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# Estimation of Advanced Glycation end-Products in Patients of Vitiligo

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## Abstract:

The goal of this study is to detect a relationship between advanced glycation end-products and the pathogenesis of vitiligo in diseased patients as compared to normal control persons using Enzyme-Linked Immunosorbent Assay (ELISA) technique on tissue skin biopsy. The present study included 30 Egyptian vitiligo patients, their age ranged from (20 to 50 years). They were recruited from Dermatology outpatient clinic at Beni-Suef University hospital. Thirty unrelated apparent healthy controls with similar demographic (matched age and sex) were taken.The studied subjects were divided into two groups as follows, Group I: (n = 30) vitiligo patients and Group II: (n = 30) unrelated apparent healthy controls. Four mm punch skin biopsy will be taken from patients( vitiligo lesion) & control and it will be kept in lysis solution for the stability of the studied parameters and will be kept frozen at -80 Celsius till analysis of advanced glycation end-products by ELISA. The result of the study showed that expression of AGEs in lesional skin of Vitiligo patients was significantly higher as compared to normal control persons. Also reveled moderate negative correlation between the expression of Advanced Glycation end-Products (AGEs) in lesional skin of vitiligo patients and Vitiligo disease activity (VIDA) score of the studied cases and that confirms that AGE levels were, instead, significantly higher in vitiligo patients with older lesions.

**Keywords**: AGEs, ROS, Vitiligo.

## 1. Introduction:

Vitiligo is an acquired mucocutaneous skin and less often hair disease defined by decrease melanocyte function and depigmentation with an approximated prevalence of 0.5–1% in most populations [1].

The pathogenetic mechanisms of vitiligo are ill-defined. Presently, research hypotheses go in three chief directions: a genetic defect in the melanocyte, an alteration in the evolution of the peripheral nervous system destruction the neural-crest derived melanocytes and, a dysregulation of the immune reaction [2].

Asingle predominant pathway is improbable to explain for all cases of melanocyte loss in vitiligo, instead, it is the result of tangled interactions of biochemical, environmental and immunological effect, in a permissive inherited surroundings. Increased level of reactive oxygen species (ROS) can damage biological processes, and this state has been referenced in active vitiligo skin. Tyrosinase activity is diminished by increased Hydrogen peroxide (H2O2) directed by oxidation of methionine residues in this cardinal melanogenic enzyme [3].

ROS overexposure in vitiligo has been manifested, and it is due to exogenous and endogenous excitations, as approved by the evidence that patients with progressive disease tend to have a remarkably decreased activity of antioxidant enzymes [4]. between these, the advanced glycation end products (AGEs) and the advanced oxidation protein products (AOPPs) represent changed macromolecules, such as fibrinogen or albumin, with adjusted function due to the exposure to oxidative pressure [5].

AGEs are lipids or proteins that become glycated due to exposure to sugars [6]. Dietary sources like animal-derived foods that are full-fat and protein are mostly AGE-rich and are prone to boost AGE formation through cooking [7].

AGEs formation occurs over aperiod of weeks so just long-living glycated proteins as albumin, collagen, haemoglobin and Low density lipoprotin (LDL) will be liable to this conversion [8].

AGEs can be an agent in aging and in the improvement or deterioration of numerous degenerative diseases, like

atherosclerosis, diabetes, Alzheimer's disease, and chronic kidney disease[9],[10].

## 2. Patients and Methods:

This was acase control study included 30 Egyptian vitiligo patients, their age ranged from (20 to 50 years). They were recruited from Dermatology outpatient clinic at Beni-Suef University hospital. Thirty unrelated apparent healthy controls with similar demographic (matched age and sex) were taken.The studied subjects were divided into two groups as follows:Group I: (n = 30) vitiligo patients and Group II: (n = 30) unrelated apparent healthy controls. Informed consent was obtained from the participants in this study after ethical committee approval from dermatology outpatient clinic at Beni-Suef University hospital. Cases were chosen randomly according to inclusion and exclusion critreria during the period from (1st may 2019 to 31 of november 2019).

### 2.1 Inclusion criteria:

• Age between 20 to 50.

• Patients with chronic white patch.

• Patients not receiving systemic or topical vitiligo treatment for at least three months.

• Both males and females will be included.

### 2.2 Exclusion criteria:

• Age below 20 and above 50.

• Patients receiving systemic or topical vitiligo treatment in the last three months.

• Patients with other autoimmune diseases.

• Patients with associated systemic or dermatological diseases.

Controls will be chosen randomly from any other outpatient clinic.

### 2.3 All patients were subjected to:

Medical history was taken from patients to show: Course of illness, Age of onset of the disease, Mode of presentation (Initial symptoms) and Positive family history. Clinical examination was done to determine the site, extent, type of vitiligo, skin type. Investigations was performed to show Lipid profile (TG, HDL, Cholesterol) and four mm punch skin biopsy was taken from patients( vitiligo lesion) & control and it was kept in lysis solution for the stability of the studied parameters and was kept frozen at -80 Celsius till analysis of advanced glycation end products by ELISA for Estimation of the levels of AGEs.

