# Influence of arginase activity with some oxidative stress parameters in Iraqi vitiligo patients.

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***Abstract***

*Arginase enzyme has drawn more attention recently because this enzyme enters in several pathways of intermediary metabolism. The purpose of this study was to measure the activity of Arginase, total antioxidant capacity, total oxidant capacity and peroxynitrite level in serum of vitiligo patients. The activity of Arginase was determined and total antioxidant capacity and total oxidant capacity were also determined while peroxynitrite levels were also determined. These methods were applied to vitiligo patients and healthy individuals who matched in age and gender with patients.*

*A highly significant increase in both serum arginase activity and its specific activity levels p=0.000 in patients group was observed in comparison with their levels in corresponding healthy group. Total antioxidant capacity in sera of patients group was highly significant lower p=0.000 when compared to the healthy group, while highly significant increase in total oxidant capacity and peroxynitrite levels p=0.000 in vitiligo patients group were observed in comparison with their levels in corresponding healthy control group. Vitiligo disease affects indirectly on the activity of arginase due to imbalance between antioxidant and oxidant system which lead to increased activity and expression of arginase.*

**Keywords:** Arginase, total oxidant capacity, total antioxidant capacity, peroxynitrite and vitiligo.

## Introduction

Vitiligo is an acquired chronic skin disease characterized by loss and disorder in melanocytes functions from the epidermis[27]. The distribution of this disease is from 0.5% to 1% of the general population[5]. The main etiology is still unclear, therefore some theories including autoinflammatory, autoimmune, neural, genetic, autocytotoxic, were proposed to explain vitiligo disase[5,27]. One of most important theory is autocytotoxic theory, which emanates from clinical observations and laboratory investigations of melanocytes, skin biopsies, serum, and circulating peripheral mononuclear cells (PMNC) of vitiligo.

The oxidative stress theory of vitiligo pathogenesis is largely based on the notion that the entire epidermis of vitiligo accumulates $H_2O_2$ that may reach to one millimole concentration[50-52]. At this concentration, $H_2O_2$ is considered to be the primary intracellular reactive oxygen species (ROS) that leads to the death of the epidermal functional melanocytes. Multiple biochemical alterations in the epidermis and melanocytes of vitiligo have been built in principle on this notion of increased epidermal $H_2O_2$ concentration[7,46,61]. Mitochondrial dysfunction seems to play a major role in vitiligo pathogenesis[17], and its increased production of $H_2O_2$ in vitiligo skin is believed to be the culprit of $H_2O_2$ -induced mitochondrial DNA damage[42] that eventually leads to melanocyte apoptosis/death[54].

The enzyme is crucially involved in various aspects of inflammation. Arginase has been shown to be either responsible for or to participate in, for example, inflammation-triggered immune dysfunction, tumour immune escape, fibrosis, immunosuppression and immunopathology of infectious diseases[8].

Arginase (L-arginine amidinohydrolase, EC 3.5.3.1) catalyzes the reaction in which L-arginine is converted to L-ornithine and urea[58]. Arginase enzyme is parel more attention because this enzyme enters in several pathways of intermediary metabolism. Arginase competes with inducible Nitric oxide synthase (iNOS; NOS2), the high output, the inducible pathway for increased production of NO, for L-Arginine, their common substrate in multiple cell types including endothelial cells. NO levels can fall, and this may be the result of enhanced Arginase activity[14]. In humans, two arginase isoenzymes have been identified, arginase 1 and arginase 2, that differ in cellular location and tissue distribution[23].

Arginase expression and activity can be regulated in many cell types including vascular endothelial cells, smooth muscle cells, and macrophages, by various cytokines [63]. The presence of at least two distinct genes for arginase in mammals has been established where arginase AI is strongly expressed in the liver and AII is expressed in extrahepatic tissues[18,21,36,56]. The hepatic arginase AI is a cytosolic enzyme that plays a fundamental role in the last step of the urea cycle and is cytokine-inducible in many cell types[9]. Arginase AI can limit substrate availability for high output NO synthesis in cells co-expressing iNOS[6, 34] and it is induced by hypoxia, lipopolysaccharide (LPS), and IL-13 in a variety of cells and tissues[31,53].

