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Original article

Detection of Serum Level of Forkhead Box O1 (FOXO1) in Patients of Vitiligo and It's Correlation with Disease Severity

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Abstract

Background: Vitiligo is an acquired epidermal pigment loss of the skin. Oxidative stress is one of the major theories in the pathophysiology of vitiligo. FOXO1 is the forkhead members of the class O (FOXO1) transcription factors, and plays an important role in cell cycle regulation, apoptosis, oxidative stress, and DNA repair. **Aim of the Work:** to detect the level of FOXO1 in patients with vitiligo. **Subjects and Methods:** Fifty patients with Vitiligo were included in the study, and fifty healthy controls matched in age and gender to Vitiligo cases. All participants were subjected to full clinical and laboratory investigations. Blood samples had been taken from all studied participants (Vitiligo cases and healthy controls) to study serum level of FOXO1 and its relationship with the pathogenesis of vitiligo. **Results:** Serum level of FOXO1 was significantly higher in Vitiligo patients as compared with healthy controls, serum level of FOXO1 was slightly higher in female's Vitiligo serum as compared with males; but with no statistically significant difference, serum level of FOXO1 was slightly higher in Vitiligo serum among patients with positive as compared with negative family history but with no statistically significant difference. There was non-statistically significant

linear correlation between serum level of FOXO1 and patients' age, disease duration and last new lesion, however, there was a statistically significant positive linear correlation between serum level of FOXO1 and Vitiligo Area Scoring Index (VASI) in studied Vitiligo patients. **Conclusion:** We can conclude that, FOXO1 might be associated with susceptibility to vitiligo, especially active vitiligo, and that increased FOXO1 levels in vitiligo patients might be associated with oxidative stress.

1. Introduction

Vitiligo is an acquired, hypomelanotic disease characterized by circumscribed depigmented macules. The absence of melanocytes from the lesional skin due to their destruction has been suggested to be the key event in the pathogenesis of Vitiligo [1].

It is the most common depigmentation disorder affecting 0.5–1% of the population worldwide [2]. Although depigmentation rarely causes any physical symptoms, vitiligo may cause psychosocial problems and impair quality of life [1].

Vitiligo itself has been classified based on clinical grounds into two major forms, namely, segmental vitiligo (SV) and non-segmental vitiligo (NSV), the latter including several variants (generalized vitiligo, acrofacial vitiligo, universal Vitiligo [2].

Non-segmental vitiligo typically evolves over time, in both distribution and extension patterns. This is the case with focal vitiligo, which may evolve into SV, into NSV, or may remain unclassifiable based on the NSV/SV classification model. For NSV, the disease may be initially classified as acrofacial but will later progress to be better classified as generalized or universal. Conversely, some cases of NSV may spare the extremities (generalized non-acrofacial vitiligo). Some cases of NSV exhibit a flexural distribution, and others a predilection for extensor aspects, suggesting different triggers or etiologies. Therefore, precisely reporting the involved sites is of importance [2].

Oxidative stress may play an essential role in activating subsequent autoimmune responses related to vitiligo [3]. Oxidative stress and autoimmunity with genetic susceptibility have been associated with the pathogenesis of vitiligo, how these 2 pathways precisely integrate with each other is not fully understood [2]. Oxidative stress is the result of elevated levels of reactive oxygen species (ROS), the most important of which are superoxide anions, hydroxyl radicals, and hydrogen peroxide. A rise in the level of ROS can damage proteins, lipids, and DNA, eventually leading to cell death [4].

Cells counteract the adverse effects of ROS by up-regulating enzymatic scavengers or DNA-damage repair genes. This response involves dephosphorylation and subsequent activation of a small family of transcription factors known as FoxOs [5].

FOXOs represent a subfamily of the Forkhead family of transcription factors. This family is characterized by a conserved DNA- binding domain (the Forkhead box or FOX) and comprises more than 100 members in humans, from FOXA to FOXS The FOXO subfamily is conserved from C. elegans to mammals but, while invertebrates have only one FOXO gene, mammals have four FOXO genes: FOXO1,FOXO3, FOXO4 and FOXO6 [6].

