INTRODUCTION

Alopecia areata (AA) is an autoimmune disease that presents as well defined, usually rounded or oval patches of non-scarring hair loss with no overt epidermal changes. The life-time risk of AA in the general population is approximately 2% ***(Villasante-Fricke and Miteva, 2015)***. AA most commonly manifests as sudden loss of hair in well-demarcated, localized area in the scalp ***(Hordinsky and Ericson, 2004).*** Several different hypotheses to what causes alopecia areata are suggested. Genetic factors seem to play an important role since there is a higher frequency of a family history of alopecia areata in people who are affected. Alopecia areata appears to also have an autoimmune factor causing the patient to develop antibodies to different hair follicle structures ***(Kalish and Gilhar, 2001)***. Cytokines may play a role in alopecia areata by inhibiting hair follicle growth. Some studies show that emotional stress may also cause alopecia areata ***(Alexis et al., 2004).***

Because of the high rate of spontaneous recovery especially in those with small areas of hair loss or with a recent onset, not all patients require pharmacological treatment. A "watch-and-wait" approach is often recommended. Psychological support may be offered if necessary. Several lines of treatment can be used e.g. topical corticosteroids and/or minoxidil, Interleukin (IL)-31 antibodies, phototherapy and 308-nm Excimer laser ***(Hon and Leung, 2011).***

Androgenetic alopecia (AGA) is hereditary and androgen-dependent, progressive thinning of the scalp hair that follows a defined pattern. While the genetic involvement is pronounced but poorly understood, major advances have been achieved in understanding principal elements of the androgen metabolism involved: androgen-dependent processes are predominantly due to the binding of dihydrotestosterone (DHT) to the androgen receptor (AR) ***(Cela et al., 2003).*** DHT-dependent cell functions depend on the availability of weak androgens, their conversion to more potent androgens via the action of 5alpha-reductase, low enzymatic activity of androgen inactivating enzymes, and functionally active AR present in high numbers. The predisposed scalp exhibits high levels of DHT, and increased expression of the AR ***(Treub, 2002).***

Conversion of testosterone to DHT within the dermal papilla plays a central role, while androgen-regulated factors deriving from dermal papilla cells are believed to influence growth of other components of the hair follicle ***(Hilmar et al., 2005).*** Current available treatment modalities with proven efficacy are oral finasteride, a competitive inhibitor of type 2 5a-reductase, and topical minoxidil, an adenosine-triphosphate-sensitive potassium channel opener which has been reported to stimulate the production of vascular endothelial growth factor in cultured dermal papilla cells ***(Trüeb, 2002).***

The vitamin D receptor (VDR), independent of vitamin D, plays an important role in hair cycling, specifically anagen initiation ***(Karrie et al., 2010).***

VDR is expressed in the epidermal component of hair follicle and dermal papilla cell and the lack of VDR results in alopecia. *In vitro* studies have supported the concept that VDR may play a vital role in the postnatal maintenance of the hair follicle. Mesodermal papilla cells and the outer root sheath of epidermal keratinocytes express VDR in varied degrees in correlation with the stages of the hair cycle ***(Reichrath et al., 1994*).** In late anagen and catagen, there is an increase in VDR, which is associated with decreased proliferation and increased differentiation of the keratinocytes. These changes are thought to promote the progression of the hair cycle ***(Reichrath et al., 1994).***

The canonical Wnt signaling pathway has been found to play an important role in follicle development. VDR, lymphoid enhancer factor (Lef1), and beta-catenin form a complex that activates the canonical Wnt pathway (***Cianferotti et al., 2007).*** However, the molecular mechanisms by which the VDR exerts these actions are not clear ***(Luderer et al., 2011).*** Mice that express the keratinocyte-specific Lef1 transgene mutation that prevents Lef1 from interaction with beta-catenin develop alopecia associated with dermal cysts and increased sebaceous glands. When VDR is absent, the synergistic activation of a Wnt response element by beta-catenin and Lef1 is prevented (***Cianferotti et al., 2007).*** The Hairless gene also plays a role in

promotion of the canonical Wnt signaling pathway ***(Beaudoin et al., 2005).*** Therefore, impairment of canonical Wnt signaling may explain the total alopecia observed in both VDR knockout mice and Hairless knockout mice ***(Cianferotti et al., 2007).***

Human studies showed that VDR is expressed in the epidermal keratinocytes of normal follicle, but reduced in the AA. β-catenin, wnt3a, and wnt5a which are associated with hair growth and strongly observed in normal follicle, also showed decreased expression in AA ***(Kim et al., 2010).*** The function of β-catenin rather than expression may be altered in VDR null hair follicles ***(Shah et al., 2006).***

