INTRODUCTION

Alopecia areata (AA) is one of the most difficult skin diseases to manage well. The pathogenesis of AA is still not fully understood and clinical phenotype and disease course is variable. Recently, the pathomechanism of AA has been thought to be a tissue-specific autoimmune disease and it has been speculated that the autoantigen is a melanogenesis-related protein, such as tyrosinase ***(Paus et al., 1993).***

It is the most common condition to cause inflammation induced hair loss ***(Gilhar et al., 2012).*** It is characterized by well demarcated patches of hair loss, which can progress to complete loss of hair from the scalp alopecia totalis (AT) or from the whole body in severe cases alopecia universalis (AU) ***(Wang et al., 2010).***

Its prevalence is 0.1% and its lifetime risk is about 2%. The disorder affects children, men, and women of all hair colors. Most patients are younger, although the disorder is uncommon below the age of 3 years. The highest prevalence is seen between the second and fourth decades of life. Up to 66% of patients are below 30 years old, while only 20% are older than 40 ***(Finner, 2011)***.

As most patients are relatively young and disease burden is commonly substantial, leading to overwhelming effects on the patient's quality of life and self-esteem. AA is considered an

organ-specific autoimmune disease stemming from loss of the hair follicle's (HFs) immune privilege; therefore, therapies are mostly immunosuppressive. Nevertheless, treatment is still a challenge in AA, and no treatment is either curative or preventive ***(Alkhalifa, 2011).***

Finding new therapies for this condition, and improving effectiveness of existing conditions, are therefore of utmost important. Platelet rich plasma (PRP) is an autologous preparation of platelets in concentrated plasma ***(Li, 2012).***

PRP was initially developed in the 1970s and first used in 1987 in an open heart surgery procedure. Since then doctors have used platelet rich plasma therapy since the mid-1990s to aid bone healing after spinal injury and soft tissue recovery following plastic surgery ***(Ferrari et al., 1987).***

Beginning in the 1990's, and likely for decades to come, growth factors have emerged as the "Holy Grail" in wound healing ***(Marx and Garg, 2005).***

It has been investigated in several disciplines in medicine for its role in wound healing especially orthopedics and dentistry ***(Rogers, 2012).***

Recently, it has also been found to be beneficial in dermatology, for example in acne scarring, wound healing and fat transplantation. It has also been shown to promote hair

survival and growth, both in vitro and in vivo ***(Uebel et al., 2006).***

The action of platelet plasma growth factors on the hair cycle has already been established. The growth factors contained in platelets of blood plasma include platelet derived growth factor, transforming growth factor-β, vascular endothelial growth factor, epidermal growth factor, connective tissue growth factor and fibroblast growth factor (FGF) ***(Uebel et al., 2006).***

They are known to activate the proliferative phase and transdifferentiation of hair and stem cells and hereby produce new follicular units. FGF is reported to promote the in vitro proliferation of papilla cells and hereby playing a key role in elongating the hair shaft ***(Katsuoka et al., 1987).***

When platelets become activated, the growth factors are released and act in tissue angiogenesis and healing process in contact with their respective receptors ***(Akiyama et al., 1996).*** In addition, their role on an implanted follicular unit has been described by Uebel et al. in 2006.

In the present study 20 AA otherwise healthy patients recruited and injected intardermally with PRP and follow up was done every month up to 9 months after the end of the study.

AIM OF THE WORK

To evaluate the efficacy and safety of Platelet Rich Plasma for treatment of AA as a new modality of therapy

Chapter One

ALOPECIA AREATA

Definition:

Alopecia areata (AA) is a nonscarring, autoimmune, inflammatory, hair loss on the scalp, and/or body ***(Wasserman et al., 2007).***

Epidemiology:

Alopecia areata (AA) is a common disease. The estimated prevalence of alopecia areata is approximately 1 in 1000 of general population, with a lifetime risk of approximately 2 percent ***(Madani and Shapiro, 2000).*** The disorder affects children, men, and women of all hair colors. Most patients are younger, although the disorder is uncommon below the age of 3 years. The highest prevalence is seen between the second and fourth decades of life. Up to 66% of patients are below 30 years old, while only 20% are older than 40 ***(Finner, 2011).***

Pathogenesis:

Introduction:

Alopecia areata is considered a tissue-restricted autoimmune disease. It commonly associated with other autoimmune diseases both within the affected persons and their families. Circulating antibodies against follicular components

are detected more frequently in people with AA ***(Tobin et al., 1997).***

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

Genetic susceptibility to the development of AA involves specific alleles of the human leukocyte antigen (HLA) region though other non-HLA genes are also likely to be involved. Susceptibility to the development of AA may be modified by environmental factors, including exposure to proinflammatory agents and possibly other modulators, including stress and diet ***(Rivitti, 2005).***