Forkhead box O1 (FOXO1), which belongs to a large family of forkhead transcription factors, participates in a wide range of cellular processes, including cell cycle arrest, DNA repair, apoptosis, oxidative stress resistance, and glucose metabolism [5].

FOXO1 is also involved in immune responses by controlling cytokine production. FOXO1 plays a role in the protection of cells from oxidative stress [7].

2.Patients and Methods:

1. Patients

Study setting:

This study had been conducted at Beni-Suef university hospital from December 2020 to October 2021 (10 months).

Study type:

This study is Case-Control Study

Study population:

Patients were assigned into two groups: Group A: Included Patients with Vitiligo disease. Group B: Normal group as control group, Age and sex matched to Vitiligo group.

Sample size: 100 subjects, 50 cases and 50 controls.

Inclusion criteria:

- All vitiligo patients regardless of their age and sex.
- Healthy control group will be age and sex matched with our patients.

Exclusion criteria:

- Patients suffering from tumors.
- Patients suffering from diabetes.
- Patients with Alzheimer's disease.

2. Methods

I) Methods of Examination: Every patient was subjected to the following;

1) Informed consent: A written informed consent was taken from every patient before participation in this study.

2) History taking:

- Personal history including:
  - Age, sex
  - Social demographic data

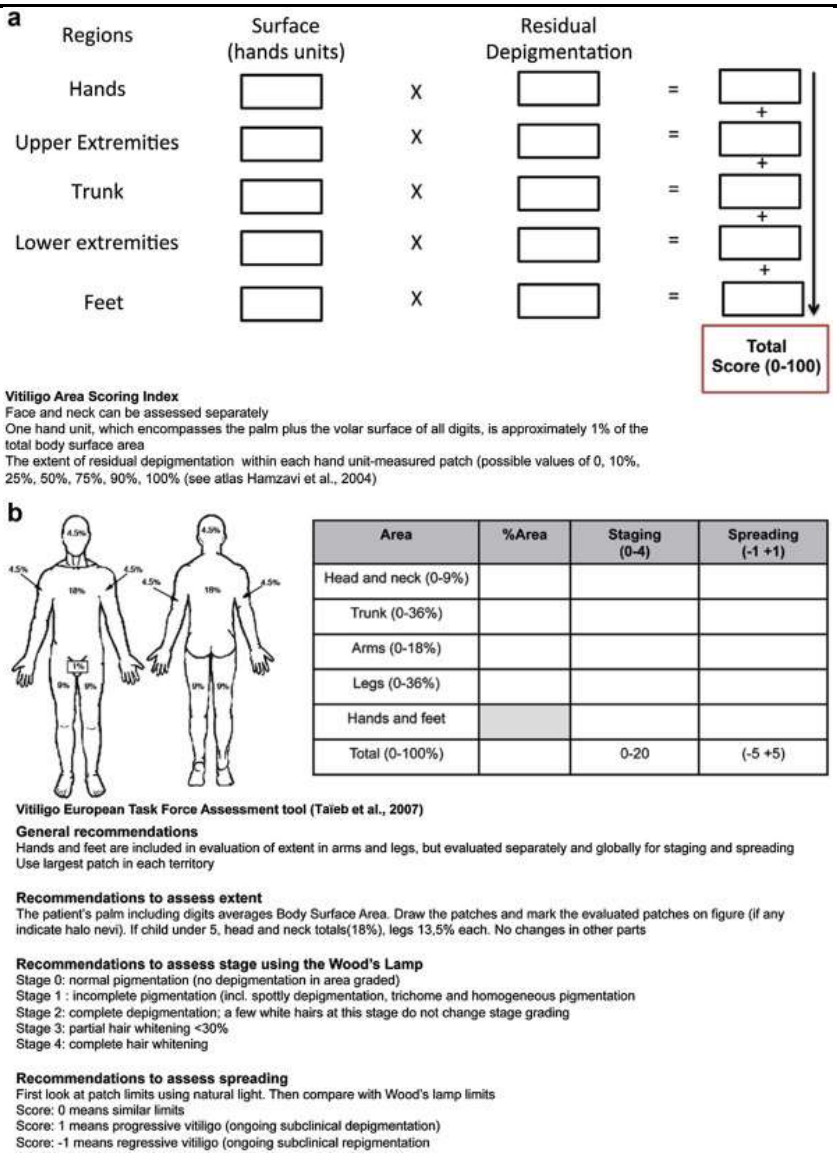
- Present history including duration of the disease.
    - Previous treatment:
    - Current treatment:
    - Associated diseases: Thyroid disease, Diabetes mellitus or others.
    - Family history of Vitiligo

