INTRODUCTION

Vitiligo is one of the most common acquired pigmentary disorders with a prevalence of 1–2% of population ***(El-Zawahry, 1964)***. The exact etiopathogenesis and mechanisms of vitiligo are not fully understood. However, an association between vitiligo and reduced serum levels of vitamin B12 and folic acid has been found ***(Lerner, 1971; Montes et al., 1992; Kim, 1999).***

Homocysteine (Hcy) level is mainly determined by the levels of folic acid and vitamin B12 ***(El-Batawi., and El-Tawil, 2001)*** because both act as cofactors of the enzyme Hcy methyl transferase for the regeneration of methionine from Hcy in the activated methyl cycle ***(Juhlin and Olsson, 1997).*** It has been reported that vitiligo is associated with elevated Hcy levels ***(Mudd et al., 1995).*** A study from Egypt also showed that serum Hcy is increased in vitiligo patients as compared with healthy subjects ***(Guttormsen et al., 1996).***

The oxidation of Hcy produces reactive oxygen species, which causes oxidative stress on melanocytes ***(Minet et al., 2000)***. Tyrosinase is a 75 KD copper-containing enzyme that initiates the melanin biosynthesis in pigmented cells ***(Brenton et al., 1966; Goth et al., 2004)***. Hcy also leads to inhibition of tyrosinase enzyme by binding with Copper, at its active site

resulting in reversible hypopigmentation ***(Kurbanov et al., 1974).***

Pigmentary dilution is observed in patients with homocystinuria, so this may further hint to the fact that an increase in local homocysteine might interfere with normal melanogenesis and play a role in the pathogenesis of vitiligo ***(Shaker and El-Tahlawi, 2008).***

AIM OF THE WORK

To investigate the role of Homocysteine in active vitiligo lesions.

VITILIGO

Definition:

Vitiligo is a common pigmentrey disorder divided into segmental and non-segmental forms. "Generalized vitiligo or non-segmental vitiligo (NSV) is an acquired chronic pigmentation disorder characterized by white patches, often symmetrical, which usually increases in size with time, corresponding to a substantial loss of functioning epidermal and sometimes hair follicle melanocytes" while "Segmental vitiligo is an acquired chronic pigmentation disorder characterized by white patches with a unilateral distribution that may totally or partially match a dermatome, but not necessarily. Other distribution patterns can be encountered that cross several dermatomes, or correspond to large areas delineated by Blaschko's lines" ***(Taïeb and Picardo, 2007).***

Epidemiology:

Vitiligo is the most prevalent pigmentary disorder that occurs worldwide with an incidence rate of between 0.1% and 2% ***(Daneshpazhooh et al., 2006; Torello et al., 2008)*** irrespective of age, race ***(Moretti et al., 2006 ; Torello et al., 2008)*** ethnic origin or skin color ***(Whitton et al., 2008).*** The prevalence has been reported as high as 4% in some South

Asian, Mexican and United States populations ***(Sehgal and Srivastava, 2007).***

Almost half of the patients present before the age of 20 years, and nearly 70-80% before the age of 30 years. Adults and children of both sexes are equally affected, although larger number of females consult the doctor probably due to the greater psycho-social perceived impact of the disease ***(Sehgal and Srivastava, 2007).***

Precipitating Factors:

Vitiligo patients often attribute the onset of their disease to a specific life event, crisis, or illness. Many can relate it to loss of a job, death of a close family member, an accident, or a severe systemic disease ***(Guerra et al., 2010).*** Psychological trauma may increase an individual's susceptibility to vitiligo ***(Manolache and Benea, 2007).***

In some patients, the onset of vitiligo follows a physical injury such as a cut or abrasion, or sun exposure; this development of focal vitiligo congruent with a site of injury is referred to as the Isomorphic Köebner Phenomenon ***(Ortonne et al., 2003; Batalla and Feal, 2010).***

Associations:

While most vitiligo patients are generally healthy, autoimmune endocrinopathies do occur in some patients.

