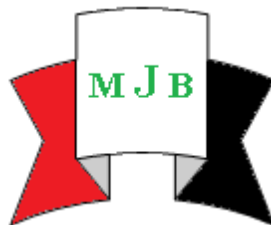


Immunological Study on Genital Wart Patients in Babylon Province-Iraq

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Abstract

The aim of this study was to immune estimation of cytokine profile including serum concentration of each (IL-6, IL-10, IFN- γ and TNF- α) in genital wart patients in Babylon Province-Iraq.

This work was applied on (40) genital wart patients who admitted to the outpatient department of Dermatology and Venereology in Merjan Medical City, Hilla-Iraq and private clinics during the period from November (2013) to March (2014).

A total of 40 patients (16 males and 24 females) were investigated with their age ranged from 2-48 years, in addition to 20 apparently healthy males and females be used as control with an age range approximately matched to genital wart patients.

Blood samples were collected from both patients and control to estimate the serum levels of the immune parameters Interleukine-6 (IL-6), Interlukine-10 (IL-10), Interferon gamma (IFN- γ) and tumor necrosis factor alpha (TNF- α) by ELISA (Enzyme linked Immunosorbent Assay) method.

The cytokine profile showed that there is a significant decrease ($P < 0.05$) in the mean serum concentration of each (IL-6, IFN- γ and TNF- α) between study groups, indicated a decreasing in Th₁ response which is responsible for cell-mediated immunity, while there was no significant difference ($P > 0.05$) in the mean serum concentration between genital wart patients and control groups for IL-10.

Key words: Genital wart; IL-6; IL-10; IFN- γ ; TNF- α ; ELISA

الخلاصة

تهدف هذه الدراسة الى التقدير المناعي المصلي لمستوى الساييتوكينات الانترلوكين-6 (IL-6)، الانترلوكين-10 (IL-10)، الانترفيرون كاما (IFN- γ) والعامل المنخر للسرطان الفا (TNF- α). لدى مرضى الفالول الجنسي في محافظة بابل-العراق.

أنجز العمل على 40 مريض (24 انثى و 16 ذكر) تراوحت اعمارهم (2-48) عاما من المراجعين لاستشارية الامراض الجلدية والتناسلية لمدينة مرجان الطبية والعيادات الخاصة وللفترة من تشرين الثاني (2013) لغاية شباط (2014) اضافة الى (20) شخصا سويا (ذكر وانثى) كانت أعمارهم مقارنة لأعمار مرضى الفالول الجنسي.

تم اخذ نماذج دم من كلا المجموعتين لغرض تحديد مستوى المعايير المناعية لكل من (IL-6)، (IL-10)، (IFN- γ)، (TNF- α) التي قيست بواسطة تقنية الامتصاص المناعي المرتبط بالأنزيم (ELISA).

أظهرت النتائج لمستوى الساييتوكينات ان هناك نقصانا معنويافي معدل تركيز كل من الساييتوكينات (TNF- α) (IL-6) , IFN- γ بين مرضى الفالول الجنسي ومجموعة السيطرة عندما كانت قيمة ($p < 0.05$) مما يدل على ان هناك نقصان في استجابة خلايا Th₁ المسؤولة عن الاستجابة المناعية الخلوية. بينما أظهرت النتائج لمجموعة السيطرة عدم حدوث فرق معنوي في معدل تركيز (الانترلوكين-10) المصلي بين مجاميع الدراسة عندما كانت قيمة ($p > 0.05$).

Introduction

Human papillomaviruses (HPVs) are a group of more than (150) related viruses. Each HPV virus in the group is given a number. HPVs are called *papilloma viruses* because some of the HPV types cause warts or papillomas, which are non-cancerous tumors [1].

Human Papillomavirus (HPV) is the single most important pathogen of the lower genital tract. Human Papillomaviruses cause benign and malignant lesions of the cervical, vaginal, vulvar, and penile mucosa and skin [2]. HPVs are epitheliotropic DNA viruses that are typed based on their DNA sequence and subgrouped into cutaneous and mucosal types, with the latter group further subclassified into low and high oncogenic risk types [3].

The papilloma viruses are attracted to and are able to live only in squamous epithelial cells in the body. Squamous epithelial cells are thin, flat cells. They are found in the surface of the skin and in moist surfaces like the vagina, anus, cervix, vulva, head of the penis, mouth, throat, trachea, bronchi, and lungs, therefore HPVs will not grow in other parts of the body [4]. Most HPV infections are asymptomatic and do not result in epithelial changes, therefore, they are not detected by either visual exam or by cytologic screening tests (Pap tests).

