INTRODUCTION

Vitiligo is an acquired, non contagious, pigmentary disease resulting from the loss of melanocytes from skin, hair and mucous membranes ***(Laddha et al., 2012).*** It affects approximately 0.1%-8.8% of the population ***(Kutlubay et al., 2011).***

The pathogenesis of vitiligo is still obscure, it is assumed to be caused by multiple etiological factors that ultimately lead to melanocyte destruction ***(Malhotra and Dytoc, 2013).***

Multiple theories are suggested in the etiopathgenesis of vitiligo including autoimmune cytotoxic T cells, oxidant-antioxidant imbalance, genetic factors, neural mechanisms or multifactorial mechanisms ***(Westerhof et al., 2011; Birlea et al., 2013; Ezzedine et al., 2015).***

Oxidative stress plays an important role in the pathogenesis of vitiligo through overproduction and/or impaired clearance of reactive oxygen species (ROS). These result in imbalance between pro-oxidant and antioxidant systems leading ultimately to melanocyte destruction ***(Karaca and Güder, 2009).*** Oxidative stress via hydrogen peroxide (H_2O_2) inhibits tyrosinase activity, down-regulates microphtalmia associated transcription factor (MITF) and α-melanocytes-stimulating hormone (α-MSH) ***(Glassman, 2011).***

Accumulation of ROS also induces lipid peroxidation, DNA damage, increased production of proinflammatory and antimelanogenic cytokines, and melanocyte apoptosis ***(Jin et al., 2010; Denat et al., 2014).***

In addition to increased free radical levels, there is lower plasma total antioxidant status (TAS) levels in vitiligo patients as compared with controls ***(Hassan et al., 2013; Mehaney et al., 2014; Gupta et al., 2016).*** TAS is the accumulative action of all antioxidants in the plasma and body fluids ***(Karacay et al., 2010).*** It was found that levels of antioxidants agents [catalase (CAT) and glutathione peroxidase (GPx), glucose-6-phosphate dehydrogenase (G6PD), superoxide dismutase (SOD), vitamins C and E] are lowered in the epidermis or serum of patients with vitiligo ***(Farahi-Jahromy et al., 2014).***

Lower TAS levels were detected in patients with active vitiligo than those with stable vitiligo ***(Gupta et al., 2016).*** However other studies showed no significant difference in mean TAS level between the two groups ***(Singh et al., 2011; Mehaney et al., 2014).***

It was suggested that oxidative stress may have a role in vitiligo onset, while autoimmunity contributes towards the disease progression ***(Laddha et al., 2014a).*** Oxidative stress induced by sub-toxic levels of H2O2 was found to stimulate secretion of interleukin-6 (IL-6) by melanocytes ***(Yao, 2012).***

AIM OF THE WORK

- Study the role of TAS and IL-6 in active and stable vitiligo patients.
- Correlate between the antioxidant defense system (TAS) and autoimmunity cytokine (IL-6).

VITILIGO OVERVIEW

Definition:

Vitiligo is an acquired, non-contagious pigmentary disease resulting from the loss of melanocytes from skin, hair and mucous membranes. It causes selective destruction of functioning melanocytes ***(Picardo, 2010; Birlea et al., 2012; Laddha et al., 2012).***

Epidemiology:

It is the most common pigmentary disorder ***(Picardo, 2010; Birlea et al., 2012; Laddha et al., 2012).*** It affects more than 0.5–1% of the population worldwide leading to devastating psychological and social problems ***(Feily, 2014).***

The course of the disease is unpredictable, it is often progressive with stable phases in between during which there is no more depigmentation. Progressive disease with extending, enlarging macules or with appearance of new depigmented lesions is classified as active vitiligo ***(Gawkrodger et al., 2010).***

It affects all people with no predilection in gender, race, or skin type ***(Kakourou et al., 2009; Alikhan et al., 2011).*** But other study found that vitiligo occurs more in dark-skinned peoples, and it is more frequent in women. It affects all ages but in more than half of cases, it occurs before the age of 20 years ***(Kakourou et al., 2009; Whitton et al., 2015).***

Precipitating factors:

- ***Psychological factors:***

Psychological trauma such as severe illness, loss of job, migration, death of a family member, or an accident may increase the susceptibility of vitiligo ***(Guerra et al., 2010).*** It was confirmed that in > 50% of the vitiligo patients, emotional stress is the triggering factor ***(Vrijman et al., 2013).***

