The Egyptian Journal of Hospital Medicine (July 2023) Vol. 92, Page 6323- 6327

Assessment of Serum Interleukin-36γ Level in Patients with Warts

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ABSTRACT

Background: The human papillomavirus (HPV) is the causative agent of harmless verrucous overgrowths called warts. Their eradication depends on effective T helper 1/cytotoxic T cells cellular immunity. Interleukin (IL)-36 agonist cytokines are secreted mainly by epithelial cells, such as keratinocytes, in response to infections or inflammatory condition. These cytokines enhance innate immune response and promote the activation of Th1 and Th17 cells.

Aim: The study aimed to evaluate IL-36γ serum level in persons with warts in comparison with healthy controls in order to investigate the potential function of Interleukin-36 in the immune response against this common skin viral infection.

Subjects and Methods: In this case-control study, participants were forty people with warts who were recruited from the Dermatology Outpatient Clinics at Suez Canal University Hospitals in Ismailia city, Egypt, as well as forty people who served as healthy control group.

Results: Serum Interleukin-36γ level was significantly greater in patients with warts than in healthy controls ($p < 0.001$). However, there was no significant relation amongst IL-36γ level & clinical data gathered from individuals affected by warts (age, gender, site, number, clinical type, and recurrence).

Conclusion: Serum level of IL-36γ was significantly increased in patients with warts compared to controls. This high serum level of Interleukin-36γ might highlight the possible role of IL-36γ in promoting the cell-mediated immune response against human papillomavirus.

Keywords: IL-36γ, Warts

INTRODUCTION

Human papillomavirus (HPV) infection results in the benign verrucous growths known as warts, which are exceedingly prevalent. Contact directly with someone who is infected or environmental exposure is the two main ways that people contract human papillomavirus infection. The different types of warts are categorized as follows: appearance or location into common warts, palmoplantar, plane, and genital warts [(1)].

In the early phases of infection, keratinocytes, dendritic cells, natural killer (NK) cells, Langerhans cells and T cells are important cells for generating an efficient adaptive immune response against HPV infection. These cells take up the antigen and carry it to lymph nodes, where T cells are exposed to it. Additionally, NK cells have the ability to totally eradicate HPV-infected cells [(2)]. Moreover, keratinocytes can be constitutively stimulated to release a number of different cytokines, including IL-1, IL-6, IL-10 and IL-18 in addition to tumor necrosis factor (TNF)-α that are implicated in the immune response against HPV [(3)].

Interleukin-36 family of cytokines includes 3 agonist cytokines: IL-36α, IL-36β, and IL-36γ [(4)]. IL-36 cytokines are mostly released by epithelial cells including keratinocytes, especially during infections and inflammatory conditions. The activation of toll like receptors on dendritic cells, macrophages, and epithelial cells by pathogen-associated molecular patterns and drugs, or other environmental factors triggers the release of IL-36 (α, β & γ). IL-36 cytokines are then activated by proteases released by neutrophil [(5, 6)].

Interleukin-36 agonists function as proinflammatory mediators that promote innate immune responses. They induce the synthesis of chemokines, IL-1, TNF-α, and antimicrobial peptides by keratinocytes, as well as interferon (INF)-γ by plasmacytoid dendritic cells & IL-1, IL-6, besides IL-23 by myeloid cells. These mediators trigger the activation of T helper (Th) 1 and Th17 cells causing the release of IL-17, IL-1, and IL-6, in addition to TNF-α. These cytokines in turn enhance more production of IL-36 agonists in a synergistic manner. In addition to this, they work in concert with one another as well as inspire epithelial cells to create a wide range of growth factors & inflammatory mediators, which both contribute along with to increase the vicious cycle of skin pathology [(5, 7)]. This study proposed to measure serum level of IL-36γ in persons with warts in contrast to the standard healthy controls to explore the possible role of IL-36γ in the antiviral immune response against human papillomavirus.

MATERIALS AND METHODS

This descriptive analytical case-control study included forty patients who attended the Dermatology Outpatient Clinic, Suez Canal University Hospitals, presenting with warts. They were evaluated by experienced dermatologist. Control subjects with age, and gender-matched and who didn't have warts and

Received: 21/2/2023
Accepted: 23/4/2023

inflammatory or auto-immune diseases were also enrolled from blood donors.

Inclusion criteria: Participants of both genders and of any age with any clinical variant of warts.

Exclusion criteria:

Patients with concomitant bacterial or viral infections, inflammatory or autoimmune disorders, febrile illnesses, chest conditions, bronchial asthma, and pregnant women. Patients who received anti-wart treatment for 1 month prior to the study.

Enrolled patients with warts were subjected to full history taking, general & dermatological examination with a special focus on the clinical variant, number, site, duration of warts, and if they had first appeared or had returned after previous treatment. The oral cavity, nails, perianal area, nasal mucosa & genitalia were also examined for the presence of warts.

