# INTRODUCTION

Alopecia areata (AA) is the most frequent cause of inflammation-induced hair loss **(*Amos et al., 2012*).** AA is a common cause of non scarring alopecia that occurs in a patchy, confluent or diffuse pattern. It may involve loss of hair from some or all areas of the body, usually from the scalp **(*Odom et al., 2006*).**

It has been hypothesized that AA is an organ–specific autoimmune disease with genetic predisposition and an environmental trigger **(*McDongah and Tazi, 2002*)**. There is an observed association between AA and various autoimmune disorders such as autoimmune thyroiditis, vitiligo, lichen planus, morphea, atopic dermatitis, pernicious anemia and diabetes mellitus ***(Amos et al., 2006).***

There is accumulating evidence suggesting that T cells and cytokines play an important role in the pathogenesis of AA **(*Ercan et al., 2004*).** The characteristic presence of lymphocytes around lesional hair follicles has also served as evidence that abnormal cell-mediated immune responses may be involved in AA. Both CD4+ and CD8+ T lymphocytes are observed peri-and intra-follicular with predominance of CD4+ T cells ***(Gilhar and Kalish, 2006).***

The development of an inappropriate immune response may stem from the inflammatory environment of the inciting event. Defects in cytokines production or signaling can lead to such autoimmune diseases **(*Randall, 2001*).** Cytokines contributing to the pathogenesis of these autoimmune disorders have been described. Recent studies have shown an association between high mobility group box 1 (HMGB1) and chronic inflammation and autoimmunity **(*Sarah et al., 2010*).**

HMGB1 is a conserved protein located in all mammalian nuclei at high concentrations. It acts as a proinflammatory cytokine in both acute and chronic inflammatory conditions such as septic shock, acute lung injury, and rheumatoid arthritis ***(Dumitriu et al., 2006).*** It is actively released from lipopolysaccharide (LPS)-, tumor necrosis factor-alfa (TNF-α)-, and interleukin (IL)-1-activated monocytes and macrophages and from other cell types. It can also be passively released from damaged dying cells during necrosis, and during the late phase of apoptosis. Extracellular HMGB1 exerts its biological actions by binding to cell-surface receptors such as receptor of advanced glycation end products (RAGE), Toll-like receptor 2 (TLR2), Toll-like receptor 4 (TLR4), and the intracellular receptor Toll-like receptor 9 (TLR9) ***(Abdulahad et al., 2011).***

There is growing interest in the association of HMGB1 with autoimmune disorders, in which it serves as a significant target antigen. Increased HMGB1 expression has been detected in several autoimmune disorders, including systemic lupus erythematosus (SLE), Sjögren's syndrome, and rheumatoid arthritis (RA) ***(Karin et al., 2005).*** In addition, regarding the cutaneous manifestations of autoimmune disease, studies have revealed increased expression of HMGB1in systemic sclerosis and cutaneous lupus erythematosus ***(Yoshizaki et al., 2009).***

Alopecia areata is considered a tissue-specific autoimmune disease and so it is possible that HMGB1 may also contribute to the pathogenesis of AA ***(Lee et al., 2013).***

# Aim of the Work

The aim of this work is to investigate the role of HMGB1 in the pathogenesis of AA which offers a new potential therapeutic target for the treatment of AA.

# Chapter (1): Alopecia Areata

## Definition:

Alopecia areata (AA) is a common form of non-scarring alopecia involving the scalp and/or body, characterized by hair loss without any clinical inflammatory signs ***(Seetharam, 2013).***

AA can present with different clinical manifestations varying from reversible patchy hair loss to complete baldness or complete body hair loss ***(Finner, 2011).***

## Epidemiology:

AA is a common non-scarring alopecia that affects 0.1-0.2% of the general population and accounts for 0.7-3% of all cases seen in dermatology practice ***(Perera et al., 2014).***

It affects both children and adults and hair of all colors. Although the disorder is uncommon in children under 3 years of age, most patients are relatively young: up to 66% are younger than 30 years of age, and only 20% are older than 40 years of age ***(Gilhar et al., 2012).***

There is generally no sex predilection, but more men were found to be affected in one study involving a group of subjects who were 21 to 30 years of age ***(Kyriakis et al., 2009).***

## Aetiology:

It was found that AA is most likely an organ-specific autoimmune disease. Gene association studies confirm a genetic predisposition. Environmental triggers have been postulated, but none have been confirmed ***(Perera et al., 2014).***

The etiology of AA has eluded investigators for years and therefore a multitude of associations have been proposed by researchers in the field of trichology. One of the strongest associations is with autoimmunity. This view has been supported by the occurrence of AA in association with other autoimmune disorders like vitiligo, lichen planus, morphea, atopic dermatitis, Hashimoto's thyroiditis, pernicious anemia and diabetes mellitus. More recently, it has been reported that there is a high prevalence of mood, adjustment, depressive and anxiety disorders in patients with AA (Fig.1) ***(Amin and Sachdeva, 2013).***