In colon cancer, VDR has been found to bind to β-catenin directly, reducing its interaction with and lymphoid enhancer-binding factor-1 (LEF1) and so reducing the transcriptional activity of β-catenin ***(Egan et al., 2010).***

Overexpression of AR in prostatic cancer cells leads to the suppression of VDR transactivation. Competition for shared coregulators between AR and VDR is one possible mechanism to explain the suppressive effect of androgen-AR signals on VDR activity. The suppression of VDR transactivation by AR signal was restored by overexpression of ARA70, but not ARA54. Together, ARA70 and ARA54 modulate VDR transactivation, and the competition for ARA70 mediates the suppressive effect of AR on VDR ***(Ting et al., 2005).*** Whether this suppressive effect of AR on VDR expression applies to androgenic alopecia was not previously investigated.

Aim of the Work

This work aims to study vitamin D receptor and β-catenin expression in both alopecia aerata and androgenetic alopecia.

Chapter One

BIOLOGY OF THE HAIR

Hair has many useful biologic functions, including protection from the environment and dispersion of sweat-gland products (e.g., pheromones). It also has psychosocial importance in our society, and patients with hair loss (alopecia) or excessive hair growth often suffer tremendously ***(Paus and Cotsarelis, 1999).***

The hair follicle, a unique characteristic of mammals, represents a stem cell-rich, phenotypic neuroectodermal-mesodermal interaction system. This factory for pigmented epithelial fibers is unique in that it is the only organ in the mammalian body which, for its entire life time, undergoes cyclic transformation from stage of rapid growth (anagen) to apoptosis- driven regression (catagen) and back to anagen, via an interspersed period of relative quiescence (telogen) ***(Paus and Foitzik, 2004).***

1. Embryology

Hair follicles start developing between 9 and 12 weeks gestational age. They are derived from the ectodermal and mesodermal layers of the embryo. The ectoderm gives rise to the hair matrix cells and the melanocytes responsible for the pigmentation of hair. Two buds form off this layer. One bud gives rise to the sebaceous gland and the other bud forms the area of attachment for the erector pili muscle. The erector pili

muscle, the hair dermal papilla, the fibrous follicular sheath and feeding blood vessels all arise from the mesoderm. Hair follicle epithelial growth continues down into the mesoderm until the follicle has reached its full size. Once this occurs, matrix cells begin dividing and pushing upward, eventually forming a hair shaft. Hair production can typically be seen by 16 to 20 weeks gestation, forming fine lanugo hair. Some of the lanugo hair will be shed around 32 to 36 weeks and after this time more substantial hair may develop on the scalp, eyebrows and eyelashes ***(Jahoda and Oliver, 1990).***

2. Types of Hair

Morphologically, there are three types of hair: ***Lanugo*** hair is the prenatal coat of fine soft unmedullated and usually unpigmented hair, which is usually shed in utero. Postnatal hair can be divided into two kinds; ***vellus*** which is soft unmedullated, occasionally pigmented and seldom more than 2 cm long; and ***terminal*** hair, which is longer, coarser and often medullated and pigmented. However, there is a range of intermediate kinds. Before puberty, terminal hair is normally limited to the scalp, eyebrows and eyelashes. After puberty, secondary sexual 'terminal' hair is developed from vellus hair in response to androgen ***(Dawber et al., 1995).***

3. Morphology of the hair follicle:

Hair is the keratinized product of the hair follicle, a tube like structure continuous with the epidermis at its upper end. The follicle is sloped in the dermis, and the longer ones extend

into the subcutaneous layer. An oblique muscle, the erector pili, runs from a point from the mid-region of the follicle wall to a point in the papillary dermis close to the dermo-epidermal junction. Above the muscle, one or more sebaceous glands, and in some regions of the body an apocrine gland also open into the follicle ***(Poblet et al., 2002).***

The hair follicle can be divided into 3 regions: the lower segment (bulb and suprabulb), the middle segment (isthmus), and the upper segment (infundibulum). The lower segment extends from the base of the follicle to the insertion of the erector pili muscle, the middle segment from the insertion of the erector pili muscle to the entrance of the sebaceous gland duct, and the upper segment from the entrance of the sebaceous gland duct to the follicular orifice ***(Shapiro, 2002).***