## 2.4 Procedures:

the kit used from (MyBioSource), No:MBS086045.

• Reagents and samples were brought to room temperature (18°C-25°C) naturally for 30min before starting assay procedures. a low - speed centrifugation was done for one or two seconds to concentrate the Standards to the bottom of the vials.

• Standard wells were set, Sample wells and Blank/Control wells, Standard 50µl added to each Standard well, Sample 50µl added to each Sample well, Sample Diluent 50µl added to each Blank/Control well. It is recommended that all Standards, samples and Sample Diluent be added in duplicate to the plate .

• One hundred µl of HRP-conjugate reagent added to each well, covered with a Closure Plate Membrane and incubated for 60 minutes at 37°C.

• The plate was washed 4 times byManual Washing or Automated Washing.

• Chromogen Solution A 50µl and Chromogen Solution B 50µl added to each well successively. Gently mixed and then protected from light to incubate for 15 minutes at 37°C.

• Fifty µl Stop Solution added to each well. The color in the wells changed from blue to yellow.

• The Optical Density (O.D.) was read at 450 nm using an ELISA reader within 15 minutes after adding Stop Solution.

## 2.5 Statistical analysis:

Data were coded and entered using the statistical package SPSS (Statistical Package for the Social Sciences) version 24. Data was summarized using mean, standard deviation, median, minimum and maximum in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. Comparisons between quantitative variables were done using the non-parametric Kruskal-Wallis and Mann-Whitney tests (Chan, 2003a). Correlations between quantitative variables were done using Spearman correlation coefficient (Chan, 2003b). P-values less than 0.05 were considered as statistically significant.

## 3. Results:

The present case control study included 30 Egyptian vitiligo patients, their age ranged from (20 to 50 years). They were recruited from Dermatology outpatient clinic at Beni-Suef University hospital. Thirty unrelated apparent healthy controls with

similar demographic (matched age and sex) were taken.The studied subjects were divided into two groups as follows, Group I: (n = 30) vitiligo patients and Group II: (n = 30) unrelated apparent healthy control.

**Table (1)**: **Sex Distribution of the Studied Population; (N= 60):**

| | | N (%) | | | |
|---|---|---|---|---|---|
| | | **Cases** | **Controls** | | |
| | | **N= 30** | **N= 30** | **TOTAL** | ***p-value***[a] |
| **Sex** | **Female** | 20 (66.7) | 20 (66.7) | 40 (66.7) | 0.608 |
| | **Male** | 10 (33.3) | 10 (33.3) | 20 (33.3) | |

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

**Table (1)** demonstrates sex distribution of studied patients; 66.7% of them were females and 33.3% were males **( figure 1)**. There was no statistically significant difference between cases and control groups regarding sex (p-value> 0.05).

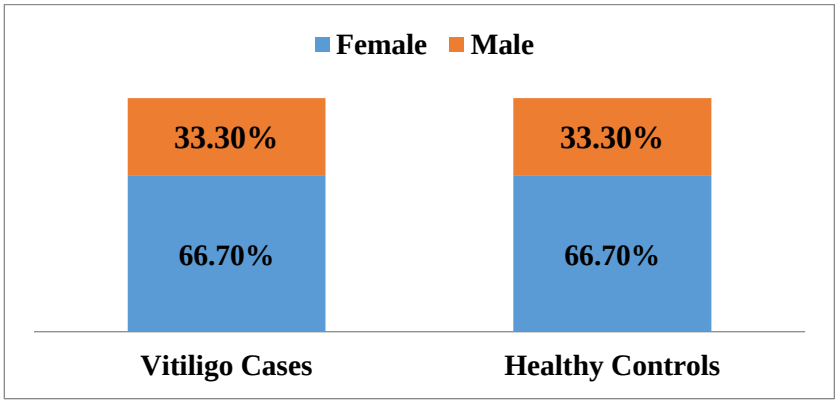


**Figure (1): Gender Distribution of the Studied Population.**

*(matched gender distribution between cases and controls)*

**Table (2): Age Distribution of the Studied Population; (N= 60):**

| | Mean ±SD | Minimum | Maximum | Range | *p-value*[a] |
|---|---|---|---|---|---|
| **Cases** | 33.57 ±10.6 | 20 | 50 | 30 | 0.459 |
| **Controls** | 33.23 ±10.1 | 20 | 50 | 30 | |

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

As illustrated in **table (2)**; the average patient age was 33.57 ±10.6 (SD) years and controls was 33.23 ±10.1 (SD) years **( figure 2)**. There was no statistically significant difference between cases and control groups regarding age.

