RESEARCH ARTICLE

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Decreased Oxidative Stress Markers in Patients with Active and Generalized Vitiligo

ABSTRACT

Objective: Oxidative stress has been shown to play a role in the pathophysiology of several diseases, making it a popular yet contentious research area. There is some evidence that selective melanocyte destruction may have developed in vitiligo patients as a result of elevated oxidative stress. The purpose of this study is to investigate the impact of oxidative stress on lipid, protein, and nucleic acid metabolism in vitiligo patients.

Methods: We used ELISA method to measure serum oxidative stress markers in patients with generalized vitiligo who had newly formed lesions in the previous three months but had not been treated, as well as healthy controls. Malondialdehyde (MDA), 2,4-dinitrophenyl hydrazone (DNPH), 8-hydroxy-2'-deoxyguanosine (8-OHdG), and uncoupling protein 2 (UCP2) levels were measured to assess the influence of reactive oxygen derivatives on lipid, protein, nucleic acid metabolism, and mitochondria, respectively.

Results: The study included 84 participants, including 64 active generalized vitiligo patients and 20 healthy controls with similar age and gender distribution. In the serum of vitiligo patients, we detected significantly lower levels of MDA (ng/mL, mean±SD=12±19; 33.4±35.9), DNPH (ng/mL, mean±SD=2±3.1; 6±7.4), 8-OHdG (ng/mL, mean±SD=11.7±17.9; 32.7±37) and UCP2 (ng/mL, mean±SD=8.7±13.7; 21.5±28.4.

Conclusions: Although there is significant evidence that oxidative stress plays a role in the pathophysiology of vitiligo, the studies should be interpreted cautiously due to the heterogeneity in the methodology, complexity of the oxidative stress pathways, and potential publication bias. Large-scale studies using a standardized methodology are required to determine how significant oxidative stress is in the core pathophysiology of vitiligo and which pathways it primarily affects.

Keywords: Deoxyguanosine, Malondialdehyde, Uncoupling Protein 2, Vitiligo.

Aktif ve Generalize Vitiligolu Hastalarda Azalmış Oksidatif Stres Belirteçleri

ÖZET

Amaç: Oksidatif stresin, birçok hastalığın patofizyolojisinde rol oynadığı gösterilmiş ve bu da onu popüler ancak tartışmalı bir araştırma alanı haline getirmiştir. Vitiligo hastalarında, artmış oksidatif stres sonucu seçici melanosit hasarının gelişmiş olabileceği yönünde bazı kanıtlar vardır. Bu çalışmanın amacı, vitiligo hastalarında oksidatif stresin lipid, protein ve nükleik asit metabolizması üzerindeki etkisini araştırmaktır.

Gereç ve Yöntem: Son üç ayda yeni oluşan lezyonları olan ancak tedavi edilmemiş generalize vitiligolu hastalarda ve sağlıklı kontrollerde, serum oksidatif stres belirteçlerini ölçmek için ELISA metodunu kullandık. Reaktif oksijen türevlerinin lipid, protein, nükleik asit metabolizması ve mitokondri üzerindeki etkisini incelemek için sırasıyla malondialdehit (MDA), 2,4-dinitrofenil hidrazon (DNPH), 8-hidroksi-2'-deoksiguanozin (8-OHdG) ve ayırıcı protein 2 (UCP2) seviyeleri ölçüldü.

Bulgular: Çalışmaya 64 aktif generalize vitiligo hastası ve benzer yaş ve cinsiyet dağılımına sahip 20 sağlıklı kontrol olmak üzere toplam 84 katılımcı dahil edildi. Vitiligo hastalarının serumunda sağlıklı kontrollere göre anlamlı olarak azalmış MDA (ng/mL, ortalama±SS=12±19; 33.4±35.9), DNPH (ng/mL, ortalama±SS=2±3.1; 6±7.4), 8-OHdG (ng/mL, ortalama±SS=11.7±17.9; 32.7±37) ve UCP2 (ng/mL, ortalama±SS=8.7±13.7; 21.5±28.4) seviyeleri tespit edildi.

Sonuç: Oksidatif stresin vitiligo patofizyolojisinde rol oynadığına dair önemli kanıtlar olmasına rağmen, metodolojideki heterojenlik, oksidatif stres yolaklarının karmaşıklığı ve olası yayın yanlılığı açısından çalışmalar dikkatli bir şekilde yorumlanmalıdır. Vitiligonun merkezi patofizyolojisinde oksidatif stresin ne kadar önemli olduğunu ve öncelikle hangi yolakları etkilediğini belirlemek için standart bir metodoloji kullanan büyük ölçekli çalışmalar gereklidir.