Measurement of serum IL-36γ level:

Human IL-36G (IL-1F9) enzyme linked immunosorbent assay (ELISA) Kit (Invitrogen, Thermo Fisher scientific, USA, Catal. No. EHIL36G) was used to assess serum IL-36γ level. This ELISA kit used a solid-phase Sandwich-ELISA as the method. The evaluation of IL-36γ level was evaluated according to the manufacturer's protocol.

Ethical approval: This study was performed with compliance with the guidelines of Helsinki Declaration. An approval was taken from the Research Ethics Committee and the Institutional Review Board of Suez Canal University on 24/02/2020 with the approval number 4112#. A written informed consent was taken from each patient before enrollment in the study.

Statistical analysis

SPSS version 20.0 (IBM Corp., Armonk, New York) was utilized in the process of data analysis. The terms used to describe qualitative data were number and percentage. The range (minimum & maximum), standard deviation, mean, median, as well as interquartile range were the statistical measures that were used to characterize the quantitative data. The significance of the findings was established at the five percent level. The chi-square test was used to compare outcomes for categorical variables among several groups. The student t-test was applied in order to normally distribute quantitative data to contrast and compare two separate study groups. The Mann Whitney test was used on quantitative data with asymmetric distributions to compare two examined groups. The ANOVA test was employed to evaluate multiple groups against one another for quantitative variables with a normally distributed distribution.

RESULTS

The study included 80 subjects allocated into group A and B. Group (A) included 40 patients with warts, 22 males (55%) and 18 females (45%), while group (B) included 40 healthy control subjects, 16 males (40%) and 24 females (60%). The mean age of patients was 25.65 ± 14.36 years, while the mean age of enrolled healthy controls was 30.60 ± 13.86 years. Patients and controls had comparable age & gender **(Table 1)**.

Table (1): Demographic data of the study cases (n=80)

	Patients with warts (n = 40)		**Controls (n = 40)**		**Test of Sig.**	**P**
	No.	**%**	**No.**	**%**		
Gender						
Male	22	55.0	16	40.0	χ^2=	0.179
Female	18	45.0	24	60.0	1.805	
Age					U= 644.0	0.133
Mean ± SD.	25.65 ± 14.36		30.60 ± 13.86			

IQR: Inter quartile range SD: Standard deviation χ^2: Chi square test U: Mann Whitney test
p: p value

As regard site of warts single site has the highest percent (85%). As regard type of warts also the single type has the highest percent (85%), as regard No. lesions one lesion has the highest percent (40%), range of duration was 1.0 – 10.0 months, As regard Recurrence of lesion the First time wart equal the Recurrent wart and the Negative family history was higher than positive family history. **(Table 2).**

Table (2): Clinical data of patients with warts (n=40)

		No.	%
Site of warts	**Single site**	**34**	**85.0**
	Face	14	35.0
	UL	14	35.0
	LL	6	15.0
	Multiple sites	**6**	**15.0**
Type of warts	**Single type**	**34**	**85.0**
	Plane	4	10.0
	Palmoplanter Common	18	45.0
	Filliform	10	25.0
	Periungual	2	5.0
	Multiple types Common and palmoplantar	**6**	**15.0**
No. lesions	1	16	40.0
	2	8	20.0
	3	6	15.0
	≥4	10	25.0
Min. – Max.		1.0 – 10.0	
Mean ± SD.		2.85 ± 2.49	
Duration (months)	Min. – Max.	1.0 – 20.0	
	Mean ± SD.	8.45 ± 4.80	
Recurrence of lesion	First time wart	20	50.0
	Recurrent wart	20	50.0
Family history	Positive	16	40.0
	Negative	24	60.0

IQR: Inter quartile range, SD: Standard deviation.

Level of interleukin 36γ in the serum of patients ranged from 29.0 to 60.0 ng/L with a mean of 39.74 ± 7.41 ng/L, while ranged from 5.0 to 12.0 ng/L with a mean of 8.70 ± 1.73 ng/L in controls. Serum level of IL-36γ was significantly increased in participants with warts equated with controls ($p < 0.001$) **(Table 3)**.

Table (3): Serum level of IL-36γ (ng/L) level among patients with warts (n=40) compared to healthy controls (n=40)

IL-36γ level (ng/L)	Patients with warts (n = 40)	Controls (n = 40)	t	P
Mean ± SD	39.74 ± 7.41	8.70 ± 1.73	25.787*	<0.001*

IQR: Inter quartile range SD: Standard deviation, t: Student t-test *: Statistically significant at $p \leq 0.05$ p: p value

There was no statistically significant link among IL-36γ serum level and persons' age, number of warty lesions or duration of disease **(Table 4)**.

Table (4): Correlation between IL-36γ (ng/L) serum level & different parameters in patients with warts (n = 40)

	IL-36γ level (ng/L)	
	R	P
Age	-0.127	0.435
No. lesions	0.144	0.376
Duration (months)	0.212	0.190

r: Pearson coefficient

Level of IL-36 in the serum was significantly higher among cases with positive family history (p=0.023). However, there was no significant relation amongst other clinical data and serum level of interleukin-36γ of individuals with warts (gender, site, clinical variant of warts, and presence of recurrent lesions) **(Table 5)**.

