INTRODUCTION

Alopecia areata (AA) is an immune-mediated disease presenting as hair loss that occurs in all ethnic groups, ages and both sexes. The estimated life time risk is 1.7% among the general population ***(Seetharam, 2013).*** It often affects children and teenagers and can cause widespread hair loss of the scalp; alopecia totalis (AT), or complete body hair loss; alopecia universalis (AU), thus it has a devastating psychological impact on patients ***(Wasserman et al., 2007).***

The clinical hallmark of AA is the sudden development of a well-circumscribed patch of hair loss on normal appearing skin. The majority of patients present with limited patches of alopecia on the scalp and occasionally in the beard, eyebrows, eyelashes, or other hair-bearing areas of the body that regrow spontaneously within 1 year, but relapses are common and many patients have more than one episode of hair loss ***(Papadopoulos et al., 2000).*** However, an estimated 7% to 10% of patients may experience more extensive and chronic forms of the disease ***(Shapiro, 1996).***

The pathophysiology of AA is considered to be T-cell mediated autoimmunity that occurs mostly in genetically predisposed individuals. In addition to disturbance of immune function, complex interactions between predisposing genetic and environmental factors act as triggers for disease progression. Infections, endocrine disorders, thyroid dysfunction and imbalance of trace elements may also trigger the onset of AA ***(Bhat et al., 2009; Masmoudi et al., 2013).***

Zinc is among the well-known trace elements that are associated with hair shedding ***(Biyukavir et al., 2005).*** It is an essential cofactor for multiple enzymes and is involved in important functional activities in the hair follicle (HF). Further, zinc is a potent inhibitor of HF regression and accelerates HF recovery ***(Plonka et al., 2005).*** As cofactors of metalloenzymes, zinc has considerable effects on nearly all aspects of the metabolism that takes place in the organs of the body, including the skin. In fact, congenital and acquired zinc deficiencies are usually expressed as a variety of skin manifestations such as acrodermatitis enteropathica, psoriasis-like eruptions, blisters, onychopathy and loss of hair ***(Rushton, 2002; Plonka et al., 2005)***.

Al-Jaff (2005) and Bhat et al. (2009) found decreased zinc levels in patients with AA. To the best of our knowledge, no previous studies have assessed or compared serum zinc levels in patients with recent onset and resistant AA. In addition, no correlations between serum zinc levels and disease duration or severity were previously studied.

AIM OF THE WORK

The aim of this study is to evaluate serum zinc level in patients with newly diagnosed and resistant lesions of AA in comparison to age and sex- matched healthy controls.

ALOPECIA AREATA

Alopecia areata is an autoimmune disease that presents as well defined, usually rounded or oval patches of non scarring hair loss on the scalp or any hair bearing surface with no overt epidermal changes ***(Hordinsky and Ericson, 2004; Ohtsuki et al., 2013).***

I- Prevalence of AA:

Alopecia areata is estimated to have a lifetime prevalence risk of about 1.7% in the general population, including males and females across all ethnic groups ***(Hon and Leung, 2011)***. It can happen at any age with peak incidence occurring in the third to fifth decades ***(Papadopoulos et al., 2000).*** It is one of the most common hair disorders of childhood ***(Wang et al., 2012).***

Positive family history among first-degree relatives has been reported to be as high as 47% for patients with early onset AA, in contrast to 1.6% for all patients ***(Yang et al., 2004).***

II- Aetiopathogenesis of AA:

The pathogenesis of AA is still unknown. Inspite of the impressive progress, there is still a long way to go to completely understand the mechanisms of the disease and to identify AA-specific targets for treatment ***(Seetharam, 2013).***

Many factors such as genetic predisposition, autoimmunity, cytokines, chemokines and stress have been suggested as causes for AA. The course of disease is not predictable and it is often associated with periods of hair loss and regrowth ***(Firooz et al., 2005).***

Alopecia areata is considered a tissue-restricted autoimmune disease. It is commonly associated with other autoimmune diseases, both within the affected persons and their families. Circulating antibodies against follicular components were frequently detected in people with AA ***(Zhao et al., 2012)***.