Anahtar Kelimeler: Deoksiguanozin, Malondialdehit, Ayırıcı Protein 2,Vitiligo.

INTRODUCTION

Vitiligo is a chronic, acquired pigmentation disorder characterized by the selective loss of epidermal melanocytes, resulting in depigmented patches on the skin (1,2). The precise mechanisms that cause melanocyte destruction are not completely understood. The oxidant-antioxidant theory proposes that an increase in reactive oxygen species (ROS) and/or a decrease in antioxidants may trigger melanocyte death, initiating or aggravating the disease (3–5).

Reactive oxygen species are produced from oxygen as a result of metabolic activity in cells, such as mitochondrial respiration or melanogenesis (2,6,7). They serve as signal molecules but can injure the cell at high concentrations (6). To counteract the detrimental effects of ROS, a variety of antioxidant defense mechanisms are available (8,9). Increased ROS and decreased antioxidants disrupt this balance, causing oxidative stress and resulting in non-specific damage to proteins, lipids, and nucleic acids (10–13). Oxidative stress has been intensively researched in recent decades and has been associated with a number of diseases, including vitiligo (10,13–17). However, a major issue is that study designs and outcomes are often varied, making generalization difficult (10,16).

ROS are difficult to quantify since they are short-lived and labile; hence, stable byproducts are measured instead (18,19). Although ROS have no specific target (18), their primary focus may shift depending on the pathophysiology of the disease, and markers should be used accordingly (15). Although an increase in total oxidants and a decrease in total antioxidants have been observed in the serum of vitiligo patients (2), the data is ambiguous as to which pathways are targeted the most during oxidative stress.

The current study was designed to investigate the impact of oxidative stress on lipid, protein, and DNA metabolism in vitiligo patients. To examine lipid metabolism, we assessed malondialdehyde (MDA) levels, an endproduct of polyunsaturated fatty acid peroxidation (17). 2,4-dinitrophenyl hydrazone (DNPH) (15,20) and 8-hydroxy-2′-deoxyguanosine (8-OHdG) (12,21) levels were measured to assess protein and DNA oxidation, respectively. We also measured uncoupling protein 2 (UCP2) levels, a protein found in the inner membrane of mitochondria that increases in response to oxidative stress in order to reduce ROS formation (11,22).

MATERIAL AND METHODS

All procedures involving human participants were in accordance with the Helsinki Declaration. The study protocol was approved by the Duzce University Health Research Ethics Committee (08/2018). All subjects provided written informed consent prior to enrollment.

Subjects: Patients were recruited from Duzce University's dermatology outpatient clinics. The study included 64 patients with generalized vitiligo aged 10–65 years old who had newly formed depigmented pathes in the previous three months but had not received any topical or systemic treatment. The control group consisted of 20 healthy people who were chosen to be similar to the patient group in age and gender. The study excluded participants who had diabetes, thyroid disease, infectious diseases, autoimmune diseases, or malignancy.

Blood Sampling & Analysis: The participants' venous blood samples were obtained in the morning under sterile settings after they had fasted for 12 to 14 hours overnight. After centrifuging the samples at 3000 rpm for 10 minutes, the sera was separated and stored at -80°C until the ELISA was performed. MDA, DNPH, 8-OHdG, and UCP2 levels were determined using a GenX microELISA device.

To perform an ELISA, serum samples were first treated with enzymes coated with monoclonal antibodies. After that, samples were incubated with biotin-labeled antibodies, and finally streptavidin-HRP was added to form immune complexes. The samples were washed to remove unbound enzyme before being treated with chromogen solutions A and B, which turned the samples blue and yellow, respectively. The concentration of markers was then determined using color chromatography (23).

Statistical Analysis: The statistical analysis software SPSS (version 26.0; SPSS Inc., Chicago, IL, USA) was used to analyze the data. The mean and standard deviation were used to Express descriptive statistics for continuous variables. The Shapiro-Wilk test was used to examine the distribution of the data. The Mann-Whitney U test was used to compare continuous and non-normally distributed data in independent groups. The median, interquartile range, mean, and standard deviation were used to express the findings. Results with a p value of less than 0.05 were considered statistically significant.

RESULTS

The study included 64 vitiligo patients and 20 healthy controls, for a total of 84 participants. Table 1 displays descriptive information on the participants' age and gender, as well as the duration of the illness in vitiligo patients.