3) Assessment of disease severity:

- Vitiligo Area Scoring Index (VASI)

Hamzavi et al. [8] have introduced a quantitative parametric score, named VASI for Vitiligo Area Scoring Index, which is conceptually derived from the PASI score widely used in psoriasis assessment [9]. The total body VASI is calculated using a formula that includes contributions from all body regions (possible range, 0–100)

$$\text{VASI} = \sum_{\text{All Body Sites}} [\text{Hand Units}] \times [\text{Residual Depigmentation}].$$



1. Blood Sample:

Blood samples were collected in BD Vacutainer K2EDTA tubes using a 21-gauge needle. Then centrifuged at 4000 rpm for 10 min, plasma was separated, and stored at −70°C.

2. Measurement of human forkhead box protein o1 (foxo1) serum level using ELISA Kit:

Blood samples were used to assay the FOXO1 level in the sample using an FOXO1 Human ELISA kit supplied by Sun Red China catalogue number 201-12-1526 according to the manufacturer's instructions. This assay has high sensitivity and excellent specificity for detection of FOXO1. No significant cross-reactivity or interference between FOXO1 and analogues was observed. Sensitivity: 2.045 ng/ml. Assay range: 3 ng/ml → 600 ng/ml, Intra-Assay: CV<9% and Intra-Assay: CV<11%.

Test principle

The kit used a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Human FOXO1 in samples. FOXO1 was added to monoclonal antibody Enzyme well which is pre-coated

with Human FOXO1 monoclonal antibody, incubation was carried out then, FOXO1 antibodies labeled with biotin was added and combined with Streptavidin-HRP to form immune complex; then incubation was carried out and washed again to remove the uncombined enzyme. Then Chromogen Solution A, B were added, the color of the liquid changed into the blue, and at the effect of acid, the color finally became yellow. The chroma of color and the concentration of the Human FOXO1 of sample were positively correlated.

Assay procedure:

1. Standard dilution: was done according to the following table:

400ng/ml	Standard No. 5	120μ l Original Standard + 120μ l Standard diluents
200ng/ml	Standard No. 4	120μ l Standard No. 5 + 120μ l Standard diluents
100ng/ml	Standard No. 3	120μ l Standard No. 4 + 120μ l Standard diluent
50ng/ml	Standard No. 2	120μ l Standard No. 3 + 120μ l Standard diluent
25ng/ml	Standard No. 1	120μ l Standard No. 2 + 120μ l Standard diluent

2. Sample Injection:

   I. Blank well: Samples, FOXO1-antibody labeled with biotin and Streptavidin-HRP weren't added. Only Chromogen solution A, B, and stop solution are allowed; other operations are the same.

   II. Standard wells: 50μl of standard, and 50μl Streptavidin-HRP were added (since the standard already has combined biotin antibody, it was not necessary to add the antibody).

   III. Test wells: 40μl sample was added, and then both FOXO1 antibody 10μl and Streptavidin-HRP 50μl were added. Then the sealing memberance was sealed and Shacked gently, incubated 60 minutes at 37 ℃.

