

## Osteopontin Level in Vitiligo Patients

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### Abstract

**Background:** Vitiligo is an acquired pigmentary disorder of unknown etiology that affects up to 1% of the general population of all skin types . It is a multifactorial disorder including genetic theory, auto-immune theory, neurogenic factors, intrinsic defect of melanocytes (self destructive theory), oxidative stress theory, reduced melanocyte survival theory, transepidermal melanocytorrhagy theory and convergence theory is also suggested. There are numerous medical and surgical treatments aiming repigmentation . Osteopontin( OPN ) is a multifunctional glycoposphoprotein secreted by many cell types, including osteoblasts, lymphocytes, macrophages, epithelial and vascular smooth muscle cells. It has been implicated in many physiological and pathological processes, such as cell-mediated immunity, inflammation, cell survival, tumor invasion and metastasis .OPN has multiple emerging roles in Th1-mediated diseases such as alopecia areata and vitiligo characterized by a prevalent of Th1 cytokine profile.

**Objective:** to evaluate serum level of Osteopontin in vitiligo patients and to correlate it with disease severity using ELISA technique. **Patients and method :** This study included 15 patients suffering from vitiligo (Group A). In addition to, 15 apparently healthy individuals of matched age and sex serving as a control group (Group B). **Results:** Serum level of OPN was found to be higher in vitiligo patients than in healthy control group and serum OPN level showed significant positive correlation with duration **Conclusion:** the present study has showed higher serum OPN in vitiligo patients in comparison to controls, as well as correlation of serum OPN with disease severity .

**Keywords:** vitiligo , osteopontin ,ELISA , depigmentation , marker

## **Introduction**

Vitiligo is an acquired chronic depigmentary disorder affecting the melanocytes, mainly in the skin and mucosa [1]. Vitiligo is characterized clinically by the presence of well defined hypoamelanotic cutaneous and mucous membrane lesions with microscopic absence of melanocyte [2].

Vitiligo is a multifactorial disorder characterized by the loss of functional melanocytes. Multiple mechanisms have been proposed for melanocyte destruction in vitiligo. These include genetic, autoimmune responses, oxidative stress, generation of inflammatory mediators and melanocyte detachment mechanisms [3]. It occurs equally in people of all skin colors and races, both males and females are equally affected. The psychological impacts of vitiligo are completely evident, especially in female patients [4]. It is classified into two major forms: nonsegmental vitiligo and segmental vitiligo. Nonsegmental vitiligo include acrofacial, mucosal, generalized, universal, mixed and rare variant [3]. Vitiligo has unpredictable course and some patients may notice spontaneous repigmentation over the depigmented areas [5]. The Wood's lamp can be used to confirm diagnosis when vitiligo is suspected. Furthermore, it allows to

distinguish vitiligo from an anaemic naevus, pityriasis versicolor and pityriasis alba. In vitiligo, because of loss of epidermal melanin, depigmented patches appear bright bluish white with sharp demarcations in the Wood's light [6].

Osteopontin (OPN) is an acidic extracellular matrix glycosylated phosphoprotein produced by immune cells, osteoblasts, hepatocytes epithelial and endothelial cells. It has been identified as an important molecule involved in tissue repair, inflammation and autoimmunity, as well as tumour growth. It was suggested that OPN plays a role in many diseases characterized by chronic inflammation including psoriasis and autoimmune diseases [7].

Osteopontin is expressed during inflammation by natural killer cells, activated T- cells and macrophages. It was originally classified as a T helper type -1 cytokine. OPN induces the expression of other proinflammatory cytokines and chemokines in peripheral blood mononuclear cells and was shown to be involved in monocyte/macrophage as well as dendritic cell migration and activation [8].

The role of OPN is enhancing differentiation of Th1 and Th17 cells and induces the production of (IL-)17. There is evidence for a key role of OPN in Th1-

mediated and Th17-mediated diseases such as psoriasis, vitiligo [9]. T-helper 17 cells have a precise role in the disappearance of melanocytes in vitiligo. Melanocyte apoptosis is considered to be one of the mechanisms of depigmentation in vitiligo, and interleukin-17 has been demonstrated to inhibit melanogenesis and accelerate melanocyte apoptosis [10].

The aim of the present study was to evaluate serum level of osteopontin in vitiligo patients by using ELISA and to correlate it with disease activity.

## **Patients and methods:**

### **Patients**

This comparative cross-sectional case-control study included 15 patients suffering from vitiligo and 15 healthy volunteers presented to Benha university hospital out-patient clinic. This study was conducted during the period from January 2021 to April 2021.

After signing an informed consent that was approved by the Research Ethics Committee of Benha Faculty of Medicine (MS - 5-9-2020).

Any patient below the age of 18 years, pregnant and lactating women was excluded from the study. Any patient suffering from any systemic disease or receiving any systemic medication, was also excluded from the study.

Included patients did not receive any systemic or topical treatment for vitiligo at least 4 weeks prior to the study. We excluded female patients and controls in their late menstrual phase at the time of venous sample or taking oral contraceptive pill in the previous 6 weeks prior to the study.

