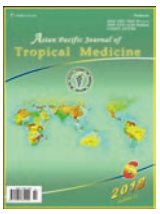

IF: 0.925

Asian Pacific Journal of Tropical Medicine

journal homepage: www.apjtm.org

doi:10.4103/1995-7645.234766

Elevated serum nitric oxide and hydrogen peroxide levels as potential valuable predictors of herpes zoster

Soheila Nasiri[1], Mehdi Hedayati[2], Seyed Mohammad Riahi[3,4], Reza M Robati[1], Marjan Khazan[1]✉

[1]*Skin Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

[2]*Cellular and Molecular Endocrine Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

[3]*Department of Public Health, Faculty of Health, Birjand University of Medical Sciences, Birjand, Iran*

[4]*Department of Epidemiology, School of Health, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

ARTICLE INFO

Article history:
Received 22 January 2018
Revision 6 April 2018
Accepted 10 April 2018
Available online 20 June 2018

Keywords:
Herpes zoster
Oxidative stress
Malondialdehyde
Nitric oxide
Hydrogen peroxide

ABSTRACT

Objective: To evaluate the biomarkers of oxidative stress in herpes zoster patients compared with control subjects. **Methods:** This study compared the nitric oxide (NO), hydrogen peroxide (H_2O_2), malondialdehyde, uric acid, and bilirubin levels between 43 herpes zoster patients and 47 age-matched control subjects. The area under the curve of the receiver operating characteristic curve was performed to evaluate the final logistic regression model. **Results:** The significant differences were observed in the serum levels of NO, H_2O_2, and malondialdehyde between the case and the control groups (P<0.001). However, no statistical differences were found in both uric acid and bilirubin levels between the groups. Additionally, the raised oxidant biomarkers were strongly associated with increased disease severity (P<0.001). Multiple logistic regression analysis with the highest area under the curve [0.98 (95% *CI* 0.95-1.00)] and the minimum number of variables showed that high levels of NO (*OR* 1.24; 95% *CI* 1.06-1.46; P=0.008) and H_2O_2 (*OR* 1.25; 95% *CI* 1.09-1.43; P=0.001) were associated with herpes zoster. **Conclusions:** High levels of NO and H_2O_2 were observed in patients with herpes zoster. Increased NO and H_2O_2 levels might be associated with herpes zoster, which needs to be confirmed by further studies.

1. Introduction

Reactivated latent varicella zoster virus in dorsal root ganglia causes herpes zoster, which is characterized by painful neuralgia and unilateral dermatomal vesicular rash[1,2]. Cell-mediated immunity and increasing age are associated with the pathogenesis and reactivation of varicella zoster virus infection. Therefore, herpes zoster occurs more frequently in people with impaired immunity and those aged over 50 years[1,3]. Recently, it has been confirmed the association of increased oxidants as well as decreased antioxidants with the pathogenesis of many age-related diseases[4-6]. Postherpetic neuralgia, complication of herpes zoster, can cause psychological distress, physical disability, impaired sleep and consequently affect the quality of life[2,7,8]. In addition, diminishing immune system due to deficiencies of antioxidant nutrients may be related to the risk of herpes zoster and postherpetic neuralgia, especially in elder people[9-10].

Oxidative stress occurs due to either excessive production of reactive nitrogen and/or oxygen species (RNS, ROS) as free radicals

First author: Soheila Nasiri, Skin Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

✉Corresponding author: Marjan Khazan, Ph.D Candidate, Researcher, Skin Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
Tel: +98(21) 22741507-12
Fax: +98(21) 22744393
E-mail: marjan_khazan@yahoo.com, khazan@sbmu.ac.ir

Foundation project: This PhD thesis is supported by Skin Research Center, Shahid Beheshti University of Medical Sciences, and this manuscript is based on PhD thesis (No.9409) of Dr. Marjan Khazan.

