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Assessment of 8-Hydroxy-2-Deoxyguanosine in Vitiligo Patients

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Abstract

Background: Acquired pigmentary condition, vitiligo, has an unclear aetiology and is likely multi-factorial, involving hereditary factors and defective metabolic pathways as well as immunological processes, melanocyte adhesion defects, and nervous system abnormalities. White macules appear on the skin as a result of the selective loss of melanocytes in the affected area. More than 2% of the world's population is infected, and it shows no preference for gender or ethnicity. Treatment results in varying degrees of improvement or worsening of symptoms. Psychological discomfort, a lowered standard of living, and an elevated risk of mental illness are all possible side effects of depigmentation. Excess levels of the oxidative stress biomarker 8-hydroxy-2-deoxyguanosine in bodily fluids are an excellent predictor of oxidative stress-related disease. In this research, 8-hydroxy-2-deoxyguanosine levels in vitiligo patients and healthy controls will be measured, and the results compared. This research was conducted at Banha University Hospital's Dermatology, Andrology, and Medical Biochemistry departments. From October 2020 to February 2021, 60 vitiligo patients were recruited from Banha University Hospital's outpatient clinic of Dermatology, Venereology. Results: The blood level of 8-OHdG was found to be substantially greater in patients than in the control group in this investigation (P 0.001). 8-OHdG, on the other hand, exhibited a reasonable degree of diagnostic accuracy for vitiligo. It has a sensitivity of 96.67 percent and a specificity of 76.67 percent for diagnosing vitiligo at cutoff values over 5 ng/ml. Serum 8-OHdG levels were shown to be significantly correlated with patient age (P 0.001), illness duration (P 0.001), and VETI score (P = 0.063) in the research. Patients with vitiligo had greater amounts of 8-OHdG than healthy individuals, according to the findings of this research. 8-OHdG levels are linked to vitilgo VETI scores, family history, and the length of the illness. 8-OHdG levels may suggest that oxidative unit is a major role in the development of vitiligo, as shown by these findings. Patients with vitiligo may benefit from the findings of this research, which might point the way toward new treatment options.

Key words: 8-Hydroxy-2, Deoxyguanosine, Vitiligo.

1. Introduction

One percent of the world's population suffers from vitiligo. There is no preference for sex or race when it comes to being impacted. Around the second or third decade of life, most people begin experiencing symptoms [1].

The skin's first line of protection against free radicals is the human epidermis. Antioxidants, like as vitamin C, are among the most common causes of vitiligo because of their ability to create reactive oxygen species (ROS). Vitiligo is characterised by an abnormality in the redox state of the epidermal melanin unit, which has been linked to an abnormal immunological response [2].

Reactive oxygen species (ROS) are produced and manifested in an imbalance with a biological system's capacity to detoxify or repair the ROS-induced damage. The generation of free radicals, which destroy all cell components, including proteins, lipids, and DNA, may result in harmful consequences when tissues are out of equilibrium [3].

For the most part, the scientific community is focused on the oxidative damage caused by 8-hydroxy-2-deoxyguanosine (8-OHdG), which originally emerged in 1984 as the first oxidative damage developing in vivo. Aerobic cellular DNA damage and oxidative stress are both biomarkers for 8-OHdG, it has been shown [5, 6, 7 and 8].

Patients with arteriosclerosis, diabetes, neurodegenerative illnesses of the brain (Parkinson's disease, Alzheimer's disease), and autoimmune diseases (rheumatoid arthritis, systemic lupus erythematosus) have higher levels of 8-OHdG. (9; 10). In this research, 8-hydroxy-2-deoxyguanosine levels in vitiligo patients and healthy controls will be measured, and the results compared.

2. Patients and Methods

Subjects

This case control study was conducted on sixty vitiligo patients and twenty age and sex matched healthy volunteers served as controls. They were recruited from the outpatient clinic of Dermatology, Venereology and Andrology Department of Benha University Hospitals.

Subjects were classified into the following groups

- Group I: sixty patients with vitiligo.
- Group II: twenty healthy individuals.

Inclusion criteria

- The study was conducted on patients suffering from vitiligo with different degrees of severity and types of vitiligo (vitiligo vulgaris, acrofacial and focal except segmental).

