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Effective role of narrow band-UVB in immunological changes of HZV: T-cells response

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Abstract

Varicella-zoster virus (VZV) infection often observed in children and usually lasts a short time. Its remains dormant in the nervous system and can reactivate causing herpes zoster (HZ). The objective of this study is to investigate the effective role of nb-UVB, on immunological response of T-regulatory cells via different types of HZV transcription factors and possible pathological mechanism. Together these data confirmed that the using of nb-UVB in treatment of VZV is associated with T-regulatory cells response changes and attenuated VZV infection.

Keywords: Varicella-zoster virus; nb-UVB; Herpes zoster; T-regulatory cells

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Introduction

VZV is an exclusively human, high neurotropic Alpha herpes virus of the Varicello virus genus, VZV causes two important related infections, primary infection causes Varicella (chicken pox), and latent infection herpes zoster HZ (shingles) [1]. HZ results as activation of VZV from nerves were it was latent after infection with chicken pox [2]. The activation of virus could be due to reduction of VZV-specific T-cells (CD4+T lymphocytes), that keep the virus latent and prevent the reactivation occur back with aging or in the causes of immune compromised, VZV high pathogenesis virus during the infection the antigen of VZV stimulate T-lymphocytes that lead to express many of immune regulation genes which involve many transcription factors functionally essential to management and initiation of immune response [3]. It includes NFAT (nuclear factor activated transcription factor), and AP-1 (activated protein transcription factor), NFAT which is consider a key regulator of T cells activation and energy [4]. NFAT transcription factor family

consist of five members: NFAT1, NFAT2, NFAT3, NFAT4, and NFAT5 that expressed in immune system cells like T and B cells, natural killer cell (NK), and also in non-immune system and tissues like heart muscle, skeleton muscle, and neurons [5]. NFAT form cooperative complex with AP-1 family which belong to C-fos, C-jun, ATF, families. AP-1 in turn control a number of cellular process including differentiation, proliferation, apoptosis, and it is regulating gene expression in response to various stimuli included cytokines, stress, bacterial and virus infection [6]. NFAT + AP-1 complex responsible in the activation of T cells (CD4 and CD8 T cell), T helper cell CD4 induced the production of pro- inflammatory cytokines which have role in acute phase of HZ infection such as IL-2, IL-6, IL-12 and INFα, γ [7]. CD8 cell play an important role in elimination of virus in addition to CD4 which have role in both latent and activated form of virus [8]. Where these cells can recognize the mediated proteins that express by VZV during it latency in ganglion nerves for that cells mediated immunity (CMI) can limit the replication and spread of VZV with nerves and not to reactivation [9]. There is association between the magnitude of CMI and cytokines production in limiting the severity of HZ in one hand, on the other hand the extent response and production of pro-inflammatory cytokines for non-infected cells and tissues lead to harmful consequences to the host, so there is need of T- regulatory cells which can be recognized from the cluster of differentiation on its surfaces include (CD4+CD25+FOXP3) [10]. The importance of T- regulatory cell lies in the control of the peripheral tolerance, preventing auto immune disease and limits the inflammatory immune response to be chronic. FOXP3 (fork head protein) produced by gene foxp3 its bind to specific region on DNA and control on many of gene expression, FOXP3 have crucial role in development and function of T- regulatory cell [11]. Both of FOXP3 and T- regulatory cells have an important role of VZV infection and many other viruses like hepatitis, Epstein bar virus and herpes virus, through regulating the excessive inflammatory response and protect the non-infected cells and tissues from destruction [12]. Where FOXP3 control the activation of T-lymphocytes and their production of pro-inflammatory cytokines by interaction with other transcription factors like NFAT, NF-KB and AP-1 which cause to decrease the inflammation, FOXP3 also express many of anti-inflammatory cytokines like IL-10, IL-35 which causes inhibition the pro-inflammatory cytokines. One of the ways that induced the T- regulatory cell expression is by exposure to nb-UVB phototherapy which includes a range of radiation spectrum ultraviolet nearly 311 nm where its less than 1% from waves of sun rays [13]. Nb-UVB use for remediation many of skin disease like skin infection, psoriasis, vitilago, eczema, fungi, itching and inflammation of nervous and skin [14]. nb-UVB have many mechanisms in regulation of immune response: induced the expression of T- regulatory cell and FOXP3, prevent the antigen presenting and apoptosis of leukocytes. nb-UVB gives