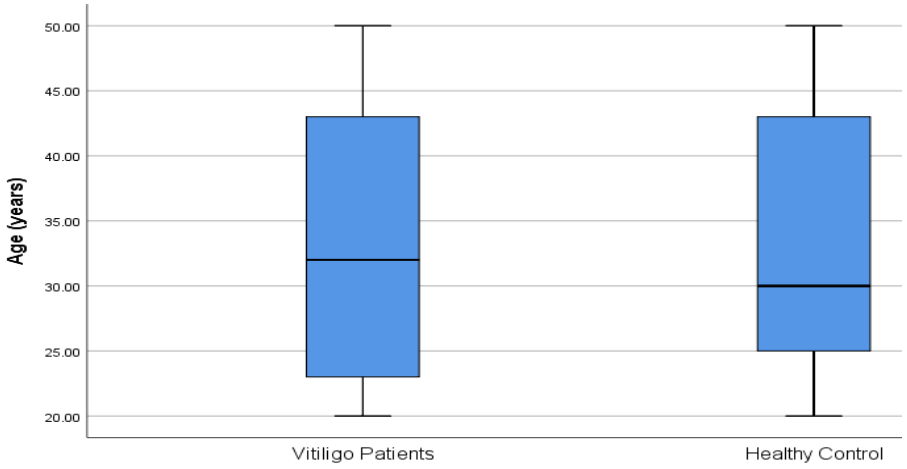


**Figure (2): Age Distribution of the Studied Population**

*(matched age distribution between cases and controls)*

**Table (3): Disease Characters of studied Vitiligo Cases; (N= 30):**

| | N | % |
|---|---|---|
| **Vitiligo Type** | | |
| **Vulgaris/Acrofacial (mixed)** | 8 | 26.7% |
| **Vulgaris** | 15 | 50.0% |
| **Acral** | 1 | 3.3% |
| **Segmental** | 2 | 6.7% |

| | | |
|---|---|---|
| **Uneversalis** | 2 | 6.7% |
| **Focal** | 2 | 6.7% |
| **Vitiligo Generalization** | | |
| **Localized** | 6 | 20.0% |
| **Generalized** | 24 | 80.0% |
| **Laterality** | | |
| **Unilateral** | 1 | 3.3% |
| **Bilateral** | 29 | 96.7% |
| **Symmetry** | | |
| **Symmetrical** | 16 | 53.3% |
| **Asymmetrical** | 14 | 46.7% |
| **Vitiligo disease activity (VIDA) score** | | |
| **Mean ±SD** | 2.27 ±1.5 | |
| **Range (Minimum-Maximum)** | 4 (0 – 4) | |
| **Vitiligo Extent Percentage (%)** | | |
| **Mean ±SD** | 23.63 ±23.9 | |
| **Range (Minimum-Maximum)** | 93 (2 – 95) | |
| **Duration of last Vitiligo Lesion (months)** | | |
| **Mean ±SD** | 5.34 ±5.23 | |
| **Range (Minimum-Maximum)** | 23.75 (0.25 – 24.0) | |

**Table (3)** illustrates that; half the studied patients had vulgaris Vitiligo type (50%) while 8 cases (26.7%) had mixed Vitiligo type, 1 case with acral type, 2 cases segmental, 2 cases universalis and 2 cases with focal Vitiligo type. Of the studied Vitiligo cases only 6 cases (20.0%) were with localized skin lesion while 24 cases (80.0%) were with generalized skin lesion. Majority of the studied cases had bilateral skin lesions and only one case had unilateral skin lesion. And regarding the lesional symmetry; 16 cases (53.3%) had symmetrical lesions and 14 cases (46.7%) had asymmetrical skin lesions.

Vitiligo disease activity (VIDA) score ranged from (0) to (+4) with a mean of 2.27 ±1.5 (SD), while Vitiligo Extent percentage ranged from (2%) to (95%) with a mean of 23.63 ±23.9 (SD). The duration of last lesion was ranged from (0.25) to (24) months with an average duration of 5.34 ±5.23 (SD).

**Table (4): Distribution of Vitiligo Lesions in the studied Cases; (N=30):**

| | N | % |
|---|---|---|
| **Site** | | |
| **All over the body** | **2** | **6.7%** |
| **Face** | **15** | **50%** |
| **Hands** | **12** | **40%** |
| **Feet** | **13** | **43.3%** |
| **Back** | **12** | **40%** |
| **Chest** | **6** | **20%** |
| **Knees** | **4** | **13.3%** |
| **Abdomen** | **5** | **16.7%** |
| **Elbow** | **8** | **26.7%** |
| **Leg** | **15** | **50%** |
| **Arm** | **6** | **20%** |

Regarding sites of lesions distribution; as illustrated in **table (4)**; only 2 patients (6.7%) had Vitiligo all over their bodies, 15 cases (50%) had vitiligo in their faces (around mouth and eyes), 12 patients (40%) had vitiligo in the hands, 13 patients (43.3%) had vitiligo in the feet, 12 patients (40%) had vitiligo in their back, 6 patients (20%) in their chest, 4 patients (13.3%) in knees, 5 patients (16.7%) had vitiligo in abdomen, 8 cases (26.7%) in elbow, 15 cases (50%) in legs and 6 patient (20%) in arms **( figure 3)**.