Though their frequency is disputed, patients should be educated with regard to their signs and symptoms. The most common association is with thyroid dysfunction, either hyper- or hypothyroidism (i.e. Grave's disease, Hashimoto's thyroditis) ***(Ortonne, 2008).***

Pathogenesis:

In contrast to the easy clinical diagnosis, the cellular mechanisms leading to the appearance of vitiligo are still uncertain and various possible pathomechanisms have been proposed, including the genetic, immune-mediated, the auto-cytotoxic and the neuronal ones which have all been considered in the convergence theory ***(Boissy and Spritz, 2009; Picardo and Taïeb, 2010).***

Vitiligo is characterized by disappearance of epidermal and/or follicular melanocytes ***(Birlea et al., 2011b)***. It is likely that melanocytes are destroyed by an as-yet unknown process. Indeed, melanocyte destruction has never been clearly demonstrated ***(Gauthier et al., 2003).*** One study reports that melanocytes are never completely absent in the skin ***(Tobin et al., 2000).***

Theories on the pathogenesis of vitiligo center on mechanisms for the destruction of melanocytes. The pathogenesis of this disorder is uncertain, but it appears to be

dependent on the interaction of genetic, immunological, and neurological factors ***(Yaghoobi et al., 2011).***

A. *The Autoimmune Hypothesis:*

It is the most important hypothesis in the pathogenesis of vitiligo. This is based on the clinical association of vitiligo with a number of disorders also considered to be autoimmune. It primarily focuses on: (1) association with other autoimmune disorders; (2) association with family history of vitiligo and autoimmune disorders; (3) presence of autoantibodies to melanocytes and autoreactive T cells; (4) presence of genetic factors, i.e. Major histocompatibility (MHC) class II alleles, Cytotoxic T lymphocyte antigen-4 (CTLA-4) gene, autoimmune susceptibility foci; and (5) positive response to immuno-suppressive therapeutic agents ***(Poojary, 2011).***

The autoimmune theory proposes that both humoral and cellular immunity co-operate in the destruction of melanocytes ***(Boissy and Spritz, 2009).***

Concerning humoral immunity, different circulating antibodies to melanocytes have been found in the sera of vitiligo patients, these seem to be related with the extent of disease. They are present in more than 90% of the patients with greater depigmentation and in 50% in the ones with minimal lesions (Non-specific autoantibodies against pigment and non-

pigment cell antigens (35, 40-45, 75, 90 and 150 kD antigens) as well as specific melanocyte autoantibodies against tyrosinase (key enzyme involved in melanin synthesis), and/or tyrosinase related proteins 1 and 2 (TRP 1, 2), Pmel17/gp100 (melanosomal matrix glycoprotein) and the surface receptor MCHR1 (melanin-concentrating hormone receptor 1) have been described in NSV patients ***(Rezaei et al., 2007; Dordic et al., 2012)***. The specific recognition of 68, 165 kDa proteins by vitiligo antibodies was also demonstrated ***(Rocha et al., 2000).***

Concerning cellular immunity, alterations in cellular immunity participate in the pathogenesis of NSV ***(Andersen et al., 2008).*** Cutaneous infiltrates at the periphery of the vitiligo lesions consist of T cells that are closely associated with the areas of melanocyte depletion ***(Van den Wijngaard et al., 2000).*** Studies have demonstrated the presence of skin-homing melanocyte-specific cytotoxic T lymphocytes (CD8+ T cells) in the peripheral blood of patients with vitiligo ***(Giovanni et al., 2011)***. ***Pichler et al. (2009)*** found an elevated ratio of $CD8^+/CD4^+$ T cells to be a sign of imbalanced lymphocyte immune response in vitiligo patients, but they did not find evidence for a pathological distribution of B cells in peripheral blood within their patients.

