The Egyptian Journal of Hospital Medicine (January 2024) Vol. 94, Page 1140-1144

Evaluation of the Gene Expression of CXCL10 in Patients with Verruca Vulgaris

Riham Abdel Mohsen Abd Elsamie [1], Ahmed Mohamed Hamed [1], Rasha Majeed Mahmood [1] * and Inas Abd Elmonem Elsayed [2]

1 Dermatology, Venereology and Andrology, 2 Medical Biochemistry and Molecular Biology Departments, Faculty of Medicine, Benha University, Benha, Egypt.

*** Corresponding author:** Rasha Majeed Mahmood, **Email:** rashamajeed94@gmail.com, **Phone:** 01090194592

ABSTRACT

Background: Verruca vulgaris, a common skin condition caused by human papillomavirus (HPV), poses significant treatment challenges. Recent research has shifted towards understanding the molecular mechanisms underlying these lesions, including the role of chemokines like CXCL10 in their pathogenesis and potential as therapeutic targets.

Objective: This study aimed to evaluate the expression of the CXCL10 gene in patients with verruca vulgaris and to evaluate its potential as a biomarker for disease presence and severity.

Methods: A prospective case-control study that included 50 patients diagnosed with verruca vulgaris and 30 healthy controls. The study measured CXCL10 gene expression levels in verruca lesions versus healthy skin biopsies using quantitative reverse transcription PCR (qRT-PCR), following RNA extraction and cDNA synthesis.

Results: The study revealed significantly higher CXCL10 gene expression in verruca lesions (3.76 ± 2.87) compared to healthy skin (1.89 ± 2.01), with a P-value of 0.008. The discriminative power of CXCL10 expression demonstrated 77% sensitivity and 60% specificity at a cutoff point of 1.339. Untreated patients showed significantly higher CXCL10 expression compared to those who received previous treatment (P = 0.031).

Conclusion: CXCL10 was significantly upregulated in verruca vulgaris lesions, highlighting its potential as a biomarker for the condition. The lack of correlation with lesion duration or size suggests CXCL10's role is more closely associated with the presence rather than the severity of the disease. The observed reduction in CXCL10 expression following treatment may open new avenues for targeted therapeutic strategies.

Keywords: Verruca vulgaris, CXCL10, Gene expression, qRT-PCR, Biomarker, Human papillomavirus.

INTRODUCTION

Verruca vulgaris, commonly known as common warts, are benign proliferations of skin and mucosa caused by the human papillomavirus (HPV). These lesions are a frequent dermatological condition encountered across all age groups, but predominantly affect the pediatric and adolescent populations [1]. Despite their benign nature, verruca vulgaris can cause significant cosmetic concern, discomfort, and, in some cases, pain, depending on their size, number, and anatomical location. Traditional treatment modalities range from topical agents and cryotherapy to laser treatments and surgical removal. However, recurrences are common, and some treatments may lead to scarring or are contraindicated in certain patient populations, highlighting the need for novel therapeutic strategies [2].

The pathogenesis of verruca vulgaris involves the interplay of viral replication, host cellular proliferation, and the immune response. Recent advances in molecular biology have shed light on the role of chemokines, small cytokines that guide cell movement, in the immune system's response to HPV infection [3]. Among these, CXCL10 (C-X-C motif chemokine ligand 10) has emerged as a molecule of interest. CXCL10 is involved in the recruitment of immune cells to the site of infection or inflammation, playing a pivotal role in the body's defence mechanism against viral infections, including HPV [4].

Investigating the expression of CXCL10 in verruca vulgaris could provide valuable insights into the immune response to HPV infection and the pathogenesis of warts [5]. Additionally, understanding the regulation and function of CXCL10 in the context of verruca vulgaris may reveal novel biomarkers for disease presence and severity, potentially leading to more targeted therapeutic interventions. Given the limitations of current treatment options and the burden of disease, there is a pressing need to explore new biomarkers and therapeutic targets that can inform the development of more effective and less invasive treatments [6]. This study aimed to evaluate the gene expression of CXCL10 in patients with verruca vulgaris.

PATIENTS AND METHODS

The present case-control research involved 50 individuals diagnosed with verruca vulgaris. These participants were selected from the Dermatology Outpatient Department specializing in Dermatology, Venereology, and Andrology at Benha University Hospitals. The recruitment spanned from October 2022 to 2023.