How to cite this article: Nasiri S, Hedayati M, Riahi SM, Robati RM, Khazan M. Elevated serum nitric oxide and hydrogen peroxide levels as potential valuable predictors of herpes zoster. Asian Pac J Trop Med 2018; 11(6):381-386.

or reduced antioxidant defense activity or both, leading to oxidative cell damage (such as DNA/protein/lipid oxidation). Both RNS and ROS are chemically reactive molecules, and as a result, they have short half-lives in biological samples; thus, stable free radicals, such as malondialdehyde (MDA), an end product of lipid peroxidation, and hydrogen peroxide (H_2O_2), which is generated by dismutation of superoxide radicals, were formed and can be measured to assess oxidative stress status[11,12]. Likewise, nitric oxide (NO) generation from *L*-arginine oxidation can cause lipid and protein oxidation and cell damage; however, NO is harmful only at high concentrations[13].

Cellular redox homeostasis is mainly maintained by the antioxidant defense activity, which is potentially affected by the concentration and function of both endogenous and exogenous antioxidants[14]. Thus, antioxidant nutrient deficiencies may cause cellular redox homeostasis imbalance to induce oxidative stress[15-17]. Uric acid (UA) and bilirubin (BIL) as potential antioxidants can be involved effectively in the neutralization of ROS/RNS and decrease oxidative stress, thereby contributing to disease prevention[18-20]. It has also been reported the vital role of oxidative stress in viral infections, autoimmune skin disorders, degenerative diseases, and aging and its related diseases[4,21-23].

However, no study has assessed the levels of MDA, NO, H_2O_2, UA, and BIL in the blood of herpes zoster patients though these have been determined in other autoimmune skin diseases[18,24-29]. Consequently, the present study aimed to measure the biomarkers of oxidative stress in herpes zoster patients compared with control subjects.

2. Materials and methods

2.1. Participants

This was a case-control study including 43 adult outpatients [15 men (34.9%), 28 women (65.1%)] aged $\geqslant$20 years with symptomatic/acute herpes zoster infections attending the Department of Dermatology of Shohada-e Tajrish Hospital, from June 2016 to June 2017. Cases were screened by serological tests for varicella zoster virus. According to the clinical diagnosis by a dermatologist, patients were recruited within 7 d of rash onset at their first visit. The control group were selected from the other Departments of Shohada-e Tajrish Hospital; the serological test results and clinical criteria of control subjects were checked by physician; and consequently, the control group consisted of 47 age-matched outpatients [18 men (38.3%), 29 women (61.7%)] with history of initial varicella zoster infection (chickenpox) and also without any inflammatory or infectious diseases.

Following were the exclusion criteria: immunosuppression; diabetes; malignancy; liver, kidney, or any chronic diseases; psychiatric disorder; experience of trauma or surgery in the previous month; treatment with steroids and/or immunosuppressive drugs; consumption of any supplements; smoking; pregnancy; lactation. The demographics and clinical data of the study participants were obtained using the preformed demographic and clinical questionnaires at the baseline visit. The informed consent was obtained from all participants, and the study received approval from the Ethics Committee of Shahid Beheshti University of Medical Sciences (Code: IR.SBMU.SRC.REC.1395.24).

The severity of herpes zoster was characterized by the number of skin lesions (papules, vesicles, or crusted vesicles), which were categorized as mild, $\leqslant$25 lesions; moderate, 25 to 50 lesions; and severe, $\geqslant$50 lesions[30]. Acute stress, as a covariate adjustment in the logistic regression model, was also defined based on the presence of reactions that commonly appear following exposure to stressful or potentially traumatic events within 4 weeks before rash onset[31].

2.2. Biochemical measurement

Fasting whole blood samples (5 mL) were collected the morning after their first visit from both the case and control groups. The serum was rapidly frozen at –80 ℃ until assayed. The measurements of serum oxidant and antioxidant biomarkers were performed using enzymatic colorimetric assay kits (Zellbio Co, Ulm, Germany; Pars Azmoun Co; respectively). The assay sensitivity for MDA, H_2O_2, NO, UA, and BIL were 0.1 μM, 5 μM, 1 μM, 0.3 mg/dL, and 0.07 mg/dL, respectively. The intra-assay coefficients of MDA, H_2O_2, NO, UA, and BIL were also less than 5.8%, 3.4%, 3.3%, 1.4%, and 2.8%, respectively.

