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Expression of Stress-associated Endoplasmic Reticulum Protein 1 (SERP1) in Patients with Vitiligo

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Abstract

Vitiligo stands out as one of the most common pigmentary skin disorders. This condition is marked by the presence of localized depigmented macules or patches, which arise due to the loss of melanocytes. The expression of stress-associated endoplasmic reticulum protein 1 (SERP1), also known as ribosome-associated membrane protein 4 (RAMP4), increased in response to stress that led to the buildup of unfolded proteins during endoplasmic reticulum (ER) stress. The aim of this study is to determine the relationship between SERP1 and the causes of vitiligo through real-time qPCR analysis. SERP1 was detected in a cohort of 30 vitiligo patients and 30 healthy controls using real-time quantitative polymerase chain reaction (qPCR). The expression of SERP1 in tissue showed a significant increase in Vitiligo skin lesions when compared to healthy skin samples from controls. SERP1 plays a role in the development of vitiligo.

1. Introduction:

Depigmented patches and macules are hallmarks of vitiligo, an inherited pigmentary disorder that may manifest on skin and mucous membranes. A disorder known as vitiligo causes a progressive loss of skin pigmentation as a result of an immune response that targets the skin's melanocytes. Onset of vitiligo typically occurs between the ages of 20 and 0.5 percent of the world's population [1].

Vitiligo has a complex etiology and several possible causes, making it a polygenic disorder. The autoimmune hypothesis has largely replaced other competing theories as the accepted explanation for autoimmune diseases [2]. Other hypothesized mechanisms include oxidative stress (OS), viral infections, poor adhesion between melanocytes, melanocyte proliferation, and neurological processes.

International consensus is that there are three primary types of vitiligo: mixed vitiligo, segmental vitiligo (SV), and non-segmental vitiligo (NSV) [3]. Depigmented patches appear on both sides of the body and usually spread out over time is generalized vitiligo, the most common kind of the disorder. SV is characterized by the lack of pigmentation in specific areas, as well as a lack of distribution across the body's midline; affected areas include the face and trunk. Combinations of SV and NSV are rare, but may occur in rare cases as mixed vitiligo [4].

The exact cause of vitiligo is often unknown, however sunlight and exposure to phenolic chemicals are among potential initiating causes. Melanocytes may undergo OS due to the causes [5].

Perilesional melanocytes in vitiligo patients often have a dilated endoplasmic reticulum (ER), which is vulnerable to OS. In order to facilitate the production of chemical bonds necessary for secondary and tertiary protein structures, a regulated environment is required in the ER, which also serves as a stress sensor for the cell. Misfolded proteins accumulate when the ED's redox balance is upset, which in turn sets off the unfolded protein response (UPR) [6].

Prolonged activation of the UPR leads to apoptosis, but it reduces endoplasmic reticulum stress by temporarily stopping global protein synthesis, increasing the production of chaperones that help with protein folding, and boosting the destruction of misfolded proteins. While UPR activity is prolonged, melanocytes may adapt to it and avoid UPR-induced cell death [7].

When the ER is under stress, stress-associated endoplasmic reticulum protein 1 (SERP1), which is also known as ribosome-associated membrane protein 4 (RAMP4), may cause misfolded proteins to accumulate [8].

SERP1 interacts with the translocon subunits (Sec 61α and Sec 61β), which help in protein synthesis by acting as a conduit for the

translocation of polypeptides across membranes. As a result, SERP1 plays a role in regulating the production of proteins in membranes. Overexpression of SERP1 protected cells from ER stress by degrading newly synthesized integral membrane proteins and promoting protein glycosylation [9]. The aim of this study is to determine the relationship between SERP1 and the causes of vitiligo.

2. Patients and Methods:

Type of the study: Case control study.

Site of the study: Dermatology Outpatient Clinic at Beni-Suef University Hospital.

Date and period of the study: February 2020- August 2020.

Study population: Outpatients attending Beni-Suef University Hospital.

Sample size: 60: 30 with vitiligo and 30 normal as control.

Calculation was done in the following formula:

$n=Z^2 \times PQ/d2$

$n=Z^2 \times PQ/d^2=(1.96)^2 * (0.01) * (1-0.01) / (0.05)^2 \approx 16$

16 Vitiligo patients were required and were duplicated for any data loss.

Participants were allocated in the study according to the following criteria:

Inclusion criteria:

1- Age between 20 to 50 years.