CD4+ CD25+ CD127− Foxp3+ regulatory T cells (Tregs) are important in maintaining self-tolerance and regulating immune responses in both physiological and disease conditions. Accumulating data indicate that a deficiency or dysfunction of Tregs is associated with impaired immune

homeostasis and the development of autoimmune diseases. To date, few papers have investigated Treg numbers or function in GV patients. One report revealed a defect in Treg cell homing to the skin, based on the finding of drastically reduced Treg numbers in vitiligo skin without any systemic drop in their abundance or activity ***(Klarquist et al., 2010).*** In contrast, a recent report identified increased numbers of Tregs in perilesional skin despite a functional defect of circulating Tregs in progressive vitiligo ***(Ben Ahmed et al., 2011).*** Impairment in Tregs leads to activation of CD8+ cytotoxic T lymphocytes in patients with generalized vitiligo ***(Lili et al., 2012).***

Multivariate analysis by ***Ezzedine et al. (2012)*** indicates that atopic diathesis lies in association with pre-pubertal onset vitiligo. This recent study concluded that vitiligo is strongly associated with personal and family history of atopy, suggesting that the predisposing immune background in vitiligo is not limited to autoimmunity.

To summarize, in cell-mediated immunity, after processing of antigens by antigen-presenting cells, antigenic peptides are presented to the T-cell receptors (TCR) of cytotoxic T lymphocytes in the context of MHC class I molecules. Cognate help (via cytokine production) by antigen specific T helper cells, in the context of antigenic peptides presented on MHC class II molecules, is required for a long-lasting cytotoxic T-cell response against melanocytes that can lead to their destruction ***(Kemp et al., 2001)***. In humoral

immunity, antigens are captured by the antigen-specific membrane immunoglobulins of B cells. The production and secretion of antigen-specific antibodies by B cells are also dependent on cognate help (via cytokine production) by antigen-specific T helper cells. Anti-melanocyte antibodies can destroy pigment cells by either antibody-dependent complement-mediated damage or antibody-dependent cell cytotoxicity (ADCC) (Figure 1) ***(Kemp et al., 2001).***

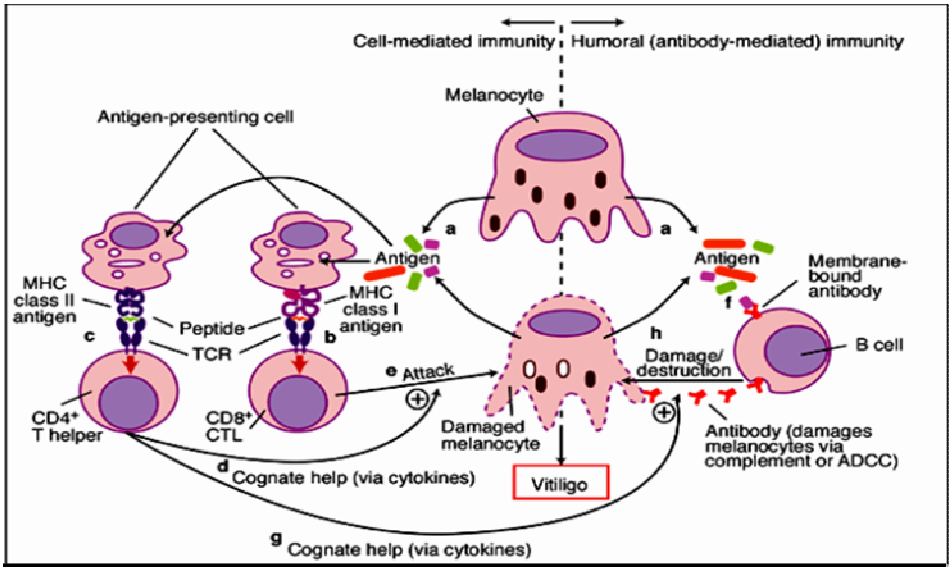

Fig. (1): Summary of the possible cellular and humoral immune mechanisms of vitiligo ***(Kemp et al., 2001).***

B. The Biochemical Hypothesis:

The biochemical hypothesis postulates that cytotoxic precursors to melanin synthesis accumulate in melanocytes, causing cell death ***(Namazi, 2005).*** Oxidative stress plays an important role in the pathogenesis of vitiligo and melanocyte

damage in vitiligo might be linked to generalized oxidative stress ***(Jalel et al., 2009).*** Sometimes, this process is referred to as the self-destruct theory of Lerner ***(Lerner, 1971).***

