Evaluation of Interleukin-4 in patients with skin warts caused by Human Papillomavirus

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Abstract
Background: Human papillomaviruses (HPV) are a class of viruses which depending on their strain, they can cause cancer or developing benign growths known as "warts", that is define as a benign epithelial keratinocyte proliferation produced by HPV infection that can affect the skin and mucous membranes. The aimed of this study was to evaluate the role of Interleukin 4 (IL-4) in the development of HPV skin wart. Subject and methods: this case-control study was conducted on 42 serum samples of man and women diagnosed with skin wart; these samples were collected from Al-Shatrah hospital from November 2021 to March 2022. Inclusion criteria include male and female patients diagnosed with skin wart based on examination of a specialist physician in dermatology. Healthy control group include 40 male and female persons negative for skin warts. IL-4 concentration was measured by ELISA in sera sample of both patients and control groups. The result showed that 30 of 42 skin wart sample was positive for HPV antibody, the mean age of patient was 24.1 and median age was 21. The mean of IL-4 concentration was high in HPV skin wart patient (14 pg/ml) compared with control (0 pg/ml). Conclusion of the present study observed that interleukin-4 may be involved in the development of skin wart caused by HPV infection.
Keywords: ELISA, HPV, IL-4, skin wart.

Introduction

HPV are a type of viruses that can cause cancer or increasing risk of development of benign growths known as warts depending on the strain (1). HPVs were classed as cutaneous or mucosal based on the anatomical detection locations (2). Cutaneous warts are an infectious skin condition that is often seen in dermatology clinics across the world (3). HPV enters a host cell and uses the host's molecular machinery to replicate (4). Initially, the HPV genome replicates alongside host cellular DNA in the basal epithelium. When basal cells differentiate into the suprabasal layers the host DNA replication machinery is suppressed, HPV-encoded E6 and E7 proteins allow continued use of the machinery and can delay epithelial cell differentiation. The combination of E6 and E7 promotes HPV replication by bypassing cell cycle checkpoints (4). Finally, HPV promotes cellular proliferation, induces blood vessel growth, and inhibits the expression of the major histocompatibility complex (5). These processes cause hyperkeratosis, small dotted vessels, and disruption of normal skin architecture, which are clinical features of cutaneous and mucosal warts (6). Each HPV strain causes a unique wart morphology, resulting in clinical manifestations of specific subtypes (7)

HPV infections are typically transmitted through direct skin to skin contact or sexual intercourse (8). Plantar warts, common warts, flat warts, and anogenital condylomata are the four main types of warts (9).

Cytokines are a type of signaling molecule used extensively in cellular communication. there are three types of cytokines; proteins, peptides, and glycoproteins. (10)

Cytokines play an important role in the defense against HPV-induced infections, modulating viral replication and polarizing the immune response toward T-helper-1 (TH1) or -2 (TH2) cell-type patterns (11). Interleukin-4 (IL-4) is a pleiotropic anti-inflammatory cytokine that primarily suppresses the proinflammatory environment. Activated T cells, mast cells, basophils, eosinophils, and NKT cells are the immune cells that produce IL-4 (12,13). IL-4 is an important regulator of humoral immunity, it stimulates T-helper cell type 2 (Th2) while inhibiting T-helper cell type 1 (Th1) and has anti-inflammatory and cytotoxic properties against tumors (14).

IL-4 has been implicated in the pathogenesis of many inflammatory diseases, including skin diseases (15), and it also plays an important role in regulating pro-angiogenic factors as well as by downregulating antimicrobial peptides (AMPs) and factors responsible for skin barrier function (16), IL-4 induced a wide spectrum of pathologies including an increased number of and Langerhans cells and mast cells in dermis and epidermis, focal deposition of collagen and a considerably reduced adipocyte layer in the dermis as well as an increased mitotic activity of keratinocytes, reflected in hyperkeratosis and acanthosis(15).

Subject and Methods

The present study designed as case control based on involving two groups of subjects, 42 patients with skin warts based on clinical examination by

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dermatologist and 40 persons without skin warts and any skin infection selected as healthy control. All patient's samples were selected from Al-Shatrah hospital through the duration from November 2021 to March 2022 within age range 6 to 68 years old. Both groups of subjects were asked special questions regarding name, age, chronic diseases and family history.