therapeutic results faster with less side effects such as skin sensitive, redness and erythematic, also it has wide positive effect in treatment. This work was aimed to determine the modulation of the gene expression of T- regulatory cells in patients with VZV treated by phototherapy and chemotherapy.

Material and Methods

Patients groups

This study was done during December 2012 - April 2015. It was carried out on 115 individuals (70 patients was infected with HZ at acute stage, 20 patients were infected with PHN and 25 healthy apparently individuals as control group). Ethical approval for the study was granted from the Ethical Committee at the University of Rochester School of Medicine and Dentistry (H-D-2007–0032). Patient's ages ranged from 7-80 years. The first visit of patients in the hospital in all most within 10 days of the development of symptoms of HZ rash, as reported by the physicians and patients, then they divided into two groups: T1 group (50 patients) who treated with phototherapy by NB-UVB cabin beside antiviral /acyclovir 800 gm five times a day for 7 days. and T2 group (40 patients) who received antiviral/ acyclovir 800 gm five time a day for 7 days with corticosteroid in severe pain cases, for both groups the VRS (variable rat score) was recorded in every visit to the hospital which determined by the dermatologist.

Designing phototherapy sessions

Sessions of phototherapy included (50) patients infected with HZ and PHN, Patients who had received treatment in Department of Dermatology in NB-UVB cabin, it is carried out on (35) men and (15) women with age range from 30-80 years. Every patients had a different degree of pain average between (0-4) point according to a variable rate score VRS, every point means a different degree of pain where (0) means no pain and (4) which means incredible pain, T1 group treated with NB-UVB (narrow band ultraviolet 311) for three times per week ,exposed locally at infected part of body while the rest of body was covered by clothing, first session started with dose 30 mj /cm (40 second) and the dose was gradually increased by 10 mj /cm at every session until reach 180 jm /cm (2 -3 minutes) as long as there is no adverse effect reported such as persisting erythematic ,burn and itching. Patients still get session until pain relief or for a maximum 18 sessions [15].

Collection of samples

Five ml of vinous blood were collected from studied group (one sample per week). 2 ml of blood kept in (EDTA) tube as anticoagulant, and 3 ml left in the room temperature at 30 minutes. Then serum was separated by centrifuge at the 4000 rpm for 5 minutes. Both of the blood samples and sera stored at 20 °C in deep freeze unit [16] until used for immunological and molecular assays.

IL-6 profile

IL-6 was assayed by Elisa using kit according to manufacture (Ray Bio® the protein array pioneer company).

Primer design and study

Primers that used in this study were GAPDH (Glycerol Aldehyde-3-Pho-sphate Dehydrate Genies). Gene primers used as Housekeeping gene, FOXP3, NFAT and AP-1 genes. Primers used as

target genes for gene expression. These primers were designed by using NCBI-Gene Bank data base and Primer 3 plus online. The primers used in quant-ification of gene expression using RT-qPCR techniques based SYBER Green DNA binding dye, and supplied by (Pioneer, Korea). As listed in (Table -1).
Data Analysis of qRT-PCR:

The results of data of qRT-PCR for target and housekeeping genes were analyzed by the relative quantification gene expression levels (fold change). Reference method that described previously [16].