- ***Physical injury:***

Sometimes vitiligo occurs following a chemical or mechanical trauma, topical preparation containing diphencyprone, accidental skin contact with plant protecting agents, abrasions, or surgical intervention on the skin. This explains the isomorphic köebner phenomenon occuring in vitiligo patients. It is observed in up to 40% of patients mostly those with non-segmental vitiligo ***(Batalla and Feal, 2010; Van Geel, 2012; Dhar et al., 2014)*** **(Figure 1).**

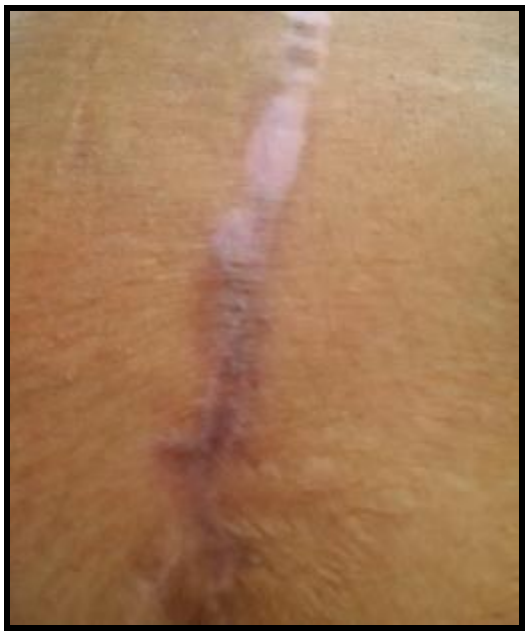

Figure (1): Koebner phenomenon: depigmented area in a scar after abdominal surgery in vitiligo patient ***(Schild and Meurer, 2016).***

- ***Life quality:***

Vitiligo is not life-threatening condition but it affects the quality of life leading to depression in the affected individual ***(Sangma et al., 2015).*** It causes decrease in dermatology life quality index (DLQI, score 0 to 30) and the global quality of life (GQOL, score 0 to 100) ***(Taieb et al., 2013; Sangma et al., 2015).*** The effect on quality of life is related to the severity of the disease, skin type, gender, age and family status ***(Bhandarkar and Kundu, 2012).***

- ***Foods:***

The type of food is strongly linked to vitiligo pathogenesis. This is due to the important contribution of ROS,

estrogen, tannin, and phenol-containing chemicals which compete with tyrosine to produce reactive quinones ***(Namazi and Chee Leok, 2009).*** It was found that mango, oak, cassava, areca nut, red chillies, cherry, raspberry, cranberry, blackberry and tea contain natural plant phenol and poly phenolic compounds (tannins), and this may aggravate the disease ***(Birol et al., 2006).***

- ***Drugs:***

It was found that vitiligo is precipitated by some drugs including anticonvulsants (carbamazepine, valproic acid), antimalarials (chloroquine), biologics (infliximab), and other drugs (clofazimine, dopamine). This occurs through 1st; activation of reactive cytotoxic T cells against melanocytes antigens, 2nd; damaging sympathetic nerves that are connected by chemical synapses to melanocytes which indirectly results in melanocytes dysfunction, 3rd direct cytotoxicity of the drugs on melanocytes ***(Curzytek et al., 2007).*** Other medications that can cause disease provocation are β-blockers, statins and tetracycline and cosmetic ingredients such as topical coenzyme Q10 (ubiquinone) ***(Schallreuter and Salem, 2010).***

- ***Pregnancy:***

The effect of pregnancy on vitiligo is variable, it may aggravate, improve, or cause no effect. However the disease usually worsens after delivery ***(Patel et al., 2003).***

Risk factors and associated autoimmune diseases

They include family history of vitiligo, personal or family history of other autoimmune diseases. Approximately 15-25% of the vitiligo patients mainly those with non-segmental vitiligo are suffering from another autoimmune disease, which is usually preceded by a history of vitiligo ***(Uncu et al., 2011; Dhar et al., 2014).***

Among the autoimmune diseases, the autoimmune thyroiditis is most commonly detected (Hashimoto's disease was seen in 88% of vitiligo cases, and Graves' disease in 12% of the cases) ***(Spritz, 2010a).*** Other associated autoimmune diseases are type 1 diabetes, Addison disease, hypoparathyroidism, myasthenia gravis, pernicious anemia, systemic lupus erythematosus (SLE), Sjogren syndrome, alopecia areata, or rheumatoid arthritis ***(Narita et al., 2011; Ezzedine et al., 2015).***