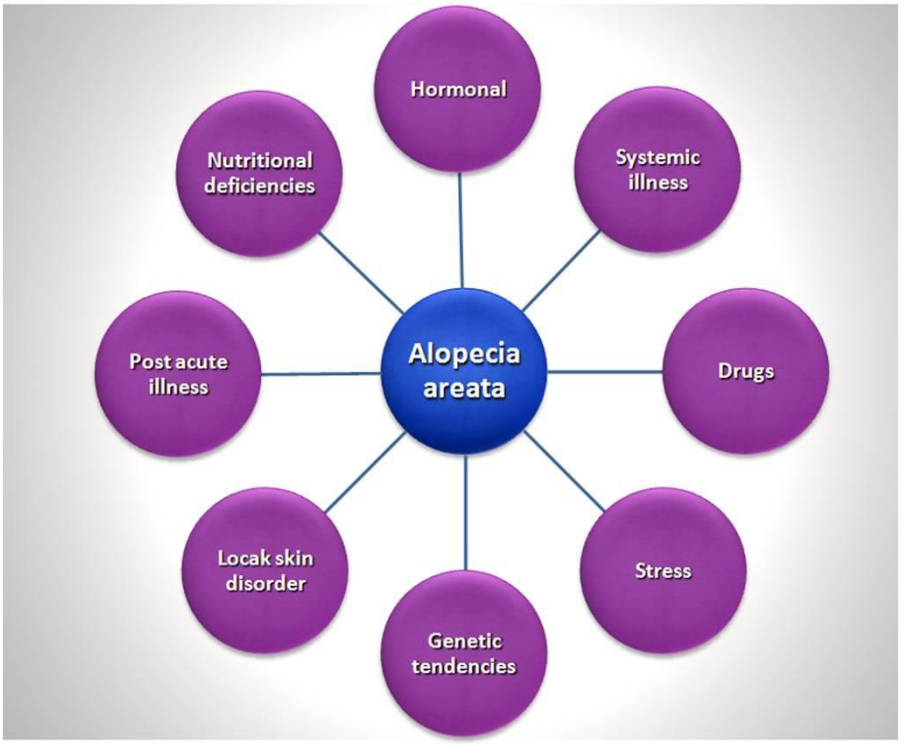


**Fig.(1):** Multifactorial etiology of alopecia areata ***(Amin and Sachdeva, 2013).***

## Predisposing Factors:

### A. Genetic factors:

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 ***(Madani and Shapiro, 2000).***

Family studies in AA have found that 28% of patients have at least one affected family member and the AA concordance rate in monozygotic twins ranges between 42 and 55% ***(Perera et al., 2014).***

Gene association studies have indicated that human leukocyte antigen (HLA) genes play a role in AA and multiple HLA class I (A, B, and C) alleles conferring susceptibility have been identified in different cohorts of AA patients. There is an aberrant expression of HLA antigens found within the follicles of AA affected patients. A much more consistent and stronger association between HLA class II alleles and AA development has been observed. HLA class II antigens are aberrantly highly expressed on AA affected hair follicles ***(Alkhalifah et al., 2010).***

HLA class I molecules are expressed on virtually all nucleated cells and platelets and present antigens to CD8+ T cells. 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, macrophages, keratinocytes, and dendritic cell and present peptides to CD4+ T cells. Because class II molecules are associated with antigen presentation, many studies have focused on this area of the HLA molecule. These associations with HLA-DR and HLA-DQ suggest a role for T cells in this disease as well as autoimmunity ***(Amin and Sachdeva, 2013).***

## B. Emotional stress:

There is a high prevalence of mood adjustment, and anxiety disorders in patients with AA ***(Wasserman et al., 2007).*** Murine stress models demonstrate nerve growth

factor (NGF), substance P and mast cell dependent inhibitory effect of stress on hair growth. In addition, NGF alone can prematurely induce catagen development ***(Peters et al., 2006).***

Acute psychic trauma before the onset of AA, higher number of stressful events in the 6 months preceding hair loss, higher prevalence of diagnosed psychiatric disorders, psychological factors and family situations in patients with AA have been reported ***(Madani and Shapiro, 2000).*** Acute emotional stress, such as death in the family or severe fright, is considered a factor precipitating or accentuating hair loss. However, there is no direct correlation found between the severity and extent of the loss of hair and the proportions of the emotional stress ***(Botchkarev, 2003).***