3. Confection: washing concentrate was diluted 30 times with distilled water as standby.

4. Washing: the membrane was removed carefully, and the liquid was drained, the remaining water was Shacked away.

5. Fifty microliters of chromogen solution A and 50μl of chromogen solution B were added to each well, gently mixed and incubated for 10 min at 37℃ away from light.

6. To stop the reaction: 50μl of stop solution were added into each well to stop the reaction (the blue was changed into yellow immediately).

7. Final measurement: blank well was taken as zero and the optical densit (OD) was measured under 450 nm wavelength which had been carried out within 15 min after adding the stop solution.

8. According to standards' concentration and the corresponding OD values, the standard curve linear regression equation was calculated and then the OD values of the sample on the regression equation had

been added to calculate the corresponding sample's concentration.

3. Ethical Considerations

The study was approved by the ethical committee of the Faculty of Medicine, Beni-Suef University**.** Informed written consent was obtained from all participants before recruitment in the study, after explaining the objectives of the

4. Statistical Analysis

The collected data were coded then entered and analyzed using the SPSS version 25 (Statistical package for social science) for windows 10.

The following tests were used:

- **Descriptive analysis** of the results in the form of percentage distribution for qualitative data and (minimum, maximum, mean and standard deviation) calculation for quantitative data.
- **Cross tabulation and Chi Square test** ($\chi 2$)**:** For comparison between categorical variables and percentage values.
- **Student t- test:** For comparison between means of two unrelated groups with a normal distribution.
- **One way ANOVA test:** For comparison between means of more than two unrelated groups with a normal distribution.
- Spearman's correlation analysis was done to evaluate linear relationship between studied FOXO1 expression and other parameters in Vitiligo patients' plasma. Correlation graphs were drawn only for significant correlation which is considered significant at $P < 0.05$. Correlation is considered positive (direct correlation) when r (correlation coefficient) had a + signal and negative (inverse correlation) in case of – signal and it is considered:
  - Weak when r = >0 – 0.35,
  - Moderate when r = >0.35 – 0.65; and
  - Strong when r = > 0.65.
- P-values equal to or less than 0.05 were considered statistically significant.
- Simple graphs were used to illustrate some information.

3. Results

The current study included 50 Vitiligo patients from both sexes. They all presented to dermatology department at Beni-Suef University hospital. The Vitiligo patients were 13 males and 37 female patients, their age ranged from 18 to 47 years, the average age was 31.04 ±9.6. And 50 healthy controls were taken, they were age and sex matched to the Vitiligo cases.

(1) Descriptive Statistics

Table (1): Sex Distribution of the Vitiligo Cases and Healthy Controls; (N= 60)

		N (%)		Total N= 60	p-value*
		Vitiligo Patients, N= 30	Healthy Controls, N= 30		
Sex	Male	13 (26.0)	16 (32.0)	29 (29.0)	0.660
	Female	37 (74.0)	34 (68.0)	71 (71.0)	

**p-value >0.05 is considered non-significant by Chi-Square test.*

Table (1) demonstrates gender distribution of the cases and the controls; the Vitiligo cases; 26% of them were males and 74% were females, while the controls; 32% were males and 68% were females. There was no statistically significant difference between the cases and the control groups regarding sex (p-value> 0.05).

Table (2): Age Distribution of the Vitiligo Cases and Healthy Controls; (N= 100)

Age (years)	Mean	SD	Minimum	Maximum	p-value
Vitiligo Patients	31.04	9.6	18	47	0.934
Healthy Controls	30.90	6.9	18	46	

*p-value >0.05 is considered non-significant by independent sample t-test.

As illustrated in table (2); the average cases age was; 31.04 ±9.6 (SD) years, while average controls age was 30.90 ±6.9 (SD) years. There was no statistically significant difference between cases and control groups regarding to age (p-value> 0.05).