Retrospective study of 2441 patients with vitiligo demonstrated that 12% of them had autoimmune thyroid disease, 8% had psoriasis, 3% had rheumatoid arthritis, and 2% had inflammatory bowel diseases ***(Sheth et al., 2013).***

Because of increased genetic susceptibility, the range of associated autoimmune diseases is wider in patients with generalized vitiligo and positive family history than those with localized sporadic disease ***(Mollet et al., 2010).***

Vitiligo can occur as a part of the autoimmune induced polyglandular endocrinopathy syndrome (APS) which is characterized by frequent association of endocrinopathies and autoimmune diseases with the presence of organ-specific autoantibodies. In type II APS (Addison's disease, hypothyroidism and / or diabetes mellitus type 1), vitiligo has been detected in 15-50% of cases with circulatory antibodies against melanocytic antigens such as melanin concentrating hormone receptor 1 (MCHR-1) ***(Amerio et al., 2010).***

In a study on 40 Italian males with vitiligo, circulating autoantibodies have been detected in 42.5 of the patients. Anti-thyroglobulin antibodies (anti-TG antibodies) were detected in 27.5%, anti-thyroperoxidase (Anti-TPO) in 22.5%, anti-smooth muscle (anti-SMA) in 17.3%, anti-nuclear (ANA), antimitochondrial (AMA) and anti-gastric parietal cells in 2.5%, respectively. Only in two cases (5%), severe thyroid disease had been detected. The circulating autoantibodies (especially anti-thyroid antibodies) were associated with low duration of the disease ***(Ingordo et al., 2011).***

Another study done on 80 Turkish patients showed that vitiligo vulgaris was found to be the most common type detected. 44 patients (55%) had autoimmune disease. Hashimoto thyroiditis occurred in 31% of the patients, alopecia areata in 12.5%, and pernicious anemia in 8.7% ***(Akay et al., 2010).***

Vitiligo pathogenesis:

The pathogenesis of vitiligo is still obscure, it is assumed to be caused by multiple etiological factors that ultimately lead to melanocyte destruction ***(Malhotra and Dytoc, 2013).***

Multiple theories are suggested in the etiopathgenesis of vitiligo including autoimmune cytotoxic T cells, oxidant-antioxidant imbalance, genetic factors, neural mechanisms or multifactorial mechanisms ***(Westerhof et al., 2011; Birlea et al., 2013; Ezzedine et al., 2015).***

1- Genetic:

Certain human leukocyte antigen (HLA) types are linked to vitiligo, they include A2, DR4, DR7 and CW6 ***(Bagherani et al., 2011).*** Family history of vitiligo is variable and is seen in one-third of the people with the disease ***(Eleftheriadou et al., 2011; Faria et al., 2014)***. Strong genetic evidence of the link between vitiligo and other autoimmune diseases has been detected ***(Spritz et al., 2010b).***

2- Neural theory:

Segmental vitiligo follows the same path as dermatome, this can be explained by dysfunction of sympathetic nervous system that inhibits melanin synthesis leading to skin depigmentation ***(Mohammed et al., 2015).*** Neuropeptides and neuronal markers such as neuropeptide Y (NPY) or calcitonin

gene-related peptide (CGRP) are highly expressed under the effect of oxidative stress at the margins of vitiligo lesions ***(Lazarova et al., 2000)***. NPY affects the formation of melanocyte dendrites and binding to keratinocytes. It also activates the immune system leading to oxidative stress through increasing catecholamine synthesis. Polymorphisms of the promoter region and exon 2 of NPY are associated with increased susceptibility to vitiligo ***(Laddha et al., 2014b).***

3- Oxidative stress theory:

Oxidative stress hypothesis suggests that there is redox disruption (oxidant-antioxidant systems imbalance) of the vitiliginous skin. This results in the excessive production of ROS ***(Khan et al., 2009; Denat et al., 2014; Colucci et al., 2015).***

Elevated levels of ROS such as H2O2 and peroxynitrite, have been demonstrated in the lesional skin biopsies of vitiligo patients. This excessive accumulation of ROS leads to lipid oxidation and DNA damage with subsequent melanocyte destruction ***(Colucci et al., 2015; Guntas et al., 2015; Li et al., 2015).***

Vitiligo patients have depressed antioxidants levels to compensate for the enhanced oxidative stress ***(Denat et al., 2014).*** Antioxidants such as CAT, GPx, G6PD levels are depressed in lesional skin biopsies of vitiligo patients ***(Colucci et al., 2015).***

4- Intrinsic theory:

It suggests that melanocytes defects in vitiligo patients lead to their death. These defects include morphologic defects, decreased adhesive properties, and growth factors deficiency ***(Picardo and Bastonini, 2015; Speeckaert et al., 2015)***.

