INTRODUCTION

Alopecia areata (AA) is characterized by nonscarring hair loss on the scalp or any hair-bearing surface. A wide range of clinical presentations can occur ranging from a single patch of hair loss to complete loss of hair on the scalp (alopecia totalis, AT) or the entire body (alopecia universalis, AU). The estimated lifetime risk of developing AA is 1.7% ***(Alex et al., 2004)*.**

The etiology of AA is unknown, although both genetic factors and environmental agents are thought to contribute to the immune disregulation leading to the final pathways of the disease. Basic research has established AA as a T cell-mediated autoimmune disease with a type 1 cytokine pattern and has clarified many of its genetic, cellular, and molecular aspects.

Perifollicular and intrafollicular mononuclear cell infiltrates directed at anagen hair bulbs are characteristic and striking histological features in early AA. The inflammatory infiltrate is composed predominantly of activated CD4+ and CD8+ T cells, together with macrophages and Langerhans cells ***(Carroll et al., 2002)***.

Tumor necrosis factor-alpha (TNF-α) possesses multifunctional activities and is one of the most important proinflammatory and proimmune cytokines. It is a potent inhibitor of hair follicle growth. An in-vitro study reported that

the histology of hair follicles maintained with inhibitory doses of TNF-α showed the following changes: condensation and distortion of the dermal papilla, marked vacuolation of the hair follicle matrix, abnormal keratinization of the follicle bulb and inner root sheath, disruption of follicular melanocytes and the presence of melanin granules within the dermal papilla ***(Philpott et al., 1996).*** However, there are some reports of failure of these TNF-α inhibitors in controlling AA ***(Price, 2003).***

Hypothesis:

Since TNF-α has been shown to be inhibitory to hair follicle growth in the in-vitro studies and the changes in hair follicles incubated with TNF-α are similar to those reported in alopecia areata, we suggest that TNF-α may play an important role in the pathophysiology of inflammatory hair disease.

AIM OF THE WORK

This Thesis is conducted to estaminate the serum level of tumor necrosis factor alpha (TNF-α) to assess its possible role in AA thus paving the way for the development of new and safe targeted therapy for AA.

Chapter (1)

ALOPECIA AREATA

I- Epidemiology:

Alopecia areata (AA) is a heterogeneous disease characterized by non scarring hair loss on the scalp or any hair- bearing surface. Prevalence in the general population is 0.1-0.2%. The lifetime risk of developing AA is estimated to be 1.7%. It is responsible for 0.7-3% of patients seen by dermatologists. All races are affected equally by AA; no increase in prevalence has been found in a particular ethnic group. Data concerning the sex ratio for AA vary slightly in the literature. In one study including 736 patients, a male-to-female ratio of 1:1 was reported. In another study on a smaller number of patients, a slight female preponderance was seen. AA can occur at any age from birth to the late decades of life. Congenital cases have been reported. Peak incidence appears to occur from age 15-29 years. As many as 44% of people with AA have onset at younger than 20 years, onset in patients older than 40 years is seen in less than 30% of patients of AA ***(Alex et al., 2004)*.**

II-Clinical Picture:

The natural history of AA is unpredictable. The condition usually is localized when it first appears. Of patients with AA, 80% have only a single patch, AA most often

affects the scalp (66.8-95%); however, it can affect any hair-bearing area e.g. beard, eyebrows or extremities. Localized AA (<50% involvement) is usually self-limited ***(Tang et al., 2004)***.

Alopecia areata is most often asymptomatic, but some patients experience a burning sensation or pruritus in the affected area.

Presence of smooth normal-colored alopecic patches is characteristic. Typically, lesions of AA consists of one or more round smooth patches in which the scalp feels slightly depressed because of loss of the supportive effects of the hair shafts. Presence of exclamation point hairs (i.e., hairs tapered near proximal end) is pathognomonic but is not always found. Positive pull test at the periphery of a plaque usually indicates that the disease is active, and further hair loss can be expected ***(Whiting, 2003)***.

A reticular pattern occurs when hair loss is more extensive and the patches coalesce. An ophiasis pattern occurs when the hair loss is localized to the sides and lower back of the scalp. Extensive AA (>50% involvement) is less common. Alopecia totalis (AT) i.e. loss of all scalp hair or alopecia universalis (AU) i.e. loss of all scalp and body hair are reported to occur at some point in 7% of patients.