Table (3): Disease Course of Vitiligo among studied Vitiligo Cases; (N= 30)

		Frequency	Percent
Disease Course	Stable	7	14.0
	Progressive	43	86.0

Table (3) demonstrates that the majority of the studied Vitiligo cases had progressive course of the disease (43) cases (86%); while only 7 cases (14%) had a stationary disease course.

Table (4): Family History of Vitiligo among studied Vitiligo Cases; (N= 30)

		Frequency	Percent
Family History	**Negative**	42	84.0
	Positive	8	16.0

Table (4) demonstrates that the majority of the studied Vitiligo cases had no family history of the disease (42) cases (84%); while only 8 cases (16%) had a positive family history of Vitiligo.

Table (5): History of Disease Duration and last new lesion among studied Vitiligo patients; (N= 50)

	Mean	SD	Minimum	Maximum
Disease Duration (years)	4.79	6.04	0.02	35.0
Last New Lesion (months)	4.74	4.3	0.25	12

Table (5) demonstrates the disease duration was ranged from (0.02) to (35) with a mean of 4.79 ±6.04 (SD) years of disease duration. The history of last new lesions among studied Vitiligo cases was ranged from (0.25) to (12) with a mean of 4.74 ±4.3 (SD) months.

Table (6): The Vitiligo Area Scoring Index (VASI) among studied Vitiligo patients; (N= 50)

	Mean	SD	Minimum	Maximum
VASI	18.15	18.0	0.50	70

The Vitiligo Area Scoring Index (VASI) score ranged from (0.5) to (70) with a mean of 18.15 ±18 (SD).

(2) Analytical Statistics:

Table (7): Comparison between controls and Vitiligo cases regarding serum level of fork-head box O1 (FOXO1)

	Mean	SD	Minimum	Maximum	p-value
Vitiligo Cases	127.79	15.77	93.50	157.20	<0.001*
Healthy Controls	33.81	6.02	21.60	52.10	

*p-value >0.05 is considered non-significant by independent sample t-test.

As demonstrated in table (7); serum level of fork-head box O1 (FOXO1) was significantly higher in Vitiligo patients as compared with healthy controls; the mean FOXO1 values (127.79 vs. 33.81) in Vitiligo and healthy controls respectively with a statistically significant p-value< 0.001.

Table (8): Relation between serum level of fork-head box O1 (FOXO1) and patients' gender in studied Vitiligo patients; (N= 50)

		N	Mean	SD	Minimum	Maximum	p-value
Gender	**Male**	13	121.47	15.59	93.50	146.20	0.229
	Female	37	130.01	15.42	101.70	157.20	

*p-value >0.05 is considered non-significant by independent sample t-test.

As demonstrated in table (8); serum level of fork-head box O1 (FOXO1) was slightly higher in female's Vitiligo serum as compared with males; however no statistically significant difference was detected in relation to gender and serum level of (FOXO1), (p-value= 0.229).

Table (9): Relation between serum level of fork-head box O1 (FOXO1) and patients' Family History in studied Vitiligo patients; (N= 50)

		N	Mean	SD	Minimum	Maximum	p-value
Family history	**Negative**	42	126.88	14.32	101.7	157.20	0.356
	Positive	8	132.56	22.52	93.50	156.20	

*p-value >0.05 is considered non-significant by independent sample t-test.

As demonstrated in table (9); serum level of fork-head box O1 (FOXO1) was slightly higher in Vitiligo serum among patients with positive as compared with negative family history; however no statistically significant difference was detected in relation between family history and serum level of (FOXO1), (p-value= 0.356).

Table (10): Relation between serum level of fork-head box O1 (FOXO1) and disease course in studied Vitiligo patients; (N= 50)

		N	Mean	SD	Minimum	Maximum	p-value
Course	**Stable**	7	122.77	15.71	108.50	146.20	0.369
	Progressive	43	128.61	15.81	93.50	157.20	

*p-value >0.05 is considered non-significant by independent sample t-test.