Extrahepatic arginase AII is a mitochondrial isoform, and its biological function has been the subject of considerable interest. It seems that this isoform provides a supply of ornithine, a crucial metabolite in the biosynthesis of

glutamic acid, proline and polyamines[1,45,55] and also is also inducible by hypoxia, LPS, TNF-, IFN-, and 8-Bromo-cGMP[10,47]. In inflammatory diseases, such as acute respiratory distress syndrome, NO production from L-Arginine via NOS is involved in the initial host response whereas L-ornithine production from L-Arginine via arginase is involved in healing [30]. Since polyamines are vital for cell proliferation, it is possible that the increased level of ornithine, due to the elevated arginase activity, may be linked to the development of carcinogenesis[42].

Our current study aimed to determine influence in the arginase activity levels prevalent among vitiligo patients and to discover the associations between the arginase activity and total antioxidant capacity, total oxidant capacity and peroxynitrite concentration in the Iraqi vitiligo patients

## Material and Methods

Two main groups were included in this study; 65 patients diagnosed with vitiligo without treatment (P group) and 40 apparently healthy controls (C group) comparable for age and gender without a history of vitiligo were involved in the current study. Each group was subdivided according to gender, where P group was divided to 33 male patients group (PM group) and 32 female patients group (PF group) while C group was divided to 20 male group (CM group) and 20 female patients group (CF group).

The samples were collected from patients who were attending to Baghdad teaching hospital in Medical city, Iraq. Venous fasting blood samples were collected from an antecubital vein (8.00-10.00 a.m.). The obtained sample was centrifuged at 3000xg for 5min and the collected serum was used freshly to measure arginase activity, protein profile, total antioxidant capacity, total oxidant capacity and peroxynitrite concentration.

In the current study patients with pregnancy, diabetic, anaemia, lactation, history of cutaneous photosensitivity, eye cataract or skin, cancer, dermatitis, psoriasis, taking a potent antioxidant, history of alcohol intake, smokers were excluded. This study protocol was approved by the Ethics Committee of the College of Science/ University of Baghdad.

**Serum arginase activity assay:** The serum arginase activity was measured according to Zofia et al method[41] which depends on the measurement of ornithine concentration as the end product of the enzymatic reaction. Levels of total protein and albumin in the serum of studied groups were measured by commercial kit from HUMAN Company. Globulin concentration in sera samples of the studied groups in this study was calculated from the following equation:

Globulin conc. (g/L) = Total protein conc. – Albumin conc.

**Serum total antioxidant capacity and total oxidant capacity:** Total oxidant status (TOS) and total antioxidant status (TAS) in the serum of studied group were measured according to methods developed by Erel[19,20].

**Serum peroxynitrite concentration:** Serum peroxynitrite concentration was determined in serum of vitiligo patients and healthy groups according to the Beckman et al cited by VanUffelen et al.[59]

## Results and Discussion

The mean ± SD ages of the two groups (P and C) was 31.52 ± 7.24 *and* 33.1 ± 8.62 year**s** respectively, there were non-significant differences ($p > 0.05$) in the mean values of total protein, albumin and globulin concentrations of all the two studied groups patients (P) and healthy control (C) as present in figure 1. A highly significant increase in both serum arginase activity and its specific activity levels p=0.000 in P group was observed in comparison with their levels in corresponding healthy C group as shown in figure 2.

Total antioxidant capacity in sera of P group was highly significantly lower p=0.000 when compared to the healthy C group (Figure 2) while highly significant increase in total oxidant capacity p=0.000 in vitiligo patients group (P group) was observed in comparison with their levels in corresponding healthy control group (C group) as shown in figure 2. The concentrations of peroxynitrite were measured in serum samples, the obtained results reflect the presence of highly significant increase in peroxynitrite concentration (p=0.000) when compared with patients group and the healthy group. These results are presented in figure 2.