The human defense against viral infection is mediated by the early reactions of innate and the later responses of adaptive immunity. The effector cells of the innate response include neutrophils, granulocytes, monocytes, macrophages and natural killer cells. They recognize, internalize and/or phagocytose the invading virus or viral associated molecular patterns, and release soluble effector molecules, cytokines, which regulate and coordinate many of their activities. There are two types of adaptive immune response, humoral and cell-mediated [5].

Cytokines are soluble molecules expressed by numerous immune cells and epithelial cells in a paracrine and autocrine fashion to regulate immune cell activities. Cytokines divided into proinflammatory, which stimulates cell-mediated response or immune-inhibitory, tumor-permissive cytokines which mediate humoral immunity [6].

Th₁, which produces IFN- γ , lymphotoxin, IL₂, IL₁₂ and TNF and leads to the activation of cell mediated immunity. On the other hand for the Th₂ the cytokine products consist of IL₄, IL₅, IL₆, IL₁₃, IL₂₅, IL₁₀, and amphiregulin, contributing to the development of humoral immune response [7,8].

Interleukine-2 and TNF- α levels (both belong to the Th₁ pattern) appear lower in HPV lesion than in healthy women [9].

Although IL₄ levels, characteristic Th₂ product, seem to increase in LSIL, as the lesion progresses they decrease slightly. Overall, in HPV lesions both Th₁ and Th₂ phenotypes are suppressed, especially Th₁, presumably due to the activity of the (T_{regs}) cells [10].

The most (T)-cell activation is caused by HPV (E₆) and (E₇) proteins, and the destruction is assisted by the upregulation of adhesion molecules like ICAM-1, VCAM-1, and E-selectin in infected cells [11].

The importance of Th₁-type cytokines in direct and indirect protection from both HPV persistence and HPV-associated disease progression has been highlighted in several studies [12, 13].

The objective of this work was to determine the cytokine profile involved in immune response against HPVs (IL-6, IL-10, IFN- γ and TNF- α) in genital wart patients in Babylon Province-Iraq.

Materials and Methods

Clinical samples

A total of 40 patients with genital wart consisting of (24) females and (16) males were involved in this study. The patient's age ranged from (2-48) years. These patients were admitted to the outpatient department of Dermatology and Venereology in Merjan Medical City, Hilla-Iraq and private clinic during the period from November (2013) to March (2014). Patients with suffering from other diseases in addition to GW were excluded.

A total of 20 males and females apparently healthy subjects of both sexes were involved as a control groups. The age range of controls was approximately matched to genital wart patients (10-40) years.

Blood samples were collected from both patients and controls a volume of five milliliters of venous blood was withdrawn by disposable syringe under aseptic technique [14]. Each blood sample was divided into two parts:

A. Two milliliters were put in a tube containing anti-coagulant (EDTA) for checking Blood group test.

B. Three milliliters were placed in a sterile plane tube and allowed to clot, and then serum was separated by centrifugation at (3000) rpm for (10) minutes. The serum has been stored by deep freezing (-20°C) until used for serological tests to estimate the concentration of (IL-6, IL-10, IFN- γ and TNF- α) according to the instructions of Bosterbio company.

Results and Discussion

Cytokine Profile for Genital Wart patients:

Concentration of Interlukin-6 (IL-6):

The concentration of this cytokine revealed that there is a significant decreasing ($P < 0.05$) in GW patients as compared to healthy control (21.13, 31.81) pg/ml respectively. Table (1) showed the results of IL-6 concentration.

Table (1): Concentration of interleukine-6 (IL-6) pg/ml for Genital wart patients and controls

Groups	Mean concentration pg/ml
GW patients (n=40)	21.13*
Healthy control (n=20)	31.81

*($p < 0.05$)

The results indicate that GW patients suffer from decreased in humoral immunity that responsible for antibody production. IL-6 is a multifunctional cytokine that regulates immune responses and acute phase reactions and mediates the host response against tissue injury. It is a proinflammatory cytokine, synthesized by mononuclear phagocytes, vascular endothelial cells, fibroblasts and other cells in response to trauma, burns, and tissue damage. The cytokine affects various processes including the immune response, reproduction, bone metabolism and aging [5].

Immune-inhibitory Th₂-type cytokines (IL₄, IL₅, IL₆, IL₈, IL₁₀), which predominantly

induce humoral immunity that eliminates the extracellular infections and toxins, which unlike intracellular pathogens are exposed to Ab in blood and other body fluids, these cytokines act as B-cells activating factors. A response that result in predominantly Th₂-helper cells will result in a proliferation of (B) cells capable of generating specific neutralizing antibody stimulates Th₂ cells that responsible for humoral immunity [6].

Concentration of Interlukin-10 (IL-10):

The concentration of this cytokine revealed that there is no significant increasing ($P > 0.05$) in GW patients as compared to healthy control (53.86, 47.24) pg/ml respectively.

