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Evaluation of the Tissue Level of CXCL10 in Patients with Verruca Vulgaris

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Abstract
Background: Verruca vulgaris is a common cutaneous viral infection characterized by epidermal hyperplasia and keratinocyte proliferation. The immune response in verruca vulgaris, particularly the role of chemokines, remains an area of ongoing investigation. CXCL10 is a chemokine with potential involvement in viral skin infections, but its tissue expression in verruca vulgaris has not been comprehensively studied. Our objective was to evaluate the tissue expression of CXCL10 within cutaneous lesions of verruca vulgaris. **Methods:** This prospective case-control trial included 50 cases with verruca vulgaris and collected lesional biopsies, along with control biopsies from healthy skin in 30 patients. Tissue CXCL-10 levels were assessed using an ELISA assay. The data was analyzed to determine the relationship between the levels of tissue CXCL-10 and verruca vulgaris, and the potential diagnostic value of CXCL-10. **Results:** Tissue levels of CXCL-10 were reported to be significantly increased in verruca lesions in comparison with healthy skin biopsies ($p = 0.035$). The sensitivity and specificity of tissue CXCL-10 in distinguishing verruca lesions from healthy skin were 70% and 54%, respectively, at a cut-off level of 248.4. **Conclusions:** Our study reveals an upregulation of CXCL10 in verruca vulgaris lesions, suggesting a potential role for this chemokine in its pathogenesis. Furthermore, the diagnostic value of tissue CXCL-10 in distinguishing verruca vulgaris from healthy skin suggests its potential as a biomarker for this viral skin infection.

Keywords: Verruca Vulgaris CXCL10, Chemokine, Tissue Expression, ELISA Assay, Biomarker, Viral Skin Infection.

1. Introduction

Verrucae are benign growths that affect the skin and mucous membranes due to papillomavirus infections. There are over 130 recognised types of human papillomaviruses (HPV) that infect the squamous epithelium, which is commonly seen on the skin or gentalia. However, each HPV type usually only targets specific areas of the body. Many HPV types can cause benign growths, often referred to as "warts" or "papillomas," in the regions they infect [1].

Warts are usually hard, small, and rough growths that have similar color to the skin. They often do not cause any symptoms, except when they develop on the soles of the feet, where they can be painful. Although warts typically manifest on the hands and feet, they can also affect other areas of the body. Multiple warts may appear, but they are not considered precancerous [2].

In response to tissue damage, chemokines are released, they play an important role in leukocyte recruitment, regulating cytokine production, the development of adaptive immune responses, and the pathogenesis of several human illnesses [3].

CXC chemokines, such as CXCL9 (IFN-γ-monokine induced), CXCL10 (IFN-γ–induced protein 10-kD), and CXCL11 (IFN-inducible T cell chemoattractant), bind to their receptor CXCR3. IFN- substantially upregulates their expression. Non-infectious and infectious tissue injuries induces CXCL10, including liver injury due to ischemia/reperfusion, chronic hepatitis C virus infection, and respiratory syncytial viral infection [4].

Moreover, CXCL10 is essential for host defence. when mice were infected with mouse hepatitis virus, blocking the interaction between CXCL10 and CXCR3 using anti-CXCL10 antisera in mice caused delayed clearance of the virus from the central nervous system and higher mortality compared to control mice [5]. Moreover, the mice infected with hepatitis virus and deficient in CXCL10 had impaired control over viral replication in the brain [6].

Furthermore, CXCL10 is implicated in infectious diseases pathogenesis and progression, including HCV-related autoimmune disorders [7], or Mycobacterium tuberculosis [8], sepsis [9], and HIV [10].

To date, no study has assessed the tissue expression of CXCL10 in patients with verruca vulgaris.

Our objective was to evaluate the tissue expression of CXCL10 within cutaneous lesions of verruca vulgaris.

2. Methodology

Subjects:

This prospective case-control study enrolled 50 patients with verruca vulgaris from the outpatient clinic of the Dermatology, Venereology, and Andrology Department at Benha University Hospitals between October 2022 and May 2023.

All participants provided a written informed consent, and the study received approval from the local ethics committee for research involving human subjects at Benha Faculty of Medicine.

Inclusion Criteria were fifty male and female patients aged 18 years or older affected by verruca vulgaris. Patients who had not received topical or systemic (immunotherapy) treatment for at least 3 months' prior skin biopsy collection.

Exclusion criteria were patients who had used any topical or systemic treatment in the previous 3 months, individuals with concurrent autoimmune diseases or malignancies, pregnant or lactating patients, patients who had received vaccination or immunomodulation within the past 3 months, patients showing signs of fever or any local or systemic infection or inflammation and any evidence of immunosuppression, including HIV.