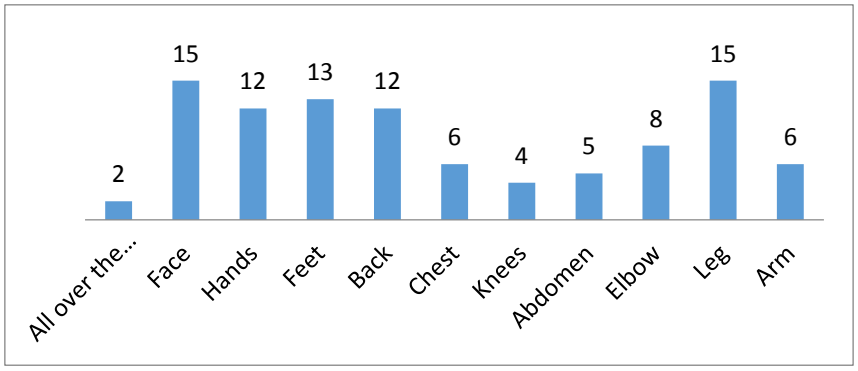


**Figure (3): Distribution of Vitiligo Lesions in the studied Cases**

**Table (5): Working Status among Studied Vitiligo Patients; (N=30):**

| | N | % |
|---|---|---|
| **Working** | 8 | 26.7 |
| **Not Working** | 22 | 73.3 |

Among studied vitiligo patients; 8 patents (26.7%) were working while 22 patients (73.3%) were not working.

**Table (6): Skin Type among Studied Vitiligo Patients; (N=30):**

| | N | % |
|---|---|---|
| **Type (III)** | 8 | 26.7 |
| **Type (IV)** | 19 | 63.3 |
| **Type (V)** | 3 | 10.0 |

Skin type in the studied Vitiligo patients was distributed as; 8 patents (26.7%) with skin type (III), 19 patients (63.3%) with skin thpe (IV) and only 3 patients (10.0%) with skin type (V)**.**

**Table (7): Family History among Studied Vitiligo Patients; (N=30):**

| | N | % |
|---|---|---|
| **Positive** | 6 | 20 |
| **Negative** | 24 | 80 |

The majority of the studied Vitiligo patients reported negative family history of Vitiligo disease, and only 6 patients (20%) had positive family history of Vitiligo.

**Table (8): Distribution of Skin Biopsy Site among Studied Vitiligo Patients; (N=30):**

| | N | % |
|---|---|---|
| **Back** | 16 | 53.3 |
| **Upper Limb** | 4 | 13.3 |
| **Lower Limb** | 10 | 33.3 |

Skin biopsies had been taken from back in 16 cases (53.3%), from upper limb in 4 cases (13.3%) and from lower limb in 10 cases (33.3%) **(figure 4).**

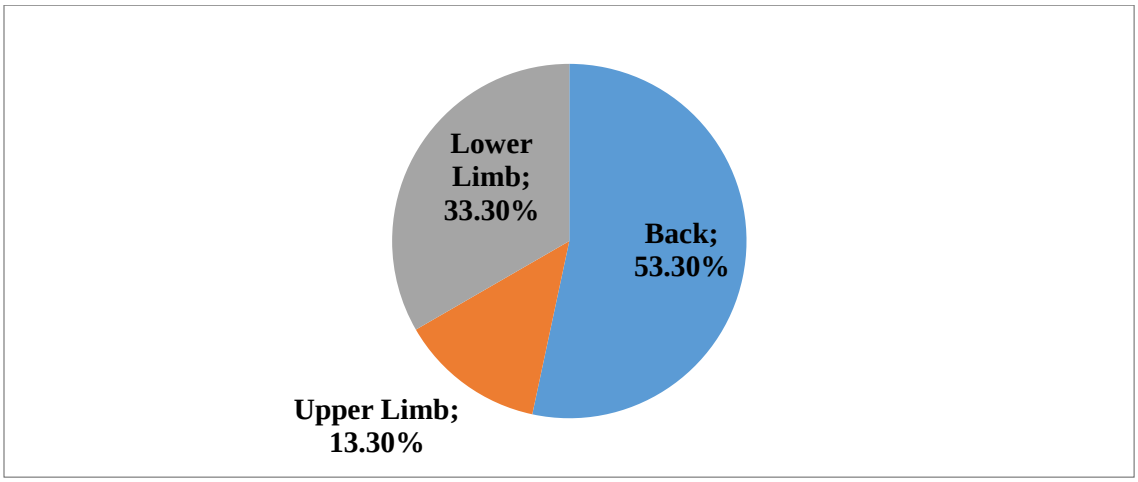


**Figure (4): Distribution of Skin Biopsy Site among Studied Vitiligo Patients.**

**Table (9): AGEs in Lesional Skin of Vitiligo Patients as Compared to Normal Control Persons; (N= 60):**

| | Cases N =30 | Controls N= 30 | *p-value* |
|---|---|---|---|
| **Mean ±SD** | 39.46 ±14.34 | 11.87 ±4.3 | **< 0.001*** |
| **Range** | 54.20 | 15.81 | |
| **Minimum** | 18.60 | 6.20 | |
| **Maximum** | 72.80 | 22.01 | |

**** P-value ≤ 0.05 is considered significant by (independent sample t-test).***

**Table (9)** demonstrates that the expression of Advanced Glycation end-Products (AGEs) in lesional skin of vitiligo patients was significantly higher as compared to normal control persons (p-value < 0.001); the mean values were (39.46 vs. 11.87) in cases and controls respectively **( figure 5)**.