Statistical analysis

Obtained data were statistically anal-yzed using the statistical package SPSS (Statistical Package for Social Sciences) version 10.0 for windows. The investigated parameters were presented in as mean ± standard error (S.E.), and differences between means were assessed by ANOVA (analysis of variance), followed by LSD (least significant difference). The difference was considered significant when the probability (P) value was ≤ 0.05 [18].

Results

Distributions of herpes zoster infection:
the results showed that infected men were higher than women for both HZ and PHN, about 67 men (74.44%) and 23 women (25.55%) as showed in the figure (1). Results also showed that the highest proportion of HZ infections within age group (41-60) by 39 (55.71%) followed by age group (≥ 60) by 16 (22.85 %) compared with the rest of groups which were the proportion of age group (1-20) are 6 (8.57%) and age group (21-40) are 9 (12.85 %) as show in the figure (2). For PHN documented results showed that the highest proportion of cases were within the age group (> 60) by 11 (65%) followed by age group (41-60) by 5 (25%) and category (21-40) are 2 (10%) and there is a lack in category (1-20) are (0 %) as showed in figure (3).

IL-6 profile

Obtained data of IL-6 levels showed a significant increase (P> 0.05) in the levels of il-6 in T2 group (87.8±24), compared to control (59.6), and T1 group (60.46±13.04), who showed no significant change (P>0.05) in comparison to control group (59.6) as showed in the figure (4 and 5).

Quantitative Reverse Transcription Real-Time PCR (RT-qPCR)

Quantitative Reveres Transcription Real-Time PCR technique was perfo-rmed for assessment of comparative quantification (gene expression analysis of FOXP3, NFAT & AP-1).

This technique was done according with the described method by (Wang & Hardy). The following thermo-cycler protocol in the following (Table -2), and figure [6, 7, 8, 9].

FOXP3 Gene Expression

The documented results showed a significant increment (P> 0.05) in the gene expression of FOXP3 for T1 and T2 group compared to the control group (1.910±0.6846), data recorded highly significant increment in T1 group (18.22±5.44) at the end of the sessions in comparison to the stage before sessions (10.5 ±2.5), while the data showed that in the T2 group there is no significant differences (P> 0.05) between the end of treatment (10.85±6.5672) and the stage

before treatment (14.5±10.6), Concluded data showed that the expression of FOXP3 at the end of treatment was higher in the T1 group than T2 group and control group as showed in table (4).

NFAT Gene expression

The obtained results showed a significant change (P> 0.05) compared to control (3.002±0.86), in other hand there was no significant difference before starting treatment and at the end of it for both groups were: T1 group gene expression for NFAT before sessions was (9.66±1.17) while at the end of sessions was (11.33±2.5), also in T2 group gene expression was (16.86±6.2) before chemotherapy treatment, and was (10.92 ±1.49) at the end of chemotherapy, as showed in table (5).

AP-1 Gene expression

Recorded results showed a significant change (P>0.05) at the gene expression of AP-1 in compared to control group (1.88 ± 1.25) in T1 group , but it was no significant difference before started sessions (10.55±2.5) and at the end of sessions (7.57±1.81), for T2 group the results showed no significant difference in NFAT gene expressions compared to control (1.88±1.25), also no significant changes before treatment which was (4.53±1.5) and at the end of treatment was (3.77±1.4), as showed in table (6).

Phototherapy results

Results showed remarkable differences between T1 group patients who are subjected to phototherapy sessions, and T2 group patients who are received chemotherapy treatment, as showed in the figure (10, 11).

The data showed that the percentage of response and improvement in (T1) group (92%) at the end of phototherapy sessions (one month later) higher than in (T2) group (60%) who relied on antiviral only (one a month later). As well as the results showed that beyond three months later than the starter of treatment which the improvement rate in T1 group was (100%) complete recovery compared with the T2 group which was (80%).