5- Autoimmune theory:

There is a strong evidence that vitiligo is an autoimmune disease ***(Sandoval-Cruz et al., 2011),*** and the autoimmune theory is well accepted as a cause of destruction of melanocytes in vitiligo patients. The immune reaction is mediated by the interaction among cellular immunity, humoral immunity, and the action of cytokines ***(Alkhateeb et al., 2010; Alikhan et al., 2011; Mohammed et al., 2015).***

- **Humoral immunity:**

Various circulating anti melanocytes antibodies have been detected in the sera of vitiligo patients, they are seen to be related to disease activity. They present in more than 90% of patients with greater extent and in 50% in patients with lesser extent of disease ***(Tahir et al., 2010).*** The role of humoral immunity in vitiligo pathogenesis will be discussed in details in chapter (3).

- **Cell-mediated immunity:**

Cell-mediated immunity in vitiligo is explained by the presence of inflammatory cells infiltrates in perilesional vitiligo skin. Decreased CD4+ to CD8+ ratio was detected in skin of vitiligo patients as compared to healthy control. CD8+ T cells against melanocytic antigens have been detected both in perilesional skin and in the blood of vitiligo patients ***(Van Den Boorn et al., 2009; Van Geel et al., 2010; Oiso et al., 2011).***

Cytokines also play an important role in vitiligo pathogenesis. There is an increased expression of tumor necrosis alpha (TNF-α) and interferon-gamma (IFN- γ) proposing that vitiligo is mediated by a T helper-1 cells (Th1) response ***(Taher et al., 2009).***

Also sera and lesional skin biopsies of vitiligo patients showed significantly higher interleukin 17 (IL-17) levels ***(Bassiouny et al., 2011).*** The levels of the regulatory T cells (Tregs) were reduced in the non-lesional, perilesional and lesional vitiligo skin ***(Klarquist et al., 2010).***

Classification of vitiligo

According to the review conducted by the Vitiligo Global Issues Consensus Conference (1) between 2011-2012, vitiligo can be classified into segmental, non-segmental, and unclassified ***(Faria et al. 2014)*** **(Table 1).**

Table (1): Classification of vitiligo ***(Faria et al. 2014)***:

Types	Subtypes
▪ **Non-segmental vitiligo**	• Acrofacial • Mucosal (more than one site affected i.e. oral and genital mucosa) • Generalized or Common • Universal • Mixed (associated with segmental vitiligo) • Rare forms
▪ **Segmental**	• Unisegmental, bisegmental or multisegmental
▪ **Unclassified or indeterminate**	• Focal • Mucosal (only one site affected)

1) **Non-segmental vitiligo (NSV):** includes acrofacial, mucosal, generalized or common, universal, and mixed forms besides rare forms.

   - **Acrofacial:** may affect the face, head, hands and feet, and prefer the perioral region and the extremities of digits.

   - **Mucosal:** affects the oral and genital mucosae. Also mucosa may also be affected in patients with acrofacial, generalized, or universal forms. When only one mucosal site is affected, it is classified as indeterminate ***(Ezzedine et al., 2012).***

- Generalized or common **(Figure 2)**: Macules / patches are often symmetrical, it may affect any part of the skin, mainly hands, fingers, face and trauma-exposed areas.
- Universal: it affects the largest area of the skin (80-90% of body surface), and represents the most common form in adulthood. It is usually preceded by generalized vitiligo
- Mixed: involvement of both segmental and non-segmental types. The segmental form often precedes NSV.
- Rare forms: vitiligo punctata, minor, and follicular. They are considered as unclassifiable.

2) **Segmental Vitiligo:** it can affect one, two or multiple segments **(Figure 3).** The unisegmental form is the most common one and consists of one or more white macules on one side of the body, it usually respects midline of the body, and there is also involvement of body hair (leukotrichia) with rapid onset of disease. Less commonly, it may affect two or more segments which may have bilateral segmental distribution, starting simultaneously or not.

3) **Unclassifiable forms or undetermined vitiligo**

- **Focal:** Isolated white macule without segmental distribution. This form can progress to segmental or NSV forms.
- **Mucosal:** when only one mucosa is affected.