Pathogenic mechanisms in alopecia areata:-

Hair follicle growth cycling modulation in alopecia areata:

There are three key phases of the hair cycle: the growth phase (anagen), the regression phase (catagen) and the resting phase (telogen). The cycling of these phases is finely coordinated by the expression of hormones, cytokines, transcription factors, and their corresponding receptors and is carefully regulated through endocrine, paracrine, and autocrine routes. The disruption of these finely tuned pathways can result in the development of hair diseases. Exogen is a hair follicle

cycle event that describes the controlled shedding of club hair fibers ***(David, 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 the hair follicle devoid of visible hair fiber, a state called kenogen ***(McElwee and Sinclair, 2008).***

In AA, significant disruption of the hair growth cycle clearly occurs, but different perturbations in hair growth develope depending on the pattern, severity, and duration of AA in each patient. There are several possible presentations of AA as follow; At First, the anagen phase of a hair follicle 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 hair follicles 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 ***(David, 2008).***

Finally, when AA is chronic, the hair follicles tend to persist in a prolonged telogen phase without an apparent attempt to return to an anagen growth phase figure (1) ***(Whiting, 2003).***

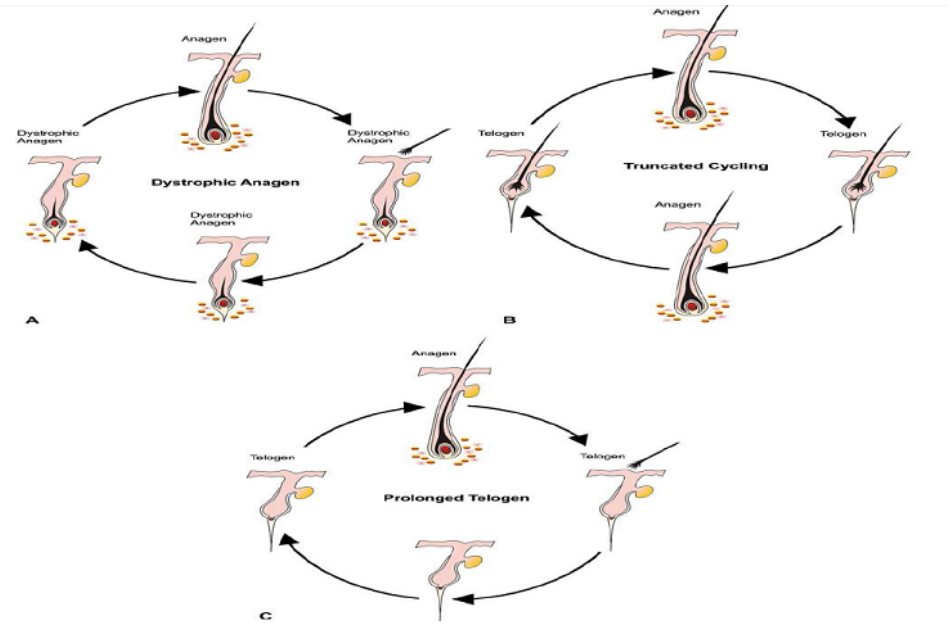

Fig. (1): Hair growth cycle patterns in alopecia areata.

A- Hair follicles 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- Hair follicles enter prolonged telogen dormancy with development of chronic alopecia areata ***(Whiting, 2003).***

Autoimmune activity in alopecia areata:

Alopecia areata is an important model of tissue specific autoimmune disease. There is evidence for loss of immune privilege coupled with T-cell mediated attack of hair follicle

autoantigens. There is also some evidence for the role of autoantibodies in the pathogenesis of AA ***(Gilhar and Kalish, 2006).***

Cell-mediated immunity:

A hallmark of AA is a peribulbar lymphocytic infiltrate that consists primarily of activated T-lymphocytes ***(Whiting, 2003& Gilhar et al., 2007).***

1. Hair follicle immune privilege:

One of the hypotheses for the development of AA is the hair follicle privilege collapse or the inappropriate presentation of antigens to the immune system during normal hair follicle cycling ***(Paus et al., 2005).***

The proximal (lower) hair follicular epithelium of normal hair follicles is deficient in expression of major histocompatibility complex-1 or 2 (MHC- I or II) molecules as well as numbers of dendritic antigen presenting cells thus; autoantigens are not recognized by CD8+ T –cells allowing normal hair growth. Also the anagen stage hair follicles retain immune privilege through the production of immunosuppressive cytokines; transforming growth factor beta (TGF-β), and insulin like growth factor-1 (IGF-1) ***(Ito et al., 2004).***

