Research Article

Estimation of the Level of Interlukien-33 and Interferon–γ in Lesional and Nonlesional Tissues of Patients with Viral Warts and in Healthy Controls.

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Abstract
Background: Viral warts are benign skin growths that are caused by Human Papilloma Virus (HPV). There are many types of warts either genital or non-genital. Interlukien-33 (IL-33) is a member of IL-1 family. It acts as alarmin molecule that provides protective antiviral immunity and plays an important role in many diseases. Interferon- γ (IFN-γ) has a well-known antiviral role and improves the expression of IL-33 in human keratinocytes in various diseases. **Aim of this work:** Estimation of the level of IL-33 and IFN-γ in lesional and non-lesional tissues of patients with viral warts and in healthy controls and evaluate their role in the pathogenesis of viral warts. **Subjects and Methods:** We did our study on two groups; 25 patients with viral warts and 25 matching relatively healthy controls. We used enzyme linked immunosorbent assay (ELISA) kits to measure the levels of IL-33 and IFN-γ in 4mm diameter skin biopsies from the lesion itself, the non-lesional skin of the same patients and from skin of healthy controls to compare between the results. **Results:** The levels of IL-33 and IFN-γ in lesional and non-lesional tissues of viral warts were significantly higher than in healthy controls. **Conclusion:** Our presenting study is suggesting that IL-33 and IFN-γ may play a role in the pathogenesis of viral warts.
Keywords: Enzyme-linked immunosorbent assay, Interferon- γ, Interlukien-33, Viral warts

Introduction
Viral warts are caused by infection with Human Papilloma Virus (HPV) which hasover 100 types. It usually causes infection by getting into the skin through areas of minimal trauma.[1]

The infection is more common among immunosuppressed individuals and school children.[2,3]

Interlukien-33 (IL-33) is a member of IL-1 family. IL-33 plays a key role in innate and adaptive immunity and plays a crucial role in multiple infectious and inflammatory disorders.[4]

Research has revealed the role of IL-33 in providing protective anti-viral immunity which is improved under the effect of Interferon-γ (IFN-γ) which increase its expression as found in cultured epithelial cells.[5]

IFN-γ has antiviral and immunomodulatory activities.

Its antiviral activity is important in early stages of viral infection. On the other hand, its immunomodulatory activities play a crucial role in later stages by coordinating the immune response to establish an antiviral state for longer term control.[6]

Our study was performed to find out whether IL-33 and IFN-γ have a role in the pathogenesis of cutaneous viral warts.

Subjects and Methods
We did our study on two groups; 25 patients with viral warts and 25 matching relatively healthy controls.

We used enzyme linked immunosorbent assay (ELISA) kits to measure the levels of IL-33 and IFN-γ in 4mm diameter skin biopsies from the lesion itself, the non-lesional skin of the same patients and from skin of healthy controls to compare between the results.

Inclusion criteria:

1. Age between 18 to 43 years old
2. Patients with viral warts .
3. Both males and females will be included .

Exclusion criteria :

1. Age below 18 and above 43 years old
2. Patients diagnosed with acute infection at time of sample taking .
3. Patients with associated other dermatological diseases.

Statistical methodology

Data were analysed and entered by using the statistical package SPSS (Statistical Package for the Social Sciences) version 24. Data were summarized using standard deviation, mean, median, minimum and maximum in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. Comparison of sex distribution among the study groups was done using the χ^2 test.

Comparisons between quantitative variables were done using the non-parametric Kruskal-Wallis and Mann-Whitney tests. Correlations between quantitative variables were done using Spear-man correlation coefficient.

Results

All patients and controls had expression of IL-33 in their skin biopsies. The mean level of IL-33 was significantly higher in the lesional biopsies from patients with viral warts compared with HCs (119.11 vs. 34.34; P <0.001) and was also significantly higher in nonlesional biopsies than in healthy control biopsies (81.20 vs. 34.34; P <0.001) (Table 1, fig. 1, 2).

Similarly, all patients and controls had expression of IFN-γ in their skin biopsies. The mean level of IFN-γ was significantly higher in the lesional biopsies from patients with viral warts in comparison with HCs (87.29 vs. 18.25; P <0.001) and was also significantly higher in nonlesional biopsies than in healthy control biopsies (51.46 vs. 18.25; P < 0.001) (Table 1, Fig. 3,4).