Verbal pain scale (VRS)

The results of the pain scale in patients with HZ, in the T1 group patient who exposed to phototherapy plus antiviral the irritable pain associated with the infected disappeared at the fastest time after the session's end (0.041 ± 0.20) compared with the group T2 (0.48 ± 0.65) and after three months later, the rate of VRS was (0±0) in T1 group compared with the T2 group which was (0.2 ± 0.4), as showed in the figure (12 and 13).

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Primer	Sequence		Reference
NFAT Gene	F	GT TGGGGAGT TGGCACTAGC	In this study
	R	GACCCGGGCT T TCTACTGG	
AP1 Gene	F	GGTGGGATAAGACCCCCTCA	In this study
	R	TCCTGCCTGCATAGCAATAGG	
FoxP3 Geng	F	TGTGCTAGGGCGGTATGAGA	In this study
	R	GCTGGGGTGCAACTATGGG	
GAPDH	F	ACGACCACTTTCTCAAGCTC	(Hayase *et al.* 2005)
	R	T TCCTCT TGTGCTCT TGCTG	

Table -1.
Primer sequences

qPCR step	Temperature	Time	Repeat cycle
Initial Denaturation	50 °C	1 hour	1
Denaturation	95 °C	20 sec	45
Annealing\Extension Detection (scan)	60 °C	30 sec	
Melting	60-95°C	0.5 sec	1

Table-2
Thermocycler protocol

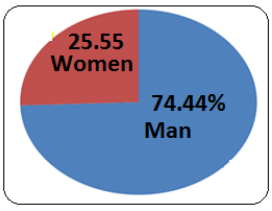

Figure 1.
Distribution of patients with HZ and PHN by (gender).

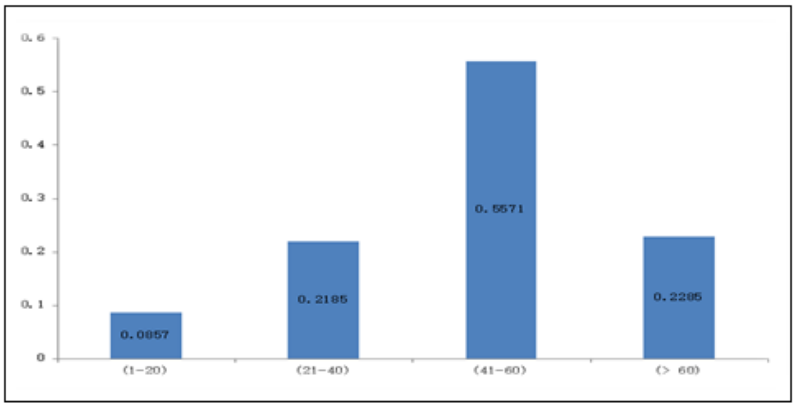

Figure 2.
Distribution of patients with herpes zoster by age group.

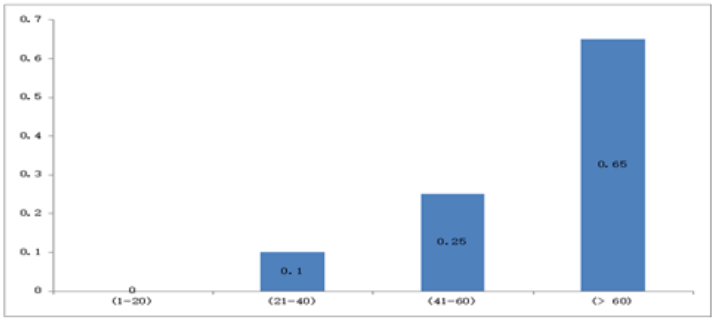

Figure 3.
Distribution of patients with PHN by age group.

