mean ± SD (Standard deviation). For the comparison between two independent groups of normally distributed variables (parametric data), independent samples *t*-test was used. The diagnostic ability of a quantitative variable in predicting a categorical outcome was determined using the receiver operator characteristic (ROC) curve. Significance was considered when p-value ≤ 0.05.

3 | RESULTS

The mean age of patients' group was 26.9 ± 5.2 years. They were 35 males (70%) and 15 females (30%), while the mean age in the control group was 28.5 ± 5.8 years, they were 32 males (64%) and 18 females (36%). There was an insignificant difference between patients and controls in age and gender (p-value > 0.05 for each).

TABLE 1 Clinical features in all studied cases

			Cases $N = 50$	
Disease duration (months)		Median, range	5.5	1–18
Number		Median, range	2	1–20
Sites	Foot	N, %	28	50%
	Hand	N, %	22	40%
	Face	N, %	3	6%
	Genital	N, %	2	4%
Types	Common wart	N, %	16	32%
	Filiform wart	N, %	2	4%
	Palmer	N, %	6	12%
	Planter	N, %	23	46%
	Plane	N, %	2	4%
	Subungal	N, %	1	2%

TABLE 2 Relation between serum level of Gal-3 and IL-17 and gender, site and types of warts in case group

		IL-17 (pg/ml)					Galectin−3 (pg/ml)			
		N	Median	Range		p	Median	Range		p
Gender	Males	35	212.2	83.2	598	0.553	17.7	6.3	22.8	0.597
	Females	15	207.6	57.8	596		17.8	6.4	22.9	
Site	Foot	28	261.4	83.2	597	0.322	17.6	7.6	22.7	0.442
	Hand	22	213.3	123.4	596		17.9	8.5	22.6	
	Face	3	403.1	212.2	594		14.45	11.2	17.7	
	Genital	2	203.9	145	593		17.8	6.3	22.9	
Type	Common wart	16	156.5	57.8	598	0.322	22.8	9.5	22.9	0.371
	Filiform wart	2	157	138.5	175.5		22.65	22.5	22.8	
	Palmer	6	233.1	222.2	597		19.2	4.5	22.6	
	Planter	23	223.2	145	592		13.45	12.2	16.7	
	Plane	2	256.5	57.8	591		16.8	7.3	21.2	

TABLE 3 Correlations of serum level of galectin-3 and IL-17 with age, disease duration, and number of warts in case group

	Galectin−3		IL-17	
	rs	*p*	*rs*	*p*
Age	0.227	0.114	−0.301	0.034
Disease duration	0.645	**<0.001**	−0.605	**<0.001**
Number of warts	0.938	**<0.001**	−0.924	**<0.001**

Note: rs, correlation coefficient.

P-values <0.01 are significant.

The median number of warts per case was 2, with a range between 1 and 20 warts. Many sites were affected, and the most common affected sites were feet (50%), followed by hands (40%), face (6%), and genitalia (4%) (Table 1).

There was a statistically significant increase in serum levels of Gal-3 (Median: 17.75 ng/ml with a range from 8.4 to 22.9 ng/ml) in patients when compared to control group (Median: 7.3 ng/ml with a range from 5.2 to 11.2 ng/ml) (p-value < 0.001). Moreover, there was a statistically significant decrease in serum level of IL-17 (Median: 211.15 pg/ml with a range from 57.8 to 598 pg/ml) in patients when compared to control group (Median: 564.5 pg/ml with a range from 128.8 to 698 pg/ml) (p-value < 0.001).

There were insignificant differences in serum level of Gal-3 and IL-17 between males and females, between different sites of warts, and between different types of warts (Table 2).

Galectin-3 serum level showed statistically significant positive correlations, while IL-17 had statistically significant negative correlations with disease duration and number of warts (Table 3). Gal-3 serum level showed a statistically significant negative correlation with IL-17 serum level in case group (Figure 1).