Inclusion criteria: Participants of any gender, aged 18 years or older, afflicted with verruca vulgaris, and who had not undergone any systemic (immunotherapy) or topical treatments for a minimum of one month prior to the acquisition of skin biopsy samples.

Exclusion criteria: Patients who used wart treatments (topical within the last month or systemic within the last three months), had autoimmune diseases, systemic conditions (diabetes, cancer and hypertension), who were pregnant or breastfeeding, and who received

Received: 25/10/2023
Accepted: 25/12/2023

vaccines or immunomodulatory therapy in the past three months, or who showed signs of fever, inflammation, infection, or immunosuppression, including HIV.

All patients were subjected to the following: Full history taking (age, sex, number of warts, the duration of the lesions, previous treatment and family history of viral warts). General examination (Complete cutaneous examination to detect site, size and number of warts).

Sampling: Tissue lesional biopsies were taken from all patients, another biopsy was taken from normal skin of matched sites in only 30 patients and served as controls. Tissue samples were preserved directly in -80 ºC till processing.

Assessment of CXCL-10 gene expression in tissue samples: Extraction of mRNA from blood samples, complementary deoxyribonucleic acid (cDNA) synthesis, qRT-PCR for detection of CXCL-10 gene expression levels

Extraction of mRNA:
This step was performed using Thermo Scientific™ Gene JET RNA Purification Kit, cat. No. ABT001 (Applied Biotechnology, Egypt) according to manufacturer's instructions.

Procedure: Subsequently, the lysate is combined with ethanol and introduced into a purification column. The presence of chaotropic salt and ethanol encourages the RNA to adhere to the silica membrane as the lysate is centrifuged through the column. Following this, contaminants are thoroughly eliminated from the membrane by cleansing the column with washing buffers. Finally, high-purity RNA is extracted under conditions of low ionic strength using nuclease-free water.

cDNA synthesis:
Using the RevertAid First Strand Complementary Deoxyribonucleic Acid (cDNA) Synthesis Kit (Thermo Fisher Scientific, USA) according to the manufacturer's instructions. RNA quantity (1 µg) was measured using a NanoDrop One/OneC UV-Vis spectrophotometer (Thermo Scientific, USA). Gentle mixing and brief centrifugation were performed. The tubes were incubated at 65 °C for 5 minutes and then placed on ice. Next, the tubes were placed in a Bio-Rad T100 thermal cycler (Bio-Rad, USA) for cDNA synthesis according to the following program: incubation at 42 °C for 60 minutes, followed by incubation at 70 °C for 5 minutes. The cDNA was immediately stored at -20 °C for later use in qRT-PCR assays. qRT-PCR for the detection of CXCL-10 gene expression was conducted using the QuantiTect SYBR Green PCR Kit (Cat. No. 204143) according to the manufacturer's instructions.[7].

Procedure: The procedure began with placing the PCR tubes in the StepOne Plus PCR System (Applied Biosystems, Singapore), and the run was initiated according to the specified program. The program started with an initial activation step for 15 minutes at 95 °C. This was followed by a cycling step, repeated for 40 cycles, consisting of denaturation at 94 °C for 15 seconds, annealing at 55 °C for 30 seconds, and then extension at 72 °C for 30 seconds. Upon completion of the program, the expression levels of the CXCL-10 gene in each sample were determined. The assay utilized the QuantiTect Primer Assay for the CXCL-10 gene (Qiagen, Germany), with B-actin serving as the target and endogenous control genes, respectively. The primer sequences for the B-actin gene were as follows: Forward: 5′-CCTGCTTCAAATATTTCCCT-3′ and Reverse: 5′-CCTTCCTGTATGTGTTTGGA-3′. The relative fold gene expression of CXCL-10 in the different samples was calculated after normalization by the B-actin gene, using the -ΔΔC(T) method [7].