As demonstrated in table (10); serum level of fork-head box O1 (FOXO1) was slightly higher among progressive Vitiligo disease patients' serum as compared with stable course of the disease; however no statistically significant difference was detected in relation between disease course and serum level of (FOXO1), (p-value= 0.369).

Table (11): Correlation between serum level of fork-head box O1 (FOXO1) and Patients' Age in studied Vitiligo patients; (N= 50)

	Age (years)	
FOXO1	R= -0.141	*p-value*= **0.330**

r Spearman's correlation coefficient analysis

Table (11) demonstrates non statistically significant linear correlation between serum level of fork-head box O1 (FOXO1) and patients' age in studied Vitiligo patients; (p-value >0.05).

Table (12): Correlation between serum level of fork-head box O1 (FOXO1) and disease duration in studied Vitiligo patients; (N= 50)

	Disease duration (years)	
FOXO1	R= 0.247	*p-value*= **0.084**

r Spearman's correlation coefficient analysis

Table (12) demonstrates non statistically significant linear correlation between serum level of fork-head box O1 (FOXO1) and disease duration in studied Vitiligo patients; (p-value >0.05).

Table (13): Correlation between serum level of fork-head box O1 (FOXO1) and duration of last lesion in studied Vitiligo patients; (N= 50)

	Duration of last lesion (months)	
FOXO1	R= 0.091	*p-value*= **0.551**

r Spearman's correlation coefficient analysis

Table (13) demonstrates non statistically significant linear correlation between serum level of fork-head box O1 (FOXO1) and duration of last lesion in studied Vitiligo patients; (p-value >0.05).

Table (14): Correlation between serum level of fork-head box O1 (FOXO1) and Vitiligo Area Scoring Index (VASI) in studied Vitiligo patients; (N= 50)

	VASI	
FOXO1	R= 0.359	*p-value*= **0.011***

r Spearman's correlation coefficient analysis

Table (14) demonstrates a statistically significant positive linear correlation between serum level of fork-head box O1 (FOXO1) and Vitiligo Area Scoring Index (VASI) in studied Vitiligo patients; (r= 0.359, p-value=0.011).

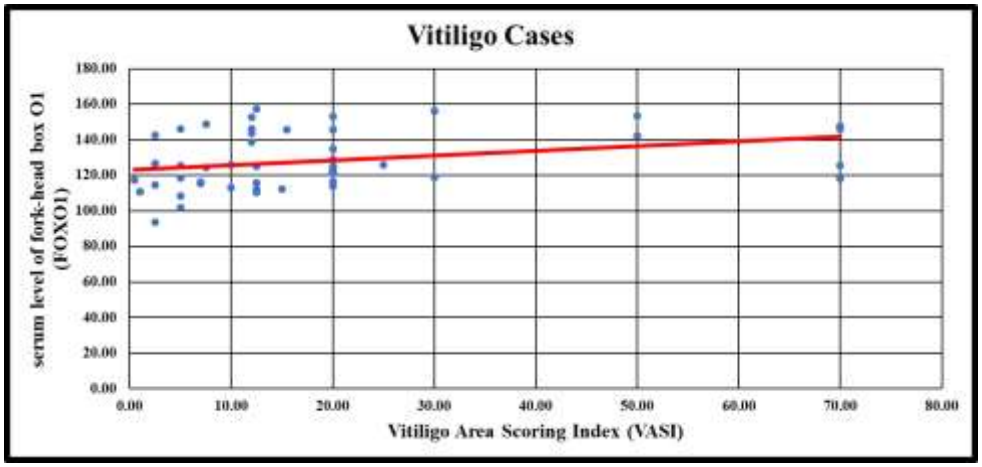


Figure (1): Correlation between serum level of fork-head box O1 (FOXO1) and Vitiligo Area Scoring Index (VASI) in studied Vitiligo patients.