Logistic regression analysis was conducted for prediction of warts susceptibility using age, sex, Gal-3, and IL-17 levels as covariates. Higher Gal-3 and lower IL-17 levels were suggested to be independent risk predictors for warts susceptibility in uni- and multivariable analyses (Table 4).

4 | DISCUSSION

The difference in the immune system response to viral warts raises the question about the unexplained role of different biomarkers in the pathogenesis of warts, so our study tried to reveal a potential role of Gal-3 and IL-17 as two contributing factors in the warts.

The results of the present study showed that the case group showed statistically significant lower IL-17 levels when compared to control group with a significant negative correlation between IL-17 serum level and both disease duration and number of warts. These results were in agreement with many studies[9,10] who showed that serum levels of IL-17 were significantly lower in patients with verruca when compared with the controls. The lower level of serum IL-17 in patients with warts in comparison with controls could be considered a contributing immunological factor that raises the risk of HPV infection.

The low serum level of IL-17 in the present study could be explained by the key role of IL-17 to maintain tissue integrity and to generate immune responses to protect against infectious microorganisms, especially at epithelial barrier sites.[11] Interleukin-17 has an important role in mediating defensive mechanism through enhancing Th1 immune responses and releasing cytokines such as IL-1β, IL-6, and TNF-α, also IL-17 acts with IL-22 to induce expression of AMPs by keratinocytes.[12]Interleukin-17 has a crucial role in clearing the HPV through enhancing the activity of cytotoxic T cell, which have the ability to eliminate invading viruses by two mechanisms; the first is through direct cytotoxicity of infected cells and the second is by releasing cytokines to elicit antiviral responses locally or systemically, and the role of IL-17 in the activation and survival of cytotoxic CD8+ T cells has been identified.[13] From the explanations, it is expected that lower serum IL-17 might be associated with increased susceptibility to viral infections like warts.

Results of the present study revealed a significant negative correlation between IL-17 serum level and both disease duration and number of warts. These results could be explained as the lack of IL-17A production resulted in higher viral shedding, more severe

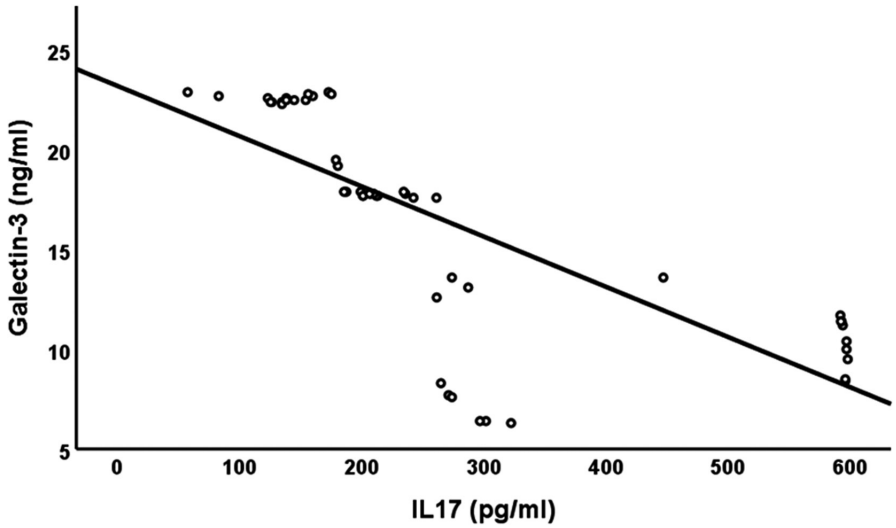

FIGURE 1 Correlations between serum levels of galectin-3 and IL-17 in case group

TABLE 4 Regression analysis for prediction of warts susceptibility

	Univariable				Multivariable			
	p	OR	95% CI		*p*	OR	95% CI	
Age	0.206	0.967	0.919	1.018				
Sex	0.756	0.909	0.498	1.659				
Galectin-3	**<0.001**	1.198	1.112	1.291	**0.003**	1.145	1.046	1.254
IL-17	**<0.001**	0.995	0.994	0.997	**0.025**	0.997	0.995	0.999