### **Methods**

All individuals were subjected to the following

#### **History taking**

Personal history included name, age, sex, address, occupation and special habits. Present history was assessed as regards to the onset, duration and course of vitiligo.

- Patients were asked about family history of vitiligo, other dermatologic diseases, systemic diseases and psychiatric diseases. Patients were asked about history of drug intake (corticosteroid, non-steroidal antiinflammatory drugs and antioxidant supplements).

#### **Clinical assessment of the dermatological disease**

Complete general examination was done including body mass index. Skin, mucous membrane, and hair examination were done to determine the extent, severity and distribution of the disease using vitiligo area severity index [11].

To evaluate serum level of Osteopontin, venous blood samples (2

ml) were collected , allowed to clot for 10-20 minutes at room temperature before centrifugation for 20 minutes at approximately 2000-3000 r.p.m. and stored at -20°C to be preserved until the time of the run of the assay. Repeated freeze/thaw cycles were avoided.

Serum level of Osteopontin was measured by ELISA kit following manufacturer manual.

Assay range for Osteopontin: 5ng/ml - 600ng /ml, sensitivity % 2.045 ng / ml.

### **Statistical analysis**

Statistical presentation and analysis of the present study was conducted, using Statistical package for Social Science (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). Descriptive data were presented as percentage (%), mean and standard deviation ( $M \pm SD$ ). For analytical data, we used Mann-Whitney test, Student T test, Fisher's exact test, Chi-square test, Spearman correlation analysis and receiver operating characteristic (ROC curve).

**The corresponding P value for each test was directly computed by the microprocessor, in which we used the one call test values:**

- Non – significant difference when  $P > 0.05$ .
- Significant difference when  $P \leq 0.05$ .

### **3.Results**

The present study included 15 vitiligo patients, and 15 healthy control subjects. The median age of vitiligo group was 41.7 years, they were 6 males (40%) and 9 females (60%). In addition to 15 healthy control group, of matched age and gender ( $p > 0.05$  for each) .Vitiligo cases showed significantly higher BMI, proportion of positive family history and relation to stress ( $p = 0.015, 0.015, 0.002$  respectively) . Median disease duration was 1.5 years, ranged from 6 months to 8 years; all cases had gradual onset, 43.3% had stationary, 56.7% had progressive course. The most frequently affected site was UL (73.3%) followed by LL (50%), head and neck (40%). Trunk was affected in 16.7%. Generalized type was found in 36.7%, acral in 30%, focal in 26.7% and acrofacial in 6.7%. Median VASI score was 7.3, ranged from 1.5 to 72. Serum osteopontin level was assayed in cases, and controls; its median level was 367.1 ng/ml, ranged from 68.9-403.8 ng/ml in cases; while its median was 66.1 ng/ml , ranged from 45.5-346.2 ng/ml.

Vitiligo group showed significantly higher level of serum osteopontin levels when compared to control group ( $p < 0.001, = 0.006$  ). For discriminating between vitiligo patients and control groups, a receiver operating characteristic (ROC)

curve of serum osteopontin level was conducted.

Serum osteopontin showed high accuracy AUC (AUC=0.936). At best cut off level

of 103.2, sensitivity was 93.3%, specificity was 80%, PPV was 82.3%, NPV was 92.3% and accuracy was 86.7% (**Table 1**)

**Table (1).** Validity of serum osteopontine level for discrimination between vitiligo cases and control groups.

	<b>Serum osteopontine</b>
<b>AUC</b>	0.936
<b>Cut off</b>	103.2
<b>Sensitivity (%)</b>	93.3
<b>Specificity (%)</b>	80
<b>PPV (%)</b>	82.3
<b>NPV (%)</b>	92.3
<b>Accuracy (%)</b>	86.7

AUC, area under ROC, receiver operating characteristic curve; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

No significant associations were found regarding serum osteopontin level according to gender, family history (FH), stress, course in vitiligo group ( $p>0.05$  for each). Generalized type was associated with highest serum osteopontin level, followed by acral, focal and the least level was found in acrofacial type. Comparing each type to

other types, generalized type was significantly associated with higher serum osteopontin level when compared to other types ( $p<0.001$ ). While focal type was significantly associated with lower serum osteopontin level when compared to other types ( $p=0.041$ ) (**Table2**).

**Table (2).** Comparison of serum osteopontine level according to other parameters in vitiligo group.

		Serum osteopontine level ng/ml			<i>p</i>	
		<b>N</b>	<b>median</b>	<b>minimum</b>		<b>maximum</b>
Gender	<b>Males</b>	12	348.9	68.9	403.8	0.227
	<b>Females</b>	18	371.8	68.9	393.4	
Family history	<b>Negative</b>	19	364.9	68.9	403.8	0.698
	<b>Positive</b>	11	369.3	68.9	387.8	
Stress	<b>Absent</b>	11	364.9	116.4	403.8	0.931
	<b>Present</b>	19	369.3	68.9	393.4	
Course	<b>Stationary</b>	13	369.3	68.9	387.8	0.426
	<b>Progressive</b>	17	351.6	68.9	403.8	
Type	<b>Generalized</b>	11	383.5	369.3	403.8	<0.001
	<b>Acral</b>	9	346.2	68.9	386.4	0.074
	<b>Focal</b>	8	331.5	116.4	371.8	0.041
	<b>Acrofacial</b>	2	220.5	68.9	372.1	0.429

Man Whitney test was used for comparison of numerical parameters.