The hair bulb is the deepest part of the follicle and is invaginated at its base by the dermal papilla. The region surrounding the dermal papilla is called the hair bulb matrix. Beside the dermal papilla which invaginates the hair bulb, there is another specialized dermal component, the dermal or connective tissue sheath surrounding the follicle ***(Messenger and McDonagh, 2000).***

The bulk of any hair fiber is composed of cortex which is surrounded by the cuticle and may also have a continuous or discontinuous core or medulla, all of which derive from highly proliferative cells in the hair bulb at the base of the follicle. Cells in the hair bulb also give rise to the inner root sheath

which surrounds the hair fiber and which disintegrates before the hair emerges from the skin. The inner root sheath is itself enclosed by the outer root sheath, which forms a continuous structure extending from the hair bulb to the epidermis (Fig. 1) ***(Oshima et al., 2001).***

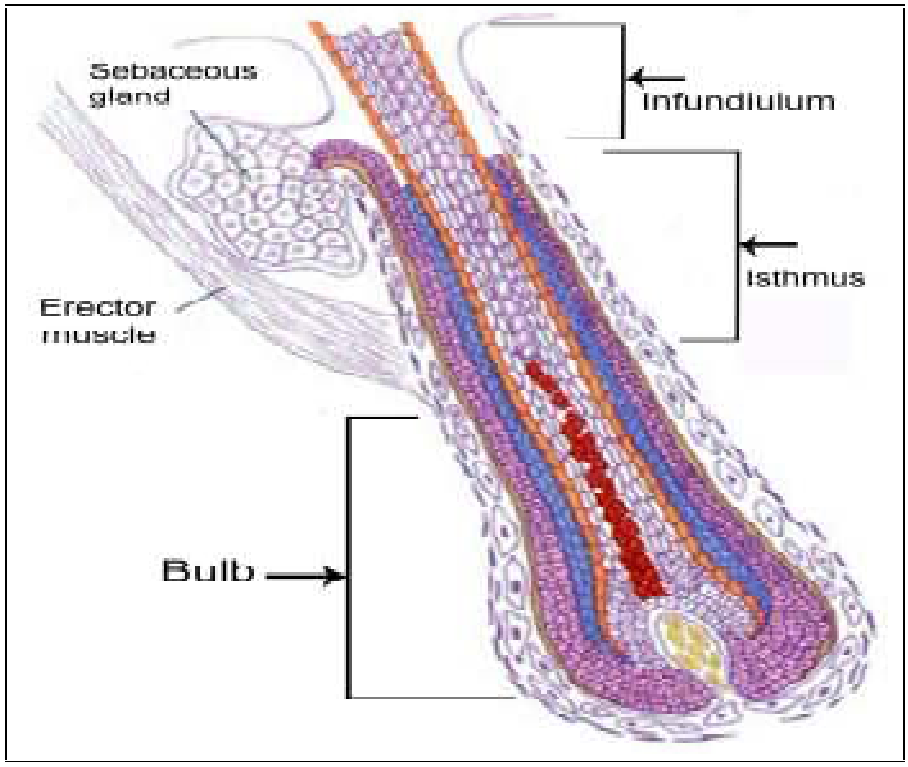

Fig. (1): Morphology of the hair follicle ***(Caserio, 1987).***

The lower part of the dermal papilla is connected to the fibrous root sheet. The hair matrix surrounds the top and sides of the dermal papilla, melanin can be found in abundance within the melanophages of the dermal papilla. Melanin is transferred from these melanocytes into the cells that make up

the hair shaft and is responsible for the color of the hair according to its quantity ***(Hashimoto, 1988).***

The hair matrix is the actively growing portion of the follicle consisting of a collection of epidermal cells that rapidly divide, move upward, and give rise to the hair shaft and the internal root sheath ***(Sperling, 1991).***

3.1. Inner root sheath: The inner root sheath (IRS) is closely apposed to the hair shaft, and, because the sheath contains no pigment, it can easily be distinguished from the hair shaft. The IRS coats and supports the hair shaft up to the level of the isthmus (Fig. 2) ***(Murphy, 1997).***

The IRS consists of 3 concentric cell layers. The layers keratinize by forming trichohyalin granules, which is unlike the hair shaft; undergoes trichilemmal keratinization. The outermost layer of the IRS (Henle layer) keratinizes first because it is lowest in the hair follicle. The innermost layer (IRS cuticle) interconnects with the cells of the hair cuticle. These 2 cuticles are completely integrated and keratinize after the Henle layer. The middle layer (Huxley layer) keratinizes after the IRS cuticle and the hair cuticle ***(Murphy, 1997).***

