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Assessment of serum level of interleukin 33 in genital warts patients

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Abstract---Background: Genital warts (GWs) are highly prevalent sexually transmitted viral illnesses that are most prevalent in young, sexually active people of both sexes. Human papillomaviruses (HPV) cause GWs. The cytokine IL-33, an IL-1 family member, stimulates immune cells necessary for type 2 immune reactions. Immune cells like CD8+ T lymphocytes, neutrophils, and macrophages, Th1 cells, and B cells, and natural killer cells that are engaged in type-1 immunity, infection, and chronic inflammation are activated in part by IL-33. Aim: This study aims to examine whether there is a difference in levels of serum IL-33 between genital warts patients and healthy controls and its correlation with genital warts' characteristics. Subjects and Methods: Levels of serum IL-33 were assessed in 40 patients with GWs and 40 healthy control persons using enzyme-linked immune-sorbent assay kits. Results: Levels of serum IL-33 were significantly lower among patients with GWs compared to the control group ($p = <.001$). Patients with recurrent warts had slightly lower levels of these substances. Additionally, a modest inverse relationship between the quantity of warts and levels of serum IL-33 was found. Conclusion: patients with genital warts had considerably lower levels of serum IL-33 than normal controls. IL-33 suggested having a role in the management of genital warts.

Keywords---genital warts, IL-33, serum level.

Introduction

Most often occurring STDs are genital warts (GWs), which are more common in young, sexually active people of both sexes (1). The causes of GWs are human papillomaviruses (HPV). More than 170 different HPV serotypes have been identified, and the ano-genital region is infected by roughly 40 different serotypes. (2). Females may get genital warts on their vulva, vaginal walls, anal canal, cervix, and the region between their external genitalia and their anus. Men's scrotums, anuses, and the tip or shaft of the penis can all develop them. Oral sex with an infected person increases the risk of developing genital warts in the mouth or throat. (3).

Genital warts can appear as a single or many pearly, filiform, or plaque-like papules. Cauliflower lesions, which might be verrucous or lobulated, are the most typical symptoms. Their Color might be the color of the skin, erythema or hyperpigmentation (4). Warts can occasionally produce discomfort, redness, or itching. Psychological discomfort may also result from a genital warts epidemic (5). In recent years, the epithelial "alarmin" defense system has been linked to IL-33, an IL-1 cytokine. IL-33 is liberated by epithelial cells in several tissues and organs, including keratinocytes, endothelial cells and immune cells. Like additional cytokines of IL-1 family, IL-33 is liberated by keratinocytes and fibroblasts, which are necrotic structural cells (6).

Epidermal keratinocytes were found to be the source of IL-33 in a subsequent examination by (Aoki et al). However, it is necessary to conduct more research into the expression of IL-33 in the infected epidermal lesion. (7). The goal of this research is to measure levels of serum IL-33 in order to find out whether there is a difference between people with genital warts and healthy controls.

Patients and Methods

Study design

The Dermatology and venereology Outpatient Clinic at the Suez Canal university hospital in Ismailia undertook an observational analytical case control research from June 2021 to January 2022. The control group was made up of age-matched 40 healthy people without genital warts while the study group was made up of 40 patients who had been diagnosed with genital warts. Participants using immunosuppressive medication concurrently, autoimmune diseases and End organ failure were enrolled out of the study.

History and examination:

Each patient underwent through local, general and medical history examinations (onset, course, and duration of the GWs; affected sites, number, and morphology of the GWs and recurrence history of the GWs). Dermatologists used well-known, plainly visible clinical indicators of GWs to make a clinical diagnosis of GWs in

each of the individuals they included.

Diagnosis of Genital warts:

The main complaint were Painless bumps, pruritus, and discharge in the genital region including the labia majora, vagina, scrotum and penile shaft. Additionally, they may be seen on the vagina's and the anus's inside surfaces. In roughly 60 % of cases, lesions appeared within 3 months after having sex with a partner who had genital warts. It was more typical to have several lesions than just one wart. Additionally, involvement of more than one affected site is common.