Methods:

All patients underwent the following procedures:

Full History Taking: This included documenting the patient's age, sex, number of warts, duration of the lesions, previous wart treatments, and any family history of viral warts. **Local Examination:** A comprehensive cutaneous examination was conducted to identify the site, size, and number of warts. **Sampling:** Lesional biopsies were taken from all patients, with an additional biopsy taken from normal skin in 30 patients to serve as controls. **Assessment of CXCL-10 in Tissue Samples:** Tissue samples were homogenized and centrifuged at 2000-3000 rpm for 20 minutes. The resulting supernatant was collected for further analysis. CXCL-10 levels were assessed using the CXCL-10 ELISA kit (Cat. No. 201-12-0413, SunRed Labs, Shanghai, China).

ELISA Assay Procedure:

Before use, all standard solutions, reagents, and samples were prepared per instructions and brought to room temperature. The assay was conducted at room temperature. The necessary number of strips was determined and inserted into frames for use. 50 µl of standard was added to the standard well. 40 µl of the sample was added to the sample wells, followed by adding 10 µl of Human CXCL-10 antibody to the sample wells, and then 50 µl of streptavidin-HRP to both standard and sample wells. A sealer was used to cover the plate, and then the plate was incubated for 60 minutes at 37°C. The sealer was removed, and the plate was washed with wash buffer five times.

During each wash, wells were soaked with 300 µl of wash buffer for 30 seconds to 1 minute. To every well, 50 µl of chromogen solution A was added, followed by 50 µl of chromogen solution B. The plate was incubated in the dark at 37°C for 10 minutes. To each well, 50 µl of stop solution was added, causing a change of the blue color into yellow. The optical density (OD value) of every well was determined using a microplate reader set to 450 nm after adding the stop solution within 10 minutes.

Calculation of ELISA Assay Results:

The OD value was taken as the vertical axis, and the standard density as the horizontal axis. A graph paper was used to draw a standard curve, and the corresponding density was calculated based on the sample OD value using the sample curve (resulting in the sample density) or the straight-line regression equation of the standard curve, which was calculated using the OD value and the standard density. The sample OD value was substituted into the equation to calculate the sample density.

Statistical analysis:

The collected data was processed using IBM SPSS, and data analysis methods depended on the data type. The Shapiro test checked data distribution normality. Descriptive statistics were applied for numerical and non-numerical data. Analytical statistics included Mann-Whitney test, Independent T Test, Kruskal-Wallis test, Chi-Square test, and Spearman correlation analysis. The Receiver Operating Characteristic (ROC) Curve assessed diagnostic measures. The significance level was $p \leq 0.05$ at a 95% confidence interval.

3. Results

The current study included 50 patients presented with verruca vulgaris, of them 30 patients were selected as to obtain healthy skin biopsies from matched sites to be regarded as control group. Mean duration of lesions in the studied patients was 3.25 ± 2.9 years, while mean size of lesions was 1.78 ± 0.8 cm^2. Twenty-six cases had single lesions while 24 cases had multiple lesions. Family history for verruca was positive in 29 cases. Main affected site was limbs in 28 patients and history of

previous treatment was positive in 29 patients. **Table 1**

Table (1) Clinical characteristics of the studied cases

Duration of lesions (years)	**Mean ± SD**	3.25 ± 2.9
	Range	0.5 – 12
Size of lesions (cm^2)	**Mean ± SD**	1.78 ± 0.8
	Range	0.5 – 3.5
Number of lesions	**Mean ± SD**	1.48 ± 0.5
	Range	1 - 4
	Single lesion	26 (52%)
	Multiple lesions	24 (48%)
Family history of verruca	**Positive**	29 (58%)
	Negative	21 (42%)
Site	**Limbs**	28 (56%)
	Trunk	9 (18%)
	Face	4 (8%)
	Generalized	9 (18%)
Previous treatment	**Present**	29 (58%)
	Absent	21 (42%)

Regarding the assessment of tissue CXCL-10 levels: Tissue levels of CXCL-10 was significantly elevated in verruca lesions in comparison with healthy skin biopsies (p = 0.035). **Table 2 and Figure 1**

Table (2) Comparison of CXCL-10 levels between the studied groups

		Verruca lesions No. (50)	**Healthy skin No. (30)**	**P value**
CXCL-10	**Mean ± SD**	569.9 ± 401.8	452.7 ± 426.6	0.035
	Range	180 - 1367	150 - 2280	

Using Mann-Whitney test, p value ≤ 0.05 is significant.