There was no detected association in the patient group between IL-33 and IFN-γ expression and either age, sex or disease duration (p-values> 0.05).

Table 1: Comparison of Interlukien-33 and Interferon-γ level.

Variable	Patients with Viral Warts N= 25 Mean ±SD	Healthy Control N= 25 Mean ±SD	P value
Interlukien-33			
Lesional skin	119.11 ±17.5	34.34 ±7.0	<0.001*
Non lesional skin	81.20 ±13.0	34.34 ±7.0	<0.001*
Interferon-γ			
Lesional skin	87.29 ±12.6	18.25 ±4.8	<0.001*
Non lesional skin	51.46 ±12.9	18.25 ±4.8	<0.001*

*** P-value ≤ 0.05 is considered significant by (Mann–Whitney U test).**

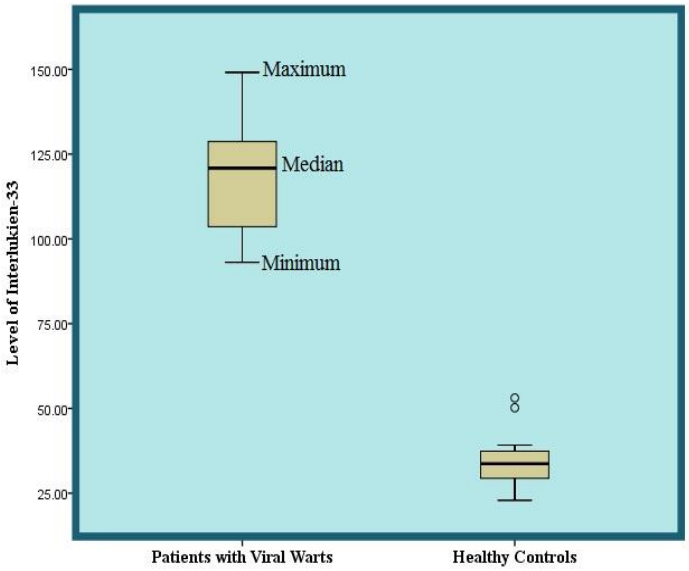

Fig. (1): Comparison of level of IL-33 among lesional skin of studied patients with viral warts and healthy control individuals.

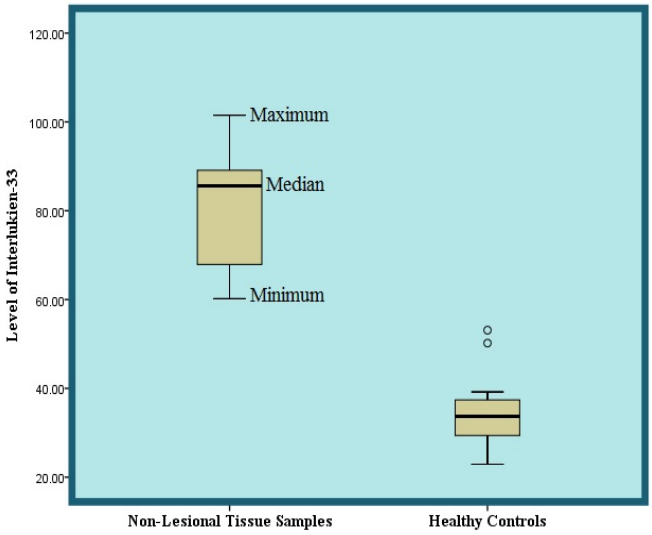

Fig. (2): Comparison of level of IL-33 among non-lesional skin of studied patients with viral warts and healthy controls.