The development of hair loss involves aberrant modulation of the hair growth cycle, resulting in dystrophic anagen HFs and/or increased frequency of telogen state follicles ***(Alkhalifah et al., 2010).***

Pathogenic mechanisms in AA:

A) Hair follicle growth cycling modulation in AA.

B) Genetic factors.

C) Autoimmune pathogenesis.

D) Infectious agents.

E) Emotional stress.

F) Oxidative stress.

G) Imbalance of trace elements.

A) Hair follicle growth cycling modulation in AA:

There are three key phases of the hair cycle: the growth phase (anagen), the regression phase (catagen), and the resting phase (telogen) ***(Vogt et al., 2008)***. Exogen is a HF cycle event that describes the controlled shedding of club hair fibers**.** This cycle is finely coordinated by the expression of hormones, cytokines, transcription factors, and their corresponding receptors. They are also regulated through endocrine, paracrine, and autocrine routes. Any disruption of these pathways can result in the development of hair diseases ***(McElwee and Sinclair, 2008)***.

In healthy individuals, shedding normally occurs during the subsequent anagen growth phase as a new hair fiber is produced. In the development of alopecia, exogen occurs in advance of renewed anagen growth, leaving a HF devoid of visible hair fiber, a state called kenogen ***(Alkhalifah et al., 2010).***

In AA, significant disruption of the hair growth cycle clearly occurs depending on the pattern, severity, and duration of AA in each patient as follows:

- First, the anagen phase of a HF can become inflamed and maintained in a dystrophic anagen state, unable to produce hair fiber of significant size or integrity ***(Freyschmidt-Paul et al., 2008)***.

- When there is a greater intensity of inflammation, the HFs may be forced into a telogen phase and may then cycle through multiple anagen and telogen phases of brief duration. Correspondingly, inflammatory cell infiltration occurs in early anagen follicles without migration to draining lymph nodes as follicles capitulate and return to telogen ***(Vogt et al., 2008)***.
- Finally, when AA is chronic, the HFs tend to persist in a prolonged telogen phase without an apparent attempt to return to an anagen growth phase (Figure 1) ***(Whiting, 2003; Freyschmidt-Paul et al., 2008; Vogt et al., 2008)***.