2- Both males and females were included.

3- Age and sex matched controls.

Exclusion criteria:

1- Age below 20 and above 50 years.

2- Patients with other autoimmune diseases.

3- Patients with associated systemic or dermatological diseases.

4- Patients who are suffering from any infection.

Controls were chosen randomly from any other outpatient clinic.

Methods:

All participants were subjected to the following**:**

1- Detailed history taking.

2- Clinical assessment to determine type, extent and sites of vitiligo.

3- One skin biopsy was taken from each patient and control subject from hidden areas. All the skin biopsies were taken using four mm punch skin biopsy using sterile 4mm punch under local anesthesia was taken from patients (vitiligo lesion) & control and it was kept in lysis solution for the stability of the studied parameters.

Ethical considerations:

The participants in the research were required to provide a written informed consent after full description and explanation of the study. The approval of the Beni-Suef University Ethical

Committee was obtained under the number: **FMBSUREC/05012020/ Saad**

Detection of SERP1 in skin tissue by Real time qPCR

Skin biopsy tissue was processed for RNA extraction followed by Reverse Transcriptase (for cDNA synthesis) and quantitative real time PCR, Table (1)

A- RNA extraction from biobsy tissue: -Total RNA was extracted from tissue homogenate using SV Total RNA Isolation system **(Thermo Scientific, USA),** according to manufacturer instructions.

B- cDNA synthesis:

The total RNA (1µg) was used for cDNA conversion using high capacity cDNA reverse transcription kit **(#K4374966, Thermo Fisher Scientific, USA)** , according to manufacturer instructions.

C-Real-time qPCR using SYBR Green I:

Real-time qPCR amplification and analysis were performed using an Applied Biosystem with software version 3.1 **(StepOne™, USA)**, according to manufacturer instructions.

Table (1): Primers Sequence of studied genes

Gene	Primer sequence
SERP1	**For-5'- AAA TCT AGG GCG ACG CTT GAC AGA-3'** **Rev-5' AAG AGG AAG GAA ACG CAA CGC AAC -3'**
GAPH	**For-5'-TGGCATTGTGGAAGGGCTCA-3'** **Rev-5'TGGATGCAGGGATGATGTTCT-3'**

Statistical analysis

The most appropriate statistical techniques to summarize, present the results and tests of significant to be used e.g. statistical package for the Social Sciences program (SPSS program).

3. Results:

Table (2): 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**	7 (23.3)	6 (20.0)	13 (21.7)	0.500
	Female	23 (76.7)	24 (80.0)	47 (78.3)	

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

Table (2) 23.3% of them were males and 76.7% were females among cases, 20% were males and 80% were females among controls, with no statistical difference (p-value> 0.05).

Table (3): Age Distribution of the Vitiligo Cases and Healthy Controls; (N=60)

Age (years)	Mean	SD	Minimum	Maximum	p-value
Vitiligo Patients	32.67	9.9	20	52	0.471
Healthy Controls	32.50	7.4	20	48	

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

As in table (3); the average cases age was; 32.67 ±9.9 years, while average controls age was 32.50±7.4 (SD) years with no statistical difference (p-value> 0.05).

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

		Frequency	Percent
Family History	Negative	25	83.3
	Positive	5	16.7

In Table (4); only 5 cases (16.7%) had a positive family history of Vitiligo among cases.

Table (5): Disease Characteristic of studied Vitiligo Cases; (N=30)

		Frequency	Valid Percent
Vitiligo Type	Acral	7	23.3
	Acrofacial	3	10.0
	Focal	9	30.0
	Mixed	4	13.3
	Non segmental	1	3.3
	Segmental	1	3.3
	Vulgaris	5	16.7
Course	Progressive	30	100
	Stationary	0	0.00
History of previous treatment	Negative	30	100
	Positive	0	0.00
History of associated skin disorders	Negative	30	100
	Positive	0	0.00
History of associated systematic disorders	Negative	30	100
	Positive	0	0.00

Table (5) illustrates that; focal Vitiligo was the most predominant among the studied Vitiligo sample; (30%), followed by Acral type in (23.3%), vulgaris was present in (16.7%), mixed type was in (13.3%), acrofacial was in (10%). All the studied Vitiligo cases had a progressive course of the disease. None of the studied Vitiligo cases had history of previous Vitiligo treatment, associated skin disorders or systematic disorders.