Abbreviations: CI, confidence interval; IL 17, Interleukin-17; OR, odds ratio.
P-values <0.01 are significant.

morbidity, and significantly compromised Th1 immune responses.[14]Regarding Gal-3, the current study showed that the case group revealed a significant higher serum galectin-3 level when compared to control group. As far we know, this is the first study to measure the changes in galectin-3 serum levels between healthy individual and patients with different types of cutaneous warts (verruca) caused by HPV. However, the Gal-3 serum level was found to be increased in other viral infections like herpes simplex virus infection[15] and in high-risk HPV-positive patients versus HPV-negative patients in head and neck squamous cell carcinoma. [16]The results of our study could be attributed to the role of Gal-3 in viral infections as viral diseases can induce overexpression of galectin-3, and Gal-3 protects the infected cell from apoptosis and having a direct action on mitochondria and on ROS formation.[17]Another explanation for the role of Gal-3 in warts is through its anti-apoptotic effect as this molecule is able to heterodimerize with b-cell lymphoma 2 protein (bcl-2) molecule, which is a well-known apoptosis suppressor; Gal-3 can promote progression of lesion by interacting with cell ligands, and it can also inhibit early stages of cell death, which are associated with disturbance of mitochondrial homeostasis. Through its antiapoptotic action, galectin 3 may participate in acanthosis, digitated epidermal hyperplasia, papillomatosis, and hypergranulosis seen in warts.[17]Lastly, angiogenesis is a well-established finding in patients with warts. Gal-3 acts as a pro-angiogenic molecule, and how galectin-3 enhances angiogenesis is not completely understood. However, Gal-3 plays a vital role in mediating the angiogenic response regulated by vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF). [18]Results of the present study revealed a significant positive correlation between Gal-3 serum level and disease duration and number of warts, which could be explained with the fact that gal-3 acts as binding mediator to facilitate virus attachment and entry and increase viral replication.[15]Regarding the relation between Gal-3 and IL-17, our results revealed a statistically significant negative correlation between Gal-3 and IL-17 serum level in case group. These results could be explained with the effect of Gal-3 on denteritic cells (DCs). Gal-3 increased level with viral infection regulates cytokine production; Gal-3 inhibits the production of IL-23 in DCs after dectin-1 or toll like receptor (TLR4) stimulation; since IL-23 is a key factor in curdlan-induced Th17 cell differentiation, it has been shown that galectin-3 inhibits the production of IL-17 axis cytokines.[19]The results of the present study agreed with Sheng,[8] who found that Gal-3 suppresses the synthesis of IL-23/IL-17A–axis cytokines by DCs, which inhibits IL-17 responses to histoplasma infection.

5 | CONCLUSIONS

Gal-3 may open a new insight into the pathogenesis of wart through its role in mediating viral infections; this role might be mediated through the decrease in IL-17 in those patients, which significantly compromise Th1 immune responses, raising the risk of HPV infections.

CONFLICTS OF INTEREST

No conflicts of interest or funding.

AUTHORS' CONTRIBUTION

All authors have read and approved the final manuscript. N.W.M., GA.A., AA.E., and A.M.H. performed the research. N.W.M. and A.M.H. designed the research study. A.A.E. and A.M.H. contributed essential reagents. GA.A., AA.E., and A.M.H. analyzed the data. N.W.M., GA.A., AA.E., and A.M.H. wrote the paper.

ETHICAL APPROVAL

The authors confirm that the ethical policies of the journal, have been adhered to and the appropriate ethical review committee approval has been received.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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How to cite this article: NW M, GA A, AA E-F, Hamed AM. Galectin-3 and Interleukin-17: A potential role in the pathogenesis of human papilloma virus infection. *J Cosmet Dermatol*. 2022;21:2618–2622. https://doi.org/10.1111/jocd.14430