Episodes of localized patchy AA usually are self-limited; spontaneous regrowth occurs in most patients within a few months, with or without treatment. In 30% of patients with AT,

complete hair loss occurred within 6 months after onset of disease. Others reported a mean progression period to AT of 4 months after onset. The natural evolution of AT is unpredictable, but recurrences of AA are expected ***(Tang et al., 2004)***.

Clinical Associations:

Atopic dermatitis is seen in 9-26% of patients with AA. Some authors have found atopy to be a poor prognostic factor for AA with more severe AA correlating with atopy ***(Kasumagić- Halilović and Prohić, 2008).*** The prevalence of thyroid disease varies among studies from 0.85-14.7%. The incidence of thyroid disease in control subjects is estimated to be 0.17-2%. Collagen- vascular diseases have been found in 0.6-2% of patients with AA, while the incidence in control subjects is 0.17%. The incidence of AA in 39 patients with lupus erythematous was 10% in contrast to 0.42% of general dermatologic patients. Vitilligo is seen with an incidence varying from 1.8-3% compared with 0.3% in control subjects ***(Werth et al., 1992).***

Diabetes mellitus was found to be more common in control subjects (1.4%) than in patients with AA (0.4%). The occurrence of AA may protect against the appearance of type I diabetes mellitus ***(Wang et al., 1994).*** Alopecia areata is seen in 6-8.8% of patients with Down syndrome. The high frequency of AA in patients with Down syndrome suggests that a genetic linkage for AA may exist on chromosome 21 ***(Barahamani et al., 2002).***

III- Aetiopathogenesis:

The pathogensis 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 ***(Norris, 2004).***

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).***

1- Genetics:

Many factors favor a genetic predisposition for AA. The frequency of positive family history for AA in affected patients has been estimated to be 10-40%. Reports of AA occurring in twins are of interest, with concordance rate of up to 55% in identical twins. There is a significantly higher incidence of a family history in patients with early onset of AA. Familial incidence of AA has been reported to be 37% in patients who had their first patch by 30 years of age and 7% with the first patch after 30 years of age ***(Madani and Shapiro, 2000).***

Within the general population, AA does not segregate as a Mendelian, monogenic trait. It is a continuous trait with varying degrees of hair loss within the affected population ***(McElwee et al., 2001).*** This suggests that AA expression

involves a complex interaction of multiple genes and is most likely a polygenic disease where several, potentially identifiable, major genes affect disease susceptibility and minor severity modifying genes may further affect the phenotype.

The role of environmental factors in initiating or triggering the condition is yet to be determined ***(McDonagh and Tazi-Ahnini, 2002).***

Several genes have been studied and a large amount of research has focused on human leukocyte antigen (HLA). Studies demonstrated that HLA DQ3 was found in more than 80% of patients with AA, which suggests that it can be a marker for general susceptibility to AA ***(Marques Da Costa et al., 2006).*** The studies also found that HLA DQ7 and HLA DR4 were present more in patients with AT and AU. Interleukin (IL)-1 cluster genes, mainly the IL-1 receptor antagonist, show a strong association with disease severity in AA and a number of other autoimmune and inflammatory diseases ***(Akar et al., 2002).*** The functional R620W variant of the protein tyrosine phosphatase nonreceptor 22 gene (PTPN22) is a general risk factor in AA with the strongest effect observed among patients with a severe type of AA, a positive family history or an early onset of disease ***(Betz et al., 2008).*** Recent studies have shown that genetic variants on the TRAF1/C5 locus (tumor necrosis factor receptor-associated factor 1, complement component 5) is involved in the aetiology of familial and severe AA ***(Redler et al., 2010).***

The association of AA with Down's syndrome ***(Barahamani et al., 2002),*** the high frequency of AA in autoimmune polyglandular syndrome type I due to mutations (single nucleotide polymorphisms) of the autoimmune regulator (AIRE) gene on chromosome 21q22.3 ***(Tazi-Ahnini et al., 2008)*** and the finding of association with MX1, another gene in the Down's syndrome region of chromosome 21 indicate this area of the genome as a promising target for future-family based investigations ***(Barahamani et al., 2002).*** Several studies have confirmed the association between AA and the atopic state, with more severe AA correlating with atopy ***(Kasumagić-Halilović and Prohić, 2008).***