Table (5): Relation among serum IL-36γ level (ng/L) level and clinical data of patients with warts (n=40)

	IL-36γ level (ng/L)	Test of Sig.	P
	N		
Gender			
Male	**22**	t=1.407	0.172
Female	**18**		
No. lesions			
1	**16**		
2	**8**	F=0.477	0.701
3	**6**		
≥4	**10**		
Site of warts			
Single site			
Face	**14**		
UL	**14**	F=2.154	0.111
LL	**6**		
Multiple sites	**6**		
Single site	**34**	t=0.120	0.905
Multiple sites	**6**		
Single type of warts			
Filliform	**10**		
Periungual	**2**		
Plane	**4**		
Palmoplanter	**18**	F=1.115	0.365
Multiple types			
Common and palmoplanter	**6**		
Single type	**34**	t=0.940	0.353
Multiple types	**6**		
Recurrence of lesion			
First time	**20**	t=1.399	0.170
Recurrent wart	**20**		
Family history			
Positive	**16**	t=2.452*	0.023*
Negative	**24**		

t: Student t-test , F: F for ANOVA test p: p value

DISCUSSION

The eradication of HPV depends on effective CD4+ Th1/CD8+ cytotoxic T cells immune response [8]. IL-36γ enhances activation of Th1 bridging between adaptive & innate immune responses [7].

To date, up to our knowledge, this is the 1st trial to assess serum levels of IL-36γ in cases with warts to highlight the possible role of IL-36γ in the antiviral immune response against these common skin viral infections.

In this study, participants with warts had significantly elevated serum IL-36γ level than controls. Thus, IL-36γ, as a pro inflammatory cytokine and an enhancer of TH1 and TH17 polarization, might be increased in patients with warts to promote the cell-mediated immune response against HPV facilitating its eradication. Nevertheless, its high level was insufficient to completely eradicate the warts.

Interestingly, prior studies revealed that the viral RNA mimic poly (I:C), initiates IL-36γ synthesis in cervical/vaginal epithelial cells and keratinocytes, supporting that this cytokine may have antiviral properties [9]. Furthermore, it was discovered that exogenous interleukin-36γ has the ability to prevent viral multiplication. IL-36γ treatment in patients with HSV-2 genital herpes resulted in the synthesis of chemokines, antimicrobial peptides, and pro-inflammatory cytokines, and was therefore recognized as a defense mechanism against HSV-2 [10].

Additionally, it has been discovered that interleukin-36γ cytokines are crucial in limiting the replication of HSV-1 in the skin. In mouse & human keratinocytes, interleukin-36γ induced an antiviral response that was regulated by STAT1 and STAT2 through increased production of type I IFN receptor's (IFNAR1), also IFNAR2 subunits, which increased cellular responsiveness to IFN [11]. In line with this anti-viral action, IL-36 cytokines prevented the mortality of alveolar macrophages in influenza-infected mice and protected against a severe influenza infection by restricting viral multiplication [12].

IL-36 cytokines have been investigated in several dermatological diseases. IL-36 cytokines serum levels and genes expression were up-regulated in patients with psoriasis than in controls, and the expression level was positively correlated with PASI [13, 14]. **Di Caprio *et al*.** [15] showed that IL-36α, β, and γ expression were increased in lesional skin of acne & hidradenitis suppurivata. **Lossius *et al*.** [16] found that IL-36γ levels were increased in acute AD as well as in chronic eczematous lesions. In Vitiligo, IL-36 tissue expression was increased in both lesional and non-lesional biopsies [17]. Furthermore, IL-36 was reported to effectively reduce the production of melanin in human melanocytes [18]. The gene expression of IL-36α was significantly higher in both lesional in addition to non-lesional scalp biopsies of individuals with alopecia areata than in heathy controls, with a significant relation between the expression in alopecia areata lesions and the disease severity [19].

This trial provides preliminary data that interleukin-36γ serum level is increased in cases with warts. Additional large scale research evaluating tissue expression of IL-36γ in warty lesions is warranted. Moreover, serum level and lesional expression of IL-36γ need to be investigated in patients with recalcitrant warts as well as pre- and post-clearance of warts with different therapeutic modalities especially immunotherapy.

CONCLUSION

Serum level of IL-36γ was significantly raised in cases with warts compared to controls. This high serum level of IL-36γ might highlight the possible role of IL-36γ in promoting the cell-mediated immune response against HPV. Thus, IL-36γ might be an appealing therapeutic option in patients with recalcitrant and multiple warts especially immunocompromised patients.

DECLARATION

- **Acknowledgements:** We would like to express our gratitude to all of the participants for their helpful cooperation and positive attitudes during this research project. Individuals who agreed to have their case information published in this work have provided their written and informed consent to do so.
- **Consent for publication:** All authors agreed to submit the work.
- **Availability of data and material:** Available
- **Competing interests:** None
- **Funding:** No fund
- **Conflicts of interest:** No conflicts of interest.

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