Serum Levels of Interleukin-17 in Patients with Human Papillomavirus Infections: A Case Control Study

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Background and study aim: Human papilloma virus (HPV) is the most common cause of cutaneous warts and mucosal papillomas and high-risk types of it are involved in developing genital, oropharyngeal, and colorectal malignancies. Interleukin-17 (IL-17), expressed by T helper 17 (Th17) cells, is crucial for inducing a protective immune mechanism against various infections. The purpose of this study was to evaluate serum IL-17 levels in patients with HPV infections compared to healthy controls.

Patients and Methods: A total of 40 patients with cutaneous warts or mucous membrane papilloma and 20 healthy controls were included in this case-control study. Using enzyme-linked immunosorbent assay (ELISA) kits, serum IL-17 levels were assessed in both groups.

Results: Serum IL-17 levels were significantly higher in patients with HPV infection compared to healthy controls (P=0.004). Moreover, there was a significant positive correlation between serum IL-17 level and lesion duration.

Conclusions: IL-17 may play a role in the chronicity of HPV infections .

INTRODUCTION

Human papilloma virus (HPV) infections are frequently manifested as cutaneous warts or mucosal papilloma. High-risk types of HPV may cause cervical, anal, and genital malignancies and may be associated with oropharyngeal and colorectal cancer **[1-4]**. HPV penetrate the basal layer through micro-abrasion and then subsequent viral genome replication in cells of the basal epithelium. HPV DNA incorporation in the host genome correlates with persistent infection progression, causing an increase in viral oncoprotein production, mainly E6 and E7 **[5-7]**. Persistent HPV infection may be attributed to the disturbance of some cytokine signaling pathways **[8]**.

Interleukin-17 (IL-17), a widely studied cytokine in immunology, which expressed by Th17 (a subset of $CD4^+$ T helpers). IL-17 includes from IL-17A to IL-17F, and the prominent member of this cytokine family is IL-17A, which mediates tissue inflammation **[9,10]**. IL-17 may participate in developing immune responses that are protective against infectious pathogens, particularly at epithelial barrier sites. Moreover, because of its pro-inflammatory properties, IL-17 is a significant modulator of immunopathology and inflammation **[11]**.

IL-17 is a critical component of the host defense system and play a role in suppressing viral infections (as in West Nile virus, adenovirus, and vaccinia virus infection). However, it is firmly aligned with promoting viral infection and related disorders as in the case of Theiler's murine encephalomyelitis virus, Coxsackie virus, dengue virus, HBV, HCV, and.

gamma herpesvirus infection **[12]**. Due to its various functions, IL-17 is now one of the immunotherapeutic targets used to treat various illnesses, including infectious diseases **[13]**.

Herein, IL-17 serum levels were measured in patients with HPV infection of the skin and mucous membrane to evaluate its protective or pathological role in these patients.

PATIENTS AND METHODS

Study design: It is a case-control study.

Study settings and sample size: 40 patients with cutaneous warts or mucous membrane papilloma of different sites, sizes, and durations were included in this case-control study, in addition to 20 healthy people of the same age and sex elected as the control; all were recruited from the outpatient clinics of the Department of Dermatology and Venereology, the Department of Tropical Medicine and the Department of Internal Medicine of Zagazig University Hospitals.

Inclusion criteria

In addition to individuals with cutaneous, oral, and perianal warts (diagnosed through clinical examination), we also included patients with esophageal and anal papillomata discovered during upper gastroduodenoscopy or colonoscopy (diagnosis confirmed by biopsy and HPV DNA detection by PCR).

Exclusion criteria

In both patients and controls, any individual with an inflammatory, autoimmune, or neoplastic disease that tends to affect IL17 levels was excluded from the study. Also, patients received any treatment for warts or papillomas were excluded from study.