2.3. Statistical analysis

The Kolmogorov-Smirnov test was used to evaluate the distribution of data that normal distribution was reported as mean±SD and examined using the Student's *t*-test and analysis of variance (ANOVA) followed by Dunnett *post–hoc* test. The correlation was evaluated using the Pearson coefficient. Linear regression analysis was also used to observe relationships between the independent variables. The associations between oxidant biomarkers and herpes zoster were assessed using multiple logistic regression. Furthermore, 95% confidence interval (*CI*) was performed to measure the association. The final model of logistic regression was evaluated by the goodness of fit test (Hosmer–Lemeshow test) and the receiver operating characteristic (ROC) curves and the area under the curve (AUC). All data were performed using the SPSS software (version 22.0.0; SPSS Inc., Chicago, IL, USA), and P-value < 0.05 was considered statistically significant.

3. Results

In this study, the mean ages of the case and control groups were (65.70±9.72) and (62.87±9.31) years respectively. There were no significant differences in the distributions of age (P=0.163) and sex (P=0.454) between the case and control groups. All cases were confirmed by serological tests. According to the herpes zoster disease severity, the number of mild, moderate, and severe cases were 5 (11.6 %), 28 (65.1%), and 10 (23.3%), respectively.

The serum levels of MDA, NO, and H_2O_2 in the cases were significantly higher than that in the control subjects (all P<0.001). The serum UA and BIL levels were also lower in the cases than in the controls; however, the differences were not significant (P=0.216, P=0.478, respectively) (Table 1).

Table 1

Comparison of serum MDA, NO and H_2O_2 levels in case and control groups.

Group	MDA (μM)	H_2O_2 (μM)	NO (μM)	UA (mg/dL)	BIL (mg/dL)
Control(n=47)	4.37±0.76	38.67±9.40	34.68±9.64	6.05±1.80	0.61±0.24
Case (n=43)	5.84±1.33	67.64±16.72	58.26±15.09	5.61±1.52	0.56±0.30
P value	<0.001*	<0.001*	<0.001*	0.216	0.478

All values represent mean±SD; MDA, malondialdehyde; NO, nitric oxide; H_2O_2, hydrogen peroxide; UA, uric acid; BIL, bilirubin. *Statistical significance of P<0.05.

According to the herpes zoster disease severity, substantial differences were found in the levels of all the three oxidants among the patients (Table 2). The mean MDA, NO, and H_2O_2 levels were observed to be significantly increased with increasing level of disease severity (P<0.001) while the differences in the mean levels of UA and BIL were not significant.

Table 2

Comparison between rash severity and oxidant biomarkers in case group.

Rash severity	MDA (μM)	H_2O_2 (μM)	NO (μM)	UA(mg/dL)	BIL(mg/dL)
Mild (n=5)	4.20±0.33	43.60±2.60	40.68±0.77	5.48±1.45	0.42±0.16
Moderate (n=28)	5.46±0.78	65.26±13.17	53.47±8.70	5.51±1.66	0.56±0.30
Sever (n=10)	7.73±0.72	86.35±7.39	80.47±6.69	5.59±1.20	0.63±0.34
P value*	<0.001	<0.001	<0.001	0.71	0.41

All values represent mean±SD; MDA, malondialdehyde; NO, nitric oxide; H_2O_2, hydrogen peroxide; UA, uric acid; BIL, bilirubin; *P<0.05, significant difference among mild, moderate, and sever rashes using Dunnett *post hoc* test.

Also, significant strong positive correlations using linear regression analysis were only found in the case group between the levels of MDA with NO and H_2O_2 (b=0.06, r^2=0.466, P<0.001; b=0.06, r^2=0.496, P<0.001; respectively), and H_2O_2 and NO (b=0.84, r^2=0.579, P<0.001) (Figure 1). However, no correlation was seen between the oxidant and antioxidant biomarkers in the control group. Also, no correlation was seen between days after the onset of rash and any of the biomarker levels.