Exclusion criteria

- Any patients with any of the following conditions were excluded from this study.
- Patients with any systemic and other dermatological diseases.
- Patients complaining of other autoimmune disease (DM, autoimmune thyroiditis).

- Pregnancy and lactation.
- Patients with known history or active malignancy.
- Patients with segmental vitiligo and those who were treated by systemic therapy for 1 month or any topical therapy for 2 weeks prior to the study.
- Administrative design

This study was approved by the Research Ethical Committee of Benha Faculty of Medicine, and was carried out according to the guidelines of the Helsinki declaration principles.

Ethical consideration

- Before taking blood samples, a written informed consent was taken from each participant.

Methods

Every participant was subjected to

Full history taking

- Personal history: Name, age, sex, occupation, residence, special habits of medical importance, marital status.
- Present history: Onset, course, duration of vitiligo.
- Past history: History of medications (type, dose and duration), and associated autoimmune diseases.
- Family history of vitiligo.

Clinical examination

- General examination of body systems was performed to discover associated medical conditions.
- Detailed dermatological examination
- Description of the vitiligo lesions including site, type.
- The clinical examination was confirmed by Wood's lamp in query cases, and assessment of vitiligo extent according to Vitiligo Extent Tensity Index (VETI) score (11).

The VETI score is a system that used to measure the extent of vitiligo by a numerical score and combines analysis of extensity and severity of vitiligo. The percentage of extension involvement (p) evaluates using the rule of nines already used in burn assessment. Five sites affected, head (h), upper limbs (u), trunk (t), lower limbs (l) and genitalia (g) are separately scored by using five stages of disease tensity (T):

- Stage 0: Normal skin.
- Stage 1: Hypopigmentation (including trichrome and homogeneous lighter pigmentation).
- Stage 2: Complete depigmentation with black hair and with perifollicular pigmentation.
- Stage 3: Complete depigmentation with black hair and without perifollicular pigmentation.
- Stage 4: Complete depigmentation with compound of white and black hair with/without perifollicular pigmentation.
- Stage 5: Complete depigmentation plus significant hair whitening.

The total body VETI was calculated using the following formula that includes contributions from all body regions:

VETI score: (Percentage of head involvement × grade of tensity) + (Percentage of trunk involvement × grade of tensity) 4+ (Percentage of upper limbs involvement × grade of tensity) 2+ (Percentage of lower limbs involvement × grade of tensity) 4+ (Percentage of genitalia involvement × grade of tensity) 0.1

The coefficients reported in this formula were based on percent of skin surface by the rule of nines. Accordingly the coefficient of head was 1 (9:9=1), trunk and lower limb was 4 (36:9=4), upper limb was 2 (18:9=2) and genitalia was almost 0.1(1:9= 0.1).

Percentage of involvement: P, Tensity: T, head: h, upper limbs: u, trunk: t, lower limbs: l, and genitalia: g.

- VETI: $(Ph \times Th) + (Pt \times Tt)4 + (Pu \times Tu)2 + (Pl \times Tl)4 + (Pg \times Tg)\ 0.1$
- $5 + 20 + 10 + 20 + 0.5 = 55.5$
- The maximum score of VETI was 55.5.

Laboratory investigations

Sampling

Five ml venous blood was collected from each participant by clean venipuncture using disposable plastic syringe on plain sterile tube (without anticoagulant) for serum separation. The tube was left at room temperature for 30 minutes till coagulation, and then was centrifuged. The resultant serum was stored at −20°C until analysis. All methods were performed according to the manufacturer's instructions.

Measurement of the serum 8-OHdG levels

Serum 8-OHdG was measured using "Human 8-OHdG ELISA Kit" Cat. No. : 201-12-1437. Provided by SHANGHAI KORAIN BIOTECH CO., LTD, Shanghai, China. http: www.korainbio.com, e-mail: support@biotechgate.com, tel. No.: +41(44) 5003848.

Assay principle

This test is a competitive protein binding assay for the measurement of 8-OHdG. It is based on the competition of 8-OHdG present in the sample with 8-OHdG coated on the well for the specific binding site.