Table (2) showed the results of IL-10 concentration.

Table (2): Concentration of interleukin-10 (IL-10) pg/ml for Genital wart patients and controls

Groups	Mean concentration pg/ml
GW patients (n=40)	53.86*
Healthy control (n=20)	47.24

*(p>0.05)

This result indicates that there is no cell-mediated immunity against viral infections that may responsible for genital wart.

The result indicates the presence of reduced cell mediated immunity in GW patients depending on the fact that elevated level of IL-10 inhibits the development of a cellular response by inhibition of IL-12 production.

These results are in agreement with [15,16] who mentioned that reduced Th₁ response and increased Th₂ response lead to suppression of cellular immunity and lesion progression. Cell mediated immunity plays a pivotal role in clearance of HPV lesion. These results are in agreement with [6] who observed that a tendency to increase IL-10 levels in patients with HPV occurred simultaneously with other infections. Also provides evidence that increases in IL-10 occur in lymphocytic infiltrates that correspond to HPV infections and are related to the Th₂-type response. [17]recently found that elevated levels of IL-10 and decreased levels of IFN- γ and TGF- β in HPV₁₆ and CIN compared to normal HPV-negative tissue. Interleukin-10 is a pleiotropic cytokine produced by myeloid cells and lymphocytes that displays both immunoregulatory and immunostimulatory effects.

The major immunobiological effect of IL-10 is the regulation of the Th₁/Th₂. IL-10

inhibits the production of other cytokines such as interleukin-2 (IL-2), interferon- γ (IFN- γ), interleukin-12 (IL-12), tumor necrosis factor- α (TNF- α) and it is also associated to major histocompatibility complex-I (MHC-I) down regulation, resulting in reduction of Th₁ response that induced cell-mediated immunity [5].

Down-regulation of cellular response may suppress eradication of virus-infected and tumor cells [18]. The increased incidence of HPV infection in individuals with cell-mediated immune response deficiencies (such as AIDS patients and transplant recipients) clearly demonstrates the crucial role that cell-mediated immune response plays in the control of HPV infections [19]. Interleukin-10 production was elevated in the group with extensive disease compared with the response in the group with localized disease and with healthy control subjects [20].

Concentration of Interferon Gamma (IFN- γ):

The concentration of this cytokine revealed that there is a significant decreasing (P< 0.05) in GW patients as compared to healthy control (0.102, 0.778) IU/ml respectively. Table-3 shows the results of IFN- γ concentration.

Table (3): Concentration of interferon gamma (IFN- γ) IU/ml for Genital wart patients and controls

Groups	Mean concentration IU/ml
GW patients (n=40)	0.102*
Healthy control (n=20)	0.778

*(p<0.05)

This result provides an evidence for viral responsibility for the induction of GW based on the protective function for this cytokine against viral diseases. The IFN- γ is secreted mainly by Th₁ cells and has a potential antiviral activity, and inducing of expression of MHC class I on virus-infected cells as a target for cytotoxic cells [5].

This result is agreed with [9] who stated that Th₁ response is decreased in HPV patients than in healthy controls. Interferon- γ can kill viruses and prevent them from reproducing. It also stimulates the body's immune system to fight viruses including

HPV that cases genital wart. Thus, interferon is given by injection just under the skin at the base of the warts [21]. [22] mentioned that IFN- γ is a possible prognostic marker for clearance of high risk HPV.

Concentration of Tumor Necrosis Factor Alpha (TNF- α):

The result of the TNF- α estimation showed a significant was decreasing (p< 0.05) in the concentration of TNF- α in GW patients when compared to healthy control (28.7, 49.1) pg/ml as mentioned in Table (4).

Table (4): Concentration of tumor necrosis factor alpha (TNF- α) pg/ml for Genital wart patients and controls

Groups	Mean concentration pg/ml
GW patients (n=40)	28.7*
Healthy control (n=20)	49.1

*(p<0.05)

This result also provides an evidence for the host susceptibility for viral infection in GW patients. This is due to the role of this cytokine against viruses. Tumor necrosis factor- α play a direct role in the induction of apoptosis in virus-infected cells [23].

This result is agreed with [9] who mentioned that TNF- α is decreasing in patients with HPV than healthy control. The importance of TNF- α is well illustrated by [24] who mentioned that the using of immunosuppressive agents including TNF- α blockers increases the risk persistent HPV infection and ultimately cervical cancer. Also, HPV vaccination strategy should be available for high-risk HPV infection or

cervical cancer in immunocompromised patients and those receiving TNF- α blockers. Cutaneous infections with HPV increase in patients who received TNF- α blocking therapy [25].

The lowering of TNF- α in GW patients may explain the low percentage of appearance of BCG scar in GW patients in this work, depending on the crucial role of TNF- α in the formation of scars.

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