Patient assessment and methods

To measure IL-17 levels, 3 ml of blood samples were collected from all participants under aseptic conditions with a clean disposable plastic syringe; the samples were placed into vacutainer serum separator tubes with polymer gel and clot activator (Greiner Bio-One GmbH, Kremsmünster, Australia) for serum separation. The samples were clotted for 30 minutes, then centrifuged for 10 minutes at 3000 rpm; then the collected serum was transferred to 1.5 ml Eippendorfs and preserved at -80°C until analysis with an ELISA kit. All samples were assessed in a single test to prevent repeated freeze-thaw cycles. Commercially available double-antibody sandwich ELISA kits were utilized in accordance with the manufacturer's procedure (cat #: 201-12- 0143, Sun red biotechnology company, Shanghai, China). The minimum detectable concentration of IL-17 is estimated to be 12.013 pg./ml. The addition of appropriately diluted serum samples and standards to the relevant precoated wells with a monoclonal antibody (capture antibody) specific for IL17 took place first, then incubation and washing, followed by the addition of a biotin-conjugated detection antibody to each well, then incubation and washing, and then Streptavidin-HRP was added; incubation was done, then washing for removal of the uncombined enzyme. On adding chromogen, Solutions A and B, the liquid color changed from blue to yellow because of the acidic effect. IL-17 color intensity and the concentration in the sample were positively correlated. Samples and standards were run in triplicate.

Statistical analysis

SPSS 26.0 for windows (SPSS Inc., Chicago, IL, USA) was utilized for collecting, tabulating, and statistically analyzing the data. To express quantitative data, mean, standard deviation, and median (IQR) were used, while for qualitative data, absolute (number) and relative frequencies (percentage) were applied. For the comparison of non-normally distributed two variable groups, the Mann-Whitney U test was conducted, while for the categorical variable percentages, the Chi-square test was performed. Spearman's rank correlation coefficient was determined, with (+) and (-) as a direct and an inverse correlation, respectively, and values around 1 and 0 as a strong and a weak correlation, respectively, for the analysis of the link between different study variables. All tests were two-sided. $P < 0.05$ is statistically significant (S), and $p \geq 0.05$ is statistically non-significant (NS).

RESULTS

Among the 40 included patients, 17 were males (42.5%), and 23 were females (57.5%). They ranged from 9 – 60 years old, with 31.8 years old as a mean. Additionally, 20 healthy subjects were included, 9 male (45%) and 11 female (55%), ranging from 11 – 57 years, with a mean of 31.75 and with NS difference in age and sex ($P \geq 0.05$) between patients and controls. The clinical data studied, including the duration, location, and number of papillomas, are shown in

Table 1. Most of included lesions were cutaneious (37.5%) and genital (27.5) warts. 4 patients with anal papilloma and 3 patients with esophageal papilloma were included **(Figure 1)**.

The mean IL-17 serum level in patients with HPV infections was 297.008 pg./ml, while it was 185.799 pg./ml in healthy controls, indicating a significant difference (P=0.004) **(Table 2) (Figure 2)**. Table 3 and Figure 3 show a significant positive correlation between papilloma duration and IL17 serum level (r = 0.56, p = 0.00017).

Table 1: Clinical characteristics of the studied patients.

Variable		N=40 (%)
Site	Cutaneous	15 (37.5)
	Genital	11 (27.5)
	Oral papillomatosis	7 (17.5)
	Anal warts	4 (10)
	Esophageal papilloma	3 (7.5)
Number of lesions	1-3	9 (22.5)
	3-5	23 (57.5)
	>5	8 (20)
Duration	Mean ± SD.	8.625 ± 5.70
	Median (Range)	7 (3 -26)

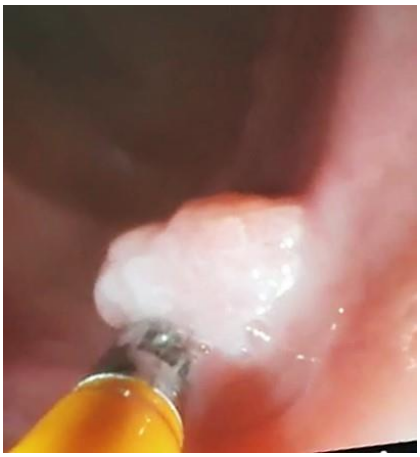

Figure (1): Esophageal papilloma.

Table 2. Comparison of IL17 levels between the case and control groups.