Table 1. The descriptive statistics for the patient and control groups.

	Vitiligo Patients	**Control Group**
N	64	20
Age (years), mean (SD)	29.1 (11.2)	27.9 (10)
Female, n (%)	30 (46.9)	9 (45)
Male, n(%)	34 (53.1)	11 (51)
Duration of illness (months), mean (SD)	108.42 (98.2)	–

Vitiligo patients had lower mean MDA levels than healthy controls (mean ±SD) = 12 ±19, 95% CI 7.2–16.8 vs. 33.4 ±35.9, 95% CI 16.6–50.2). Vitiligo patients also had significantly lower DNPH (mean ±SD = 2 ±3.1, 95% CI 1.2–2.7 vs. 6 ±7.4, 95% CI 2.5–9.5), 8-OHdG (mean ±SD = 11.7 ±17.9, 95% CI 7.2–16.2 vs. 32.7 ±37, 95% CI 15.3–50), and UCP2 (mean ±SD = 8.7 ±13.7, 95% CI 5.3–12.2 vs. 21.5 ±28.4, 95% CI 8.2–34.8) levels (Table 2).

Table 2. Comparison of malondialdehyde (MDA), 2,4-dinitrophenyl hydrazone (DNPH), 8-hydroxy-2′-deoxyguanosine (8-OHdG) and uncoupling protein 2 (UCP2) levels between vitiligo patients and control group.

	Vitiligo Patients		**Control Group**		**p***
	Median (IQR)	[Min.–Max.]	Median (IQR)	[Min.–Max.]	—
Malondialdehyde, ng/mL	4.4 (5.5)	[1.5 – 92]	12.9 (72.4)	[2 – 92.3]	0.003
2,4-dinitrophenyl hydrazone, ng/mL	0.9 (0.7)	[0.5 – 19.7]	2.1 (10)	[0.5 – 26.1]	0.011
8-hydroxy-2′-deoxyguanosine, ng/mL	4.7 (2.8)	[2.6 – 96.4]	10.6 (64.9)	[3.9 – 114.2]	0.002
Uncoupling Protein 2, ng/mL	3.9 (2)	[3 – 83.4]	5.8 (25.8)	[2.9 – 111.6]	0.045

* Mann-Whitney U test

DISCUSSION

We found significantly lower levels of MDA, DNPH, 8-OHdG, and UCP2 in vitiligo patients compared to healthy controls. There are conflicting findings in the literature, with various oxidative stress markers being reported as increased or decreased in vitiligo patients (2,10,17).

Malondialdehyde: MDA is formed as a result of ROS peroxidation of polyunsaturated fatty acids and is widely accepted as a biomarker of oxidative stress (14). Although it is frequently used, preanalytical (sampling, storage, artifact formation, etc.) and analytical factors (choosing the appropriate measurement method) should be taken into account in order to achieve accurate results (14). Speeckaert et al. conducted a meta-analysis and concluded a significant increase in MDA levels in vitiligo patients, but they cautioned that the high heterogeneity due to study designs may introduce bias when interpreting results (17). Another meta-analysis found that MDA levels were significantly higher in patients with active or stable vitiligo, and further subgroup analysis revealed that this significance was maintained in serum, plasma, whole blood, and skin samples but not in erythrocytes (24). They also reported similar sample size and heterogeneity limitations, as well as publication bias in MDA-related studies (24).

2,4-dinitrophenyl hydrazine: DNPH is generated by the derivatization of protein side chain carbonyl groups by 2,4-dinitrophenylhydrazine and is one of the most commonly used protein oxidation markers (15,20). While the samples are easy to store and analyze due to their chemical stability, the disadvantage is that the measurement cannot differentiate which ROS are produced and which proteins are damaged specifically (15). Given that proteins have distinct biological activities and that oxidative stress-induced diseases are triggered by the loss of function in a specific protein, protein carbonyl levels alone are insufficient to explain specific disease processes (15). However, it is still widely used and has been linked to various disorders such as Alzheimer's, diabetes, and inflammatory bowel disease (15).

A study indicated that DNPH levels in the whole blood of active and stable vitiligo patients were high but comparable to healthy controls, while no significant level was reported (25). According to the same study, plasma levels of another protein oxidation marker, advanced oxidation of protein products, are comparable as well (25). Other studies have found that serum levels of advanced oxidation of protein products are comparable (26) or increased (27,28) in vitiligo patients. The oxidised form of tyrosinase protein, which plays an important role in melanin synthesis, was found to be significantly higher in vitiligo patients (28). Studies on protein oxidation products in vitiligo patients are scarce, and they are hampered by heterogeneity in study designs, which is a common limitation of oxidative stress studies.