4.Discussion:

Vitiligo is an acquired depigmenting disease characterized by milky white patches of skin due to local loss of the epidermal melanocytes. It occurs with a frequency of 0.1–2% worldwide [10]**.** Although its pathophysiology is still unknown, diverse theories have been proposed, including autoimmune, neural, oxidative stress, apoptosis, and genetic factors [11].

Recent studies have suggested that oxidative stress might play a prominent role in the pathogenesis of vitiligo [12]. Oxidative stress is defined as a disruption of the delicate balance between the formation of reactive oxygen species (ROS) and the antioxidant defense system [13]. ROS, especially superoxide anions and hydrogen peroxide

(H2O2), can cause lipid peroxidation and protein and DNA oxidation, as well as alter apoptotic pathways, thereby inducing cellular injury. It has been suggested that some enzyme defects during melanogenesis might cause oxidative stress, with a consequent accumulation of H2O2 [14].

Forkhead box class O (FOXO) proteins are a subclass family of the Forkhead box (FOX) transcription factors, including FOXO1, FOXO3A, FOXO4, and FOXO6, which play a crucial role in diverse cellular processes such as cell cycle regulation, apoptosis, oxidative stress and DNA repair [15]. Studies have shown that FOXO1 have important roles in the regulation of oxidative stress [16]. The FOXO1 gene is located on chromosome 13 and translates into FOXO1 protein, which contains 4 functional domains, including the nuclear localization signal domain (NLS), the nuclear export signal (NES), the transactivation domain (TA) and the Forkhead domain (FKH). FOXO1 modulates numerous targets, such as genes involved in apoptosis and autophagy, anti-oxidative enzymes, cell cycle arrest genes, and metabolic and immune regulators [17], ***Wang et al. [18],*** rendering it a super transcription factor with complex activities. However, to the best of our knowledge, there have been no reports regarding the relationship between FOXO1 genetic variants and the risk of vitiligo. Considering the possible effects of oxidative stress and apoptosis in the pathophysiology of vitiligo, we hypothesized that FOXO1 might be associated with risk of vitiligo. For this reason, we conducted the current study with an aim to detect the level of FOXO1 in patients with Vitiligo disease as compared with healthy controls.

It was a case-control study, included 50 Vitiligo patients from both sexes, all presented to dermatology department at Beni-Suef University hospital, and 50 healthy controls were taken, they were age and sex matched to the Vitiligo cases.

The average patient age was 31.04 ±9.6 (SD) years. Gender distribution of studied patient was showed a female preponderance with female to male ratio of (2.8:1); and this was in accordance with certain other studies in this regard which confirm female preponderance [19]. The observed female predominance can be explained by their emotional fragility and the most major cosmetic concern of parents that is the case in our context.

Family history of Vitiligo appears to lie between 15 and 20 % in most studies [20]. In a Chinese survey of 815 probands, family history was 15.7 % [21]. In a Gujarat survey, 20.4 % had a family history of vitiligo [22]. The lowest family history rate identified while making this study was 3.43 % in a cohort of 30,000 patients from Mumbai [23]. In the current study, the majority of the studied Vitiligo cases had no family history of the disease and only 8 cases reported a positive family history of Vitiligo. Majority of the studied Vitiligo cases had a progressive course of the disease. None of the studied Vitiligo cases

had neither history of previous Vitiligo treatment nor history of associated skin disorders.

Considering the roles of FOXO1 in oxidative stress and apoptosis, we mainly examined the relationship between FOXO1 serum levels and vitiligo. In our study, serum level of FOXO1 was significantly higher in Vitiligo patients as compared with healthy controls (p-value <0.001). The underlying mechanism is difficult to explain, as there are no published studies about serum/plasma FOXO1 levels in Vitiligo patients. However, we think that the high serum levels of FOXO1 might be associated with increased oxidative stress in the Vitiligo patients. In response to various types of cellular stress stimulus or exogenous agents, the transcriptional activity of FOXO1 can be regulated by post-transcriptional modifications, such as phosphorylation, acetylation, and ubiquitylation [24, 25].