IL-33 assessment:

Antecubital veins of the patients and control subjects were used to collect blood samples (5 mL) under aseptic circumstances. The sera were put in separator tubes. After allowing blood samples to coagulate for 20 minutes, they were centrifuged at 3500 rpm for about 20 minutes. Each sample of serum was taken outside, divided into two aliquots using 1ml cryo-tubes and kept cold until the test. In order to avoid repeated freeze– thaw cycles, a single measurement was used to evaluate each serum sample. Human IL-33 was tested using Commercial ELISA kits in accordance with the manufacturer's recommendation (using ELISA Thermo Fisher Scientific Multiskan EX Microplate Reader, Oy, FI-01621, and Vantaa, Finland).

Ethical consideration

The Suez Canal university faculty of medicine's research ethical committee approved this work. Informed written consent were taken from patients after illustration of benefits and risks.

Statistical Analysis

Using SPSS 26.0 for windows (SPSS Inc., Chicago, IL, USA), all data were gathered, tabulated and statistically examined. Qualitative data were analyzed using number and percent. Quantitative data were described using range (minimum and maximum), mean, median and standard deviation. Every statistical comparison used a two tailed significance test. P-values below 0.05 indicate a significant difference, those below 0.001 indicate a highly significant difference, and those above 0.05 indicate a Non-significant difference.

Results

Table (1): Demographic characteristics among the study population

	Diseased group (No = 40)		Control group (No= 40)		Test of Sig.	p
	n	%	n	%		
Sex					X2 =	0.653
- Male					0.202	

No (%)	17	43%	19	48%		
- Female						
No (%)	23	58%	21	53%		
Age (years)					t = 0.177	0.86
Mean ± SD.	32.42 ± 9.22		32.08 ± 8.4			
Median (IQR)	32.5 (20.0 - 58)		32.5 (21 - 59)			
Range (Min-Max)	38 (20 - 58)		38 (21 - 59)			

χ^2 : Chi- Square test SD: standard deviation

IQR: interquartile range

t: Independent T test

p: p value for comparing between the studied groups

P-value > 0.05: Non significant; P-value < 0.05: Significant; P-value < 0.001: Highly significant

Table (1) showed Demographic characteristics among the study population. Regarding sex, there wasn't a significant difference between both of the studied groups ($p=0.653$). Age in Diseased group ranged from 20 to 58 with mean $\pm$ SD = 32.42 ± 9.22 while the Age in Control group ranged from 21 to 59 with mean $\pm$ SD = 32.08 ± 8.4 with no statistical significant difference ($p=0.86$) between them.

Population data regarding Number of GWs

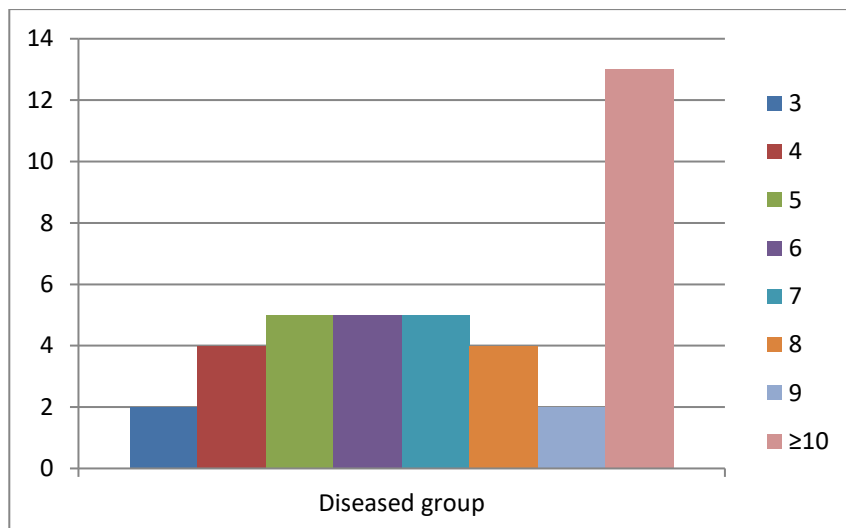


Figure (1): Bar chart showing study population data regarding Number of GWs.