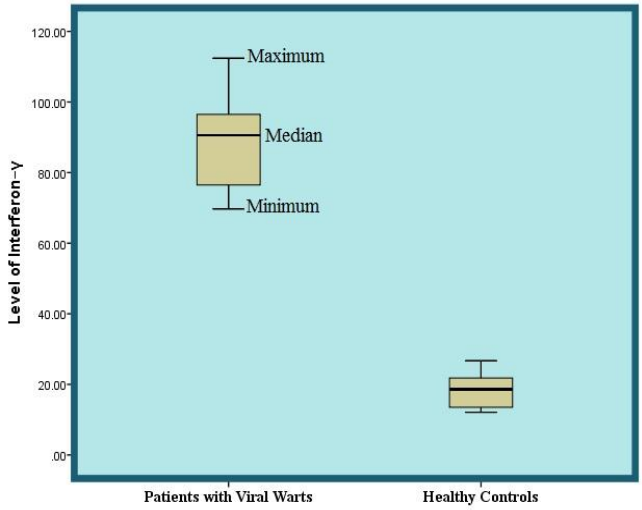

Fig. (3): Comparison of level of IFN–γ among lesional skin of studied patients with viral warts and healthy controls.

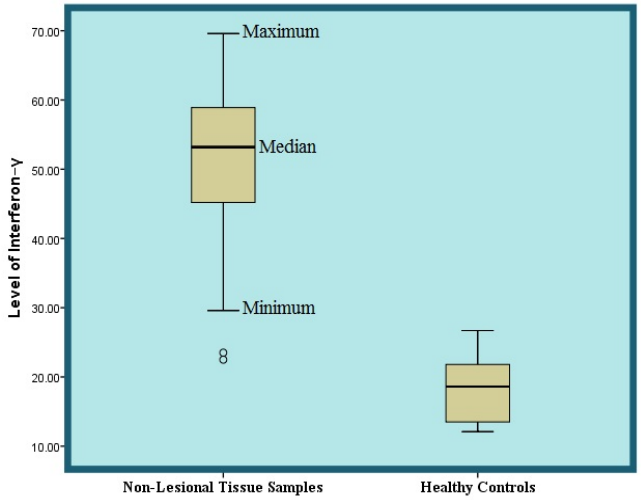

Fig.(4): Comparison of level of IFN–γ among non- lesional skin of studied patients with viral warts and healthy control.

Discussion

Viral warts are benign epithelial growths that are caused by a family of DNA viruses called Human papilloma viruses (HPVs). Lesions appear either on skin or mucous membranes.[7] It is a very common condition among school children and adults.[3]

It is well known that host immune defense mechanisms play a great role in fighting and eliminating initial HPV infection in large percentage of individuals affected by it.[8]

IL-33 is a proinflammatory cytokine that has different isoform products which are biologically active. IL-33 is known to be associated with Th2 immunity. Multiple evidences now support the role of two of IL-33 isoforms in facilitating the production of protective CD8 T cell and Th1 immunity against various pathogens. For that reason, a better studying and understanding of the IL-33 isoforms will update us on how to make use of them and utilize them in developing, for example, vaccine adjuvants for immune therapy.[9]

Research had made clear the vital role of IL-33 in the immune system as it was found that it plays a great role in driving protective antiviral immunity. In addition to that, researchers had shown also that IFN-γ plays an important role in improving IL-33 expression in the cultures of epithelial cells.[5]

Our study included 25 patients diagnosed clinically with viral warts and 25 healthy controls. Those patients were diagnosed at dermatology clinic at Beni-Suef University Hospital.

The aim of our study was to estimate the level of the IL-33 and IFN-γ in patients with viral warts; lesional and non-lesional in the same patients and in healthy controls skin biopsies to detect any possible role of these cytokines in the pathogenesis of the viral warts.

In our current study, we found that the levels of IL-33 in patients with viral warts were significantly higher when compared to its levels in skin biopsies of healthy controls where the mean values were (119.11 vs. 34.34) in patients with viral warts and in healthy controls respectively; (p-value <0.001).

Wang et al., (2014)[5] reported that IL-33 was expressed in endothelial cells and epithelial cells in HPV-positive cervical tissues. Additionally, IL-33 protein levels were positively correlated with IFN-γ mRNA

levels in patients infected with HPV cervical infection. Moreover, In vitro, IFN-γ was also found to up regulate IL-33 expression in human epidermal keratinocytes (NHEKs) in a dose-dependent manner.

On the other hand, in a study done by **Jin et al., (2018)**[10] on skin biopsies from three patients with herpes viral infection and one patient with herpes zoster infection and with two patients with verruca vulgaris found that strong nuclear and mild cytoplasmic staining of IL-33 was detected in the epidermal keratinocytes of the lesional skin samples with HSV and VZV infection. Though, IL-33 was not detected in VV lesions. Very weak cytoplasmic staining was observed in the basal layers of VV samples.