The kit used a double-antibody sandwich ELISA to assay the level of Human 8-OHdG in samples. Serum containing 8-OHdG was added to the well which was precoated with Human 8-OHdG monoclonal antibody, 8-OHdG antibodies labeled with biotin, and combined with Streptavidin- Horseradish peroxidase was added to form immune complex.

Assay procedure

- All reagents, standard solutions and samples were prepared as instructed. All reagents were brought to room temperature before use. The assay was performed at room temperature.
- A 50µl standard was added to standard well.
- A 40µl of sample were added to sample wells and 10µl anti-8-OHdG antibody were added to sample wells, then 50µl streptavidin- Horseradish peroxidase were added to sample wells and standard wells (Not blank control well) and mixed well. The plate was covered with a sealer and incubated 60 minutes at 37°C.

- The sealer was removed, and the plate was washed 5 times with wash buffer by automated washing. The plate was blotted onto paper towels.
- A 50µl of substrate solution A was added to each well and then 50µl of substrate solution B was added to each well. Plate was covered with a new sealer and incubated for 10 minutes at 37°C in the dark.
- A 50µl stop solution was added to each well, the blue color was changed into yellow immediately.
- The optical density value was determined of each well using a microplate reader set to 450 nm within 10 minutes after the stop solution was added.

3. Results

The 8-OHdG was measured in serum of all patients and control. **Table (1)** showed the median 8-OHdG was significantly higher in patients (7 ng/mL) compared to controls (4.9 ng/mL); **(P** <0.001).

Table (1) 8-OHdG level in both groups**.**

		Cases (n = 60)	Controls (n = 20)	Z	P
8-OHdG (ng/mL)	Median (range)	7 (4.5 - 20.6)	4.9 (1.1 - 7.1)	-5.189	<0.001

Mann-Whitney U test was used

The 8-OHdG in **Table (2)** showed an overall significant difference between types of vitiligo (**P** = 0.047).

Table (2) 8-OHdG regarding different study parameters

		8-OHdG (ng/mL)	Z	P
Type of vitiligo	Focal	6.3 (4.5 - 16.9)	KW = 6.119	0.047
	Vulgaris	7.8 (5.6 - 20.6)		
	Acrofacial	6.8 (5.1 - 19)		

Z=Mann-Whitney U test, KW= Kruskal Wallis test

Table (3) and Chart (1, 2) presented a significant positive correlation between the 8-OHdG level in both age of the patients (r = 0.454 and P <0.001) and disease duration (r = 0.587 and P <0.001). Also, showed non-significant correlation between 8-OHdG level and VETI score (r = 0.241 and P = 0.063).

Table (3) Correlation between 8-OHdG with other parameters

	8-OHdG	
	r	P
Age (years)	.454*	<0.001
Duration of disease (years)	.587*	<0.001
VETI	0.241	0.063

Spearman's correlation was used

R; Correlation coefficient

*Significant

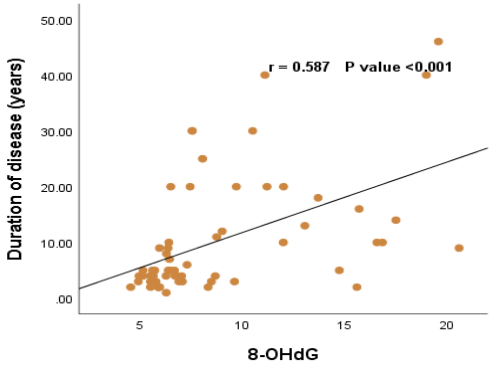

Chart (1) Correlation between 8-OHdG with disease duration**.**

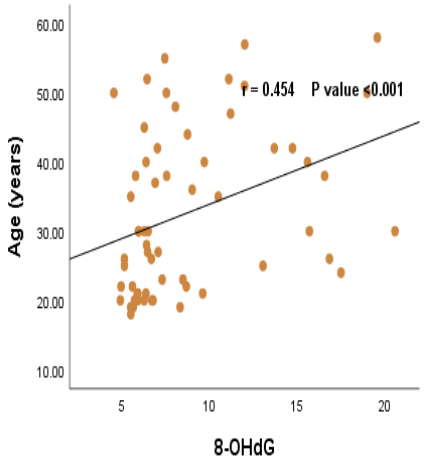

Chart (2) Correlation between 8-OHdG with patients' age.0

ROC analysis was performed for serum 8-OHdG level in diagnosing vitiligo. It showed an excellent significant AUC of 0.889 with a 95% confidence interval ranged from 0.820 to 0.968 (P <0.001). The best cut-off point was > 6.12, at which sensitivity, specificity, PPV, and NPV were 71.7%, 90%, 95.6%, and 51.4%, respectively Table (4) and Chart (3).