Genome-wide searches have been utilized for identification of chromosomal regions associated with disease risk. The identification of chromosomal regions with such genome-wide screens is only a first step, and more additional effort is required to map the risk to specific genes. In conclusion, many studies reveal that AA is a polygenic disease, with certain genes correlated with susceptibility and others with severity. Most likely, there is an interaction between genetic and environmental factors that trigger the disease ***(Ying, 2007).***

2- Autoimmunity:

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 support that AA is an organ-specific autoimmune disease;

- T lymphocytes that have been shown to be oligoclonal and autoreactive are predominantly present in the peribulbar inflammatory infiltrate.
- AA frequently occurs in association with other autoimmune diseases, such as thyroiditis and vitiligo.
- 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 multiple structures of anagen hair follicles in AA patients.
- The beneficial use of immune modulating drugs, including corticosteroids and contact sensitizers in the management of AA ***(Hordinsky and Ericson 2004).***

Immune privilege:

The hair follicle 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 major histocompatibility complex (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.
- 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)*.**

Also, it has even been speculated that the rich endowment of the hair follicle'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)*.**

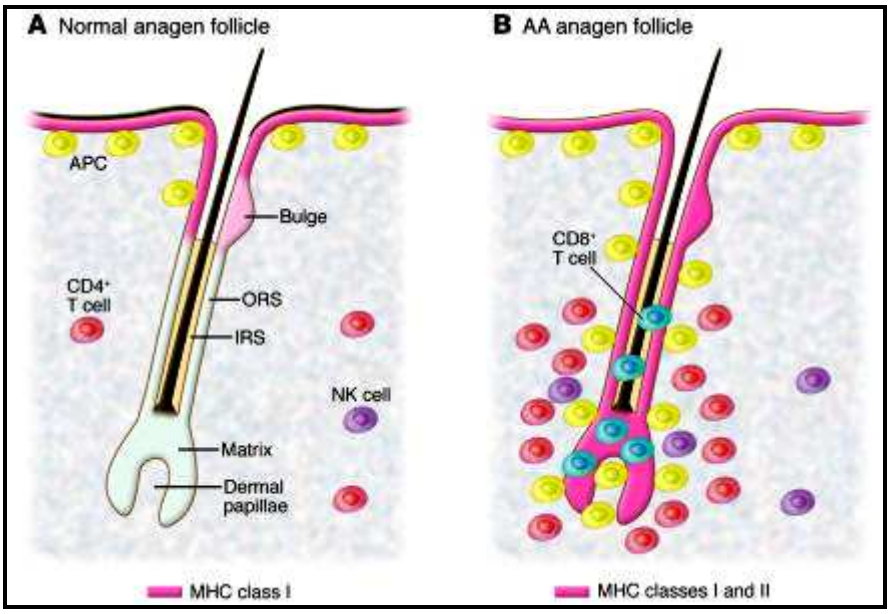

Figure (1): ***(Gilhar et al., 2007)***.

(A) A normal anagen (growing) hair follicle and **(B)** a hair follicle in AA are shown. MHC class I molecules are expressed on the epidermis, and on the most superficial (distal) portion of the normal hair follicle epithelium. The inferior (proximal) portions of the hair follicle are immune privileged and deficient in expression of MHC classes I and II as well as APCs. By contrast, the AA anagen hair follicle expresses MHC class I and II molecules throughout the follicular epithelium, including the portion adjacent to the dermal papilla of the hair follicle. Active AA also exhibits a perifollicular infiltrate of $CD4^+$ T cells and an intrafollicular infiltrate of $CD8^+$ T cells. IRS, inner root sheath; ORS, outer root sheath.

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 INF-γ which induces expression of MHC class I molecules on follicular cells, leading 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 antibody, macrophages, expression of Fas L (Factor of apoptosis signal ligand), apoptosis and damage to follicular cells ***(Norris, 2004).*** Moreover, there is evidence that anti-hair follicle antibodies that are modulated during the disease process, can occur before clinically detectable hair loss, and may be reduced in titer during successful treatment ***(Tobin, 2003).*** Also, RNA analyzed from biopsies of human AA patients showed increased expression of the CD1a Langerhans cell specific marker, suggesting that an ongoing immune response involving APC may also be involved in human AA ***(Carroll et al., 2002)***.