8-hydroxy-2′-deoxyguanosine: Guanine is the most easily oxidized nucleic acid base, and the oxidation product 8-OHdG is a widely established biomarker of ROS-induced DNA damage (29). 8-OHdG can be produced as a result of DNA damage in the nucleus or mitochondria (29,30). Despite using different analytical methodologies, studies in vitiligo patients observed higher amounts of 8-OHdG in whole blood (31), mononuclear leukocytes (32), serum (33), and skin (33) samples. Both nuclear and mitochondrial DNA are damaged in vitiligo patients (34), and a genetic polymorphism in the apurinic endonuclease 1 enzyme, which is responsible for the DNA base excision repair pathway, has been linked to the risk of vitiligo (35). It has been postulated that mitochondrial DNA damage causes instability, which results in increased ROS production and a compensatory rise in mitochondri and mitochondrial DNA quantities (31).

Although the results of the studies in this field are similar, the paucity of data and significant differences in the study designs, such as differing analytical methods, appear to be limitations.

Uncoupling Protein 2: Uncoupling proteins are transport proteins found in the inner membrane of mitochondria (36). UCP1 was initially identified as a protein that generates heat rather than ATP when transferring H+ ions from the intermembranous area to the mitochondrial matrix (37). UCP2, on the other hand, has been discovered as a transport protein that upregulates in response to oxidative stress caused by ROS, lipid peroxidation products, and other alkenals, and restricts mitochondrial ROS generation (11,22,38,39). UCP2 is a marker of increased oxidative stress that has been linked to cancer (37), diabetes, obesity, degenerative diseases, and aging (22). Although UCP2 proteins have been found to function in the skin (40), data on UCP2 levels in vitiligo patients is limited. Although our study demonstrated a significant decrease in serum UCP2 levels in active vitiligo patients compared to healthy controls, more research is needed to draw conclusive results.

Strengths and Limitations: While the study's strengths include the simultaneous evaluation of common ROS targets such as lipids, proteins, and nucleic acids, using analytical methods consistent with those previously reported in the literature, and enrolling a large number of active vitiligo patients, there are some significant limitations that should be acknowledged in our research and oxidative stress research in general.

The heterogeneity of methodology, which is a common problem in oxidative stress research, is a major limiting factor that we encounter in both independent studies and meta-analyses (16,24). The lack of standardization in the enrollment of the patient (active or stable, localized or generalized vitiligo) and control groups, the sampling (plasma, serum, whole blood, skin, etc.) and storage preferences, and most importantly, the selection of analytical methods makes it difficult to interpret the results together (14,16).

There are several oxidative stress indicators, and they may be implicated in the underlying pathophysiology of various diseases; establishing which one is associated with which disease is a work in progress. Due to the lack of a defined analytical method and the inability to determine reference values, oxidative stress markers are not suitable for routine use or diagnosis (14,16). Furthermore, aside from the unique underlying pathogenetic processes of the diseases, oxidative stress markers may rise owing to non-specific factors (alcohol, tobacco, drugs, air pollutants, UV radiation, certain foods, metals, and industrial solvents, etc.) (8,16,19). It should be noted that in studies with small sample sizes, there may be increases in oxidative stress markers associated with unanticipated non-specific processes in both the patient and control groups, and caution should be exercised when drawing conclusions. Larger sample size studies employing mutual analytical methods will aid in understanding the role of oxidative stress in the pathophysiology of vitiligo.

CONCLUSION

In recent decades, reactive oxygen derivatives and oxidative stress research have become popular yet contentious topics. There have been several reports of elevated oxidative stress markers in systemic disorders such as diabetes and chronic kidney disease (16), as well as skin diseases such as vitiligo, psoriasis, atopic dermatitis, lichen, and urticaria (17). Although there is substantial evidence that oxidative stress has a role in vitiligo, it is unclear whether increased ROS markers are primarily responsible or specific for the disease's underlying pathophysiology (16,17). Although the majority of research is done on serum or plasma samples, studies that analyze the oxidative stress markers in skin samples (41) may be considered a more realistic model for oxidative stress research due to the ease of access to the tissue where the pathophysiology primarily occurs. Furthermore, research on both skin and blood samples in the same subjects will pave the way for a better understanding of the disease process. More research is needed to determine the precise mechanism of oxidative stress in the pathophysiology of vitiligo. The data obtained might lead to future targeted antioxidant therapies.

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