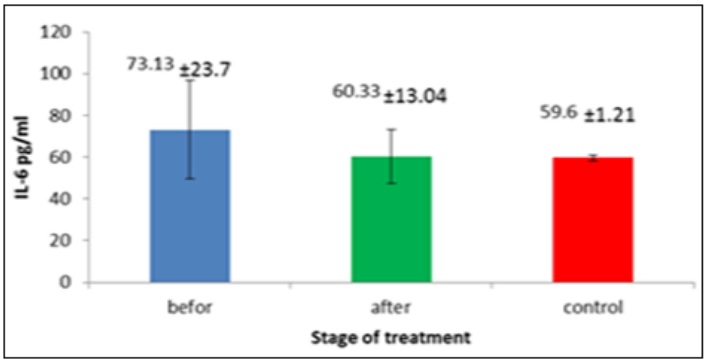

Figure 4.
Levels of IL-6 at T1 group and control.

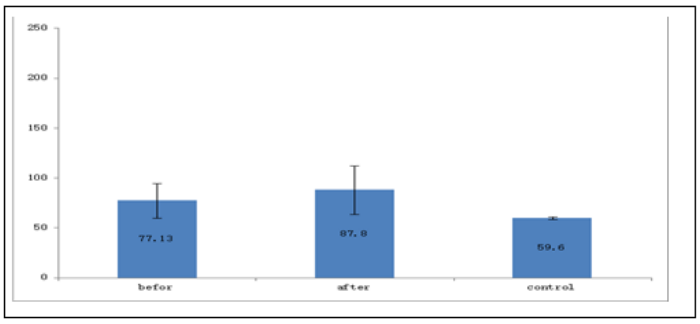

Figure 5.
Levels of IL-6 at T2 group and control.

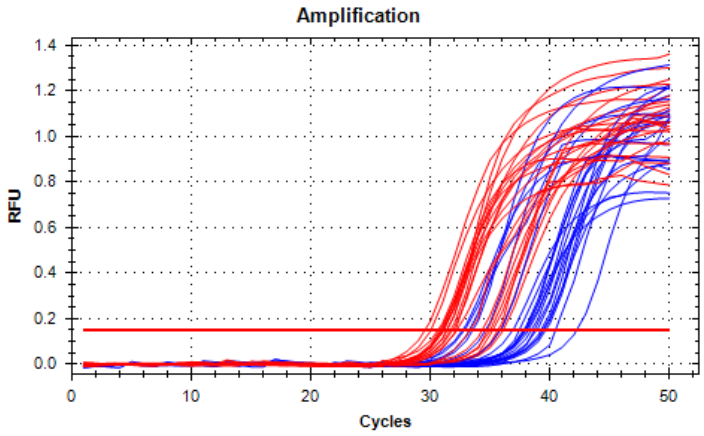

Figure 6.
Real-Time PCR amplification plot of FoxP3 gene patientsgroup samples (red plot) and healthy control group samples (blue plot).

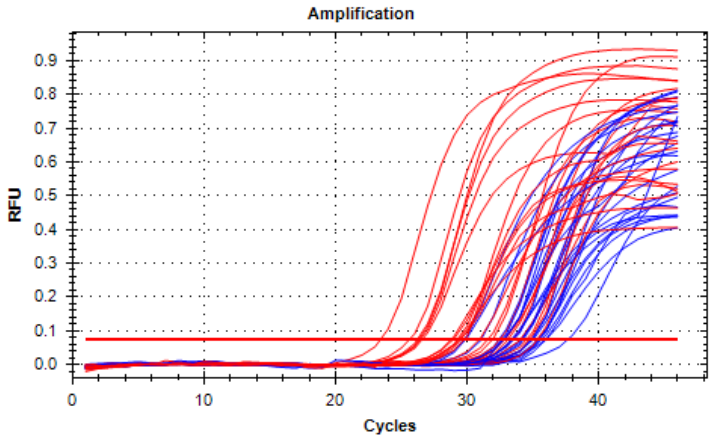

Figure 7.
Real-Time PCR amplification plot of NFAT gene in patients group samples (red plot) and healthy control group samples (blue plot).