In our recent study, we also observed that levels of IL-33 in lesional skin of patients with viral warts were significantly higher when compared with non-lesional skin of the same patients where the mean values were (119.11 vs. 81.20) in lesional and non-lesional skin in patients with viral warts respectively; (p-value <0.001).

Furthermore, Levels of IL-33 in non-lesional skin of patients with viral warts were found to be significantly higher as compared with normal healthy controls where the mean values were (81.20 vs. 34.34) in non-lesional skin of patients with viral warts and normal healthy controls respectively; (p-value <0.001).

In a study done by Abu El-Hamd et al., (2019)[11] to estimate serum levels of IL-21 and IL-33 between 45 patients with genital warts (GWs) and its comparison with 45 relatively healthy controls using commercially ELISA kits . The result was that levels of serum IL-21 and IL-33 were significantly lowered amongst patients with GWs in comparison with the healthy controls. These low levels of both cytokines may have a role in development, severity, persistence, and recurrence of GWs which depends mainly on the deceased cell-mediated immunity. This result is inconsistent with our work and that maybe as we estimated the level of IL-33 in tissue biopsies not in serum. Also the variation in the technique and the subjects may played a role in this disparity of the results.

In our work, we also found that levels of IFN-γ in patients with viral warts were significantly higher in comparison with its levels in healthy controls where the mean values were (87.29 vs. 18.25) in patients with viral warts and normal healthy controls respectively; (p-value <0.001).

It is well known that IFN-γ has an important antiviral role and that was proved by this study done by **Banno et al., (2003)**[12] in which they found that IFN-γ starts a well-ordered molecular program boosting host antiviral defenses, obstructing viral entry into healthy keratinocytes needed for viral replication, suppressing kertinocytes proliferation and hindering differentiation that may get in the way with the epidermal tropism of viruses that require differentiating cells for growth as in case of HPV.

Level of IFN–γ in lesional skin of patients with viral warts was significantly higher as compared with non-lesional skin of the same patients where the mean levels were (87.29 vs. 51.46) in lesional and non-lesional skin in patients with viral warts respectively; (p-value <0.001).

Level of IFN–γ in non-lesional skin of patients with viral warts was significantly higher as compared with normal healthy controls where the mean values were (51.46 vs. 18.25) in non-lesional skin of patients with viral warts and normal healthy controls respectively; (p-value <0.001).

Nofal et al., (2017)[13] in a study done on 54 patients with multiple verruca vulgaris. A statistically significant relation was found in between the levels of IFN-γ and the response of warts to in tralesional injection of Candida antigen, suggesting that it may be used as a good predictor of the therapeutic response of warts to this line of therapy. A blood sample

was taken from patients before starting therapy with candida antigen and cultured. Evaluation of levels of IFN-γ in treated cases was done and they found that there is a relation between treated cases and elevation of IFN-γ. The exact mechanism of therapy by candida antigen is still unclear but is thought to be mediated by stimulation of cytokines secreted by Th1 cells such as IFN-γ.

But on the other hand, we found that there is no detected correlation between level of IL-33 and level of IFN-γ in lesional warts among studied cases; (p-values> 0.05).

In a study done by Meephansan et al., (2012)[14] found that when NHEKs are treated with IFN-γ, it induces production of IL-33 by these cells. Real-time PCR (RT-PCR) showed that IFN-γ significantly increased the expression of IL-33 mRNA in comparison with untreated cells in a dose dependent manner. These results indicated that the cytokine IL-33 has a vital role in inflammatory skin diseases especially in those diseases where IFN-γ and TNF-α are secreted in high levels.

Level of IL-33 was significantly highest among cases with skin type (III) as compared with other skin types (p-value= 0.033). However; no detected significant differences between other skin types. Level of IFN–γ among studied lesional warts had no significant differences in relation to different skin types; (p-values> 0.05).

No detected association between level of IL-33 and IFN–γ in lesional warts and course of disease; (p-values> 0.05). Also there is no detected association between level of IL-33 and IFN–γ in lesional warts and sex of studied cases; (p-values> 0.05).

No detected correlation between level of IL-33 and IFN–γ in lesional warts and age of studied cases; (p-values> 0.05).