Serum samples was collected from both patients and controls and then stored at (-20 ℃) until they delivered to the laboratory of Pathological Analysis Department at Collage of Science, Thi-Qar University, where ELISA used for estimation the concentration level of IL-4.

Ethical approval

This study subjected to the qualifications of ethical considerations and according to the form prepared for this purpose by the Iraqi Ministry of Health. Also, the research got the agreement by the committee of ethical standards at the College of Science, Thi-Qar University, one of the colleges belonging to the Ministry of Higher Education and Scientific Research, Iraq. In addition, Informed consent was obtained from all patients and healthy persons before taking samples.

Statistical analysis

The statistical analysis of this prospective study performed with the statistical package for social sciences (SPSS) 20.0 and Graphpad prism Version 7. Numerical data were tested for normality testing using Shapiro-Wilk test found that the data were abnormally distributed. The data described as median and 5-95 confidence interval and Mann–Whitney U test used for comparison between two groups while Kruskal wallis test used for comparison among more than two groups. Categorical data were described as count and percentage. Chi-square test or Fisher exact test used to estimate the association between variables. The lower level of accepted statistically significant difference is bellow or equal to 0.05.

Relative risk used to estimate the potential risk of pathogen associated with incidence of disease (17).

Results

Sex group

The results divided the patient of the current study into 14 (46.7%) female and 16 (53.3%) males with skin warts, table (1)

Table (1): Distribution of Study groups according to sex.

Parameter		Study group		P value
		Control	Patient	
Sex	Female	17	14	0.089^{NS}
		42.5%	46.7%	
	Male	23	16	
		57.5%	53.3%	
	Total	40	30	
		100.0%	100.0%	

NS: Non-significant difference.

Age group

The results of the present study revealed that the median of age for patients was 21 years old and the mean of age was 24.1 years old, control median age was 23 years old and mean age was 24.5 years old the median age of male patient with skin warts was 19.5 years old, mean 19.8 years old and median age of female patient with skin warts was 22.5 years old, mean age 29 years old table (2)

Table (2): Distribution of patient and control groups according to age.

			Study group		P value
			Control	Patient	
Age (years)	Female	Median	21	22.5	<0.001**
		Mean	21.4	29	
		Percentile 05	19	11	
		Percentile 95	26	65	
	Male	Median	24	19.5	0.004 *
		Mean	26.5	19.8	
		Percentile 05	20	6	
		Percentile 95	43	45	

*Significant difference

Results of serological diagnosis of HPV.

The results of the present study showed presence of anti-HPV antibody in 30 (71.4 %) of 42 patients diagnosed to have skin warts, 14 of 30 have anti-IgM antibody only, 2 of 30 have anti-IgG antibody only while 14 of 30 have both anti-IgM and IgG antibodies. Table (3)

Table (3): Percentage of anti-HPV IgM and IgG antibodies in skin wart's patients

Type of anti-HPV antibody	Skin wart's patients
IgM only	14
	46.6%
IgG only	2
	6.6%
IgM and IgG	14
	46.6%

1. Detection of anti-HPV IgM Antibody

Skin wart's patients showed to have anti-HPV IgM antibody in 28 (93.3%), while all control individuals were negative, table (4).

Table (4): Detection of anti-HPV IgM antibody in patient and control groups.

Parameter		Study group	
		Control	Patient
HPV IgM	Positive	0	28
		0.0%	93.3%
	Negative	40	2
		100%	6.7%
P value			<0.001
RR			21.00
CI 95 %			6.805 to 66.13

2. Detection of anti-HPV IgG Antibody

The result showed detection of anti-HPV IgG antibody in 16 (53.3%) of skin wart's patients, while all controls were negative, table (5).

Table (5): Detection of anti-HPV IgG antibody in patient and control groups.