Alterations in oxidative stress mediated by reactive oxygen species (ROS) accumulation cause a significant increase in the transcriptional activity of FOXO1, and this has a major impact on the expression of superoxide dismutases (SODs) and Catalase, which are key antioxidant enzymes and are always reported as oxidative stress markers or evidence because of the elimination of reactive oxygen species (ROS)-mediated injuries [26]. This phenomenon suggests that FOXO1 acts as a sensing element in the anti-oxidative signaling pathway. In addition, it was suggested that low-level FOXO1 might be involved in the reduced transcription of antioxidant enzymes in many diseases, such as spindle cell lipomas, and mammary type myofibroblastomas [27].

In the current study we reported a statistically significant positive linear correlation between serum level of FOXO1 and Vitiligo Area Scoring Index (VASI) in studied Vitiligo patients, this significant correlation could be explained by the reported significant positive correlation between oxidatve stress and VASI score in Vitiligo patients [28]. However, further studies are recommended to assess this correlation.

In the current study, there was a significant linear moderate positive correlation between tissue expression of STAT and Vitiligo disease activity (VIDA) score among studied Vitiligo patients; (r= 0.547, p=0.002). This was opposite to ***Samaka et al. [29],*** study who reported; there was no significant relationship between STAT3 expression and VIDA score.

In the current study we found non-statistically significant association between FOXO1 serum level in Vitiligo patients with their gender, age, disease duration, duration of last lesion, or disease course. Further larger studies are recommended in order to confirm or refute these results

FOXO1 has been reported to protect cells against ROS such as H2O2 by activating various ROS detoxifying enzymes [30]. However, the subtle mechanisms of the effects

of FOXO1 remain elusive and even conflicting in the pathogenesis and progression of Vitiligo disease. The determination of these questions potentially has implications for further research regarding FOXO1 signalling and the identification of targeted drugs.

Limitations in our study include the small number of participated patients (cases and controls), and it was a short-term study. On the other hand, one of the limitations was the inability to make a comparison between FOXO1 serum level and different severities of the Vitiligo disease due to relatively small number of participants.

To the best of our knowledge, this is the first study to investigate the influence of FOXO1 serum levels in vitiligo patients. In conclusion, our results demonstrate, FOXO1 might be associated with susceptibility to vitiligo, especially active vitiligo, and that increased FOXO1 levels in vitiligo patients might be associated with oxidative stress. Our observations confirm that oxidative stress is implicated in the pathophysiology of vitiligo, through increased protein oxidation and impaired antioxidant defenses. Therefore, we suggest that additional treatments might be useful for reducing the oxidative stress status of vitiligo patients. However, further studies with larger samples are required to elucidate the molecular mechanism responsible for the relationship between FOXO1 and oxidative stress and vitiligo.

5. Conclusion and Recommendations :

Oxidative stress is reported in the development of numerous diseases, which is attributable to the accumulation of reactive oxygen species (ROS). Vitiligo is an acquired epidermal pigment loss of the skin. Oxidative stress is one of the major theories in the pathophysiology of vitiligo. FOXO1 is the forkhead members of the class O (FOXO) transcription factors, and plays an important role in cell cycle regulation, apoptosis, oxidative stress, and DNA repair.

Our study indicates that FOXO1 may associated with pathophysiology of vitiligo, especially with extensive forms of Vitiligo. Further studies with larger samples are required to elucidate the role of FOXO1 in vitiligo.

Based on the currently observed elevated FOXO1 serum level in Vitiligo patients, we recommend the followings:

- Further studies with larger samples are required to elucidate the molecular mechanism responsible for the relationship between FOXO1, oxidative stress and vitiligo.
- Additional research is also necessary to discover if FoxO1 can be utilized for drug targeting and development of novel therapeutics for Vitiligo disease.

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