Variables	Case group (n=40)	Control group (n=20)	P value
IL17 (pg./L):			
Mean ± SD	297.008 ± 164.385	185.799 ± 41.470	**0.004***
Median (IQR)	243.5 (183.1-366.9)	180.6 (163.8-196.0)	

*Mann Whitney test; Bold values highlight the significant results; IL-17: interleukin-17

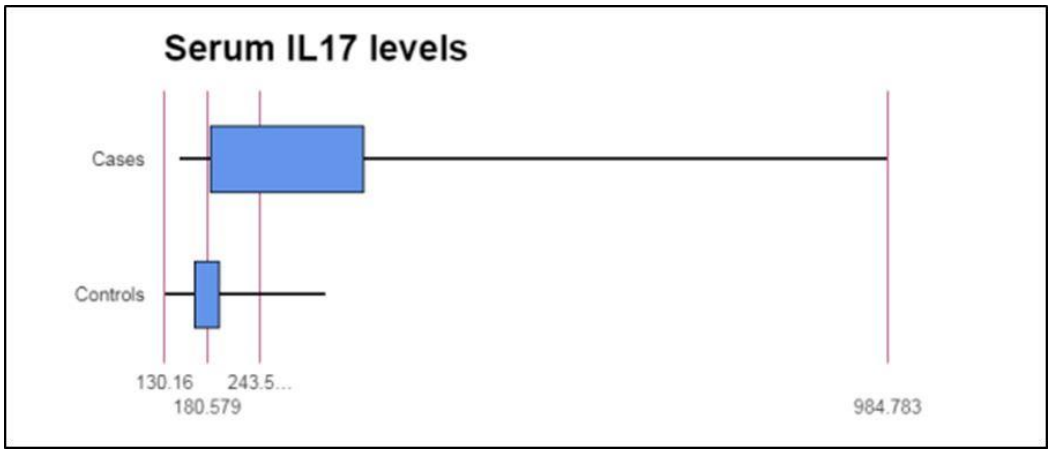

Figure (2): Boxplot comparing levels of IL17 between the studied groups.

Table 3. Correlation between papilloma duration and serum IL17 level among cases (n=40).

Variables		Duration
IL17 (pg./L)	r	0.56*
	p	**0.00017***

*Spearman's rank correlation coefficient; Bold values highlight the significant results.; IL-17: interleukin-17

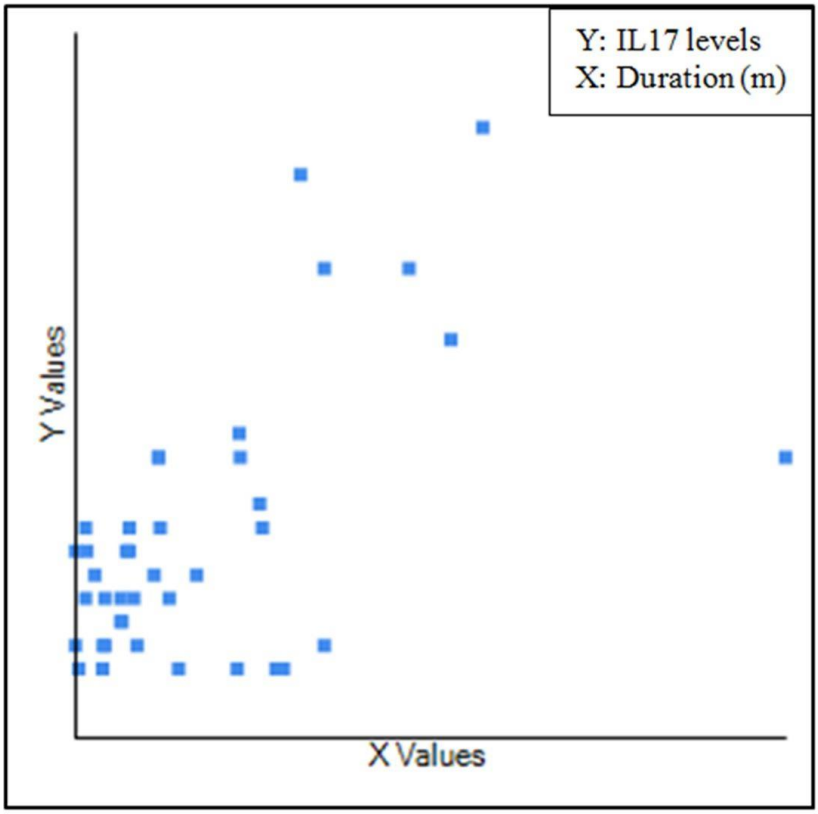

Figure (3): Dot plot graph representing the correlation between serum IL17 levels and papilloma duration among patients.