It has been shown that in vitiligo patients a metabolic defect in tetrahydrobiopterins homeostasis due to oxidative stress leads to overproduction of 6- and 7 tetrahydro-biopterins. This defect results in hydrogen peroxide overproduction ***(Grando et al., 2006; Dell'Anna et al., 2007).*** One consequence of hydrogen peroxide accumulation in vitiligo epidermis is the oxidative degradation of the porphyrin active site of catalase, resulting in low levels of this enzyme in vitiligo ***(Spencer et al., 2007a).*** Compared with control patients, cells of vitiligo patients have lower levels of glutathione, which helps prevent free radical mediated injury ***(Hazneci et al., 2005).*** It is suggested that the imbalance in the oxidant-antioxidant system might play such a role in vitiligo. Research at the molecular level has also demonstrated deficiency of antioxidant substances in vitiliginous skin. The free radicals are cytotoxic to melanocytes and inhibit tyrosinase ***(Shameer et al., 2005).***

Jain et al. (2011) observed significantly high levels of superoxide dismutase in active vitiligo cases as compared to stable vitiligo and healthy controls. However, in his study glutathione peroxidase (GPx) did not show any significant association in the active and stable cases of vitiligo. Previous studies reveal an inconsistent pattern in the level of GPx. ***Passi***

et al. (1998) reported an increased level of GPx whereas ***Agrawal et al. (2004)*** reported a decreased level in vitiligo cases.

Many studies suggest that NO is involved in the inhibition of cell proliferation, differentiation, and apoptosis and thus it may contribute to the pathogenesis of various autoimmune diseases including vitiligo ***(Al Khateeb et al., 2003).*** NO is synthesized by a group of enzymes called NO synthase (NOS). NOS catalyses the production of NO an L-citrulline from L-arginine, O2 and NADPH. NOS family consists of three isoforms: neuronal NOS, endothelial NOS, and inducible NOS ***(Li et al., 2002).*** inducible NOS gene polymorphisms may play an important role in the genetic susceptibility to the development of vitiligo ***(Zhang et al., 2011).***

C. The Neural Hypothesis:

Segmental vitiligo often occurs in a dermatomal pattern. This observation led to a neural hypothesis which proposes that some chemical mediators released from peripheral nerve endings cause decreased production of melanin ***(Grimes, 2004).***

The close associations between the skin, immune system, and nervous system, along with specific changes demonstrated in vitiligo patients, support a pathogenic mechanism of vitiligo that involves neuroimmunologic factors, the release of which

can be governed by mental stress ***(Yu et al., 2012).*** It also proposes that elevated levels of some neurotransmitters and catecholamine degrading enzymes injure melanocytes ***(Namazi, 2005).*** Other studies have reported ultra-structural evidence of axonal damage ***(Al-Abadie et al., 1995)***, supporting this hypothesis.

Neuropeptides and nerve growth factors are critical regulators of emotional response and may precipitate the onset and development of vitiligo in certain predisposed individuals. More studies are required to investigate whether a direct link exists between genetics, mental stress, and neurogenic factors in vitiligo ***(Yu et al., 2012).***

D. Other Possible Etiological Factors:

A new theory is emphasizing that depigmentation in vitiligo patches results from a chronic detachment of melanocytes that is proposed to be designated as "*melanocytorrhagy"*, which is possibly related to increased susceptibility to mechanical and other types of stresses. This theory involves that *in vivo* friction of perilesional nonsegmental vitiligo skin induces the detachment of living melanocytes from the basal layer followed by transepidermal migration and eventual death of detached pigment cells (Figure 2) ***(Gauthier et al., 2003; Yaghoobi et al., 2011; Kumar and Parsad; 2012).***