Table (6): History of Disease Duration and last new lesion among studied Vitiligo patients; (N=30)

	Mean	SD	Minimum	Maximum
Disease Duration (years)	5.45	7.6	0.05	35.0
Last New Lesion (months)	5.08	5.2	0.25	24

Table (6) demonstrates the disease duration was ranged from (0.05) to (35) with a mean of 5.45 ±7.6 years of disease duration.

The history of last new lesions among studied Vitiligo cases was ranged from (0.25) to (24) with a mean of 5.08 ±5.2 months.

Table (7): Vitiligo disease activity (VIDA) and Vitiligo Area Scoring Index (VASI) among studied Vitiligo patients; (N=30)

	Mean	SD	Minimum	Maximum
VIDA	+2.37	+1.4	0	+4
VASI	240.00	±413.25	5.00	2000

VIDA score ranged from (0) to (+4) with a mean of 2.37±1.4. VASI ranged from (5) to (2000) points with an average index of (240.0 ±413.00) points.

Table (8): Comparison between controls and Vitiligo skin regarding Expression of SERP1

SERP-1	Mean	SD	Minimum	Maximum	p-value
Vitiligo Patients	5.29	1.4	2.70	8.03	**<0.001***
Healthy Controls	1.03	0.04	0.97	1.20	

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

As demonstrated in table (8); Tissue expression of SERP1 was significantly higher in Vitiligo skin lesions as compared with healthy skin biopsies taken from controls; the mean SERP-1 values (5.29 vs. 1.03) in Vitiligo skin lesions and healthy skin from cases and controls respectively with a statistically significant p-value< 0.001.

Table (8): Relation between SERP1 tissue expression and patients' gender in studied Vitiligo patients; (N=30)

	Gender	N	Mean	SD	Minimum	Maximum	p-value
SERP-1	**Male**	7	5.91	1.54	3.80	8.03	0.187
	Female	23	5.10	1.34	2.70	6.90	

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

As demonstrated in table (8); tissue expression of SERP-1 was slightly higher in male Vitiligo skin lesions as compared with females; however no statistically significant difference was detected in relation between gender and tissue expression of SERP-1 (p-value=0.187).

Table (9): Relation between SERP1 tissue expression and patients' Family History in studied Vitiligo patients; (N=30)

	Family History	N	Mean	SD	Minimum	Maximum	p-value
SERP-1	**Negative**	25	5.41	1.34	3.20	8.03	0.326
	Positive	5	4.72	1.76	2.70	6.80	

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

As demonstrated in table (9); tissue expression of SERP-1 was significantly higher in Vitiligo skin lesions in patients with positive as compared with negative family history; however no statistically significant difference was detected regarding SERP-1 tissue expression and family history; (p-value=0.326).

Table (10): Correlation between SERP1 tissue expression and Patients' Age in studied Vitiligo patients; (N=30)

	Age (years)	
SERP-1	r=0.0028	*p-value*=**0.882**

r Spearman's correlation coefficient analysis

Table (10) demonstrates no detected significant linear correlation between tissue expression of SERP-1 and patients' age in studied Vitiligo patients; (p-value >0.05).

Table (11): Correlation between SERP1 tissue expression and History of Last New Lesion in studied Vitiligo patients; (N=30)

	Last New Lesion (Months)	
SERP-1	r=-0.192	*p-value*=**0.310**

r Spearman's correlation coefficient analysis

Table (11) demonstrates no detected significant linear correlation between tissue expression of SERP-1 and last lesion duration in studied Vitiligo patients; (p-value >0.05).

Table (12): Correlation between SERP1 tissue expression and History of Disease Duration in studied Vitiligo patients; (N=30):

	History of Disease Duration	
SERP-1	r=-0.415	*p-value*=**0.022***

r Spearman's correlation coefficient analysis

Table (12) demonstrates a significant linear moderate negative correlation between tissue expression of SERP-1and history of disease Duration in studied Vitiligo patients; (p-value=0.002).