Table (4) ROC analysis for 8-OHdG in diagnosing vitiligo

ROC characteristics	
AUC (95% CI)	0.889 (0.820 - 0.968)
Best cut off	> 6.12
Sensitivity	71.7%
Specificity	90%
PPV	95.6%
NPV	51.4%
P-value	< 0.001

AUC; Area under curve
95% CI; 95% confidence interval
PPV; Positive predictive value
NPV; Negative predictive value

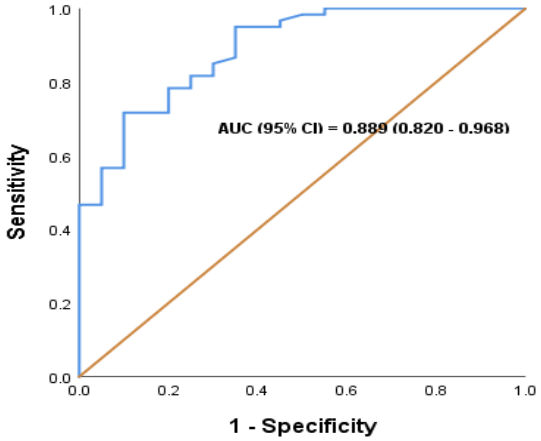

Chart 3: ROC analysis for 8-OHdG in diagnosing vitiligo

Multivariate logistic regression analysis in **Table (5)** was done for the prediction of vitiligo. It revealed that 8-OHdG was a significant independent predictor for vitiligo (**P** = 0.001), controlling for the effect of age and gender (OR = 4.106 and 95% confidence interval ranged from 1.849 to 9.118).

Table (5) Multivariate logistic regression model for vitiligo prediction

	OR (95% CI)	P-value
8-OHdG	4.106 (1.849 - 9.118)*	0.001

*Adjusted for age and gender
OR; Odds ratio
95% CI; 95% confidence interval

4. Discussion

A statistically significant difference between patients and controls was found for 8-OHdG levels, with patients' median values being 7 ng/mL and controls' at (4.9 ng/mL) (P 0.001).

There has only been one previous study that looked at the 8-OHdG levels in vitiligo patients, and the current study agrees with that study, finding that a higher level of 8-OHdG was associated with an increased risk of vitiligo. The authors also found that the APE1gene, specifically the APE1-148Glu variant, was linked to an increased risk of vitiligo. As a result, it has been postulated that the APE1-Asp148Glu polymorphism leads to a hereditary propensity to vitiligo in human melanocytes. An adjusted odds ratio of 2.32 was found, with a 95% confidence interval of 1.22–4.44 (P=0.001). Serum 8-OHdG levels were considerably greater in vitiligo patients than in controls.

Patients with alopecia areata were studied by El-Taweel et al. [13] for their 8-OHdG blood levels. More 8-OHdG was found in patients than in controls, and there was a strong association between 8-OHdG levels and the severity of a condition. Patients' (8-OHdG) blood levels were (21 7.1 ng/mL) and controls' (P=0.018).

There was a substantial difference in 8-OHdG levels between patients and healthy controls (21.10 vs. 7.42 pg/ml, P 0.001), which was in line with the findings of Korkmaz et al. Syafrita et al., [15] showed that patients with post-ischemic stroke depression had substantially higher levels of 8-OHdG than the control group (4.39 2.19 vs. 3.08 0.73 ng/mL, P 0.001).

An higher 8-OHdG level was found in psoriasis patients by Basavaraj et al. [16], who found it to be significant (P=0.034). For example the study by Shimamoto et al. [17] found that the mean 8-OHdG levels in patients with both p. vulgaris and atopic dermatitis were significantly higher than those in healthy controls (Mean SD=57.2 15.1 ng/mL), while the mean levels in healthy controls were significantly lower (Mean SD=37.1 9.8 ng/mL), (P= 0.042).