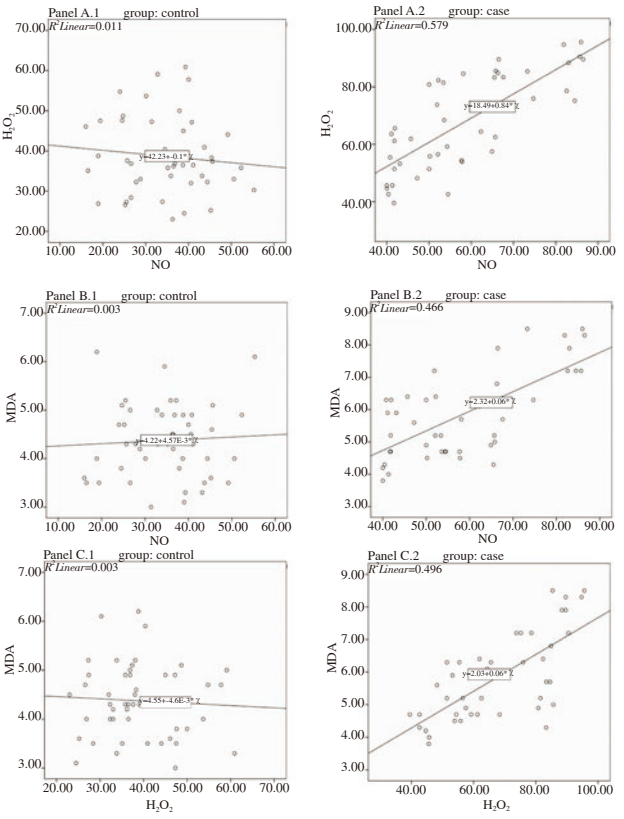

Figure 1. Correlation between oxidant biomarkers in case and control groups.

Based on an assumption of a causal relationship, the multiple logistic regression analysis, ROC curve with the highest AUC [0.98 (95% *CI* 0.95-1.00)] (Figure 2) was considered as the best model to show the association between the oxidant biomarkers and herpes zoster. A strong significant association of herpes zoster was seen with high levels of NO (P=0.008) and H_2O_2 (P=0.001) after adjustment for acute stress (Table 3). The Hosmer-Lemeshow test was not significant for the final model (P=0.99). The specificity, sensitivity, and accuracy of the final model were 93.6%, 88.4%, and 91.1%, respectively. The association of herpes zoster with increased levels of NO and H_2O_2 remained significant even after adding MDA in the last logistic model because no crucial change was observed in the AUC score. In addition, after including UA and BIL in the logistic regression univariate analysis, it was not even significant.

Table 3

Association between oxidant biomarkers and herpes zoster in multivariate logistic models.

Model	NO		H_2O_2		MDA	
	OR (95% *CI*)	P value*	*OR* (95% *CI*)	P value*	*OR* (95% *CI*)	P value*
Model 1†	1.24 (1.06-1.46)	0.008	1.25 (1.09-1.43)	0.001	-	-
Model 2	1.20 (1.06-1.37)	0.005	1.18 (1.07-1.30)	0.001	1.78 (0.62-5.09)	0.279

CI, confidence interval; *OR*, odds ratio; MDA, malondialdehyde; NO, nitric oxide; H_2O_2, hydrogen peroxide; *statistical significance of P<0.05; †adjusted for acute stress in the model 1; AUC for the model 1 equal to 0.98 (0.95-1.00).