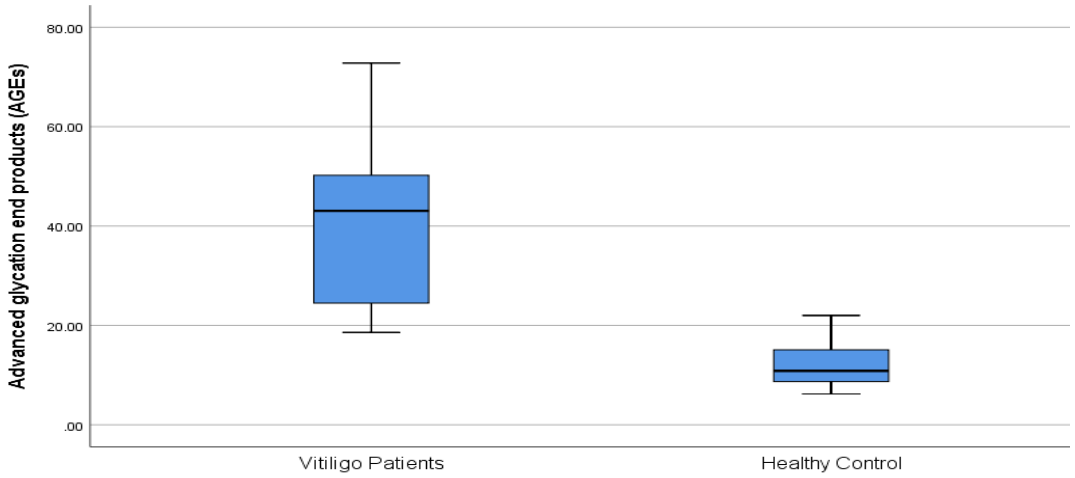


**Figure (5): AGEs in Lesional Skin of Vitiligo Patients as Compared to Normal Control Persons.**

**Table (10): Correlation between The Expression of AGEs in Lesional Skin of Vitiligo Patients and Age of the studied cases; (N=30):**

| | **Age of the studied cases** | |
|---|---|---|
| **Advanced Glycation end-Products (AGEs) Expression** | *r* = -0.102 | *p-value* = 0.591 |

***r Pearson correlation***

**Table (10)** demonstrates no detected correlation between the expression of AGEs in lesional skin of vitiligo patients and age of the studied cases; where (p-value> 0.050).

**Table (11): Relation between The Expression of AGEs in Lesional Skin of Vitiligo Patients and sex distribution of the studied cases; (N=30):**

| | **Females** | **Males** | ***P-value*** |
|---|---|---|---|

| | N= 22 | N= 8 | |
|---|---|---|---|
| **Mean ±SD** | 39.16 ±13.2 | 40.06 ±17.2 | 0.875 |
| **Minimum** | 18.60 | 22.80 | |
| **Maximum** | 56.90 | 72.80 | |
| **Range** | 38.30 | 50.00 | |

** P-value ≤ **0.05** is considered significant by (Mann–Whitney U test).*

**Table (11)** showed that there were no detected relation between sex and expression of AGEs in lesional skin of vitiligo patients; p-value >0.05.

**Table (12): Relation between The Expression of AGEs in Lesional Skin of Vitiligo Patients and Vitiligo Generalization among the studied cases; (N=30):**

| | Localized N= 6 | Generalized N= 24 | *P-value* |
|---|---|---|---|
| **Mean ±SD** | 38.02 ±19.7 | 39.82 ±13.2 | 0.788 |
| **Minimum** | 23.90 | 18.60 | |
| **Maximum** | 72.80 | 58.10 | |
| **Range** | 48.90 | 39.50 | |

**** P-value ≤ 0.05 is considered significant by (Mann–Whitney U test).***

**Table (12)** showed that There were no detected relation between Vitiligo Generalization and expression of AGEs in lesional skin of vitiligo patients; p-value >0.05.

**Table (13): Relation between The Expression of AGEs in Lesional Skin of Vitiligo Patients and Vitiligo Symmetry among the studied cases; (N=30):**

| | Symmetrical N= 6 | Asymmetrical N= 24 | *P-value* |
|---|---|---|---|
| **Mean ±SD** | 41.27 ±14.5 | 37.38 ±14.4 | 0.468 |
| **Minimum** | 22.80 | 18.60 | |

| | | | |
|---|---|---|---|
| **Maximum** | 72.80 | 58.10 | |
| **Range** | 50.00 | 39.50 | |

** P-value ≤ **0.05** is considered significant by (Mann–Whitney U test).*

**Table (13)** showed that There were no detected relation between Lesional Vitiligo symmetry and expression of AGEs in lesional skin of vitiligo patients; p-value >0.05.