Gender in present study showed that mean levels of serum arginase activity and arginase specific activity in PM showed the significant increase at p=0.011 and p=0.016 respectively and in PF group there was a significant increase at p=0.006 and p=0.007 respectively when compared with corresponding healthy group (CM and CF groups respectively). Meanwhile, the levels of total antioxidants capacity and total oxidant capacity in patients and healthy control groups were altered, the result showed a significant decrease in total antioxidants capacity in both PM and PF groups p=0.015 and p=0.028 respectively, while highly significant increase in total oxidants capacity in both MP and PF groups p=0.000 and p=0.000 respectively was observed when compared with corresponding healthy groups (CM and CF groups).

It is clear from table 1 when that peroxynitrite concentration between male and female of vitiligo patients with their corresponding healthy groups was observed when significant increase in peroxynitrite concentration is present in PM and PF groups (0.002 and p=0.009 respectively).

The results of comparison among serum of male with female of vitiligo patients show both arginase activity and arginase specific activity as non-significant increase p=0.786 and p=0.79 respectively and non-significant in peroxynitrite

concentration, total antioxidants capacity and total oxidant capacity p=0.99, p=1 and p=0.994 respectively.

The cuerrnt study was in agreement with several authors for the TAC and TOC systems in vitiligo. They found that patients with vitiligo had significantly lower TAC and higher TOC than controls group[2,4,33]. Other researchers illustrated that there was impairment in the antioxidant system of vitiligo which leads to low level of TAC in both serum and tissue.

Also patients with vitiligo have been shown to have various alterations in oxidant, and antioxidant parameters in both tissues and blood such as increase in $H_2O_2$ [2], increase in lipid peroxidation products such as malondialdehyde[16], low level of vitamins C and E[16], increased or decreased[3,16,28] activity of superoxide dismutase[25,39], low activity of catalase[3,16] and glutathione peroxidase activity showed conflicted result: it may be normal[40], increased[39] or decreased[16,36] activity. These alterations in results may be due to difference of samples (tissue and blood), type, and duration of this disease. Our skin also acts as a barrier between the environment and the body, it is represented as the first defense exposed to a different array of physical, chemical, and biological agents, many of which either are inborn oxidants or catalyze the generation of ROS[22].

In the last decades, many studies suggested that hypersensitivity to OS is a crucial role in determining melanocyte degeneration[2,16,24,29,32].

Vitiligo is disordered or impaired melanocyte cells, the cause of this disease is not understood yet, and several hypotheses have been proposed to explain its pathogenesis including autoimmune, neural, autocytoxic, self-destruction and inherent defect theories[5,27]. So a single mechanism is not responsible for all cases of melanocyte damage in vitiligo, and OS is considered a possible pathogenic event in melanocyte loss which believed that the formation of ROS in the melanocytes destroys melanocyte and leads to this disease[29].

TAC and TOC are essential for establishing the health status of the body[37], therefore decreased antioxidant levels and increased oxidant levels may play essential roles in the damage of melanocytes observed in vitiligo patients[49]. The above reason can explain the decreasing in TAC and increasing in TOC levels in sera of vitiligo patients indicated in the present study. that is because a shift in the equilibrium between antioxidant-oxidant levels which leads to impaired blance between these impotrant parametes. Meanwhile the global antioxidant capacity of patients with vitiligo might have been exhausted by a defense mechanism against oxidant processes.

Results presented here demonstrate that arginase activity and its specific activity in the sera of vitiligo patients are markedly increased when compared with apparently healthy individuals. Also, their levels were increased in both PM and PF patients group when compared with CM and CF respectively while no one mentioned the effect of arginase enzyme in the serum of the vitiligo disease. The increase in arginase activity in our study can be explained depending on two theories autocytoxic and autoimmune as follows:

1. As mentioned above, the patients with vitiligo had significantly lower TAC and high TOC than control as revealed by our study and others studie[2,4,24,33].