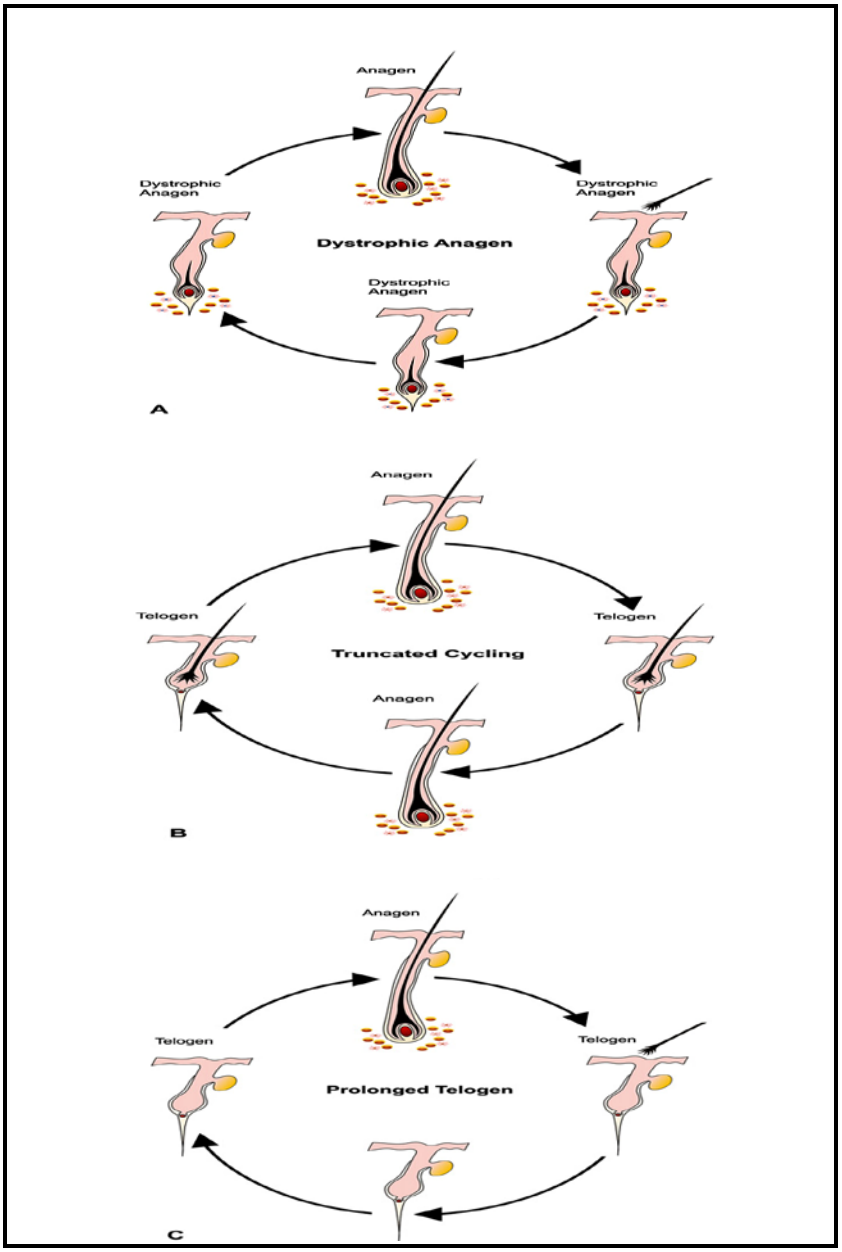

Figure (1): Hair growth cycle patterns in AA: A. HFs held in dystrophic anagen by mild inflammatory insult unable to produce significant hair fiber. B. Anagen growth phases truncated by moderate inflammatory insult resulting in rapid cycling and brief hair fiber growth. C. HFs enter prolonged telogen dormancy with development of chronic AA ***(Whiting, 2003).***

B) Genetic factors:

Alopecia areata, similar to other autoimmune diseases, is polygenic i.e. there are multiple susceptibility genes that interact with environmental factors ***(Duncan et al., 2013).***

- ***Histocompatibility leukocyte antigen associations:***

The Histocompatibility Leukocyte Antigen (HLA) system is the name of the antigens encoded by the Major Histocompatability Complex (MHC) in humans; the super locus containing a large number of genes related to immune system function in humans. This group of genes resides on chromosome 6, and encodes cell-surface antigen-presenting proteins and many other genes. Different classes of MHC antigens are essential elements in different immune functions ***(Valluri et al., 2005).***

The aetiology of AA and its association with other autoimmune conditions had been investigated for decades, among which HLA associations have been the focus of investigation ***(Haida et al., 2013).***

Studies have indicated a strong association between HLA antigens and AA ***(Risch, 2000)***. HLA class I molecules are expressed on virtually all nucleated cells and present antigens to CD8+ T cells. Multiple HLA- I (A, B, C) alleles have been identified in different groups of AA patients ***(Lu et al., 2006)***.

HLA class II molecules have three main subclasses (DR, DQ, and DP); they are found on specific immune cells

including B cells, activated T cells and macrophages, keratinocytes and dendritic cells. HLA-II molecules present antigens to CD4+ T cells ***(Hordinsky and Ericson, 2004).***

Strong associations have been established between AA and HLA class II genes: HLA-DQB1*0301 (DQ7), HLA-DQB1*03 (DQ3), and HLA-DRB1*1104 (DR11). HLA-DQB1*03 appears to be a susceptibility HLA marker for all forms of AA, whereas the HLA alleles DRB1*0401 (DR4) and HLADQB1*0301 (DQ7) are considered markers for severe longstanding AT/ AU ***(Martinez-Mir et al., 2007)***. An increase in the frequency of DQ3 which was greater in AT/AU than in patchy alopecia was found ***(Barahmani et al., 2010; Forstbauer et al., 2012).***

Notch proteins are cell surface receptors of short range intercellular signaling. Notch genes (Notch1-4) are important in adult tissues in a variety of situations, including angiogenesis, hair growth and T-cell maturation ***(Leong et al., 2002)***. The human Notch4 gene is located on chromosome 6p21.3. Several associations have been reported between Notch4 gene polymorphisms and mild to severe AA, particulary with polymorphisms at positions +1297 and +3063 ***(Deftos and Bevan, 2000; Tazi-Ahnini et al., 2003)***.