A breach in immune privilege and exposure of unique hair follicle antigens through several triggers, such as emotional

factors, skin microtrauma, or infectious agents aided by a possible underlying immune predisposition may result in targeting by the skin immune system ***(Paus et al., 2005).***

2. Role of T cells in alopecia areata:

Alopecia areata is a Th1 autoimmune condition mediated by both CD4+ and CD8+ T- cells ***(Gilhar et al., 1998).***

Histologically, the skin of mice and rats with AA shows an infiltration of CD4+ and CD8+ T cells, macrophages, and dendritic cells in and around the hair follicles ***(Zhang and Oliver, 1994).***

When AA affected mouse skin is grafted to severe combined imunodeficient (SCID) mice, in the absence of CD4+ and CD8+ T cells, hair regrowth is observed in grafted skin and no loss of hair is observed in the host skin ***(McElwee et al., 1998).*** This is a strong indication that CD4+ and CD8+ T cells are directly involved in the hair loss promotion mechanism. Further, partial restoration of hair growth is observed by depleting CD4+ or CD8+ T cell subsets by using monoclonal antibodies in AA affected mice ***(Lu et al., 2006).***

Most significantly, subcutaneous injection into normal haired mice of lymphocyte cell subsets isolated from AA affected mice shows that these cells induce the disease. CD8+ cells quickly induced localized patches of hair loss. CD4+ cells did not induce local hair loss, but they were shown to activate the host's immune system to promote multiple AA patches after

several weeks. It was concluded that CD8+ cells are the direct modulators of hair loss while CD4+ cells play a classic ‘‘helper’’ role in AA onset ***(McElwee et al., 2005).***

A further SCID-human mouse model has been developed. AA affected human skin regrows hair after grafting to SCID mice. Using a cell transfer method, hair loss can be reinduced by injecting patient CD4+ and CD8+ T cells into the human skin graft ***(Gilhar et al., 2002).***

3. Melanocyte autoantigens and alopecia areata:

Alopecia areata patients show an increased incidence of autoimmune diseases including pigmentary defects. Remarkably, white or greying hair follicles are relatively spared in AA while regrowing hair shafts are usually white before they become repigmented. The sudden appearance of fulminant AA affects mostly pigmented hair follicles. Thus, only pre-existing grey or white hair is observed. This phenomenon is known as overnight greying.

These are evidences that point to follicular melanocyte as a possible important target in the autoimmune process of AA ***(Gilhar and Kalish, 2006).***

Humoral immunity:

Circulating antibodies to follicular structures have been found in both human and animal models of AA, but they have not been found to be pathogenic in either. These autoantibodies are not specific and target multiple structures in anagen hair

follicles ***(Tobin et al., 1997)***. It had been suggested that these autoantibodies are a marker for CD4+ T cell recognition of hair follicles but their role in the pathogenesis of AA is not clear ***(Kalish and Gilhar, 2003)***. Moreover, hair-follicle-specific immunoglobulin G (IgG) autoantibodies have been found in increased concentrations in the peripheral blood of AA-affected individuals compared with non-affected humans ***(Tobin, 2003).***

Cytokines in alopecia areata:

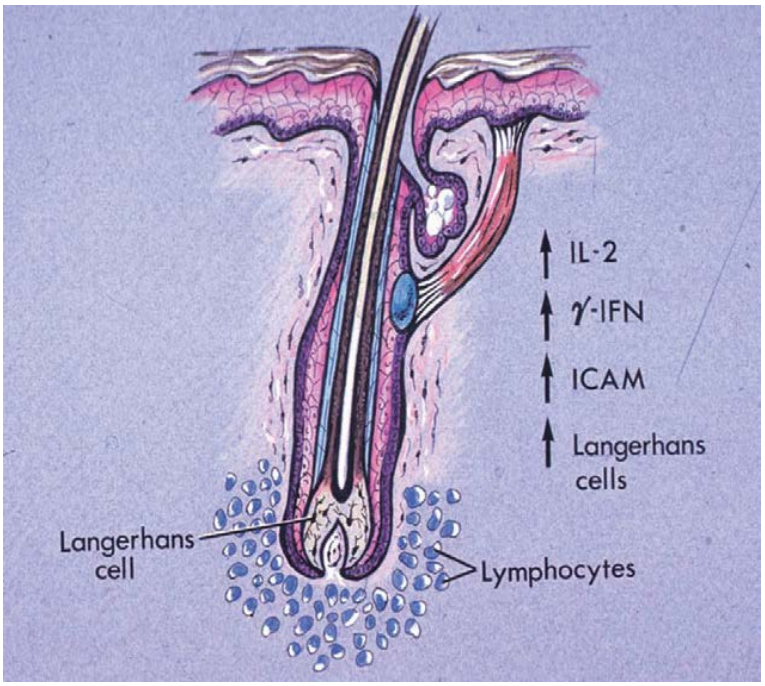

Fig. (2): Pathogenesis of alopecia areata. Langerhans cells present epitopes to peribulbar lymphocytes, followed by a cascade of immunological events with an increase of interleukin-2 (IL-2), gamma interferon (γ-IFN), and intercellular adhesion molecules (ICAM) (courtesy of Jerry Shapiro, Department of Dermatology and Skin Science, University of British Columbia,Vancouver, BC, Canada) ***(Otberg, 2011).***

Interfeon-gamma (IFN- γ):

Interferon-gamma is the main cytokine known to be aberrantly expressed in alopecia areata through a CD4+ Th1 mediated response. IFN-γ is produced by perifollicular or follicular antigen presenting cells and among several actions it also deprives dermal papilla cells of their ability to maintain anagen hair growth, as shown in human studies ***(Sato-Kawamura et al., 2003).*** It has been shown that serum levels of IFN-γ are significantly higher in patients with alopecia totalis (AT) or alopecia universalis (AU) compared to controls, but no significant difference has been found in levels of IFN-γ between patients with localized alopecia areata and those with more extensive forms ***(Arca et al., 2004).***

MIG (monokine induced by IFN-γ) is a cytokine that is elevated in human alopecia areata and its level correlates with disease activity, increasing in expanding lesions and vice versa, making it a useful marker of monitoring of the disease status and response to treatment**.** MIG mRNA is mostly found in mononuclear cells in the peri- and intrabulbar infiltrate and also in the follicular papilla ***(Kuwano et al., 2007).***

Also, another chemokine leading to recruitment of mononuclear cells is IP- 10 (interferon inducible protein-10) which is also induced by IFN-γ. Experiment in IP-10 is much less expressed compared to MIG but accounts for the

persistence of Th1 response in alopecia areata, perpetuating the recruitment of lymphocytes ***(Benoit et al., 1994).***

Interleukins (ILs):

Studies have shown that IL-1 is a very potent inducer of hair loss and a significant human hair growth inhibitor in vitro**.** ***Hoffman et al (1994)*** have demonstrated that during induced murine hair cycle, IL-1α and IL-1β increase profoundly with the onset of spontaneous catagen phase, while they peak during telogen phase and are associated with increased expression of the signal transducing type I IL-1 receptor. In human scalp areas affected by alopecia areata, an excessive expression of IL-1β is detected particularly at the early stages of the disease, while susceptibility to the disease and severity are determined by polymorphisms of the IL-1- receptor antagonist and IL-1α ***(Hoffmann, 1999).***

Teraki et al. in (1996) showed that patients with severe forms of alopecia areata have an increased frequency of the IL-1β 1, 2 genotype. Serum levels of IL-1α and IL-4 are significantly elevated in patients with localized alopecia areata, while IL-2 and IFN- γ are mainly elevated in extensive disease states, possibly implying that the progression to the extensive form may be mediated by Th1 cytokines.

It is also considered that disequilibrium in the production of cytokines with a relative excess of proinflammatory and Th1

types, versus antiinflammatory cytokines, such as IL-4 and IL-10 may be involved in the persistence of alopecia areata lesions, as shown in human scalp biopsies ***(Bodemer et al., 2000)***.

Finally, in agreement with above, it has been shown that steady-state levels of IL-10 mRNA increase after successful diphenylcyclopropenone (DPCP) treatment, making IL- 10 an important inhibitor of Th1 cytokine production ***(Hoffmann et al., 1994).***

Tumor necrosis factor-alpha (TNF- α)

Tumor necrosis factor-alpha is well known to play a major role in the pathogenesis of alopecia areata. TNF-α is synthesized in epidermal keratinocytes along with several other cytokines and is known to be a very potent inhibitor of keratinocyte proliferation ***(Ansel et al., 1990).***

In vitro studies have shown that TNF-α, along with IL-1α and IL1-β causes vacuolation of matrix cells within the follicle bulb and a decrease in the size of the matrix, as well as disorganization of follicular melanocytes and abnormal differentiation and keratinization of the precortical cells and the inner root sheath ***(Philpott et al.,1996).***

Tumor necrosis factor-alpha levels in the skin correlate positively with plasma Adrenocorticotrophic hormone (ACTH) levels and cutaneous ACTH receptor expression levels under