Parameter		Study group	
		Control	Patient
HPV IgG	Positive	0	16
		0.0%	53.3%
	Negative	40	14
		100%	46.7%
P value			<0.001
RR			3.857
CI 95 %			3.586 to 40. 940

Table (6): Estimation of IL-4 concentration in HPV positive patient and control groups

	Study group	N	Mean	Std Deviation	Std. Error Mean	P value
Serum IL-4 (pg/ml)	Control	40	0.015	0.003	0.001	0.003
	HPV positive Patient	30	14.05863	28.903299	4.885549	

Estimation the level of IL-4.

The detection of IL-4 by ELISA revealed the level of concentration in HPV skin wart's patients (Mean=14.05 pg/ml). Statistically, there was significant difference (P < 0.003) in the level of IL-4 of patient when compared with control group, table (6).

Discussion

Skin warts are benign epithelial keratinocyte proliferations produced by HPV infection that can affect the skin and mucous membranes (18). Many cytokines play as essential component of the immunological milieu following HPV infection, and they may impact the infection's prognosis. (19).

The result of the present study showed that HPV-skin wart is more frequent in male than female (53.3 %: 46.7 %), this result agreed with the findings of the researchers such as; in Baquba Teaching Hospital, Diyala Provence which indicated that HPV skin wart was the most prevalent (39.1%) and affecting the young adult males predominantly (20). On the other hand, Aldiwaniyah province Iraq there was a study observed that HPV skin wart was more common in female (60%) than male (40%) (21).

However, regarding the results of the current study there was an explanation why male be more prevalence than female in HPV skin warts, this may be attributed to males being physically more active compared to females (22), also exposure of males to outdoor activities causing trauma and breaks in stratum corneum providing entry points to HPV (23).

The result of the present study showed that HPV-skin warts infection occurs within age group 6-65 years old, median age 21 years old, while mean age 24.1 years old, this result was agreed with the finding of study in Iran University of Medical Sciences, Tehran, there was a study showed the age of the participants with HPV cutaneous warts was in the range of 5 to 46, the mean age was 24.67 years old (24). On the opposite side, in Al Diwaniyah, Iraq the age range of Iraqi patients with HPV cutaneous warts was 20 to 55 years and mean age was 35.81 years (21). Regarding the results of the current study there was an explanation why HPV skin wart occurs in children and young adults, this could be due to increased exposure of extremities and limbs to physical activities and friction (22).

In the present study, the detection of anti-HPV IgM antibody alone was more prevalent (46.6%) than anti-HPV IgG (6.6%) of 30 serum sample of HPV-skin wart, while patient that have both IgM and IgG was (46%), this finding agreed with the study in Northern Ireland, that observed about (41.5%) of patient with planter wart was positive for HPV2 IgM antibody and (45.6%) patients with warts at other sites (25). On the other hand, in Iraq, Public Hila Hospital, showed the outcome of a study confirmed that 99% of samples positive to IgG anti HPV test (26)

However, the antigenic stimulation varies from case to case this could explain the differences in immune response and, most likely, the presence of IgM antibodies in the sera of some of the patients lacking IgG. Many studies have found a link between the IgG-antibody response to the virus and the resolution of warts (27, 28) IgG antibodies have the importance in conferring immunity to reinfection (28).

In the current study the level of IL-4 concentration in serum of HPV-skin warts patient was elevated than in controls. The mean level was 14.05 pg/ml compared to 0.015 in control ($P < 0.003$), very few studies assessed serum level of cytokines in patients with HPV-skin wart and almost of these studies did not agree with our study. A study in Zagazig, Egypt showed there were a non-significant change in the level of IL-4 between the patient and control groups (29), same to that a study in South Korea observed lower expression of IL-4 in patients compared with controls (30).

However, the role of IL-4 in HPV-skin wart pathogenesis can be explained, it has been involved to play an significant role in the pathogenesis of numerous inflammatory diseases including skin diseases (14), it also plays a critical role in regulating pro-angiogenic factors as well as by downregulating antimicrobial peptides (AMPs) and factors responsible for skin barrier function (15), IL-4 induced a wide spectrum of pathologies including an increased number of mast cells and Langerhans cells in dermis and epidermis, focal deposition of collagen and a considerably reduced adipocyte layer in the dermis as well as an increased mitotic activity of keratinocytes, reflected in acanthosis and hyperkeratosis (14)

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