A possible explanation of the pathogenic mechanisms provoked by emotional conditions lies in the production of neuromediators capable of interfering with the immunity. Some studies have revealed a decrease in the expression of calcitonin gene related peptide (CGRP) and substance P in the scalp of alopecia areata patients. CGRP has an anti-inflammatory action, and its decrease in alopecia areata could favor the characteristic follicular inflammatory phenomena. Substance P is capable of inducing hair growth in mice and its decrease in alopecia areata could be a contributing factor to the reduced proliferation of pilar follicles ***(Amin and Sachdeva, 2013).***

## C. Atopy:

The frequency of atopy is significantly high in patients with AA ***(Kasumagic-Halilovic and Prohic, 2008).*** Studies reported frequencies of atopy in AA patients ranging from 1 % to 52% ***(Tan et al., 2002),*** although other studies reported atopy in 60.7% of patients ***(Goh et al., 2006).***

## D. Hormonal factors:

In a mouse model study, the development of AA was strongly associated with higher central and peripheral hypothalamic-pituitary-adrenal (HPA) tone. The AA affected mice had a significantly blunted systemic HPA response to acute physiologic stress and a decreased habituation response to constant psychological stress ***(Alkhalifah et al., 2010).***

***Arı et al., (2012)*** reported a possible relation between prolactin and certain autoimmune or lymphocyte associated diseases suggest that prolactin may also play part in progression of the autoimmune process which causes follicular damage in alopecia areata.

## E. Environmental factors:

It is likely that there are many potential environmental inputs with different factors involved in AA development for different individuals. Infectious agents and vaccinations have all been cited as possible triggers for AA. In the mouse model, dietary soy oil increases resistance to AA development, suggesting that diet might also play a role in AA susceptibility ***(Alkhalifah et al., 2010).***

A precipitating factor can be found in 15.1% of patients with AA. Major life events, febrile illnesses, drugs, pregnancy, trauma, and many other events have been reported, but no clear conclusions can be drawn. Furthermore, most patients with AA fail to report a triggering factor preceding episodes of hair loss ***(Bolduc et al., 2009).***

## Pathogenesis:

Hair follicles are the only organs in the human body that undergo extensive, lifelong, cyclic transformation. They switch from a period of very rapid growth, pigmentation, and hair-shaft production (anagen, the active-growth phase, with classification ranging from stages I to VI) to a short, apoptosis-driven phase of organ involution (catagen). After catagen, the hair follicle enters a period of relative quiescence (telogen) before it reenters anagen. This regenerative cycle is made possible by an abundance of keratinocyte and melanocyte stem cells located in the so-called bulge area (Fig.2a) ***(Gilhar et al., 2012).***

In the past, AA was believed to be of infectious or neurotrophic origin. Recent research studies have indicated that AA is an inflammation-driven disease and is likely an autoimmune disorder. The association of AA with other autoimmune diseases has been reported. The presence of inflammatory lymphocytes around and within affected hair follicles and the ability to promote hair regrowth with the use of immunosuppressive agents is consistent with an

autoimmune hypothesis. The infiltration of antigen presenting cells (APCs) such as macrophages and Langerhans cells both around and within the dystrophic hair follicles has also been observed. This is potentially consistent with a response to autoantigens within the hair follicles and attraction of these APCs ***(Alkhalifah et al., 2010).***

Autoimmunity is believed to play a central role in the development of AA. Patients with AA have been found to have an increased frequency of hair follicle-specific auto-antibodies. The inferior portion of the normal hair follicle is an "immune privileged" site which is protected from surveillance by T cells. Major histocompatibility complex (MHC) class I and II, molecules that bind and present pathogens to the immune system, are not expressed in normal hair follicle epithelium. In AA, however, this immune privileged site is compromised. There is an increased MHC I and II complexes and increased expression of adhesion molecules ICAM-2 and ELAM-1 surrounding the perivascular and peribulbar hair follicular epithelium ***(Perera et al., 2014).***

Although the physiological function of immune privilege with respect to hair follicles is not yet evident, we do know that several autoantigens associated with pigment production are highly immunogenic (as seen in vitiligo and halo nevi). Therefore, one plausible theory is that melanogenesis- associated autoantigens generated during active hair-shaft pigmentation pose a constitutive risk of

attracting autoreactive CD8+ T cells already present ***(Meyer et al., 2008).*** As with other tissues protected by classic immune privilege (e.g., the anterior chamber of the eye, the central nervous system, and the fetal trophoblast), down-regulation of MHC class I molecules may serve to reduce the risk that follicle-associated autoantigens will be presented to CD8+ T cells (Fig.2b) ***(Gilhar et al., 2012).***