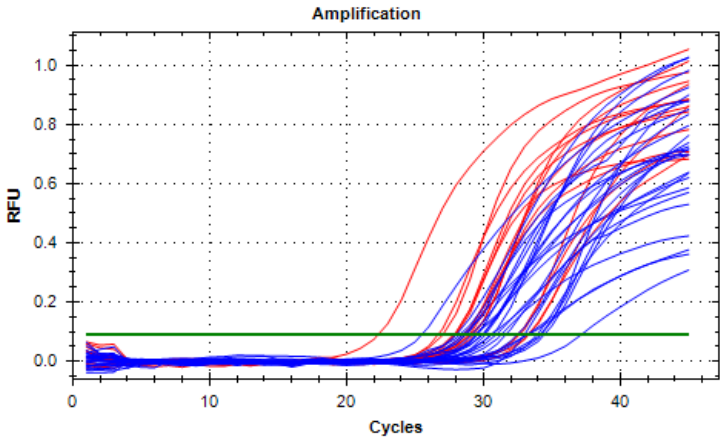

Figure 8.
Real-Time PCR amplification plot of API gene in patients group samples (red plot) and healthy control group samples (blue plot).

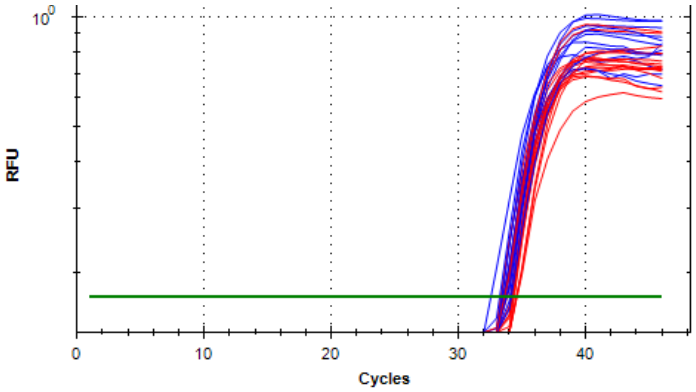

Figure 8.
Real-Time PCR amplification plot of API gene in patients group samples (red plot) and healthy control group samples (blue plot).

Treatment type	Stage of treatment		
	Before treatment	At mid period of treatment	At the end of treatment
Phototherapy	10.55±2.51	9.60±3.25	18.22±5.44
Chemotherapy	14.55±10.64	7.89±5.82	10.85±6.56
Control	1.91±1.25	1.91±1.25	1.91±1.25

Table 4.
Gene expression of FOXP3 by RT-qPCR

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Treatment type	Stage of treatment		
	Before treatment	At mid period of treatment	At the end of treatment
Phototherapy	9.66±1.17	8.93±1.31	11.33±2.55
Chemotherapy	7.66±3.27	6.50±1.91	10.92±1.49
Control	3.002±0.86	3.002±0.86	3.002±0.86

Table 5.
Gene expression of NFAT by RT-qPCR

Treatment type	Stage of treatment		
	Before treatment	At mid period of treatment	At the end of treatment
Phototherapy	10.55±2.516	9.60±9.60	7.57±1.81
Chemotherapy	4.53±1.53	5.34±1.08	3.77±1.47
Control	1.88±1.25	1.88±1.25	1.88±1.25

Table 6.
AP-1 gene expression by RT-qPCR

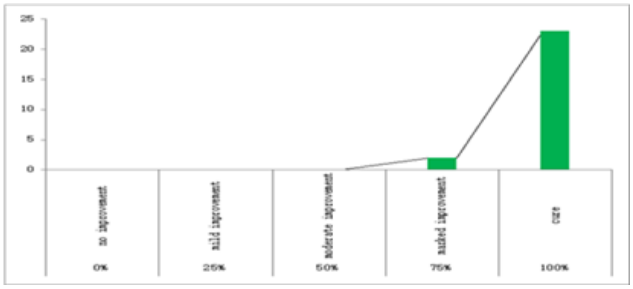

Figure 9.
Percentages of the degree of improvement in VRS at T1 group befor sessions.