3.2. Hair shaft: It consists of 3 layers. The outermost layer of the hair shaft (cuticle) interlocks with the IRS cuticle, which leads to a firm attachment between the hair shaft and the IRS. As a result, they move upward in the follicular canal as a single unit ***(Caserio, 1987).***

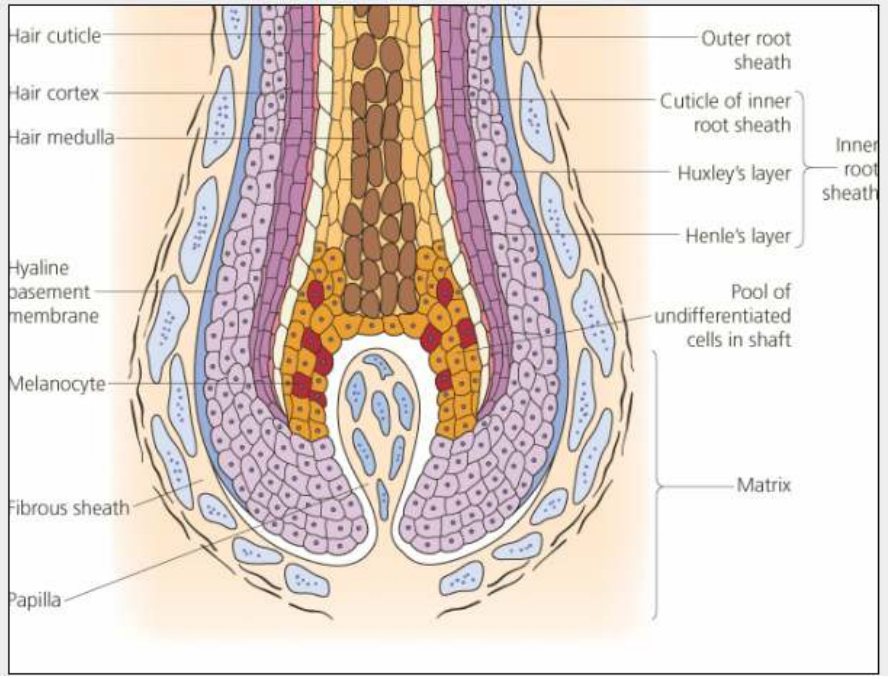

Fig. (2): Structure of the Hair Follicle ***(Murphy, 1997).***

The middle layer of the hair shaft (hair cortex) constitutes the bulk of the hair and consists of cells that keratinize gradually as they move upward from the hair matrix. Unlike the IRS, which keratinizes by forming trichohyalin granules (soft keratin), the hair cortex cells keratinize without forming granules (trichilemmal keratinization). The keratin produced is termed hard keratin ***(Tiede et al., 2007).***

The innermost layer (medulla) is frequently difficult to visualize. Routine light microscopy is typically used to visualize this layer because this layer is discontinuous in many cases and is often completely absent ***(Hashimoto, 1988).***

3.3. Outer root sheath: The outer root sheath (ORS) covers the IRS as it extends upward from the matrix cells at the lower end of the hair bulb to the entrance of the sebaceous gland duct. The ORS is thinnest at the level of the bulb and thickest in the middle portion of the hair follicle. The ORS does not keratinize below the level of the isthmus (in contrast to the IRS). However, at the level of the isthmus where the IRS disintegrates, the ORS keratinizes without forming granules (trichilemmal keratinization), which is similar to the keratinization of the hair cortex ***(Murphy, 1997).***

At the level of the infundibulum, keratinization of the ORS changes to normal epidermal keratinization with formation of the granular cell layer and stratum corneum. The basal cell layer of the ORS contains inactive amelanotic melanocytes, these inactive melanocytes can become melanin-producing cells after skin injury ***(Tiede et al., 2007).***

3.4. Glossy (vitreous) layer: It is the eosinophilic acellular zone surrounding the ORS. This layer is continuous with the epidermal basement membrane and is similarly periodic-acid-Schiff (PAS) positive and diastase resistant. Unlike the epidermal basement membrane, the glossy layer is much thicker and is visible with routine stains. During the catagen phase, the vitreous layer becomes much thicker and corrugated, which is a distinguishing feature of catagen phase hairs ***(Murphy, 1997).***

3.5. Fibrous root sheath: It is the outermost layer of the hair follicle and surrounds the vitreous layer. It consists of thickened collagen bundles that coat the entire hair follicle. The root sheath is continuous with the dermal papilla at its lower end and with the papillary dermis above it ***(Shapiro, 2002).***