Number of patients with (7) GWs in the study population was 5 (13 %). Number of patients with (8) GWs in the study population was 4 (10 %). Number of patients with (9) GWs in the study population was 2 (5 %). Number of patients with (≥10) GWs in the study population was 13 (33 %). Figure (1)

Study population data regarding affected site

Number of patients with Affected Libia in the study population was 9 (23%). Number of patients with Affected Libia and anus in the study population was 8 (20 %). Number of patients with Affected Libia and Thigh in the study population

was 6 (15 %). Number of patients with Affected Penis in the study population was 4 (10 %). Number of patients with Affected Penis and groin in the study population was 8 (20 %). Number of patients with Affected Penis and scrotum in the study population was 3 (8 %). Number of patients with Affected Penis, groin, and scrotum in the study population was 2 (5 %). Figure (2)

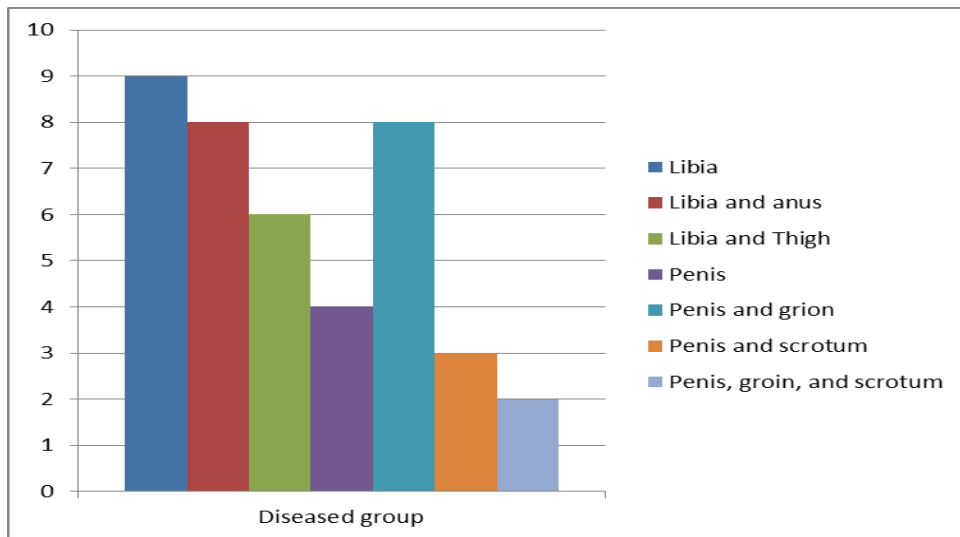


Figure (2): Bar chart showing study population data regarding affected site.

Table (2): Incidence of recurrence among the diseased group

	Diseased group (No= 40)	
	n	%
Recurrence		
- yes		
No (%)	2	5%
- no		
No (%)	38	95%

Table (2) showed Incidence of recurrence among the diseased group. Number of patients with Recurrence in the study population was 2 (5%).

Table (3): Measurements of IL-33 among the study population

	Diseased group (No = 40)	Control group (No = 40)	Test of Sig.	p
IL-33			t = - 11.156	<0.001
Mean $\pm$ SD.	14.89 $\pm$ 5.43	32.83 $\pm$ 8.59		
Median (IQR)	15.31 (11.27 - 18.5)	31.96 (26.83 - 37.95)		
Range(Min-Max)	24.79 (4.22 - 29.01)	45.62 (10.34 - 55.96)		

t: Independent T test SD: standard deviation

IQR: interquartile range

p: *p* value for comparing between the studied groups

P-value more than 0.05: Non significant; *P*-value less than 0.05: Significant; *P*-value less than 0.001: Highly significant

Table (3) showed Measurements of IL-33 among the study population. IL-33 in Diseased group ranged from 4.22 to 29.01 with mean $\pm$ SD = 14.89 ± 5.43 while in Control group the IL-33 ranged from 10.34 to 55.96 with mean $\pm$ SD = 32.83 ± 8.59 with highly statistical significant difference ($p = <.001$) between the two groups.