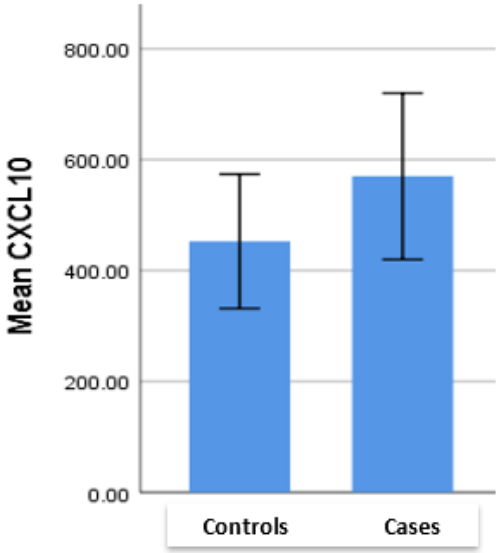

Fig. (1) Comparison of CXCL-10 levels between the studied groups

Tissue level of CXCL-10 showed good discriminative power in verruca lesions compared to healthy skin at a cut-off level of 248.4, with specificity of 54% and sensitivity of 70%. **Table 3**

Table (3) ROC curve analysis of CXCL-10 in verruca lesions

AUC	**P value**	**CI**	**Cut-off point**	**Sensitivity**	**Specificity**
0.640	0.036	0.519-0.762	248.4	70%	54%

AUC: area under the curve, CI: confidence interval

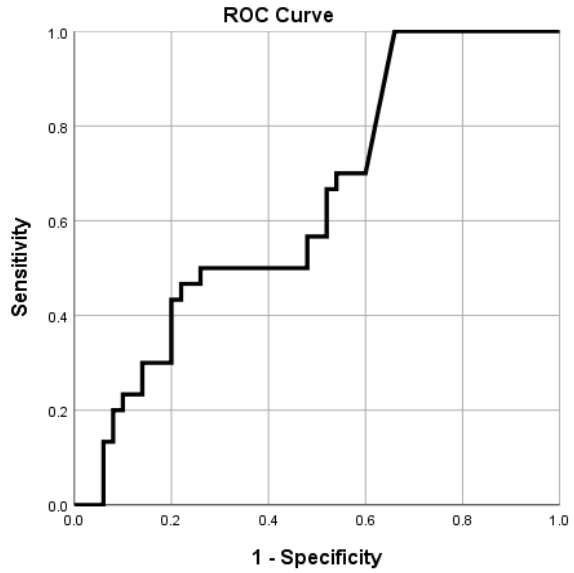

Fig. (2) ROC curve of tissue CXCL-10

Correlations of tissue CXCL-10 with patients' age, duration of lesions and lesions' size revealed no significant results. **Table 4**

Table 4: Correlations of tissue CXCL-10 with patients' age, duration of lesions and lesions' size

		Tissue CXCL-10
Age	**Rho value**	-0.028
	P value	0.848
Duration of lesions	**Rho value**	-0.029
	P value	0.842
Size of lesions	**Rho value**	0.246
	P value	0.085

Using Spearman correlation test, p value ≤ 0.05 is significant.

Relations of tissue CXCL-10 to gender, number of lesions, family history of verruca, site of lesions and previous treatment revealed that patients with multiple lesions had significantly higher tissue CXCL-10 levels compared to patients with single verruca lesions. No other significant difference was detected. **Table 5** and **Figure 3**

Table (5) Relations of tissue CXCL-10 to gender, number of lesions, family history of verruca, site of lesions and previous treatment

		CXCL-10 Mean ± SD	P value
Gender	**Male**	463.6 ± 349.5	0.372*
	Female	437.7 ± 523.9	
Number of lesions	**Single lesion**	331.8 ± 249.3	0.045*
	Multiple lesions	564.3 ± 522.1	
Family history of verruca	**Positive**	431.7 ± 473.8	0.196*
	Negative	481.7 ± 360.4	
Site	**Limbs**	454.5 ± 352.4	
	Trunk	469.1 ± 434.9	0.739#
	Face	774.8 ± 1018.9	
	Generalized	287.5 ± 426.7	
Previous treatment	**Present**	536.2 ± 511.3	0.189*
	Absent	337.4 ± 236.1	

*Using Mann-Whitney test, #Using Kruskal-Wallis test, p value ≤ 0.05 is significant