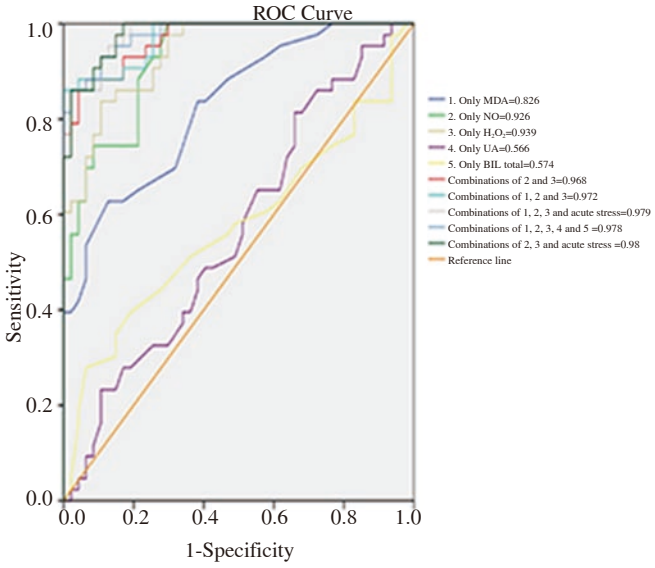

Figure 2. ROC curves for prediction models to discriminate herpes zoster.

4. Discussion

In this study, differences in the serum levels of MDA, NO, and H_2O_2 were significant between the case and the control groups; the differences in the levels of UA and BIL were not significant between the groups. Additionally, there were statistically significant correlations between MDA, NO, and H_2O_2 in the case group. According to the severity of herpes zoster, all the mean levels of oxidant biomarkers differed significantly.

The disruption in the balance between free radical production and antioxidant activity can lead to oxidative stress status, which plays a main role in the pathogenesis of viral infection and is also generated by phagocytic cells under inflammatory conditions[32,33]. The findings of this study indicate the presence of oxidative stress in herpes zoster patients compared with the controls as evidenced by the higher levels of NO, H_2O_2, and MDA and, though not significant, lower levels of UA and BIL.

NO production, either directly or indirectly, is potentially associated with various diseases; however, its deleterious effect depends on the concentration and the site where it is released[13]. For instance, Zolini *et al.* reported that NO produced by macrophages contributes to the control of herpes simplex 1 (HSV-1) infection via the immune response[34]; in contrast, another study showed that NO could cause or potentiate the harmful effects in the virally infected host[35]. In this study, the level of NO was shown to be significantly higher in herpes zoster patients compared with control subjects. It might be due to the multiple roles of NO during viral infections that can modulate both immunological host responses and nonspecific inflammatory reactions, which leads to enhanced NO production, as has been shown in other studies[13,35,36]. Additionally, overproduction of H_2O_2 as one of the main endogenous ROS can occur under disease conditions through several different mechanisms[37]. In accordance, this study found drastically higher levels of H_2O_2 in the cases than controls, which can possibly be due to the immune responses to viral pathogen through inflammatory pathways and T-cell mediated macrophage activation; however, under oxidative stress condition, excessive H_2O_2 can lead to cell damage and death and, consequently, contribute to carcinogenesis. Furthermore, the logistic regression showed that the increased H_2O_2 and NO levels were strongly associated with herpes zoster.

The synergistic effect of H_2O_2 and NO eventually lead to excessive production and accumulation of RNS/ROS that can potentially trigger cell death processes due to elevated MDA resulted from increased lipid peroxidation[37,38]. In this study, MDA levels were significantly higher in the cases compared with controls. This may be explained by either excessive RNS/ROS production or disruption of the antioxidant defense mechanisms or both of them, which lead to increased MDA formation. This result is confirmed by the strong correlation between the oxidant biomarkers in the case group. This finding concurs with other studies that showed MDA, as a lipid peroxidation indicator, was mainly implicated in the pathogenesis of various diseases[26,29,39].

On the other hand, UA as well as BIL are regarded as scavengers of peroxynitrite and can contribute to preventing lipid peroxidation[19,40]. BIL also protects cells against excess H_2O_2[41]. Similar to previous studies[42,43], our results showed that the lower level of UA and BIL in herpes zoster patients compared with control subjects, though not significant, might be related to a compensatory response to elevated oxidative stress; the lack of significant difference may be due to the small sample size and needs further clarification.

Moreover, similar to a prior report[26], this study showed that the high NO, H_2O_2, and MDA levels were associated with rash severity. However, in contrast to another study finding[44], there was no relationship between the disease duration and oxidant levels, which may be because the oxidant levels had changed before the onset of symptoms, although this study has not and cannot show a causal relationship between oxidant levels and pathogenesis of herpes zoster.