This down-regulation of MHC class I molecules, however, entails the risk that the hair follicle may be attacked by natural killer (NK) cells, since NK cells are primed to recognize and eliminate MHC class I−negative cells. To reduce this risk, healthy hair follicles appear to downregulate the expression of ligands that stimulate the activation of NK-cell receptors (NKG2D) and secrete molecules that inhibit NK-cell and T-cell functions, such as transforming growth factors β1 and β2, α melanocyte−stimulating hormone, and macrophage migration inhibitory factor ***(Gilhar et al., 2012).***

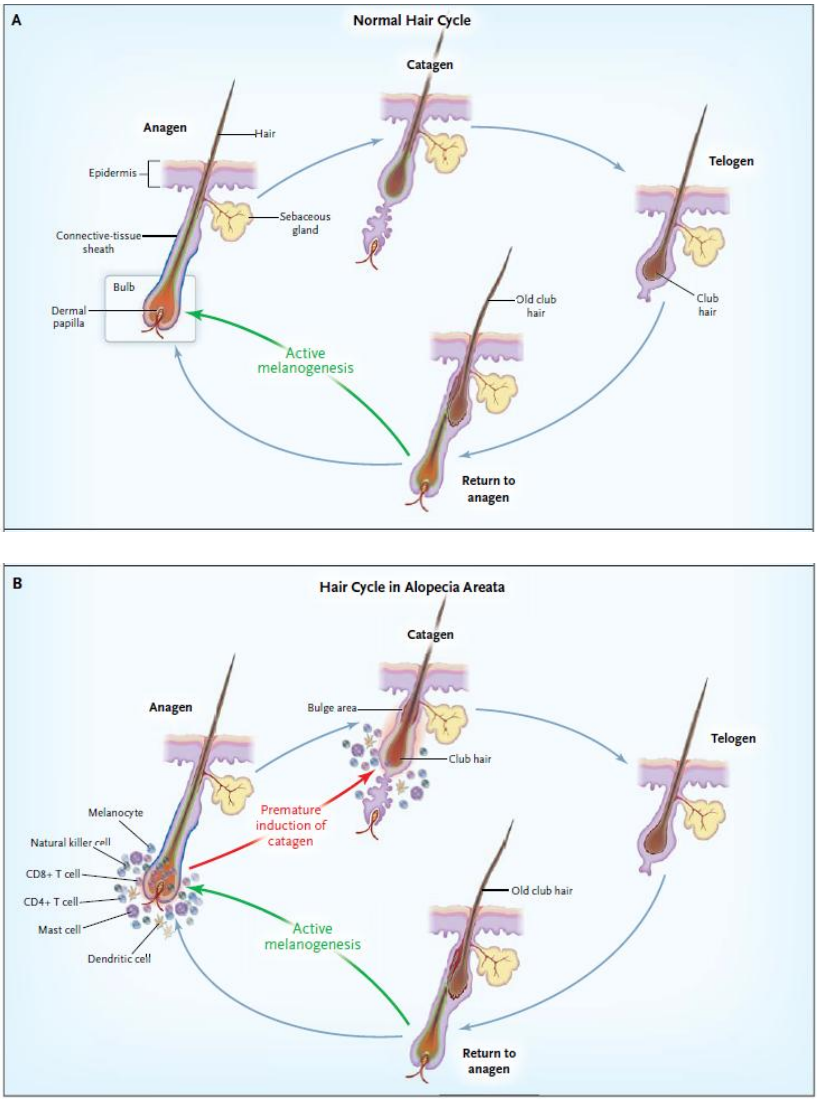


**Fig. (2 a, b):** The Normal Hair Cycle and Disordered Cycling in Alopecia Areata ***(Gilhar et al., 2012).***

## Histopathology:

The histopathological features vary depending on the stage of the disease. It can be divided into 4 stages: acute, subacute, and chronic and recovery ***(Perera et al., 2014).***

In the acute stage, Terminal hairs are surrounded by bulbar lymphocytes, which can take the classical (but not commonly observed) appearance of a 'swarm of bees' (Fig.3a) ***(Perera et al., 2014).***

In the subacute stage, large numbers of catagen hairs, followed by telogen hairs, can be observed. The percentage of catagen/telogen is markedly increased (Fig.3b) and often exceeds 50% of the total follicles. Some remnant inflammation may persist in or around fibrous streamers as the follicles ascend to telogen level ***(Alkhalifah et al., 2010).***

In the chronic stage, there are decreased terminal and increased miniaturized hairs. Inflammation may be variable. Immunofluorescence studies have shown deposits of C3, IgG and IgM on the basement membrane of the inferior part of the hair follicle ***(Perera et al., 2014).*** Chronic lesions are characterized by the presence of nanogen follicles (an intermediate stage between terminal and vellus anagen) (Fig.3.c) ***(Alkhalifah et al., 2010).***

In the recovery stage, the terminal to vellus ratio reverts to normal, the percentage of anagen hairs increases, and there is little or no inflammation ***(Alkhalifah et al., 2010).***