Ethical considerations: The study was done after being accepted by The Research Ethics Committee, Benha University. All patients provided written informed consents prior to their enrolment. The consent form explicitly outlined their agreement to participate in the study and for the publication of data, ensuring protection of their confidentiality and privacy. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis

The collected data from the study were meticulously revised, coded, tabulated, and analyzed using the Statistical Package for Social Sciences (SPSS) version 25.0 (IBM Corp., 2017). Data presentation and analysis varied according to the nature of each parameter, incorporating the Shapiro test for normality checks. Descriptive statistics included means, standard deviations, medians, and ranges for numerical data, alongside frequencies and percentages for categorical data. Analytical statistics employed the Independent T-test, Mann-Whitney test, Chi-Square test, Kruskall-Wallis test, and Spearman's correlation analysis to evaluate differences, relationships, and associations between study groups and variables. The study further utilized the ROC curve to determine the diagnostic sensitivity and specificity, categorizing AUC values into excellence, good, fair, poor, or failed based on their range from 0.9 to 0.5, with a significance level set at $p \leq 0.05$ and a 95% confidence interval.

RESULTS

The current study included 50 patients presented with verruca vulgaris, of them 30 patients were selected to obtain healthy skin biopsies from matched sites to be

regarded as control group. Demographic data were illustrated in table (1).

Table (1): Demographic data of included patients

Age (years)	**Mean ± SD**	33.27 ± 8.4
	Range	18 - 47
Gender N (%)	**Male**	29 (58%)
	Female	21 (42%)
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	18 (36%)
	Face	4 (8%)
Previous treatment	**Present**	29 (58%)
	Absent	21 (42%)

SD: standard deviation.

Tissue gene expression of CXCL-10 was significantly higher in verruca lesions compared to healthy skin biopsies (p = 0.008) (Table 2).

Table (2): Comparison of CXCL-10 gene expression between the studied groups

		Verruca lesions No. (50)	**Healthy skin No. (30)**	**P-value**
CXCL-10	**Mean ± SD**	3.76 ± 2.87	1.89 ± 2.01	**0.008**
	Range	0.56 – 11.18	0.02 – 7.91	

Using Mann-Whitney test, p value ≤ 0.05 is significant.
Tissue gene expression of CXCL-10 showed good discriminative power in verruca lesions compared to healthy skin with sensitivity of 77% and specificity of 60% at a cut-off relative quantification of 1.339 (Table 3).

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

AUC	**P value**	**CI**	**Cut-off point**	**Sensitivity**	**Specificity**
0.731	**0.008**	0.582-0.880	1.339	77%	60%

AUC: area under the curve, CI: confidence interval

Correlations of tissue CXCL-10 gene expression with patients' age, duration of lesions and lesions' size revealed non-significant results (Table 4).

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

		Tissue CXCL-10 gene expression
Age	**Rho value**	0.067
	P value	0.744
Duration of lesions	**Rho value**	-0.150
	P value	0.464
Size of lesions	**Rho value**	-0.070
	P value	0.734

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

Relations of tissue CXCL-10 gene expression to gender, number of lesions, family history of verruca, site of lesions and previous treatment revealed that patients who didn't receive any treatment for verruca lesions had significantly higher tissue CXCL-10 gene expression compared to patients who received previous treatment for verruca lesions. No other significant difference was detected (Table 5).

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

		CXCL-10 Mean ± SD	**P value**
Gender	**Male**	4.35 ± 3.04	0.060 *
	Female	2.15 ± 1.54	
Number of lesions	**Single lesion**	3.36 ± 3.01	0.217 *
	Multiple lesions	4.31 ± 2.7	
Family history of verruca	**Positive**	2.93 ± 1.95	0.223 *
	Negative	4.59 ± 3.43	
Site	**Limbs**	4.53 ± 3.1	0.156 #
	Trunk	1.84 ± 1.03	
	Face	2.34 ± 2.39	
Previous treatment	**Present**	2.71 ± 1.85	**0.031 ***
	Absent	5.44 ± 3.45	

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

DISCUSSION

In the current study, tissue gene expression of CXCL-10 was significantly higher in verruca lesions compared to healthy skin biopsies. In addition, tissue gene expression of CXCL-10 showed good discriminative power in verruca lesions compared to healthy skin with sensitivity of 77% and specificity of 60% at a cut-off relative quantification of 1.339.

The current study also revealed that patients who didn't receive any treatment for verruca lesions had significantly higher tissue CXCL-10 gene expression compared to patients who received previous treatment for verruca lesions.

To the best of our knowledge, there was scarcity in data involving tissue expression of CXCL-10 in verruca vulgaris patients. **Ahnaf *et al.*** [5] conducted similar work and evaluated the tissue expression of CXCL10 within cutaneous lesions of verruca vulgaris. The study included 50 cases with verruca vulgaris and collected lesional biopsies, along with control biopsies from healthy skin in 30 patients. Similar to our results, Tissue levels of CXCL-10 were reported to be significantly increased in verruca lesions in comparison with healthy skin biopsies.