The risk of herpes zoster in general population is estimated to be about 20%-30% during their lifetime, reaching 50% in people over 80 years old[45]. Raised oxidative stress may diminish the effectiveness of immune functions and, consequently, enhance the incidence of infections in older populations[3,9,21]. In addition, long-term accumulation of ROS/RNS in the dorsal root ganglion may be responsible for the neuronal damage, thus leading to inflammatory hyperalgesia as well as central and peripheral nerve

injury-induced neuropathic pain[46,47]. Since the risk of herpes zoster and postherpetic neuralgia may be associated with antioxidant micronutrient deficiencies, the increased intake of antioxidant nutrients may support the antioxidant defense activity to counteract the oxidative stress, particularly in elderly individuals[9,48,49]. The high intake of antioxidants is also related to high plasma concentration of endogenous and exogenous antioxidants which neutralize oxidants[20]. Additionally, enhanced or prolonged free radical levels may cause decreased activity of antioxidant defense systems, leading to the development of diseases and aging. Thus, it has been suggested that antioxidant therapeutic approach along with zoster vaccine may contribute to reduced oxidative stress damage and consequently prevent herpes zoster and subsequent postherpetic neuralgia.

Our study has some limitations. A temporal relationship cannot be established between the exposure and the outcome. Oxidative stress status is associated with nutrient status[9,10] that are mentioned as study design limitations. Food frequency questionnaire was not assessed in the study populations. Additionally, assessment of the oxidative stress state in herpes zoster patients required to evaluate a complete list of oxidative stress biomarkers. More studies are required to make statements on the biomarker changes in postherpetic neuralgia.

In conclusion, an imbalance in oxidative stress due to markedly high serum levels of NO, H_2O_2, and MDA was found in patients with herpes zoster. Elevated NO and H_2O_2 might be associated with clinical manifestations of herpes zoster, which needs to be confirmed by further studies.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Acknowledgements

This PhD thesis is supported by Skin Research Center, Shahid Beheshti University of Medical Sciences, and this manuscript is based on PhD thesis (No.9409) of Dr. Marjan Khazan.

References

[1] Saguil A, Kane S, Mercado M, Lauters R. Herpes zoster and postherpetic neuralgia: Prevention and management. *Am Fam Physician* 2017; **96**(10): 656-663.

[2] Schmader K. Herpes zoster. *Clin Geriatr Med* 2016; **32**(3): 539-553.

[3] Shirane R, Tang H, Hayashi K, Okuno Y, Iso H, Asada H, et al. Relationship between cell-mediated immunity to varicella-zoster virus and aging in subjects from the community-based Shozu herpes zoster study. *J Med Virol* 2017; **89**(2): 313-317.

[4] Davalli P, Mitic T, Caporali A, Lauriola A, D'Arca D. ROS, cell senescence, and novel molecular mechanisms in aging and age-related diseases. *Oxid Med Cell Longev* 2016; **2016**: 3565127.

[5] Field CJ, Johnson IR, Schley PD. Nutrients and their role in host resistance to infection. *J Leukoc Biol* 2002; **71**(1): 16-32.

[6] Weber D, Stuetz W, Toussaint O, Debacq-Chainiaux F, Dolle MET, Jansen E, et al. Associations between specific redox biomarkers and age in a large European cohort: The MARK-GE Project. *Oxid Med Cell Longev* 2017; **2017**: 1401452.

[7] Johnson RW, Alvarez-Pasquin MJ, Bijl M, Franco E, Gaillat J, Clara JG, et al. Herpes zoster epidemiology, management, and disease and economic burden in Europe: A multidisciplinary perspective. *Ther Adv Vaccines* 2015; **3**(4): 109-120.

[8] Schmidt-Ott R, Schutter U, Simon J, Nautrup BP, von Krempelhuber A, Gopala K, et al. Incidence and costs of herpes zoster and postherpetic neuralgia in German adults aged 50 years: A prospective study. *J Infect* 2018; **18**(1): 30053-30057.