Table (13): Correlation between SERP1 tissue expression and Vitiligo Extent Tensity Index (VETI) among studied Vitiligo patients; (N=30)

	VETI	
SERP-1	r=-0.010	*p-value*=**0.956**

r Spearman's correlation coefficient analysis

Table (13) demonstrates no detected significant linear correlation between tissue expression of SERP-1 and **VETI** in studied Vitiligo patients; (p-value >0.05).

Table (14): Correlation between SERP1 tissue expression and VIDA among studied Vitiligo patients; (N=30):

	VIDA	
SERP-1	r=-0.010	*p-value*=**0.956**

r Spearman's correlation coefficient analysis

Table (14) demonstrates no detected significant linear correlation between tissue expression of SERP-1 and **VIDA** in studied Vitiligo patients; (p-value >0.05).

4. Discussion:

Sudden loss of skin pigmentation is known as vitiligo. The gradual loss of hair follicle melanocytes and other melanocytes in the interfollicular epidermis characterizes this disorder [9].

The ER is the next target of OS. The translocon, which aids in protein synthesis by aiding the translocation of polypeptides across membranes, is believed to be interacting with SERP1 via its subunits (Sec 61α and Sec 61β). As a result, SERP1 plays a role in regulating the production of proteins in membranes. Overexpression of SERP1 protected cells from endoplasmic reticulum stress by degrading newly synthesized integral membrane proteins and promoting protein glycosylation [10]. Examining the relationship between stress-associated endoplasmic reticulum protein 1 and the cause of vitiligo was the main goal of this study.

Thirty patients from the dermatology outpatient clinic at Beni-Suef University Hospital served as subjects in a case-control study.

There were 23.3% men and 76.7% females among the Vitiligo patients, and 20% men and 80% females among the controls. With a p-value greater than 0.05, there was no discernible difference in sex between the experimental and control groups. With a mean age of 32.67 ± 9.9 years, the cases were older than the controls, who had an average age of 32.50 ± 7.4 years. With a p-value greater than 0.05, there was no discernible age difference between groups.

Research by Li et al. [11] supported our results; they found no age or sex-related statistical differences between the control group and the patients. Cases had an average age of 24.7 ± 13.6 years and controls 26.4 ± 13.3 years (P = 0.667). Males made up 55.3% of the cases and 44.7% of the controls, whereas the corresponding figures for the cases and controls were 54.1% and 45.9%, respectively (P=0.655).

A total of 122 patients, or 61 each group, participated in the study by Al Houssien et al. [12]. There was no difference in the gender distribution between the cases and controls; 72% of the participants were female and 28% were male. The average age of the cases was 45 ± 19 years, whereas the average age of the

controls was 40 ± 17 years. Families with vitiligo account for about 30% of cases. When trying to diagnose vitiligo, a biopsy may be required to rule out other hypopigmented or depigmenting diseases. When looking at the afflicted skin under a microscope, it becomes clear that there are no melanocytes and no epidermal pigmentation at all. Vitiliginous lesions may include superficial lymphocytic infiltrates around the edges, which might be a sign of a cell-mediated process that targets melanocytes [13].

The majority of the Vitiligo patients studied in this study did not have a history of the disorder in their families. Out of 25 cases (83.3%), none had a known family history of Vitiligo, while only 5 cases (16.7%) had a positive family history.

Our results were in line with those of Wang et al. [14], who found that 153 individuals (15.3%) had a vitiligo history in their family, whereas 847 (84.7%) did not. Thirty percent of the people in the study had focal vitiligo, whereas twenty-three percent had acrofacial, seventeen percent had vulgaris, thirteen percent had mixed, and ten percent had no visible spots at all. The analyzed cases of Vitiligo all followed a downward spiral.

In the current study; the average duration of the disease was 5.45 ± 7.6 years, however it ranged from 0.05 to 35 years. The average duration of vitiligo cases was 5.08 ± 5.2 months, ranging from 0.25 to 24 months. Among the cases studied by Chen et al., [15] , 803 (or 80.3% of the total) had active vitiligo, whereas 197 (or 19.7% of the total) had stable vitiligo. Having one or more first- to third-degree relatives afflicted by vitiligo was considered a family history of the illness in patients (n=152, 15.2%). 598 patients (or 59.8% of the total) were considered to have early-onset vitiligo if the onset age was less than 20 years. A total of 35 had autoimmune diseases.