Table (4): Univariate analyses of IL-33 as a risk factor for genital warts and genital warts recurrence with odds ratios and 95% confidence intervals (CI)

	Genital warts			
	OR	95% CI		P
		Lower	Upper	
IL-33				
	0.995	0.947	1.047	0.857
	Genital warts recurrence			
	0.815	0.623	1.067	0.136

OR: Odds ratio

CI: Confidence Interval

p: *p* value

Table (4) showed Univariate analyses of risk factors for genital warts with Odds ratio of IL-33 was 0.995, and the 95% Confidence Interval (CI) was ranged from 0.947 to 1.047, and for genital warts recurrence with Odds ratio of IL-33 was 0.815, and the Confidence Interval was ranged from 0.623 to 1.067.

Pearson's correlation coefficients (*r*) between IL-33 and genital warts. Pearson's Correlation Coefficient (*r*) between IL-33 and genital warts was 0.626. Figure (3)

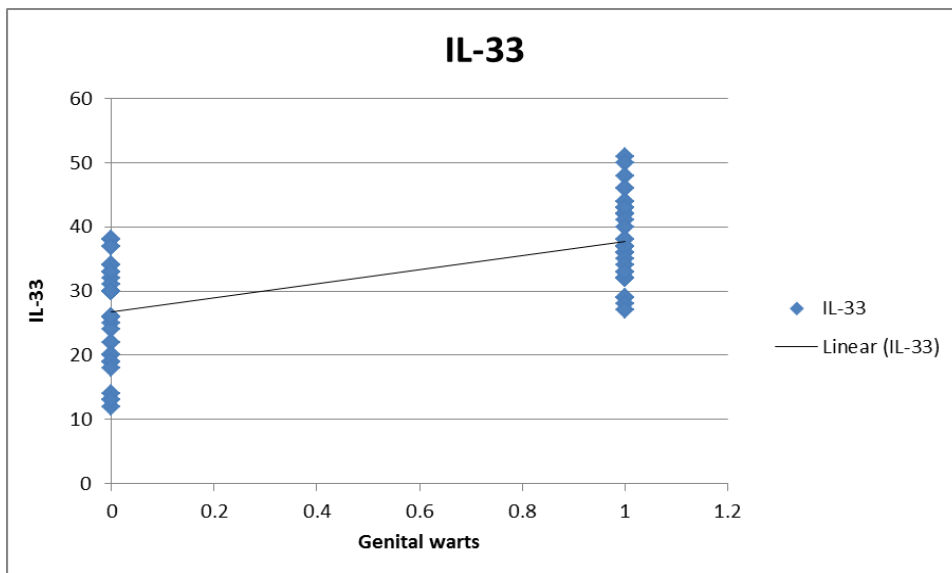


Figure (3): Scatter plot graph showing correlation between IL-33 and genital warts

Table (5): Pearson's correlation coefficients (r) between IL-33 and Genital warts characteristics

	IL-33	
	Pearson's correlation coefficients (r)	P
Number of GWs		
	-0.211	0.878
Recurrence of GWs		
	-0.202	0.211

Table (5) showed Pearson's correlation coefficients (r) between IL-33 and Genital warts characteristics. Pearson's Correlation Coefficient (r) of IL-33 and Number of GWs was -0.211 . There was a low negative Correlation between IL-33 and Number of GWs. Pearson's Correlation Coefficient (r) of IL-33 and Recurrence of GWs was -0.202 . There was a low negative Correlation between IL-33 and Recurrence of GWs.

Discussion

Research had made clear the important role of IL- 33 in the immune system as it was found that it plays a great role in driving protective antiviral immunity (8). This is an observational analytical case-control study that was conducted in the Dermatology Outpatient Clinic in the university hospital of Suez Canal in Ismailia. 40 participants with genital warts who were diagnosed based on clinical evaluation and 40 matched healthy controls were participating in this research. Our study found that serum IL-33 had significantly lower levels among patients who have genital warts compared to the control group where the level of serum IL-33 in Diseased group ranged from 4.22 to 29.01 with mean $\pm$ SD = 14.89 ± 5.43 while in Control group the IL-33 ranged from 10.34 to 55.96 with mean $\pm$ SD = 32.83 ± 8.59 with highly statistically significant difference ($p = <.001$) between these groups.