**Table (14): Relation between The Expression of AGEs in Lesional Skin of Vitiligo Patients and Vitiligo Type among the studied cases; (N=30):**

| | **Mean ±SD** | *p-value* |
|---|---|---|
| **Vulgaris/Acrofacial (mixed); n=8** | 38.05 ±15.1 | 0.564 |
| **Vulgaris; n=15** | 39.05 ±13.4 | |
| **Acral; n=1** | 42.90 | |
| **Segmental; n=2** | 51.30 ±30.40 | |
| **Uneversalis; n=2** | 49.60 ±1.83 | |
| **Focal; n=2** | 25.00 ±1.6 | |

**** P-value ≤ 0.05 is considered significant by (Independent Sample Kruskal Wallis test).***

**Table (14)** showed that There were no detected relation between Vitiligo type and expression of AGEs in lesional skin of vitiligo patients; p-value >0.05.(fig 6).

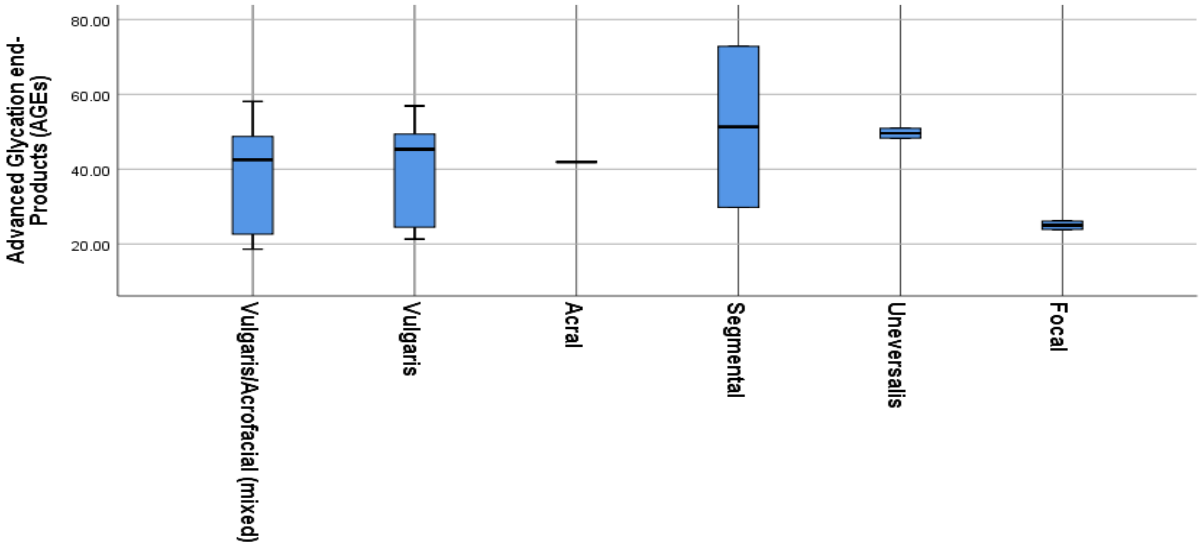


**Fig (6): relation between Vitiligo type and expression of AGEs in lesional skin of vitiligo patients.**

**Table (15): Relation between The Expression of (AGEs) in Lesional Skin of Vitiligo Patients and Skin Type among the studied cases; (N=30):**

| | **Mean ±SD** | *p-value* |
|---|---|---|
| **Type (III); n=8** | 37.08 ±12.6 | 0.349 |
| **Type (IV); n=19** | 38.72 ±15.5 | |
| **Type (V); n=3** | 50.43 ±7.9 | |

**** P-value ≤ 0.05 is considered significant by (Independent Sample Kruskal Wallis test).***

As showed in **table (15)** There were no detected relation between skin type and expression of (AGEs) in lesional skin of vitiligo patients; p-value >0.05.

**Table (16): Relation between The Expression of (AGEs) in Lesional Skin of Vitiligo Patients and Skin Biopsy Site; (N=30):**

| | **Mean ±SD** | *p-value* |
|---|---|---|

| | | |
|---|---|---|
| **Back; n=16** | 37.40 ±13.1 | 0.414 |
| **Upper Limb; n=4** | 37.82 ±14.8 | |
| **Lower Limb; n=10** | 43.41 ±16.7 | |

**** P-value ≤ 0.05 is considered significant by (Independent Sample Kruskal Wallis test).***

As showed in **table (16)** There were no detected relation between skin biopsy site and expression of (AGEs) in lesional skin of vitiligo patients; p-value >0.05.