Vaccaro et al. [16] found that vitiligo might last anywhere from 1 to 54 years, on average 10.45 ± 10.1 years, and would impact an average of 57 ± 25.2% of the afflicted body surface area (BSA), with a range of 35% to 90%. According to Xu et al. [17], 58 percent of patients started experiencing symptoms before the age of 20. In this group, eighty percent were actively sick. The majority of individuals (92%) had vitiligo that was not segmental.

Of the individuals studied by Ebrahim et al. [18], 47.5% had vitiligo vulgaris, 40% had acrofacial vitiligo, and only 12.5% had localized vitiligo. The range for the afflicted BSA was 22% to 26%, with an average of 11.73±6.57%. According to the VASI, 22.5% of patients showed no change and 77.5% showed a clear upward trend.

Vitiligo tends to proceed in a random fashion. While the illness usually worsens or settles down gradually, it might happen suddenly. A fresh depigmented macule may appear, an

existing lesion may centrifugally enlarge, or both may occur simultaneously to cause vitiligo to spread [16].
Our results are at odds with those of Karadag et al. [19] and Dash et al. [20], who both suggested that vitiligo vulgaris is the leading kind.

The average VIDA score in our study was 2.37 ± 1.4, and it ranged from 0 to +4. The mean score of the VASI was 240.0 ± 413.00 points, and it ranged from 5 to 2000 points. Half of the patients in the study by Sorour et al. [21] required an increase in the duration of the condition. It is possible that the planned VASI score was 4.7±13.7 and the intended VIDA score was 1.15±1.9.
We found that Vitiligo skin lesions had significantly higher tissue expression of SERP1 compared to healthy skin biopsies from controls. The mean SERP-1 values were 5.29 in Vitiligo skin lesions and 1.03 in healthy skin from cases and controls, respectively, with a p-value of less than 0.001. Although SERP-1 tissue expression was somewhat higher in male lesions than in female ones, there was no statistically significant difference. Although there was no statistically significant difference in SERP-1 tissue expression relative to family history, there was a marked increase in tissue expression of SERP-1 in Vitiligo lesions among individuals with a positive family history compared to those with a negative one. There was no statistically significant correlation ($p>0.05$) between the age of patients, the duration of the last lesion in patients and VETI with SERP-1 tissue expression. In the Vitiligo patients studied, there is a significant linear moderate negative correlation between SERP-1 tissue expression and the most recent cession (p-value=0.002). Research has shown that SIRT1 protects cells against stress-related diseases by deacetylating proteins that regulate cellular processes such as metabolism, inflammation, replicative senescence, and stress response [22].

5. Conclusions:

The expression of SERP-1 in the lesional skin of vitiligo patients was markedly elevated compared to the controls. A substantial linear moderate negative connection was also revealed between SERP-1 tissue expression and illness length history in the investigated vitiligo individuals. Based on these data, we concluded that SERP-1 is a novel biomarker implicated in the etiology of vitiligo. However, more investigations into the diagnostic and prognostic significance, as well as the use of SERP-1 as a clinical biomarker in the therapy of vitiligo, are warranted.

6. References:

1- Abdelmoneim T A, Aljarbou A N, Alfarouk K O (2018): The Prevalence of Vitiligo Variants in Association with Helicobacter pylori Infection. American Journal of Dermatology and Venereology; 7(1), 1-5.

2- Iannella G, Greco A, Didona D (2016): Vitiligo: pathogenesis, clinical variants and

treatment approaches. Autoimmunity Reviews;15(4), 335-343.

3- Picardo M, Dell'Anna ML, Ezzedine K (2015): Vitiligo. Nat Rev Dis Primers;1,150-200.

4- Rahman R and Hasija Y (2018): Exploring vitiligo susceptibility and management: a brief review. Biomedical Dermatology;2(1), 20-28.

5- Picardo M and Bastonini E (2015): A new view of vitiligo: looking at normal-appearing skin. Journal of Investigative Dermatology; 135(7), 1713-1714.