Both vitiligo and psoriasis are characterised by the presence of CD49a-expressing TRM cells (Tissue Resident Memory T cells). CD8+ CD49a+ TRM cells produce effector molecules perforin and granzyme B, as well as IL-15 stimulation, and are ready for cytotoxic response [18]. When treating vitiligo by targeting TRM cells, you may be able to reverse the condition by increasing the number of healthy TRM cells in the body. This discovery might provide new insight into the relationship between memory-dependent immunity and oxidative stress [19].

8-OHdG levels in acne vulgaris were measured by Izmirli et al. (20). The 8-OHdG level was found to be greater in patients (21.0 4.3 ng/mL) compared to the controls (10.07 4.01 ng/mL) in the research described. (P 0.0001).

Patients with oral squamous cell cancer and oral lichen planus showed considerable increases in 8-OHdG levels, according to Nandakumar et al. [21]. 8-OHdG levels in the control group were 6.59 ng/dL, compared to 13.89 ng/dL in the patients (P 0.0001).

Serum 8-OHdG levels were shown to be significantly correlated with patient age and illness duration in this investigation, but not with VETI score.

According to Dalle-donne et al., [22], 8-OHdG levels may be affected by age, viral infections, smoking habits, and other systemic disorders such as diabetes, hypertension, or cardiovascular disease, which is in accordance with their findings. One such research backs the idea that oxidative damage builds up over time and contributes to the progression of age-related illnesses. DNA damage caused by RONS is thought to accumulate with age, and Liguori et al. [23], corroborated that with a rise in 8-OHdG levels with ageing.

Heart failure patients had greater 8-OHdG levels, whereas females tend to have higher 8-OHdG levels than males. However, Nagao et al. [24] disagreed with this finding and discovered a favourable link between 8-OHdG levels and patient age. For males, the mean and SD of 8-OHdG levels were (35.5 ng/mL) (35.5 ng/mL) for patients, whereas for women, the mean and SD were (32.1 ng/mL) (25.0 ng/mL) (P 0.005).

The 8-OHdG was examined by Basavaraj et al. [16] in psoriatic patients to see whether it was associated with the severity of the illness. The mean 8-OHdG levels were found to be considerably higher, which is in line with the findings. 8-OHdG levels and illness severity were shown to have a strong positive connection in the research. For comparison, the 8-OHdG levels in the control groups were 1.18 0.93 ng/mL for the mild, 3.46 0.82 ng/mL for the moderate, 3.68 0.67 ng/mL for the severe, and 4.86 1.7 ng/mL for the extreme (P 0.05).

Lee et al., [25] found that 8-OHdG levels were greater in SLE patients than in healthy controls; however, they disagreed with the treatment's impact on 8-OHdG levels. A statistically significant difference (P 0.001) was found between the two values.

According to Gao et al. [26], a study looking at the effects of ionising radiation on 8-OHdG revealed significantly higher levels of 8-OHdG in patients than in controls. This finding was further supported by the finding that 8-OHdG levels were positively correlated with the age of patients and their duration of exposure to oxidative stress. Compared to the controls, patients had an average blood 8-OHdG level of (131.42 ng/mL) (P 0.001).

Cellai et al., [27] conducted an epidemiological investigation to examine the link between 8-OHdG and asbestos exposure. There were higher levels of 8-OHdG in patients than healthy controls, and this finding was also supported by finding that the highest levels of 8-OHdG were strongly related to the amount of time patients had been exposed to oxidative stress. The mean 8-OHdG levels in patients were significantly higher than those in healthy controls (P = 0.001) than those in healthy controls.

Increased levels of 8-OHdG are an indication of oxidative DNA damage. Because of this, it is fair to assume that oxidative stress indicators and levels of antioxidant enzymes may play an important role in the development of vitiligo.

5. Conclusion

Patients with vitiligo had greater amounts of 8-OHdG than healthy controls, according to this research. 8-OHdG levels are linked to vitilgo VETI scores, family history, and the length of the illness. 8-OHdG levels may suggest that oxidative unit is a major role in the development of vitiligo, as shown by these findings. Patients with vitiligo may benefit from the findings of this research, which might point the way toward new treatment options.

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