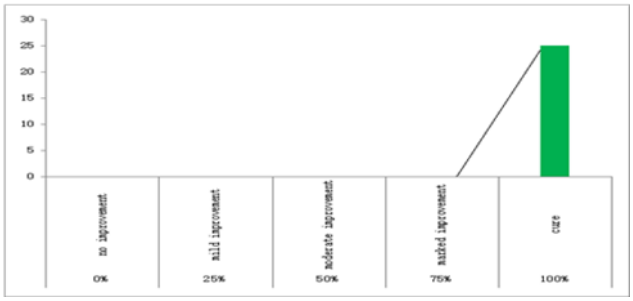

Figure 10.
Percentage of the degree of improvement in VRS in T1group after three months later.

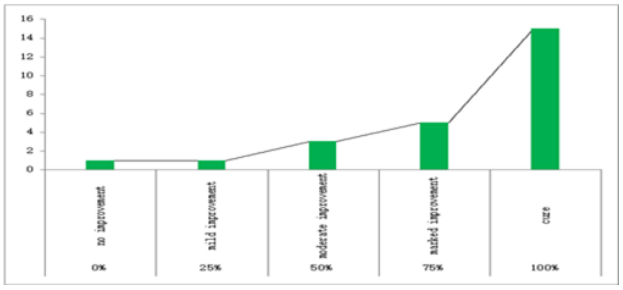

Figure 11.
Percentage of the degree of improvement in VRS inT2group at the end of chemotherapy.

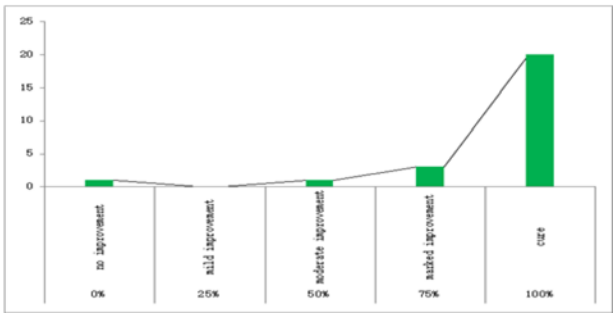

Figure 12.
Percentage of the degree of improvement in VRS inT2group after three months later.

Discussion

From the obtained results, it was clear that nB-UVB represent a positive effective tool in treated patients with HZ and PHN. The recovery from HZ infection was 100% in patients who subjected nB-UVB session and which never developed to PHN in all cases as mentioned in the study of (Jalali et al., 2006).

When they used the broad band ultraviolet rays in treatment. The current study also showed the role of nB-UVB in reduction the nerve pain in people living with PHN, as reported in the study of (Eman, 2011). In study of (Knappe, 2013), showed that light rays can effect on the nerve ending in the superficial dermises and epidermis by reducing the cutaneous nerve density, in addition light rays have ability to improve the damage nerve ending by regeneration ending damage. nB-UVB also play fundamental role as antimicrobial by inhibiting the replication of viruses through the light rays which can penetrate skin and raised the levels of vitamin D which induce human beta defense and microbial peptide II-37 (Jeremy *et al*, 2013). Through reactivation of latent VZV from sensory ganglia and transport to the skin which followed by skin lesion formation, inflammatory response formed include immune mediators that associated with the release of paracrine secretion such as complement, interferon, histamine, substance p, and pro-inflammatory cytokines (Young, 2015). In current results it's clear that pro-inflammatory IL-6 expression was unregulated in patients