6- Almanza, A., Carlesso, A., Chintha, C., Creedican, S., Doultsinos, D., Leuzzi, B., & Papaioannou, A. (2019). Endoplasmic reticulum stress signalling–from basic mechanisms to clinical applications. The FEBS journal, 286(2), 241-278.

7- Xu W and Wang X (2019): Detection of melanocyte lineage-specific genes in vitiligo lesions. Experimental and therapeutic medicine; 17(6), 4485-4491.

8- Ma, Q., Wu, X., Wu, J., Liang, Z., & Liu, T. (2017). SERP1 is a novel marker of poor prognosis in pancreatic ductal adenocarcinoma patients via anti-apoptosis and regulating SRPRB/NF-κB axis. International journal of oncology, 51(4), 1104-1114.

9- Tian J N, Yang C C, Chuang C K (2019): A Dengue Virus Type 2 (DENV-2) NS4B-Interacting Host Factor, SERP1, Reduces DENV-2 Production by Suppressing Viral RNA Replication. Viruses; 11(9), 787-785.

10- Ezzedine, K., Eleftheriadou, V., Whitton, M. & Geel, N. V. (2015).Vitiligo. Lancet 386, 74–84 .

11- Li, K., Shi, Q., Yang, L., Li, X., Liu, L., Wang, L., & Gao, T. W. (2012). The association of vitamin D receptor gene polymorphisms and serum 25- hydroxyvitamin D levels with generalized vitiligo. British Journal of Dermatology, 167(4), 815-821.

12- Al Houssien, A. O., Al Houssien, R. O., Al Ajroush, W., & Al Kahtani, H. S. (2017). Chronic diseases among vitiligo patients: A case control study. Saudi medical journal, 38(4), 400-465.

13- Frisoli MI, Harris JE. (2017). Vitiligo: mechanistic insights lead to novel treatments. J Allergy Clin Immunol; 140: 654-662.

14- Wang, M., Law, M. E., Castellano, R. K., & Law, B. K. (2018).The unfolded protein response as a target for anticancer therapeutics. Critical reviews in oncology/hematology, 127, 66-79.

15- Chen, J. X., Shi, Q., Wang, X. W., Guo, S., Dai, W., Li, K., & Gao, T. W. (2014). Genetic polymorphisms in the methylenetetrahydrofolate reductase gene (MTHFR) and risk of vitiligo in Han Chinese populations: a genotype–phenotype correlation study. British Journal of Dermatology, 170(5), 1092-1099.

16- Vaccaro, M., Cicero, F., Mannucci, C., Calapai, G., Spatari, G., Barbuzza, O., & Gangemi, S. (2016). IL-33 circulating serum

levels are increased in patients with non-segmental generalized vitiligo. Archives of dermatological research, 308(7), 527-530.

17- Xu, M., Liu, Y., Liu, Y., Li, X., Chen, G., Dong, W., & Xiao, S. (2018). Genetic polymorphisms of GZMB and vitiligo: a genetic association study based on Chinese Han population. Scientific reports, 8(1), 1-5.

18- Ebrahim, A. A., Hamed, A. M., Ahmed, E. S., & Khudhair, A. S. (2018).Evaluation of serum interleukin-33 in nonsegmental vitiligo. Benha Medical Journal, 35(1), 54-70.

19- Karadag AS, Tutal E, Ertugrul DT, Akin KO, Bilgil SG. 2012. Serum holotranscobalamine, vitamin B12, folic acid and homocysteine levels in patients with vitiligo. Clin Exp Dermatol; 37: 62–64.

20- Dash R, Mohapatra A, Manjunathswamy BS. (2015). Anti-thyroid peroxidase antibody in vitiligo: a prevalence study. J Thyroid Res;145-146.

21- Sorour, N. E., Alkady, O. H., El Fallah, A. A., Mansour, A. I., & Abdelwahab, W. W. (2020). Evaluation of S100B Serum levels in non-segmental vitiligo patients. Benha Journal of Applied Sciences, 5(1 part (2)), 1-7.

22- Saunders LR, Verdin E.(2007). Sirtuins: critical regulators at the crossroads between cancer and aging. Oncogene 26: 5489-504.