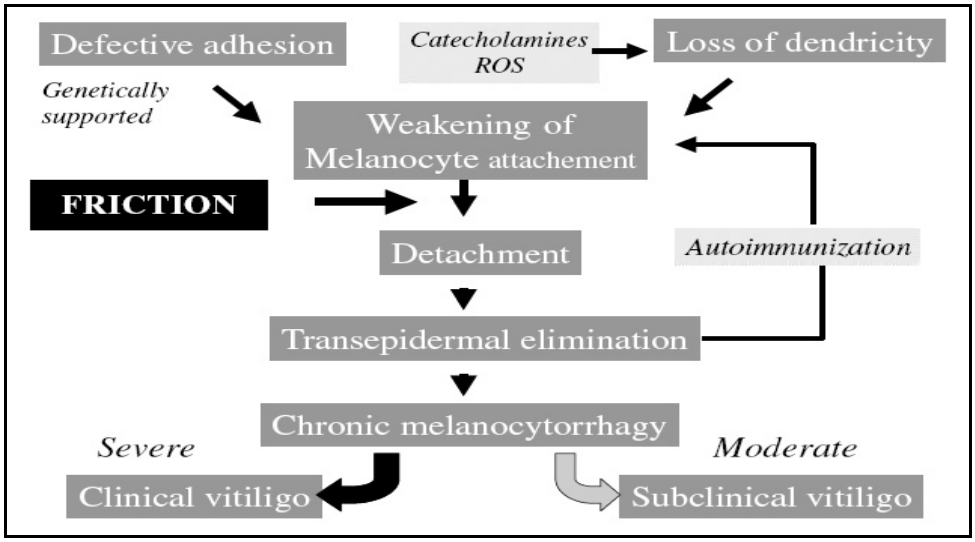

Fig. (2): Melanalocytorrhagy: Proposal of a new integrated theory for non-segmental vitiligo ***(Gauthier et al., 2003).***

The baseline expression of adhesion molecules may differ in vitiligo patients as compared to controls ***(Kumar et al., 2011).*** Recently, it has been shown that in unstable vitiligo patients, melanocytes were poorly attached to Type IV collagen, whereas stable vitiligo melanocytes and control melanocytes were firmly adhered to Type IV collagen ***(Kumar et al., 2011).*** More importantly, dendrites of perilesional unstable vitiligo patients were small with clubbed ends. Dendrite of these melanocytes seems to be retracted, whereas the dendrites of control and stable vitiligo patients were normal in shape and size ***(Kumar and Parsad, 2012)***.

The Newest Hypothesis about vitiligo proposed by ***Bagherani et al. (2012)*** states that most of the suggested pathogeneses of vitiligo can be attributed to lack of one factor, Zinc-α2-Glycoprotein (ZAG), based on: (i) ZAG as a

keratinocyte-derived factor influences melanocyte proliferation and dendricity. Moreover, ZAG can be considered as a marker of maturation and differentiation of cells, thus in vitiligo, the lack of ZAG is expected to inhibit melanocyte proliferation; (ii) ZAG can be effective in prevention of vitiligo by immunoregulation ***(Hassan et al., 2008)***, so its deficiency can be incriminated in autoimmune hypothesis; (iii) ZAG is effective in attaining the properties of cell adhesion between cells and extracellular matrices, thus ZAG seems to have a role in the new theory of melanoctorrhagy; (iv): In whitish patches of vitiligo, the expression of TNF-α is high ***(Yaghoobi et al., 2011).*** Some studies revealed that TNF- α can decrease ZAG expression and secretion; (v): Some findings have shown that the minimal level of ZAG is required for melanin production ***(Hassan et al., 2008).***

As a hypothesis, ***Bagherani et al. (2012)*** suggested that zinc might be effective in the treatment of vitiligo. Some studies have shown that zinc can precipitate ZAG ***(Yaghobi et al., 2011). Bagherani et al. (2012)*** concluded that zinc, by precipitating circulating ZAG in the site of vitiligo, can be effective in treatment of this disease.

The apoptosis hypothesis proposes that cytokines such as interleukin-1 (IL-1), interferon gamma (IFN-γ) or tumor necrosis factor-alpha (TNF-α), released by lymphocytes, keratinocytes and melanocytes can initiate apoptosis of melanocytes ***(Huang et al., 2002; Yaghobi et al., 2011).*** Based