with acute HZ and PHN and this is come in conformity with previous studies that found IL-6 increment in acute phase of HZ and PHN which influence directly on the nerve ending by destruction these ends, and this situation lead to neurological and also lead to developed the HZ infection to PHN (Guptarak *et al*, 2013). So it's obvious that nB-UVB had useful effect in down regulation the expression of IL-6 in order to prevent the excessive inflammatory response and to prevent the chronicity of infection, and that was clear from the obtained results between T1 group which had reduction in IL-6 levels at the end of phototherapy sessions and T2 group who had significant increment in the IL-6 levels at the end of chemotherapy treatment, as come in the results of (Hong, 2015). which used UVB plus electro acupuncture and observe the reduction of IL-6 at the end of sessions. The collected results showed that no significant changes in the gene expression of NFAT and AP-1 before starting treatment and at the end of treatment for both T1 and T2 groups, several earlier studies differs among in their results about the effect of activated transcription factors and the role of their gene expression on the TH1 cells (CD4, CD8 lymphocytes) during the infection of HZ some of studies reported that TH1 cells decreased and it is assumed the HZ incidence, while other studies mentioned the opposite (Sheng *et al*, 2009). In both conditions increasing or reduction has no association with the development of HZ to PHN (Malayige &Gathsauric, 2007). But it may associated with viral load and the role of both of CD4 and CD8 T cells mediate viral clearance during acute illness (Gwela, 2013). Which may approve that excessive inflammatory responses come from the mediator immune response for damage of infected nerve and not from the T lymphocytes mediator against the virus? In this study the use of nB-UVB to regulate the excessive inflammatory immune responses by induced T-regulatory cells and FOXP3 which consider the key of T- regulatory cells (Elis *et al*, 2014). That was clear from the obtained results which showed a highly significant elevation in the gene expression of FOXP3 for T1 group at the end of phototherapy sessions compared with chemotherapy group. FOXP3 have ability to expressed anti-inflammatory cytokines such as IL-10 which have crucial role in modulation the role of pro-inflammatory cytokines like IL-2, IL-6, and IL-12 by regulate or suppress it, which in turn leads to decrease the inflammation in nerve and prevent the development of HZ infection to PHN.

Conclusion:

Nb-UVB may provide a potential tool in the management of HZ and prevent to be developing to PHN. By activation the role of T-regulatory cells which have critical role for maintaining immune tolerance and immune homeostasis by protecting against devastating autoimmune disease and overwhelming inflammation, through induced the expression of foxp3 gene is the central molecule in the function of T regulatory cells, both in the context of maintenance of immune tolerance and also in regulation of response. Therefore, this transcription factor is very important to play a crucial role in the generation of T regulatory phenotype. Foxp3 may play a

role in immunopathology due to potent suppressive effect on T-cell activation and effector function by suppression of the expression of transcription factors NFAT, AP-1 which resulted in production of pro-inflammatory cytokines (IL-2, IL-5, IL-6, IL-12), and other immune mediators which produced from the damaged nerves infected with VZV. Foxp3 suppress these pro-inflammatory cytokines through inducing the expression of IL-10. In addition nb-UVB act as antimicrobial as long as rays can penetrate layers of the skin and kills the virus, and also have the ability to re-building the damaged nerves ends.

Future research

Recommended that future studies take a large number of patients with a broader measure of the pro-cytokines and anti-cytokines such as IL-2, IL-10, IL-17. with more likely types of genes that are related to theT- regulatory cells in addition to the work of Histological study from infected nerve infection varicella zoster virus.

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Competing interests

Author declare that We have no competing interests.

Authors' contributions

MYK- was involved in conception and design of the study, contributed surgical expertise, involved in data collection and drafting of the final manuscript. GOK- was involved in conception and design of the study, contributed surgical expertise and offered a critical review of the manuscript for intellectual content. JEM- was involved in conception and design of the study, offered surgical expertise and also data collection. SGA-contributed surgical expertise and offered a critical review of the manuscript for intellectual content. KA-offered surgical expertise and data collection. BT- offered surgical expertise and data collection. EDY: Offered a critical review of the manuscript for intellectual content. All the authors have read and approved the final version of the manuscript.

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