3.6. Suprabulb region: It extends from the hair bulb to the isthmus and consists of components of the hair shaft, IRS, ORS, vitreous layer, and the fibrous root sheath ***(Caserio, 1987).***

3.7. Isthmus: It is the shortest segment of the hair follicle, extending from the attachment of the erector pili muscle (bulge region) into the entrance of the sebaceous gland duct ***(Hashimoto, 1988).***

3.8. Infundibulum: It is the upper portion of the hair follicle above the entry of the sebaceous duct. Surface epidermis lines the infundibulum ***(Hashimoto, 1988).***

4. The scalp hair:

The scalp hair is a fiber 60 to 80 nm in diameter, each hair grows steadily, approximately 1 cm per month, and continuously for 3 to 5 years (anagen phase). Growth then stops and is followed by a brief transient stage (catagen) lasting 2 to 4 weeks, and a 2 to 4 months resting stage (telogen) during which old hair is shed. With the onset of the anagen stage, new hair starts to grow from each follicle. The growth process functions individually in each follicle, therefore, hairs are not

shed simultaneously. At any given time, some hairs are growing; some are resting, and some being shed normally, of approximately 150, 000 scalp hairs, 90% are in the anagen phase, and the remaining 10% are in the catagen and telogen phases, with 50-100 hairs being shed daily ***(Wolfram, 2003)***.

5. Hair Cycling:

Traditionally, three phases of the growth cycle are recognized: a growth phase (anagen phase I–VI), a regression phase (catagen), and a resting phase (telogen) (Fig. 3) ***(Muller-Rover et al., 2001).***

These cyclic transformations are controlled by finely tuned changes in the local signaling milieu, based on changes in the expression of cytokines, hormones, neurotransmitters and their receptors as well as transcription factors and enzymes, which act via endocrine, paracrine or autocrine routes. The hair cycle includes a complex remodeling and regeneration of the complete non-permanent portion of the hair follicle. It is not just the hair follicle epithelium, but also the mesenchyme, the extracellular matrix, the vasculature and innervation, and the hair-follicle-associated cell populations that undergo dramatic changes ***(Blume et al., 2008).***

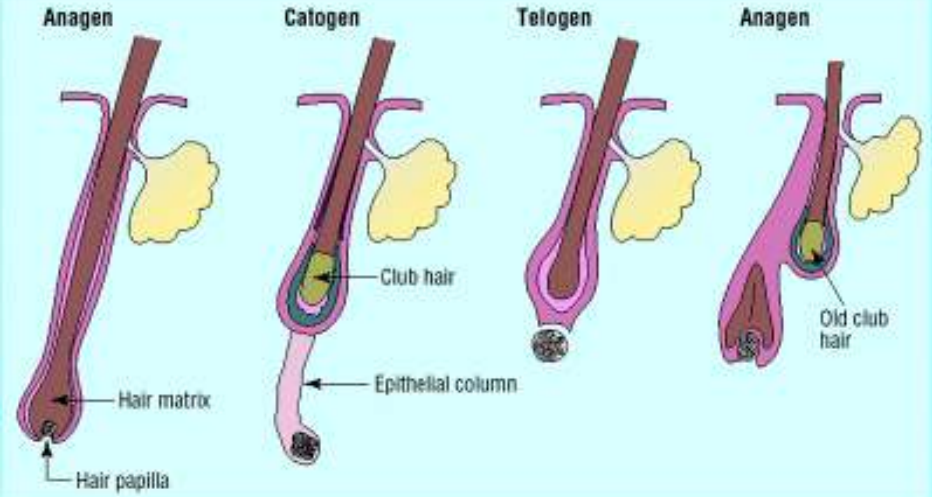

Fig. (3): Normal hair cycle. In a normal individual, the hair follicle undergoes a repetitive sequence of growth and rest known as the hair cycle. Each telogen hair is replaced by a new anagen hair ***(Rathnayake and Sinclair, 2010).***

Cycles of hair growth in human beings are not synchronized; each hair enters phases of the growth cycle at a different time ***(Ellis et al., 2002)***.

5.1. Phases of the Hair Cycle:

5.1.1. Anagen: The phase of active hair growth. It is subdivided into six substages (I to VI), the first five of which are collectively called proanagen. They are defined by progressively higher levels of new hair-tip position within the follicle. The sixth stage, metanagen, is defined by emergence of the hair shaft above the skin surface (Fig. 4) ***(Blume et al., 2008)***.

- Anagen I-growth of the dermal papilla and onset of mitotic activity in the germ-like overlying epthelium.