DISCUSSION

HPV is a non-enveloped and double-stranded DNA virus, causing infections to skin basal keratinocytes or mucosal membranes, as well as being a high-risk factor for developing cervical malignancy at the squamocolumnar junction cells, which harbor a multipotent residual embryonic cells, similar cells are present at the gastro-esophageal squamocolumnar junction and

were linked to Barrett's metaplasia **[14]**. Furthermore, the anorectal junction is a squamocolumnar junction like the cervix but with distinct topographical differences in comparative microanatomy **[15]**.

Multiple common warts are common skin conditions caused by HPV. HPV only completes its life cycle in differentiated squamous cells. When the virus enters the proliferating basal epithelial cells, it induces hyperproliferation of keratinocytes through induction of several cytokines including IL-17 **[9, 16]**. Persistent HPV infections are common, as the virus has evolved some mechanism for evading the immune system. Despite that, regression may occur, representing effective cell-mediated immunity (CMI) against HPV infection **[17, 18]**. Some studies have reported that IL-17 might hinder this reaction by inhibiting Th1 cell differentiation **[19]**.

Herein, the possible IL17 role in HPV-infected patients was assessed, revealing a significant increased IL17 serum level within the case group than controls, as well as a significant correlation between serum IL17 levels and the lesion duration. This elevated level may be due to prolonged and persistent HPV infection that induces an inflammatory response, which is believed to contribute to disease progression rather than the induction of an effective immune response **[20]**. Furthermore, it is generally expected that viral load increases with a much longer duration and, blocked cellular immunity against HPV in long-duration warts **[21-22]**.

In consistency with our results, *Li et al.* **[23]** concluded that HPV infection stimulates IL17 release by activating the signal transducer and activator of the transcription 3 pathway (Stat3), and this consistent with the positive feedback loop hypothesis in which HPV triggers the release of IL-17, enhancing keratinocyte proliferation and viral replication **[9]**. Similarly, Alkady *et al.* **[24]** indicated that the mean serum IL-17 of the case group was (141.1 ± 155.5 pg./ml) while the control was (49.97 ± 22.36 pg./ml), with a significant difference ($p < 0.001$). The cases group was significantly high in mean IL-17 than the control. However, in contrast to our study, they reported a non-significant negative correlation between lesion number and duration with IL-17 ($p > 0.05$).

Hou et al. **[25]** reported that Th1-type cells are the primary type responsible for the viral immune response. Th1 cytokines, such as IFN-γ, possess a decisive antiviral role and antagonize Th17 cell development **[25-27]**. Unlike this defense mechanism, HPV can avoid antiviral type I and II IFN responses **[25, 28]**, which facilitate its persistence in the host by raising IL-17 levels, which suppress antiviral immune response through direct inhibition of IFN-γ expression in T cells, but antiviral immunity is usually enhanced when IL-17 is neutralized **[16, 29, 30]**. Furthermore, IL-17 inhibits effective antiviral immunity by interfering with cytolytic T-cell-function, resulting in long-term viral infection. During viral infection, this IL-17-mediated feedback loop promotes viral persistence, contributing to the pathogenesis of virus-induced chronic disorder **[25]**. As a result, we can conclude that there is a vicious cycle of viral-induced IL17 production and IL17-mediated viral persistence.

A recent study strongly supports the association between HPV infection and high IL-17 levels, in which patients, after several months of treatment with secukinumab, significantly decreased cutaneous HPV lesions **[31]**.