**Table (17): Correlation between The Expression of (AGEs) in Lesional Skin of Vitiligo Patients and Vitiligo disease activity (VIDA) score of the studied cases; (N=30):**

| | **Vitiligo disease activity (VIDA) score** | |
|---|---|---|
| **Advanced Glycation end-Products (AGEs) Expression** | *r* = - 0.348 | *p-value* = 0.049 |

***r Pearson correlation***

**Table (17)** demonstrates moderate negative correlation between the expression of (AGEs) in lesional skin of vitiligo patients and Vitiligo disease activity (VIDA) score of the studied cases; (r= -0.348, p-value= 0.049) **(figure 7 )**.

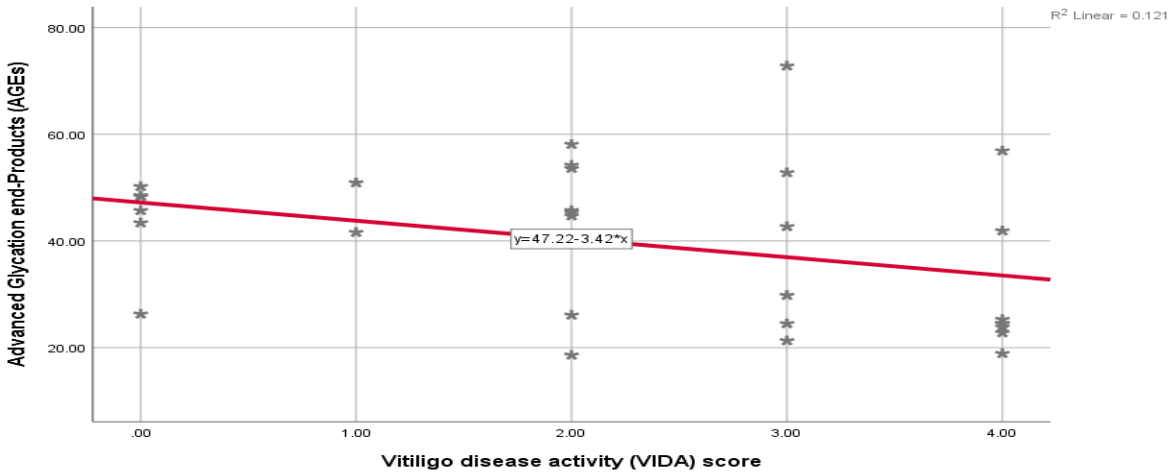


**Figure (7 ): Correlation between The Expression of (AGEs) in Lesional Skin of Vitiligo Patients**

**and Vitiligo disease activity (VIDA) score.**

**Table (18): Correlation between The Expression of (AGEs) in Lesional Skin of Vitiligo Patients and Duration of Last Vitiligo Lesion among the studied cases; (N=30):**

| | Duration of Last Vitiligo Lesion | |
|---|---|---|
| **Advanced Glycation end-Products (AGEs) Expression** | *r* = 0.268 | *p-value* = 0.152 |

***r Pearson correlation***

**Table (18)** demonstrates no detected correlation between the expression of (AGEs) in lesional skin of vitiligo patients and Duration of Last Vitiligo Lesion among the studied cases; (p-value> 0.050).

**Table (19): Correlation between The Expression of (AGEs) in Lesional Skin of Vitiligo Patients and Vitiligo Percentage among the studied cases; (N=30):**

| | Vitiligo Percentage | |
|---|---|---|
| **Advanced Glycation end-Products (AGEs) Expression** | *r* = 0.099 | *p-value* = 0.603 |

***r Pearson correlation***

**Table (19)** demonstrates no detected correlation between the expression of (AGEs) in lesional skin of vitiligo patients and Vitiligo Percentage among the studied cases; (p-value> 0.050).

## 4. Discussion:

Vitiligo is an acquired pigmentary mucocutaneous disease defined by patchy loss of skin pigmentation caused by exclusive loss of melanocytes [11]. Etiological hypotheses of vitiligo include autoimmune, oxidative pressure, and inherited theories [12]. In vitiligo, melanocytes are mainly liable to oxidative stress due to the pro-oxidant state produced during melanin formation and to the inherited antioxidant defects [13].

Between these oxidative factors are AGEs that are acomplex and different group of altered proteins or lipids that nonenzymatically become glycated and oxidized after interaction with aldose sugars [6,14] .

Oxidative damage in vitiligo can be considered a critical step in disease advancement, as it has been suggested as a induction for continuous damaging of the melanocyte level, then leading to production of autoantigens and melanocyte alteration

[15], Additionally new reports propose that reactive oxygen species injury induces the liberation of IL-23 and IL-33 that, successively, induces a Th2 response and cause a proinflammatory loop [16].

The exact role of ROS in vitiligo is tangled, Increased level of ROS in vitiligo disease has been demonstrated, and it is due to exogenous and endogenous excitations, Approved by the evidence that patients with progressive disorder tend to have a decreased action of antioxidant enzymes [4], and functional insufficiency of the Nrf2-ARE/HO-1 pathway [17].

In this present study we aimed to detect AGEs in vitiligo skin of diseased patients as compared to normal control persons to investigate the possible role of AGEs in the pathogenesis of vitiligo using ELISA technique.