The free radical such as ROS that generates from melanocytes is produced by its mitochondria which are regarded as the main source of free radicals and play a pivotal role in cell survival and apoptosis[62]. ROS are considered normal byproducts of aerobic metabolism, namely, superoxide anion, hydroxyl anion, *NO* and $H_2O_2$. The superoxide anion is primarily metabolized by the mitochondrial Mn-SOD and the cytosolic Cu/Zn-SOD, generating $H_2O_2$[43].

The widely accepted current OS theory of vitiligo pathogenesis is largely based on the notion that the entire epidermis of vitiligo accumulates $H_2O_2$ that may reach one millimole concentration[50-52]. At this concentration, $H_2O_2$ is considered to be the primary intracellular ROS that leads to the death of the epidermal functional melanocytes. Multiple biochemical alterations in the epidermis and melanocytes of vitiligo have been built in principle on this notion of increased epidermal $H_2O_2$ concentration[46, 7, 61]. Mitochondrial dysfunction seems to play a major role in vitiligo pathogenesis[17] and increased production of $H_2O_2$ in vitiligo skin is believed to be the culprit of $H_2O_2$-induced mitochondrial DNA damage[54] that eventually leads to melanocyte apoptosis/death.

In animal, Thengchaisri et al[57] studied the effect of $H_2O_2$ on the arginase enzyme by treating coronary arterioles of porcine with $H_2O_2$ for 60 minutes. The results from his experiment were increased arginase I mRNA by 2-fold and increased its activity without altering eNOS expression. The increase in arginase enzyme may be due to rapidly conversion of $H_2O_2$ to hydroxyl radical shown to activate mammalian p38 mitogen-activated protein kinases (P38 MAPKs) by a wide range of cellular stresses as well as in response to inflammatory cytokines[44,64] and also can activate cAMP62 pathways. Both P38 MAPKs and cAMP can cause arginase induction in some cells.[13,60] It is speculated that these ROS-induced signaling cascades may be involved in the upregulation of arginase expression.

Therefore, $H_2O_2$ appears to upregulate the gene and protein expression of arginase I in the coronary arteriolar wall, especially in endothelial cells. At the present time, the mechanism underlying the upregulation of arginase remains unclear. Another free radical caused an increase in the activity of arginase is ONOO-. The unregulated activity of nitric oxide synthase produced more NO as published

recently[15] and also increases in OS, TOC levels (which mean increase in ROS), It has been shown that $H_2O_2$ may lead to an increase in other ROS such as superoxide[11] which can react rapidly with any available NO to form ONOO- and this reaction occurs more rapidly than SOD converting superoxide ($H_2O_2$)[38] leading to an increased cellular redox stress[26].

Sankaralingam et al[48] reported the effect of arginase II expression and its activity by ONOO- in the vasculature of women with preeclampsia vasculature. In addition, endothelial cells incubated with plasma from women with preeclampsia show enhanced arginase II expression and activity as well as increased superoxide levels and evidence for increased ONOO- formation. They proved that:

1) FeTPPS is a ONOO- scavenger in the presence of the preeclamptic plasma to investigate if ONOO- generated by preeclamptic plasma was responsible for enhanced arginase expression and its activity. The result was significant reduction in the arginase expression that was induced by preeclamptic plasma suggesting that ONOO- generated by preeclamptic plasma is responsible for the upregulation of arginase.

2) When exogenous ONOO- was used, arginase expression and activity were upregulated.

In addition, another study of Thengchaisri et al[57] showed increased arginase activity and arginase I expression in bovine aortic endothelial cells by oxidative species ONOO- and $H_2O_2$ through the pathway involving PKC activation of p115-Rho GEF and subsequent RhoA/Rho kinase [12] also was in agreement with Sankaralingaml et al[48] and Thengchaisri et al[57]. The dominance of tissue expression of arginase I or II may depend on organ, disease state, and species involved[35].