Brunet-Possenti et al. **[9]** reported the beneficial influence of anti-IL-17 therapy on cutaneous warts and genital HPV infections; they also suggested the promising therapeutic option of IL-17 inhibitors for patients with chronic HPV infection, this showing an HPV infection-IL-17 relationship. Multiple studies have reported the relationship between HPV and IL17 in patients with various affection sites, such as cervical cells and breast carcinoma; the findings of these studies may be consistent with the results of our research, as they observed that HPV might be associated with increased IL17 serum level or overexpression in infected tissue **[32-35]**.

In the study by Bonin et al. **[32]**, IL-17A was measured in serum and exfoliated cervical cells from infected individuals with high-risk HPV, but without neoplastic tumors. They discovered a more significant increased IL-17 serum level in patients than in non-infected cases; the concentration of IL-17 in control people was (5.73 ± 1.8 pg./mL) versus (25.84 ± 5.71 pg./mL) in infected patients ($p < 0.05$). Furthermore, they suggested that patients with high-risk HPV infections had high stimulation of systemic IL-17 release and that elevated serum levels of IL-17 may affect viral persistence through

immunosuppressive pathways and accelerate cancer development.

Also, *Gosmann; et al.* **[33]** concluded that increased IL-17 levels might be correlated to HPV16/18 infections and cervical epithelial neoplasia. Nevertheless, IL-17 role in premalignant epithelial disorders and viral persistence is still unknown.

Xue et al. **[34]** stated that there were slightly high IL-17 levels in high-risk HPV infections patients than in control group, in their study of Th17 and IL17 effects on cervical lesions progression.

Khodabandehlou et al. **[35]** showed that HPV could induce expression of proinflammatory cytokines, including IL-17, in breast cancer cases than HPV-negative breast cancer cases and controls. This suggested the important role of HPV in IL-17 induction in these patients. Additionally, *Nassar et al.* **[36]** concluded that IL-17 level declined with Candida antigen treatment in patients with multiple warts, with a more significant decline in patients who had a complete response following treatment, suggesting elevated IL-17 concentration in cutaneous wart patients.

Against our study, *El-Hamd et al*. **[37]** in their study, IL-17 in cases of cutaneous wart had significantly lower levels than in controls, with mean serum levels of 89.4 pg./L in cases, while 123.44 pg./L in controls ($P < 0.01$). Similarly, Ghanem et al. **[38]** observed that persistent wart patients had significantly lower serum levels of IL-17 than controls, with a mean serum level of 490 pg./mL in patients, while 770 pg./mL in controls ($P < 0.001$).

Factors associated with the study variables, including the selected population, the number of investigated patients, the affection duration, the various forms of papillomas, and the general condition of the controls and associated diseases, all may affect the level of IL-17and may explain the differences in the results of research.

CONCLUSIONS

IL-17 may have an important role in immune evasion and persistence of HPV infection by inhibiting Th1 response and stimulating keratinocyte proliferation, indicating that IL-17 inhibitors may be a promising therapeutic option for chronic HPV infection patients, as reported in two previous studies **[9, 31]**. Moreover, treatment of chronic HPV infection can reduce risk of developing cervical, genital, colorectal and oropharyngeal malignancies. Further studies on IL-17, including a large population, are needed to find if serum IL-17 levels are elevated in certain HPV genotypes and for verification of its ability to be used as a target in the treatment of chronic HPV infection.

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Conflict of interest: None.

Ethical consideration

All participants handled informed consent before being included. This study was approved by the institutional ethics committee of the Faculty of Medicine of Zagazig University (ZU-IRB #9801/11-1-2022).

HIGHLIGHTS

- Human papilloma virus (HPV) infections are frequently manifested as cutaneous warts or mucosal papilloma. Unfortunately, High-risk types of HPV infections may be implicated in the development of different malignancies as cervical, anal, genital malignancies and have been reported to be associated with oropharyngeal and colorectal cancer.
- Persistent HPV infection may be contributed to the disturbance of some cytokine signaling pathways. Among these cytokines involved in HPV persistence, IL-17 appears to play an important role.
- In the current study, we detected that serum IL-17 levels were significantly higher in patients with HPV infection compared to healthy controls and there was a significant positive correlation between serum IL-17 level and lesion duration.
- IL-17 have a role in persistence of HPV infections, and this make it among the immunotherapeutic targets for the treatment of a wide range of infectious diseases.

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