[9] Chen JY, Chang CY, Lin YS, Hu ML. Nutritional factors in herpes zoster, postherpetic neuralgia, and zoster vaccination. *Popul Health Manag* 2012; **15**(6): 391-397.

[10] Lesourd B. Nutritional factors and immunological ageing. *Proc Nutr Soc* 2006; **65**(3): 319-325.

[11] Droge W. Free radicals in the physiological control of cell function. *Physiol Rev* 2002; **82**(1): 47-95.

[12] Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem Biol Interact* 2006; **160**(1): 1-40.

[13] Khazan M, Hdayati M. The role of nitric oxide in health and diseases. *Scimetr Int J Med Sci* 2014; **3**(1): 1-10.

[14] Poljsak B, Suput D, Milisav I. Achieving the balance between ROS and antioxidants: When to use the synthetic antioxidants. *Oxid Med Cell Longev* 2013; **2013**: 956792.

[15] Rahal A, Kumar A, Singh V, Yadav B, Tiwari R, Chakraborty S, et al. Oxidative stress, prooxidants, and antioxidants: The interplay. *Biomed Res Int* 2014; **2014**: 761264.

[16] Alpert PT. The role of vitamins and mMinerals on the immune system. *HHCMP* 2017; **29**(3): 199-202.

[17] Pae M, Wu D. Nutritional modulation of age-related changes in the immune system and risk of infection. *Nutr Res* 2017; **41**: 14-35.

[18] Yousefi M, Rahimi H, Barikbin B, Toossi P, Lotfi S, Hedayati M, et al. Uric acid: A new antioxidant in patients with pemphigus vulgaris. *Indian J Dermatol* 2011; **56**(3): 278-281.

[19] Hatfield GL, Barclay LR. Bilirubin as an antioxidant: kinetic studies of the reaction of bilirubin with peroxyl radicals in solution, micelles, and

lipid bilayers. *Org Lett* 2004; **6**(10): 1539-1542.

[20]Mironczuk-Chodakowska I, Witkowska AM, Zujko ME. Endogenous non-enzymatic antioxidants in the human body. *Adv Med Sci* 2017; **63**(1): 68-78.

[21]Reshi ML, Su YC, Hong JR. RNA viruses: ROS-mediated cell death. *Int J Cell Biol* 2014; **2014**: 467452.

[22]Fimognari C. Role of oxidative RNA damage in chronic-degenerative diseases. *Oxid Med Cell Longev* 2015; **2015**: 358713.

[23]Baek J, Lee MG. Oxidative stress and antioxidant strategies in dermatology. *Redox Rep* 2016; **21**(4): 164-169.

[24]Barikbin B, Yousefi M, Rahimi H, Hedayati M, Razavi SM, Lotfi S. Antioxidant status in patients with lichen planus. *Clin Exp Dermatol* 2011; **36**(8): 851-854.

[25]Nassiri S, Malekzad F, Sarlak M, Saeedi M, Hedayati M, Qaisari M. Interplay among antioxidants and oxidants in psoriasis. *Iran J Dermatol* 2009; **12**(2): 56-59.

[26]Bakry OA, Elshazly RM, Shoeib MA, Gooda A. Oxidative stress in alopecia areata: A case-control study. *Am J Clin Dermatol* 2014; **15**(1): 57-64.

[27]Ji H, Li XK. Oxidative stress in atopic dermatitis. *Oxid Med Cell Longev* 2016; **2016**: 8.

[28]Lin X, Huang T. Oxidative stress in psoriasis and potential therapeutic use of antioxidants. *Free Radic Res* 2016; **50**(6): 585-595.

[29]Rekha VR, Sunil S, Rathy R. Evaluation of oxidative stress markers in oral lichen planus. *J Oral Maxillofac Pathol* 2017; **21**(3): 387-393.

[30]Nagasako EM, Johnson RW, Griffin DR, Dworkin RH. Rash severity in herpes zoster: Correlates and relationship to postherpetic neuralgia. *J Am Acad Dermatol* 2002; **46**(6): 834-839.