**Table 1**
**Mean of arginase activity, arginase specific activity and Peroxynitrite level in serum samples according to gander.**

| Samples | Group | Arginase activity (U/L) | P value | Specific activity of arginase (U/g) | P value | Peroxynitrite (µmole/L) | P value | TAC | P value | TOC | P value |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Male | CM (n=20) | 8.93 ± 2.14 | 0.011 | 0.129 ± 0.031 | 0.016 | 4.43 ± 2.35 | *0.002* | 1.49 ± 0.29 | 0.015 | 9.94 ± 4.93 | 0.000 |
| | PM (n=33) | 15.4 ± 4.76 | | 0.216 ± 0.067 | | 8.61 ± 2.61 | | 1.09 ± 0.31 | | 14.12 ± 5.52 | |
| Female | CF (n=20) | 5.9 ± 1.55 | 0.006 | 0.085 ± 0.022 | 0.007 | 3.78 ± 0.89 | *0.009* | 1.51 ± 0.23 | 0.028 | 8.24 ± 3.88 | 0.000 |
| | PF (n=32) | 13.77 ± 6.94 | | 0.194 ± 0.097 | | 8.33 ± 3.28 | | 1.08 ± 0.39 | | 13.06 ± 4.79 | |
| Vitiligo patients | PM (n=33) | 15.4 ± 4.76 | 0.786 | 0.216 ± 0.067 | 0.79 | 8.61 ± 2.61 | *0.99* | 1.09 ± 0.31 | 1 | 14.12 ± 5.52 | 0.994 |
| | PF (n=32) | 13.77 ± 6.94 | | 0.194 ± 0.097 | | 8.33 ± 3.28 | | 1.08 ± 0.39 | | 13.06 ± 4.79 | |

TAC (total antioxidant capacity), TOC (total oxidant capacity), CM (male healthy group), CF (female healthy group), PM (male patients group), PF (female patients group), PT1 (before treatment patients), PT2 (after treatment patients)
$p<0.05$ significant, $p<0.001$ highly significant, $p>0.05$ non-significant