CXCL-10 can play a dual role in DNA viral infections, acting as either a protective agent or a detrimental factor, influenced by the immune status of the host and the specific type of infection. It has been identified as a critical component in the body's initial defence mechanism against viral attacks, by drawing in and stimulating NK cells. Moreover, the presence of CXCL-10 increases following a DNA viral infection as a result of IFN-gamma being produced by dendritic cells and NK cells [6]. HPV was found to increase the expression of CXCL-10 in infected tissues in humans. CXCL-10 induced JAK-STAT, which aid HPV escape from immune system response. Previous literature reported different explanations of CXCL-10 increase in HPV infected tissues. **Chen *et al.*** [8] reported that HPV was found to increase the expression of CXCL-10 in infected tissues in human as well as in HPV transgenic murine samples. CXCL-10 induced Janus kinase/signal transducers and activators of transcription (JAK-STAT) and programmed death-ligand 1 (PD-L1) pathways. These mechanisms were supposedly helping HPV to escape immune response attack.

On the other hand, these findings are contradicting to previous results reported by **Liu *et al.*** [9] who reported that CXCL-10 was increased in HPV infected tissues as an immune response. Immune system acts through downregulating the expression of proliferating cell nuclear antigens and increases the expression of HPV oncoproteins E6 and E7. This leads to an increase in the apoptotic rate and thus getting rid of HPV infected cells.

Gene expression of CXCl-10 was previously discussed in other dermatological diseases. In inflammatory skin diseases with infective predisposition as in atopic dermatitis and eczema, the role of CXCL-10 expression was high-lightened. **Kasraie *et al.*** [10] reported that bacterial toxins especially from Staphylococcus aureus strongly induced CXCL10 in macrophages at both the mRNA and the protein levels. These toxins stimulated macrophages and induced the migration of human CD4+ lymphocytes via the CXCL10 receptor (CXCR3). As a result, the bacterial toxins aid in the inflammatory process of atopic dermatitis and eczema. HPV cause immune chemotaxis in the pathogenesis of verruca vulgaris that is similar to the mechanism mentioned above. That could be explained by increased CXCL-10 gene expression in patients included in our study.

Wang *et al.* [11] observed comparable results in vitiligo, a skin condition characterized by inflammation, highlighting an increased expression of the CXCL-10 receptor (CXCR3) mRNA in the peripheral blood mononuclear cells of affected individuals. In cases of progressive vitiligo, there was a notable rise in the percentages of circulating CXCR3+CD4+ and CXCR3+CD8+ T cells compared to healthy controls. However, only CXCR3+CD8+ T cell levels were elevated in individuals with non-progressing vitiligo. Additionally, histological examinations revealed a significant presence of CXCR3+ cells within the lesions of vitiligo, underscoring the ligand's involvement in the condition. Similar results were recorded by **Richmond *et al.*** [12] who reported that CXCL-10 expression in vitiligo skin lesions strongly correlates with disease activity and severity.

In their study on bullous pemphigoid, **Riani *et al.*** [13] explored the role of CXCL-10 within the cytokine/protease inflammatory cycle linked to this condition. They found that keratinocytes, fibroblasts, and immune cells at the sites of skin lesions in patients express higher levels of CXCL10 than in controls. Neutrophils and monocytes in those with the disease increased secretion of matrix metalloproteinase (MMP-9) in response to CXCL-10, triggered via the ERK, p38, and PI3K pathways. This mechanism, illustrates how MMP-9 facilitates viral spread in verruca vulgaris and also points to the broader role of MMP-9 as a type IV collagenase. MMP-9's activity in degrading extracellular matrix proteins and triggering cytokines and chemokines underscores its critical function in the inflammation and fibrosis observed across various diseases, highlighting the interconnected pathways influencing tissue remodeling [14, 15].

Recently, **El-Domyati *et al.*** [16] also demonstrated the role of CXCL-10 in immune cells attraction in vitiligo. Elevated CXCL10 expression in lesional skin correlated with increased leukocytic infiltrate, disease duration and its higher level in the serum. Moreover, changes in CXCL-10 serum levels in patients treated with psoralen plus UVA (PUVA) phototherapy, narrowband UVB (NB-UVB) phototherapy, and systemic steroids correlated with changes in the intralesional CXCL-10 expression levels in repigmented skin. The greater the decrease in CXCL-10 expression the better response of patients to treatment.
Finally, this study had some limitations, as it was done over a small sample of patients and also was performed in a single center.