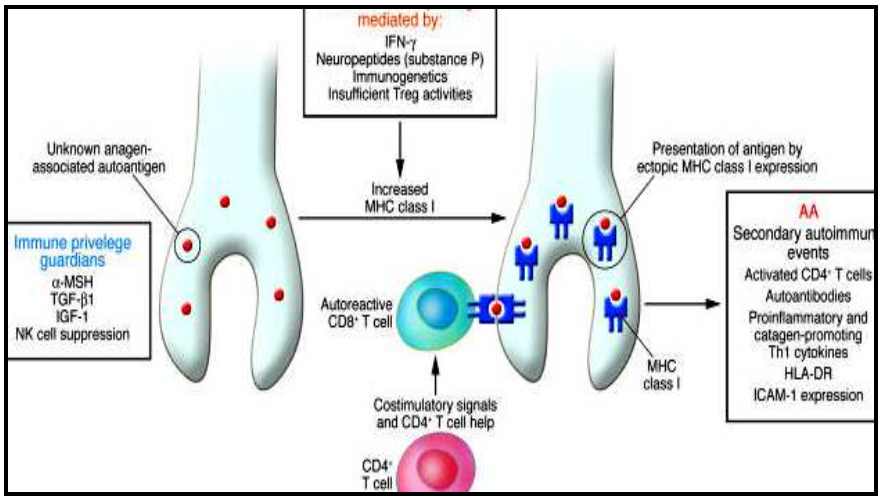

Figure (2): *(Gilhar et al., 2007).*

Proposed pathogenesis of AA.

Cytokines and cellular factors responsible for maintaining immune privilege are listed in the left box. Those factors believed to mediate loss of immune privilege and initiation of disease are listed in the middle box. Loss of immune privilege is associated with expression of MHC class I molecules, which are capable of presenting hair follicle autoantigens to T lymphocytes. Secondary autoimmune amplification circuits that may help establish or amplify the pathology are listed in the right box.

Researches suggest a self contained disease cycle involving four key events: (1) Failure of the putative anagen stage hair follicle immune privilege and exposure of hair follicle located AA inciting epitopes to the immune system; (2) Antigen presentation, costimulation, and activation of responsive lymphocytes by antigen presenting cells; (3) Activated inflammatory cell migration to, and infiltration of hair follicles; (4) The subsequent disruptive actions of the inflammatory cell infiltrate on the hair follicles ***(McElwee et al., 2003).***

Affected hair follicles terminate the anagen phase prematurely and regress via the induction of massive apoptosis of the lower portion of the follicle (catagen phase), resulting in a resting hair follicle (telogen phase). Hair follicles may then reenter the anagen phase, but in the presence of lymphocytic infiltrate, anagen is terminated prematurely, resulting in miniaturized hair follicles. AA represents a disorder of hair follicle cycling in a dual sense: It almost exclusively attacks anagen hair follicles and then greatly disturbs hair follicle cycling as such by "catapulting" anagen follicles into catagen. Therefore, one potential therapy proposed to halt disease progression involves arresting hair follicles in the telogen stage of the hair cycle ***(Paus et al., 2005).***

The humoral factors, especially autoantibodies, may tell us something about the hair follicle (HF) antigens targeted in AA, and/or may even be important in perpetuating the disease.

Anti-HF antibodies have been detected by direct immunofluoresence (DIF), indirect immunofluoresence (IIF) an By immunoblotting analysis of serum antibodies ***(Tobin et al., 1998).*** DIF revealed deposits of Immunoglobulin (Ig) G predominantly on the lower HF glassy membrane/basal lamina. Circulating IgG antibodies reacted with multiple components of anagen HF. The precortex, the site of significant keratinocyte and melanocyte differentiation, was the most commonly targeted component. In this way, the humoral factors may disturb cell differentiation and compromise HF formation ***(Tobin et al., 2003).***

Candidate autoantigens that have been identified include the **44/46 kDa hair-specific keratin** (expressed in the precortical zone of anagen hair follicles) and **trichohyalin** (an important intermediate filament-associated protein expressed in the IRS of the growing hair follicle) which is believed to be necessary for correct alignment of keratin filaments of the IRS and medulla. It is likely that defects of IRS differentiation (an anagen specific event) may result in defective HF formation ***(Tobin et al., 2003).*** There is also evidence that AA preferentially target pigmented hair and spare white hair. AA-like lesions can be induced in mice by inducing CD8 (+)T-cell mediated immunity to **hair follicle melanocytes.** Regrowing hair is often initially white, indicating a selective targeting of hair bulb melanocytes ***(Nagai et al., 2006)***.