- Chromosomal linkage studies:

Different analysis studies about AA susceptibility in human population suggested potential gene loci located on chromosomes 2, 6, 14, and 21 ***(Galbraith et al., 1984; Tarlow et al., 1994; Colombe et al., 1995).*** Moreover, there is an increased frequency of AA in Down's syndrome with up to 9%

of patients affected because the Down's syndrome region on chromosome 21 may potentially include genes involved in the pathogenesis of AA ***(Tazi-Ahnini et al., 2000; Sureshbabu et al., 2011).***

Familial clustering is a common phenomenon in autoimmune diseases and in multifactorial conditions ***(Kurtev and Iliev, 2005).*** It has been suggested that AA is inherited as a multifactorial trait ***(Alzolibani et al., 2012).*** Researchers have also proposed that this is an autosomal dominant condition with limited penetrance ***(Kurtev and Iliev, 2005).***

De Andrade et al. (2000) examined the sex of individuals from 72 affected sib pairs with AA or AT/AU, the incidence was similar among sisters and brothers. They concluded that AA is not related to X-linked inheritance and it affects both sexes equally.

The influence of family history on occurrence risk of AA has also been reported. There has been a wide range reported in family histories, from 10% to 50% of families ***(Xiao et al., 2006).*** In a sample size of 348 severely affected patients, researchers reported that 16% of participants had a first-degree relative with AA, 7% had one affected parent, whereas 3% had an affected sibling ***(Harper, 1998).*** Others reported a positive family history in 42% of patients in a sample size of 800 ***(Kurtev and Iliev, 2005).*** In a small study involving 36 families, positive family history was reported in 50% of them. The proportion of affected first-, second-, and third-degree relatives revealed that the

risk in first-degree relatives was the highest (13.9%), followed by second-degree (4.2%), and third-degree relatives (1%) ***(Harper, 1998).*** Thus, as a multifactorial condition, the risk of AA is highest in the closest relatives, and falls with more distant relationships ***(De Andrade et al., 2000).***

Another way to approach the inheritance of AA is to study identical (monozygotic) twins. AA has been reported in sets of identical twins. Results revealed that among 13 sets of identical twins, 6 male sets were concordant for AA or AT/AU and 7 sets were discordant, including 4 male and 3 female-sets ***(Martinez-Mir et al., 2007).***

C) Autoimmune pathogenesis:

Alopecia areata is believed to be due to an anti-hair bulb autoimmune process in which CD4 and CD8 lymphocytes affect the peribulbar area ***(Abramovits and Losornio, 2006).*** Strong direct and indirect evidence supports that AA is an organ-specific autoimmune disease:

- Alopecia areata is a T-lymphocyte mediated autoimmune condition that occurs in genetically susceptible individuals ***(Petukhova et al., 2010)***. T lymphocytes that have been shown to be oligoclonal and autoreactive are predominantly present in the peribulbar inflammatory infiltrate ***(Hordinsky and Ericson, 2004).***

- Alopecia areata frequently occurs in association with other autoimmune diseases, such as thyroiditis and vitiligo ***(Thomas and Kadyan, 2008).***
- Lesional scalp from AA patients grafted onto nude mice regrows hair coincident with a loss of infiltrating lymphocytes from the graft ***(Kalish and Gilhar, 2003).***
- High levels of autoantibodies to follicular structures have been found in both human and animal models of AA ***(Sundberg and King, 1996).***
- The beneficial use of immune modulating drugs, including corticosteroids and contact sensitizers has been found in the management of AA ***(Hordinsky and Ericson, 2004).***

- Immune privilege:

The HF has a distinct immune system that differs from its surrounding skin ***(Madani and Shapiro, 2000).*** The anagen hair bulb meets the criteria of an immunoprivileged tissue, of which the anterior eye chamber, testis, brain, and fetotrophoblast are the best studied examples. They sequester auto- or alloantigens from immune recognition. Peripheral tolerance may also be induced against auto- and/or alloantigens that escape from such territories of relative immune privilege ***(Gilhar et al., 2007).***