We performed this case-control study at dermatology outpatient clinic at Beni-Suef University hospital during the period from (1st MAY 2019 to 31 of Novembar 2019) and the study included 60 participants; divided into two matched groups as 30 cases with vitiligo and 30 normal controls.The average patient age was 33.53 ±10.6 (SD) years. Gender distribution of studied patient was showed a female preponderance with female to male ratio of (2:1); and this was in accordance with certain other studies in this regard which confirm female preponderance [18]. The higher frequency of Vitiligo in females is in consistence with certain other studies in this regard [19]. Among studied vitiligo patients only (26.7%) were working, Variable skin types (26.7% type III ,63.3% type IV ,10% Type V ).

The vitiligo clinical types were variable and distributed as acral, focal, segmental, mixed (valgaris,acrofacial), universalis and valgaris subtypes. 80% of the studied Vitiligo cases in the present study were with generalized skin lesion.

In our study 80% of cases reported negative family history. And no patients of our studied group reported association of previous treatment of Vitiligo. Majority of

the studied cases had bilateral skin lesions and only one case had unilateral skin lesion, And regarding the lesional symmetry; 16 cases (53.3%) had symmetrical lesions and 14 cases (46.7%) had asymmetrical skin lesions.

The analysis of AGEs in the punch skin biopsy from our studied population (patients with Vitiligo lesion & control skin) reveled that; expression of AGEs in lesional skin of Vitiligo patients was significantly higher as compared to normal control persons (p-value < 0.001). Punch skin biopsy sites include (back, upper limp ,lower limp).

Our findings were supported by the Mario et al., 2017 [20]. The research team investigated the AGEs and AOPPs involvement in vitligo disease. In their study; they performed a case–control study by spectrophotometry and spectrofluorimetry, in 47 patients with generalized non-segmental vitiligo and 47 sex and age matched controls. Remarkably increased levels of both AOPPs (p\0.0001) and AGEs (p\0.0001) were determined in vitiligo patients compared to controls. In vitiligo patients serum levels of AOPPs and AGEs were directly related to duration, extension, and disease activity.

They also found that there was a positive relation of AOPPs with duration, extension, and disease activity. (q = 0.48, 0.37 and 0.55, respectively; p\0.05 in all cases); another relation was determined between AGEs serum levels and the same clinical parameters (q = 0.45, 0.32 and 0.53, respectively; p\0.05 in all cases).

On the opposite, another earlier research found that there was no difference between levels of AOPP in 53 vitiligo patients compared to 20 healthy controls, but proved altered proportion between the levels of prooxidant and antioxidant component,and ferric-reducing antioxidant power (FRAP) [21]. Our study also reveled moderate negative correlation between the expression of AGEs in lesional skin of vitiligo patients and VIDA score of the studied cases; (r= -0.348, p-value= 0.049).

Our findings were supported by the by Mario et al.,2017 [20]. The research team found that AOPP levels were Remarkably increased in patients with progressive than with stable vitiligo (p\0.0001), Althouge this was not the state for AGEs (p = 0.94). AGE levels were Remarkably increased in older lesions of vitiligo (p\0.01) or also have Hashimoto thyroiditis (p\0.01); they were also increased in patients with concomitant psoriasis or diabetes, in spite of statistical

significance was not reached in these cases, probably because of the small sample size (n = 3 for diabetes and n = 2 for psoriasis). they revealed no significant correlation of age or gender with AGE or AOPP levels was determined.

These studies indicate That ROS, and especialy AGEs and AOPPs, could be one of the principal biomarkers to evaluate the onset and activity of vitiligo, due to the potential effect as direct stimulus of cell injury and also as autoimmunity triggers.

In our present study we had no detected correlation between the expression of AGEs in lesional skin of Vitiligo patients and age of the studied cases, no correlation with the duration of the last Vitiligo lesion. Also no correlation with gender of the studied cases, vitiligo generalization ,vitiligo symmetry, vitiligo type, skin type of patients,,vitiligo percentage, skin biopsy site.

**5. Conclusion and Recommendations:**

Our findings confirmed that expression of AGEs in lesional skin of Vitiligo patients was significantly higher as compared to normal control persons.Also reveled moderate negative correlation between the expression of Advanced Glycation end-Products (AGEs) in lesional skin of vitiligo patients and Vitiligo disease activity (VIDA) score of the studied cases and that confirms that AGE levels were, instead, significantly higher in vitiligo patients with older lesions.

Further investigations for detection of AGEs in vitiligo skin by other methods and detection of its exact relation to pathogenesis of vitiligo are needed in this concern as vitiligo treatment may be directed to reduce AGEs production or inhibition of its work.Further investigations are needed to detect other types of ROS in vitiligo skin and how effective the anti oxidant medication to treat vitiligo.

AGEs are widely investigated in non dermatological diseases so further investigation of these important molecules are needed in other inflammatory skin diseases. We recommend further studies on the role of AGEs in vitiligo disease but on large number of patients, and may be using other techniques.

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