Serum osteopontine level showed significant positive correlation with disease duration and VASI score

( $p=0.019, 0.040$  respectively); but not with age, or BMI ( $p>0.05$  for each) (Table3).

**Table (3).** Correlations of serum osteopontine level with age, BMI, duration, severity in vitiligo group.

	Serum osteopontine level	
	<i>rs</i>	<i>p</i>
Age / years	0.026	0.891
BMI /kg/m <sup>2</sup>	0.178	0.347
Duration / years	0.424	0.019
VASI	0.377	0.040

*rs*, Spearman’s correlation coefficient.

### Discussion

The present study is a case control study included 15 patients suffering from vitiligo in addition to 15 apparently healthy individuals of matched age and sex were serving as a control group. In the current work, the preponderance of vitiligo in women was observed, they were 9 females (60%) and 6 males (40%) . This is agreed with another study , 68.7% were females, while 31.3% were males [12]. In addition, previous study has showed that among 30 recruited vitiligo patients, 66.7% were women and 33.3% were men [13].The number of female vitiligo patients were found to be higher than male because women notice the change in appearance and approach the doctors sooner than men and of the social stigma in the community, young females tend to report earlier due to matrimonial anxiety [14]. The mean age of vitiligo cases in the present study was

41.7 years. A great variation of age was noticed among different studies, one study's results showed that the age of vitiligo patients ranged from 5 to 68 years with average age of 31.5 years [15]. And another study reported the age of vitiligo cases ranged from 15 to 50 years also their mean age was 31years[13].

The present study showed that the vitiligo cases were significantly associated with stress . This is in a correlation of other study which suggested environmental and psychological stressors to trigger the onset and progression of vitiligo[16]. And another study has showed that vitiligo patients have high levels of perceived stress. In patients predisposed to vitiligo, metabolic and psychological stress might influence the onset and progression of vitiligo [17]. And as

regard BMI, the present study has showed that vitiligo is strongly associated with high BMI . Also other study has demonstrated higher rates of obesity in vitiligo patients compared to controls [18]. The present study showed that the vitiligo cases were significantly associated with positive family history(36.7 %) . Similar associations were reported in previous study ( 31 %) [19] . The present study showed that generalized type of vitiligo was found in 36.7% of patients and focal type in 26.7%. While in other study, 40% had generalized form of the disease while 15% had focal disease [13].

In the present study, by evaluating serum level of OPN in Vitiligo group , there was significantly higher level of serum OPN level when compared to control group .Its median level was 367.1 ng/ml ranged from 68.9-403.8 in cases. to the best our knowledge the current study was first study to asses serum level of OPN in relation to vitiligo and disease severity .This result agreed with other studies about role of OPN in dermatological diseases mainly autoimmune disorders. Other studies showed that OPN plasma levels in patients with alopecia areata (85.25 ng/ml ) (  $p=0.008$  ) were significantly higher than in healthy controls and it could be hypothesized that OPN has a

role in the pathogenesis of AA, amplifying the inflammatory cascade [20]. As regards psoriasis, OPN level was significantly higher in psoriatic patients(31.65ng/ml) ( $p<0.001$ ) than controls[21]. Another study considered plasma OPN could be used as a biomarker for diagnosis of sarcoidosis, disease activity and response to treatment plasma OPN higher in sarcoidosis patients than control [22] .

Elevated serum concentrations of osteopontin was also detected in some diseases as acute disseminated allergic contact dermatitis [23], chronic urticarial [24]. Others reported that osteopontin level was higher in the systemic sclerosis than healthy control group[25] Also, increased levels of OPN have been reported in the serum and plasma of systemic lupus erthematosus patients and their use has been suggested in monitoring systemic lupus erthematosus severity and poor outcome [26].

In the current study serum osteopontin level showed significant positive correlation with disease duration ( $p=0.019$  ) . Other studies showed that significant positive correlation between serum OPN level in Alopecia areata (AA)and duration of disease [27] .

In the current study, there are no significant correlation found regarding serum OPN level with age. However, a

previous study showed that upon aging, the expression of OPN in the murine bone marrow is reduced, and suggested a critical role for reduced OPN for hematopoietic stem cells aging [28].

OPN levels in the current study, was considered as independent predictors of vitiligo severity.

There are no significant associations were found regarding serum OPN level in the present study as regarding family history, stress, course in vitiligo group. And that agreed with other study in alopecia areata, no significant differences were observed between the mean plasma level of osteopontin and age, gender, family history in alopecia areata [29].

This study has some limitations due to small sample size as well as the limitations of inclusion and exclusion criteria of the participants. It is recommended that multiple future studies will be undertaken for best detection of serum level of OPN and correlation of this level with severity of the disease.

### **Conclusion:**

The present study has showed higher serum OPN in vitiligo patients in comparison to controls, as well as correlation of serum OPN with disease severity.

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