[31]World Health Organization. *WHO guidelines approved by the guidelines review committee. Guidelines for the management of conditions specifically related to stress.* Geneva: World Health Organization; 2013.

[32]Mittal M, Siddiqui MR, Tran K, Reddy SP, Malik AB. Reactive oxygen species in inflammation and tissue injury. *Antioxid Redox Signal* 2014; **20**(7): 1126-1167.

[33]Ivanov AV, Bartosch B, Isaguliants MG. Oxidative stress in infection and consequent disease. *Oxid Med Cell Longev* 2017; **2017**: 3496043.

[34]Zolini GP, Lima GK, Lucinda N, Silva MA, Dias MF, Pessoa NL, et al. Defense against HSV-1 in a murine model is mediated by iNOS and orchestrated by the activation of TLR2 and TLR9 in trigeminal ganglia. *J Neuroinflammation* 2014; **11**: 20.

[35]Uehara EU, Shida Bde S, de Brito CA. Role of nitric oxide in immune responses against viruses: Beyond microbicidal activity. *Inflamm Res* 2015; **64**(11): 845-852.

[36]Akaike T, Maeda H. Nitric oxide and virus infection. *Immunology* 2000; **101**(3): 300-308.

[37]Gough DR, Cotter TG. Hydrogen peroxide: A Jekyll and Hyde signalling molecule. *Cell Death Dis* 2011; **2**: 213.

[38]Girard PM, Arbabian A, Fleury M, Bauville G, Puech V, Dutreix M, et al. Synergistic effect of H_2O_2 and NO_2 in cell death induced by cold atmospheric He plasma. *Sci Rep* 2016; **6**: 29098.

[39]Marrocco I, Altieri F, Peluso I. Measurement and clinical significance of biomarkers of oxidative stress in humans. *Oxid Med Cell Longev* 2017; **2017**: 6501046.

[40]Squadrito GL, Cueto R, Splenser AE, Valavanidis A, Zhang H, Uppu RM, et al. Reaction of uric acid with peroxynitrite and implications for the mechanism of neuroprotection by uric acid. *Arch Biochem Biophys* 2000; **376**(2): 333-337.

[41]Baranano DE, Rao M, Ferris CD, Snyder SH. Biliverdin reductase: A major physiologic cytoprotectant. *Proc Natl Acad Sci USA* 2002; **99**(25): 16093-16098.

[42]Li X, Tong Q, Xie D, Chen Z, Pan S, Zhang X, et al. Low serum uric acid levels in patients with acute central nervous system viral infections. *Neuroreport* 2017; **28**(18): 1250-1254.

[43]Liu J, Li M, Wang X, Yi H, Xu L, Zhong XF, et al. Serum uric acid levels in patients with infections of central nervous system. *Acta Neurol Belg* 2016; **116**(3): 303-308.

[44]Abdel Fattah NS, Ebrahim AA, El Okda ES. Lipid peroxidation/antioxidant activity in patients with alopecia areata. *J Eur Acad Dermatol Venereol* 2011; **25**(4): 403-408.

[45]Harpaz R, Ortega-Sanchez IR, Seward JF. Prevention of herpes zoster: Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 2008; **57**(5): 1-30.

[46]Grace PM, Gaudet AD, Staikopoulos V, Maier SF, Hutchinson MR, Salvemini D, et al. Nitroxidative signaling mechanisms in pathological pain. *Trends Neurosci* 2016; **39**(12): 862-879.

[47] Yowtak J, Lee KY, Kim HY, Wang J, Kim HK, Chung K, et al. Reactive oxygen species contribute to neuropathic pain by reducing spinal GABA release. *Pain* 2011; **152**(4): 844-852.

[48]Thomas SL, Wheeler JG, Hall AJ. Micronutrient intake and the risk of herpes zoster: A case-control study. *Int J Epidemiol* 2006; **35**(2): 307-314.

[49]Chen JY, Chu CC, Lin YS, So EC, Shieh JP, Hu ML. Nutrient deficiencies as a risk factor in Taiwanese patients with postherpetic neuralgia. *Br J Nutr* 2011; **106**(5): 700-707.