Level of IL-33 showed negative slight correlation with disease onset (months); (r= -0.385, p-value= 0.049). On the opposite side; IFN–γ had positive moderate correlation with disease onset (months); (r= -0.385, p-value= 0.016) among lesional warts of studied cases No detected correlation between level of IL-33 and IFN–γ in lesional warts and age of studied cases; (p-values> 0.05).

Conclusion and Recommendations

There was elevation of the level of IL-33 and IFN-γ in lesional tissues of patients with viral warts and these levels were higher than their levels in non-lesional tissues of the same patients, but in the same time, the levels of both cytokines in lesional and non-lesional tissues were higher than in healthy controls skin tissues.

These results suggest that during the viral infection with HPV. These cytokines are fighting the infection by putting the body in an antiviral state and trying to cure the body from viral warts.

We recommend further studies on more patients of the antiviral role of IL-33 and IFN-γ in the pathogenesis of viral warts.
Estimating the level of IL-33 and IFN-γ in the serum of patients with viral warts and in healthy control to compare.

Clinical trials are suggested to be done on treating viral warts via using these two cytokines and trying to use them in predicting the success of different lines of therapy of viral 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.

References

1. Loo, S. K. F., & Tang, W. Y. M. (2009): Warts (non-genital). BMJ clinical evidence, 2009.
2. Nai-Ming, L., & Yuk-Ming, T. W. (2007): Warts (non-genital). BMJ clinical evidence, 2007.
3. Bruggink, S. C., de Koning, M. N., Gussekloo, J., Egberts, P. F., ter Schegget, J., Feltkamp, M. C., ...

&Eekhof, J. A. (2012):- Cutaneous wart-associated HPV types: prevalence and relation with patient characteristics. Journal of Clinical Virology, 55(3), 250-255.

4. Liew, F. Y., Girard, J. P., &Turnquist, H. R. (2016):- Interleukin-33 in health and disease. Nature Reviews Immunology, 16(11), 676.
5. Wang, L., Li, H., Liang, F., Hong, Y., Jiang, S., & Xiao, L. (2014): Examining IL-33 expression in the cervix of HPV-infected patients: a preliminary study comparing IL-33 levels in different stages of disease and analyzing its potential association with IFN-γ. Medical Oncology, 31(9), 143.
6. Schroder, K., Hertzog, P. J., Ravasi, T., & Hume, D. A. (2004): Interferon-γ: an overview of signals, mechanisms and functions. Journal of leukocyte biology, 75(2), 163-189.
7. Stanley, M. A. (2012): Epithelial cell responses to infection with human papilloma virus. Clinical microbiology reviews, 25(2), 215-222.
8. Westrich, J. A., Warren, C. J., & Pyeon, D. (2017): Evasion of host immune defenses by human papillomavirus. Virus research, 231, 21-33.
9. Villarreal, D. O., & Weiner, D. B. (2015):- IL-33 isoforms: their future as vaccine adjuvants?.
10. Jin, M., Komine, M., Tsuda, H., Oshio, T., &Ohtsuki, M. (2018): Interleukin-33 is expressed in the lesional epidermis in herpes virus infection but not in verruca vulgaris. The Journal of dermatology, 45(7), 855-857.
11. Abu El-Hamd, M., Assaf, H. A. R., Sedky, A., & Mohammad, S. H. (2019): Possible role interleukin-21 and interleukin-33 in patients with genital warts. Dermatologic therapy, e13063.
12. Banno, T., Adachi, M., Mukkamala, L., & Blumenberg, M. (2003): Unique keratinocyte-specific effects of interferon-gamma that protect skin from viruses, identified using transcriptional profiling. Antiviral therapy,8(6),541-554.
13. Nofal, A., Marei, A., Amer, A., & Amen, H. (2017)**:** Significance of interferon gamma in the prediction of successful therapy of common warts by intralesional injection of Candida antigen. International journal of dermatology, 56(10), 1003-1009.
14. Meephansan, J., Tsuda, H., Komine, M., Tominaga, S. I., & Ohtsuki, M. (2012): Regulation of IL-33 expression by IFN-γ and tumor necrosis factor-α in normal human epidermal keratinocytes. Journal of Investigative Dermatology, 132(11), 2593-2600.