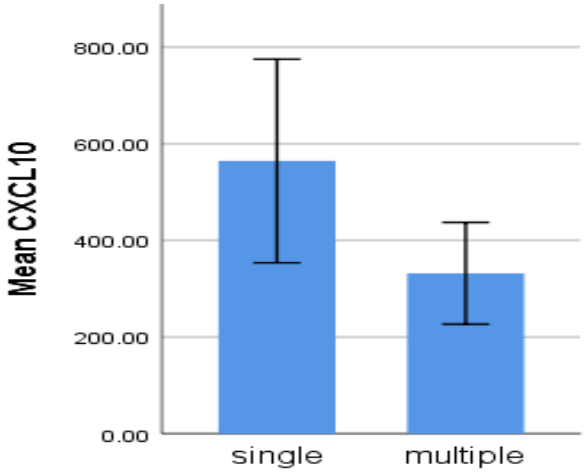

Fig. (3) Relations of tissue CXCL-10 to number of lesions

4. Discussion

Verruca vulgaris is a benign chronic condition resulting from HPV infection in the skin or mucosa. Despite several reported pathogenic mechanisms for its recurrence and relapse [11], the specific causes remain unclear. Among the potential factors contributing to verruca development are chemokines, with interferon-inducible protein 10 (IP-10/CXCL10), an α-chemokine, standing out due to its known antitumor properties related to its monocyte and T lymphocyte chemotactic activity [12]. Yet, the role of CXCL-10 in verruca vulgaris and its clinical implications remained unexplored. Therefore, our objective was to assess the tissue expression of CXCL10 in cutaneous verruca lesions.

The study involved 50 patients with verruca vulgaris, with 30 healthy skin biopsies from the same patients serving as a control group. Notably, the most commonly affected site was the limbs, mirroring the vulnerability of these areas to HPV infection due to their frequent exposure [13]. Tissue CXCL-10 levels were significantly elevated in verruca lesions than in healthy skin biopsies, possibly indicating a dual role for CXCL-10 in DNA viral infections, based on viral type and host immune status. It can either play a protective role or contribute to pathogenesis by promoting immune escape mechanisms [14].

Notably, patients with multiple lesions exhibited significantly higher tissue CXCL-10 levels than those with single verruca lesions. However, no significant correlations were observed between the levels of CXCL-10 and lesion size or duration, suggesting that multiple lesions reflect a higher viral load [15]. Tissue CXCL-10 levels demonstrated good discriminative power in verruca lesions, with sensitivity at 70% and specificity at 54% at a cut-off level of 248.4.

Given that verruca vulgaris is typically a clinical diagnosis, CXCL-10 could serve as a prognostic marker for evaluating treatment responses, with a more substantial decrease in CXCL-10 levels indicating improved viral particle clearance and treatment efficacy. While this study is the first to assess tissue CXCL-10 levels in verruca vulgaris patients, various prior studies have explored CXCL-10 in different dermatological conditions. For instance, research by Yang et al. (2017) [16] investigated the role of CXCL-10 in vitiligo and nevi, demonstrating its accumulation in skin lesions of these conditions, along with a positive correlation between increased H2O2 levels and CXCL10 in vitiligo and halo nevus.

In parallel, Richmond et al. (2017) [17] found that CXCL-10 expression in vitiligo skin lesions strongly correlated with disease activity and severity, with keratinocytes identified as the primary CXCL-10 producers. These findings align with the notion of cellular immunity and keratinocyte dysfunction playing crucial roles in verruca development [18]. Similarly, studies have reported elevated CXCL-10 levels in the skin lesions of vitiligo [19] and the hair follicles of acute-phase alopecia areata lesions [20]. In psoriasis, skin biopsies have revealed high cellular infiltration of natural killer (NK) cells, often located in the mid and papillary dermis, along with increased TNF-α and IFN-γ production [21].

Moreover, patients with atopic dermatitis exhibited significant up-regulation of CXCL-10 in skin biopsies [22]. In bullous pemphigoid, CXCL-10 levels were notably higher in-patient blister fluids, particularly among neutrophils and monocytes, indicating its role in triggering the secretion of matrix metalloproteinase-9 (MMP-9), which is related to tissue remodelling in various diseases. MMP-9 directly activates cytokines and chemokines and degrades extracellular matrix proteins [23].

Beyond dermatological diseases, CXCL-10 has also shown potential in melanoma control, reducing tumor growth in mice and melanoma invasion in vitro [24]. These findings collectively emphasize the multifaceted role of CXCL-10 in various dermatological conditions and its potential as a crucial player in verruca vulgaris pathogenesis.

5. Conclusion

In the current study the tissue expression of CXCL10 within cutaneous lesions of verruca vulgaris was studied and evaluated. Tissue levels of CXCL-10 were significantly elevated in verruca lesions in comparison with healthy skin biopsies. In addition, patients with multiple lesions had statistically significantly higher tissue CXCL-10 levels compared to patients with single verruca lesions. Therefore, tissue CXCL-10 showed beneficial role in understanding the pathogenesis of verruca vulgaris.

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