CONCLUSIONS

The current study revealed that tissue gene expression of CXCL-10 was significantly higher in verruca lesions compared to healthy skin biopsies. The current study also revealed that patients who didn't receive any treatment for verruca lesions had

significantly higher tissue CXCL-10 gene expression compared to patients who received previous treatment for verruca lesions. This reflects the significant role played by CXCL-10 in the pathogenesis of verruca vulgaris.

Financial support and sponsorship: Nil.
Conflict of Interest: Nil.

REFERENCES

1. **Lipke M (2019)**: An armamentarium of wart treatments. Clin Med Res., 4: 273-93.
2. **Muršić I, Včev A, Kotrulja L *et al.* (2020)**: Treatment of Verruca vulgaris in Traditional Medicine. Acta Clinica Croatica, 59: 745-50.
3. **Mukherjee A, Wanjari U, Gopalakrishnan A *et al.* (2022)**: Exploring the Molecular Pathogenesis, Pathogen Association, and Therapeutic Strategies against HPV Infection. Pathogens, 12 (1): 25.
4. **Alaziz M, Najim N (2021)**: Histological differential diagnosis of verruca vulgaris: A systematic review. International Journal of Clinical and Diagnostic Pathology, 4 (3): 26-31
5. **Ahnaf O, Hamed A, Abd Elsamie R *et al.* (2023)**: Evaluation of the Tissue Level of CXCL10 in Patients with Verruca Vulgaris. Benha Journal of Applied Sciences, 8: 41-7.
6. **Elemam N, Talaat I, Maghazachi A (2022)**: CXCL10 Chemokine: A Critical Player in RNA and DNA Viral Infections. Viruses, 14: 2445.
7. **Livak K, Schmittgen T (2001)**: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods, 25: 402-8.
8. **Chen X, He H, Xiao Y *et al.* (2021)**: CXCL10 Produced by HPV-Positive Cervical Cancer Cells Stimulates Exosomal PDL1 Expression by Fibroblasts via CXCR3 and JAK-STAT Pathways. Front Oncol., 11: 629350.
9. **Liu T, Li J, Deng Z *et al.* (2022)**: Increased serum levels of CCL3, CXCL8, CXCL9, and CXCL10 in rosacea patients and their correlation with disease severity. J Dermatol., 49: 525-33.
10. **Kasraie S, Niebuhr M, Kopfnagel V *et al.* (2012)**: Macrophages from patients with atopic dermatitis show a reduced CXCL10 expression in response to staphylococcal α-toxin. Allergy, 67: 41-9.
11. **Wang X, Wang Q, Wu J *et al.* (2016)**: Increased expression of CXCR3 and its ligands in patients with vitiligo and CXCL10 as a potential clinical marker for vitiligo. Br J Dermatol., 174: 1318-26.
12. **Richmond J, Bangari D, Essien K *et al.* (2017)**: Keratinocyte-Derived Chemokines Orchestrate T-Cell Positioning in the Epidermis during Vitiligo and May Serve as Biomarkers of Disease. J Invest Dermatol., 137: 350-8.
13. **Riani M, Le Jan S, Plée J *et al.* (2017)**: Bullous pemphigoid outcome is associated with CXCL10-induced matrix metalloproteinase 9 secretion from monocytes and neutrophils but not lymphocytes. J Allergy Clin Immunol., 139: 863-72.e3.
14. **Miyoshi N, Tanabe H, Suzuki T *et al.* (2020)**: Applications of a Standardized Green Tea Catechin Preparation for Viral Warts and Human Papilloma Virus-Related and Unrelated Cancers. Molecules, 25: 2588.
15. **Laronha H, Caldeira J (2020)**: Structure and Function of Human Matrix Metalloproteinases. Cells, 9: 1076.
16. **El-Domyati M, El-Din W, Rezk A *et al.* (2022)**: Systemic CXCL10 is a predictive biomarker of vitiligo lesional skin infiltration, PUVA, NB-UVB and corticosteroid treatment response and outcome. Arch Dermatol Res., 314: 275-84.