This immune privilege is generated and maintained by a number of mechanisms including:

- Down-regulation or absence of MHC class Ia expression.
- Local production of potent immunosuppressants such as transforming growth factor (TGF)-ß1, interleukin (IL)-10 and melanocyte stimulating hormone (MSH)
- Functional impairment of antigen presenting cells (APC)s
- Absence of lymphatics
- Construction of extracellular matrix barriers to hinder immune cell trafficking
- Expression of non-classical MHC class Ib molecules

(Ito et al., 2004)

It has even been speculated that the rich possession of the HF's connective tissue sheath with mast cells may contribute to maintaining a low-level constitutive immune privilege of this skin appendage ***(Waldmann, 2006)***. This immune privilege serves mainly to sequester anagen-associated autoantigens from immune recognition by autoreactive CD8+ T cells ***(Paus et al., 2003).***

An **immune privilege collapse** model was proposed to explain autoimmunity in AA. In this model, infections, bacterial superantigens, or follicular damage trigger the release of IFN-γ which induces expression of MHC class I molecules on follicular cells. This leads to the induction of both CD8 positive cytotoxic cells and MHC class II molecules, leading to induction of CD4 helper, and then to downstream autoimmune phenomenon with generation of autoreactive T cells. Eventually there is spread of the immune response with antibodies, macrophages, expression of factor of apoptosis signal ligand (Fas L), apoptosis and

damage to follicular cells (Figure 2) ***(Norris, 2004; Rudnicka and Lukomska, 2012).***

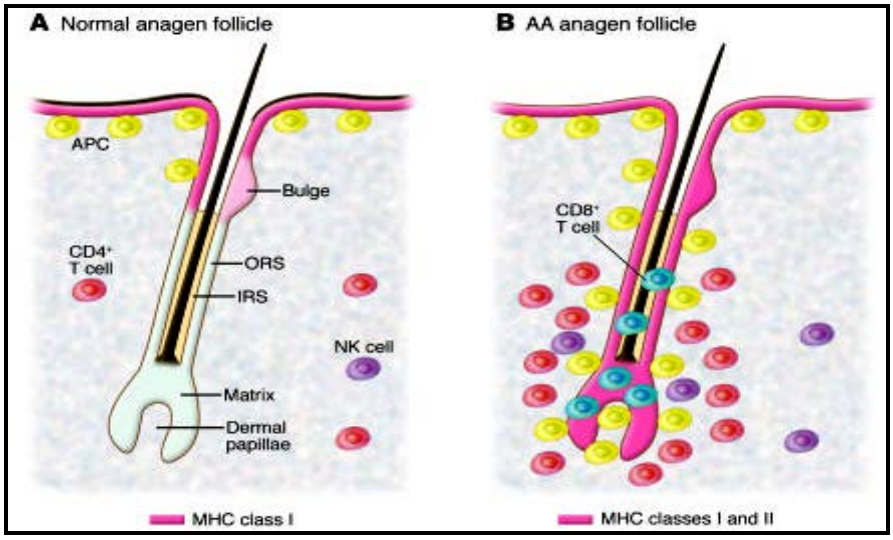

Figure (2): A model of immune privilege collapse in AA pathogenesis *(Gilhar et al., 2007).*

There is evidence that anti-HF antibodies that are modulated during the disease process, can occur before clinically detectable hair loss, and may be reduced in titer during successful treatment ***(Kutner and Friedman, 2013).***

It was suggested that the autoantibodies directed against follicular autoantigens are markers for CD4+ T cell recognition without having a direct role in disease pathogenesis ***(Gilhar et al., 2002; Zhang et al., 2013).*** They play a role in secondary immune response cascades that may be triggered by the primary immunopathogenesis events ***(Paus et al., 1994; Paus et al., 2005).***

Cytokines and cellular factors like α-MSH, TGF- ß1, insulin like growth factor-1 (IGF)-1 in addition to NK cell suppression are