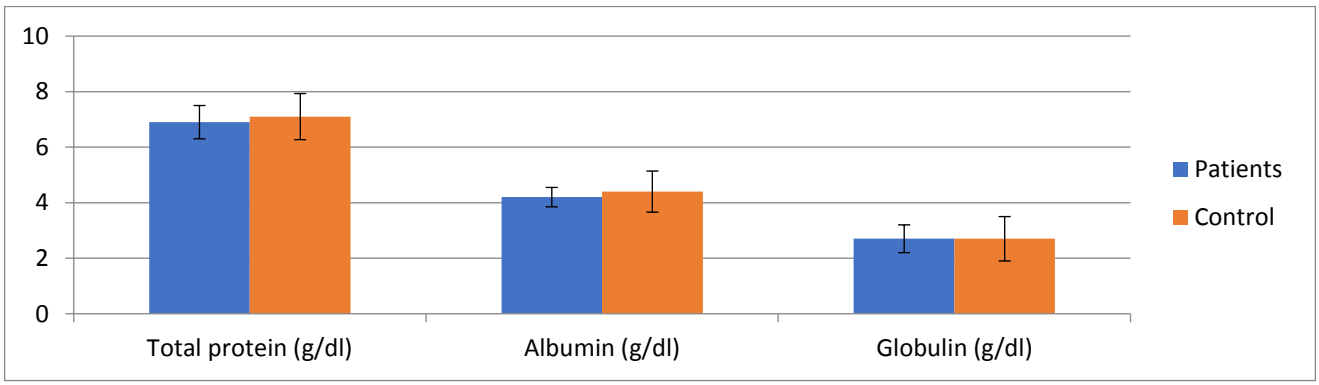


**Figure 1: Mean of total protein, albumin and globulin concentrations of the two studied groups.**

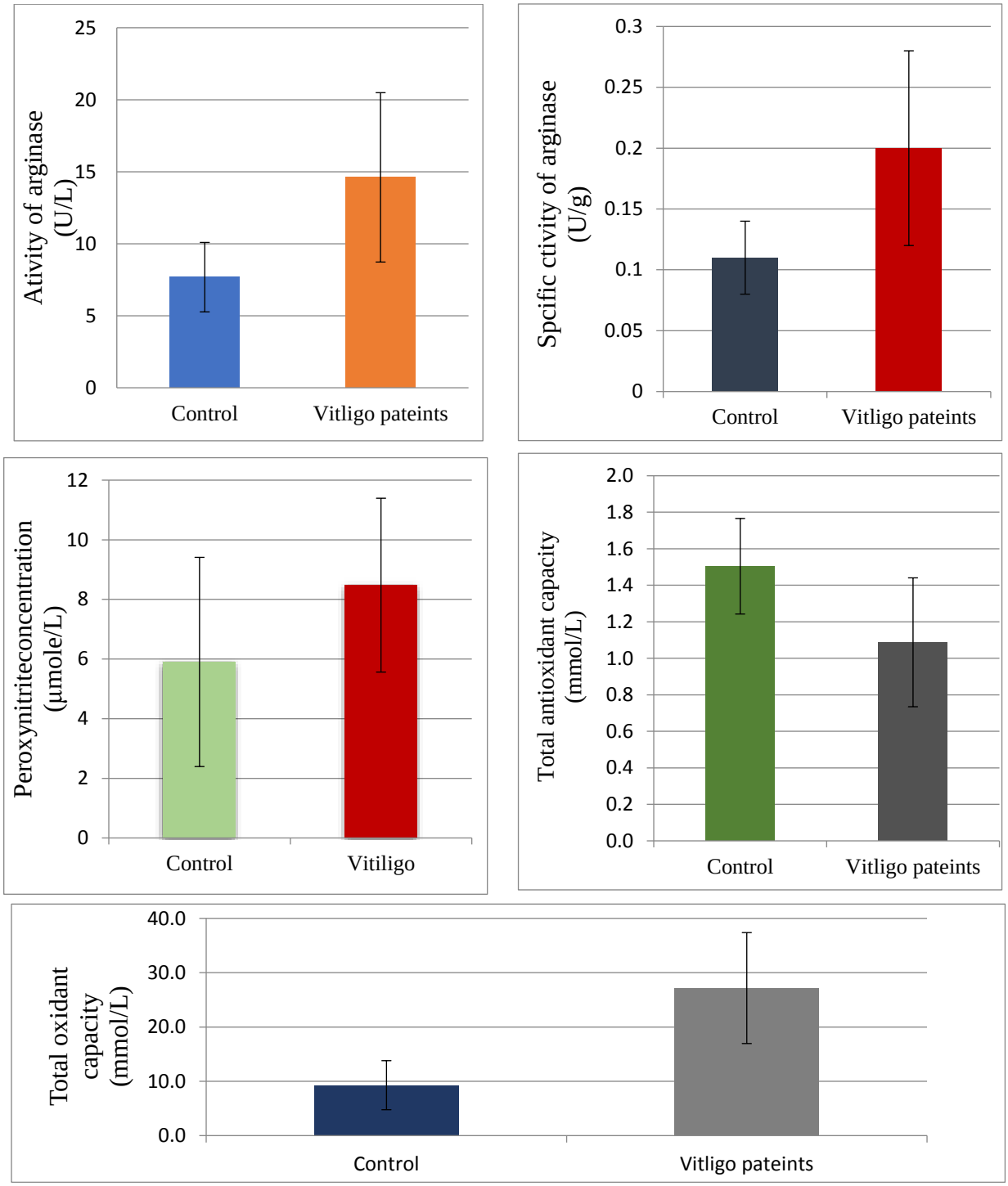


**Figure 2: Comparison of serum arginase activity, arginase specific activity, peroxynitrite concentration, total antioxidants capacity and total oxidant capacity in sera of the vitiligo patients and control groups.**

## Conclusion

We measured arginase activity in the vitiligo and healthy individuals. Out of the result from the present study, one can conclude that vitiligo disease caused alterations in the activity of arginase activity and imbalance between antioxidant and oxidant system leading to increase in arginase activity and expression of arginase. Increased arginase activity led to increase both oxidative stress and inflammation and results in more destruction or dysregulation in melanocyte cells.

## Acknowledgement

The authors are thankful to all the staff of the Baghdad teaching hospital, Dermatology department.

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