Immune cells involved in type-2 immune responses are activated by IL-33 cytokine of IL-1 family members. Immune cells, like CD8+ T cells, Th1 cells, neutrophils, B cells, macrophages and nature killer cells involved in type-1 immunity, infection and chronic inflammation are also activated by IL-33. The results of the present study were corroborated by Abu El-Hamd et al., (2019) observation that noted that GWs patients had significant lower levels of blood IL-21 and IL-33 than did the controls (p below .0001). These findings might be promoted by Wang et al., (2014) who found that IL-33 was observed in the cervical endothelial and epithelial cells of patients who are HPV-positive. The level of IL-33 protein in tissues of the cervix was significantly decreased in severe intraepithelial neoplasia of the cervix.

In addition, Kulhan et al., (2019) discovered a relationship between a rise in cervical HPV infections and a fall in the levels of IL-33 and suppressor of tumorigenicity 2 (ST2). There was a statistically significant positive connection between IL-33 and ST2 in the HPV-positive patients. Furthermore, in accordance with Jin et al (2018), verruca vulgaris lesions have very little IL-33 expression in

their epidermis. This study detected a slightly negative correlation between IL-33 and number of genital warts among the studied diseased group. There is a possibility that presence of several infections is decided by immunologic mechanism. That might come in accordance with Chaturvedi AK, Myers L, Hammons AF, et al. (2005) who declared that women with HIV who have immunosuppressed immune systems had a significant frequency of numerous infections.

Additionally, patients with recurrent genital warts had somewhat lower levels of serum IL-33, according to this study. Increased HPV load of infection and resistance to treat genital warts may be due to decreased adaptive and innate antiviral cellular immune responses. That might be explained by Theiler RN, et al., (2010) who found that HIV-positive women who are sexually inactive have a greater incidence of recurrent HPV infection than HIV-negative controls, and research in HIV-positive women imply that immune suppression is necessary for reactivation, which may explain why it is uncommon in healthy people. Patients with HIV infection showed considerably reduced serum levels of IL-33.

According to recent data, IL-33 is crucial for host defence against several infections., (Humphreys et al., 2008; Alves-Filho et al., 2010; Jones et al., 2010). Our study couldn't detect significant differences between serum levels of IL-33 and different morphological patterns or affected sites of genital warts. Dubina M, (2009) stated that although external genital warts can appear singly as a keratotic papule or plaque, they tend to occur in big clusters. Genital warts frequently start out on the skin as little, indistinct 1 to 2 mm flesh-colored papules and may keep this appearance for the length of the infection. It is advised to conduct a study using a large sample size and a variety of genital warts with different morphological patterns.

Regarding the demographics of the study population, the current study found no statistically significant difference in the two studied groups' age ($p = 0.860$) or sex ($p = 0.653$) distributions. These findings may point to an involvement of IL-33 in the immune response to genital warts. Low serum levels of IL-33 may contribute to lowered immunity, which raises the risk of GW development and recurrence as well as HPV infections. To the best of our knowledge, this study was the first to identify risk factors for the occurrence and recurrence of genital warts using Univariate analysis.

Sample size is small. In addition, we solely examined the levels of serum IL-33. If these factors were examined in the intra-lesion and serum samples, we might obtain more precise results. It is recommended that more research be done to determine how IL-33 is expressed in genital wart lesions and how its concentration changes as a result of various genital wart treatment approaches. Additional research is needed to determine whether systemic and local IL-33 injections can be used as GWs medicinal treatments.

Conclusion

Clinical trials are suggested to be done on treating genital warts via using IL-33 and trying to use it in predicting the success of different lines of therapy of genital

warts especially immunotherapy in case of multiple and recalcitrant warts. We also recommend studying the use of IL- 33 as adjuvant in vaccines against various strains of HPV. Our findings need to be confirmed by additional research with a larger sample size and longer follow-up in order to identify more risk factors for genital warts.

Declarations

Consent for Publication: I attest that all authors have agreed to submit the work.

Availability of data and material: Available

Competing interests: None

Funding: No fund

Conflicts of Interest: The authors claim that they have no conflicts of interest regarding the publication of this paper

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