INTRODUCTION

Graying "or Greying", depigmentation or failure of hair pigmentation has been variously referred to as 'canities', 'achromachia', 'achromotrichia', the condition of absence of pigment from hair or feathers. It is apparently caused by failure of the hair follicles to deposit pigment in the hair at the time the hair is being formed ***(Frost, 1948).***

Although there is no precise definition for the premature canities, it is defined as the occurrence of the hair graying before the age of 20 in whites, 25 in Asians, and 30 in Africans ***(Naieni et al., 2011).***

It is a genetically determined phenomenon that is usually age related and can be accelerated by some exogenous factors ***(Arck et al., 2006 and Naieni et al., 2011).***

The scalp is subjected to intrinsic or physiologic aging factors and extrinsic or premature aging due to external factors. Intrinsic factors are related to individual genetic and epigenetic mechanisms with interindividual variation. Prototypes are familial premature graying, and androgenetic alopecia (AGA). Extrinsic factors include ultraviolet radiation, air pollution, smoking, nutrition, and lazy lifestyle. Experimental evidence supports the hypothesis that oxidative stress plays a major role in premature skin and hair aging ***(Trüeb, 2006).***

Premature canities has also been shown to be associated with a cluster of autoimmune disorders such as vitiligo, pernicious anemia, autoimmune thyroid diseases, and some rare premature aging syndromes like Werner's syndrome ***(Naieni et al., 2011).***

Melanocyte aging may be associated with reactive oxygen species-mediated damage to nuclear and mitochondrial DNA with resultant accumulation of mutations with age, in addition to dysregulation of anti-oxidant mechanisms or pro/anti-apoptotic factors within the cells ***(Tobin and Paus, 2001).***

In addition to pigments, lipids and trace elements, proteins mainly compose 65 to 95% of human hair. Most of trace elements don't exist in hair alone, but bind with proteins or fatty acids ***(Tsai et al., 2000).***

Gray hair results when insufficient nutrients are supplied to the scalp to maintain normal melanin production. Several nutrients are responsible to convert the amino acid tyrosine into melanin. The most common deficiency is with copper (Cu) as grey hair contains much less copper, magnesium (Mg) and calcium (Ca) than naturally coloured hair. While ***Bertazzo et al.*** reported lower concentrations of Cu in white hairs in comparison to black ones and no significant difference in zinc (Zn) concentrations of different hair colors ***(Bertazzo et al., 1996), Allergi et al.*** demonstrated lower concentrations of both Zn and Cu in white hairs ***(Allergi et al., 1990).***

AIM OF THE WORK

The aim of this study was to assess the correlation between serum iron, zinc, copper concentrations and premature canities.

Chapter 1

HAIR FOLLICLE EMBRYOGENESIS

Hair in mammals is composed of the hair follicle and the hair shaft. Follicles are formed only once in the lifetime of a mammal that is born with a fixed number of follicles which does not change with time. The hair follicle is formed as a small finger-like structure from the embryonic epidermis. The embryonic epithelium and mesenchyme of mammals have the necessary elements that induce the formation of follicles. At the pre-natal stage, the primitive epithelium and the underlying mesoderm send out growth signals and the follicles begin to be formed from the primitive epidermis. Complete hormonal or neural circuits are not required for the hair follicles to begin its formation. This has also been proved when hair follicles have been found to grow in organic cultures of embryonic skin ***(Pacini and Bonelli, 1991).***

In humans, development of hair follicles first begins on the head in the fourth fetal month of gestation, particularly on the eyebrows along with the lower and upper eyelid of an embryo. From these first few hair follicles on the face, the development of the hair follicles gradually expands symmetrically to cover the entire embryo saving for the palms of hands and soles of the feet. Skin areas further away from the head, especially those on the limb extremities, take longer to develop embryonic hair follicles. It can take up to 110 days before hair follicles can be

seen to start developing on the arms and legs ***(Holbrook and Minami, 1991).***

Once the necessary signals are received, the epithelium grows downwards into the dermis, forming a small plug that joins with the dermal papilla at the base **"Fig.1"**. The dermal papilla is a mesenchymal condensation composed of specialized fibroblasts. The fibroblasts control the matrix cells "that arise from epidermis" and in this way determine the size of the hair. About 5 million hair follicles are formed in mammals at birth with one million on the head of which 100,000 cover the scalp. The distribution of the hair follicles and their spacing is genetically predetermined and does not change throughout the lifetime of the mammal ***(Hearle, 2000).***

The hair follicles begin to develop between 9 and 12 weeks gestational age and are derived from the ectodermal and mesodermal layers of the embryo **"Fig. 1"**. The ectoderm gives rise to the hair matrix cells and the melanocytes responsible for the pigmentation of hair. Two buds form off of this layer, one gives rise to the sebaceous gland and the other forms the area of attachment for the erector pili muscle. The erector pili muscle itself, the hair dermal papilla, the fibrous follicular sheath and feeding blood vessels all arise from the mesoderm ***(Greco et al., 1989).***

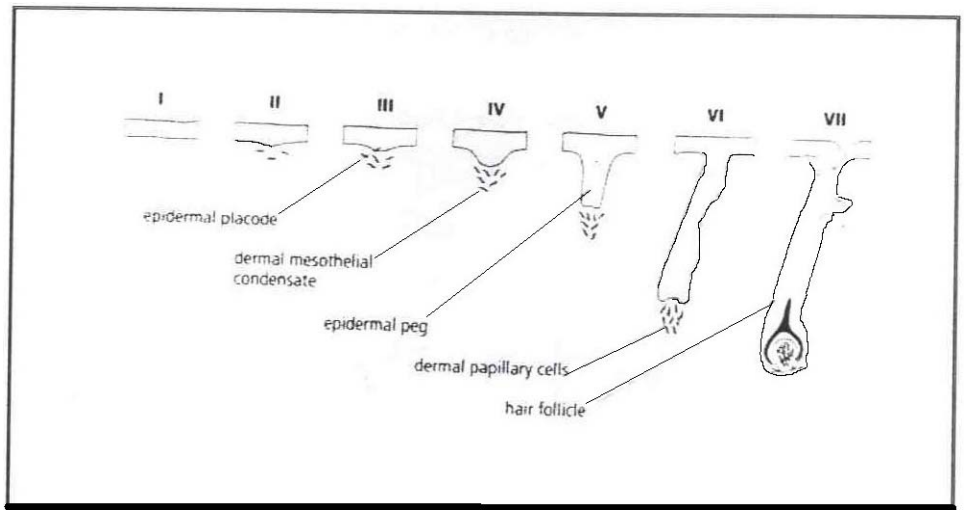

Fig. (1): Embryology of hair development ***(David, 2004).***

The dermal papilla cells begin to aggregate in the dermis just below the epidermis. For humans, this initial aggregation begins when the embryo is approximately 60 days old. The dermal papilla cells at this stage are only loosely collected and are long, spindle shaped cells. The development of a dermal papilla marks the site for future development of a hair follicle ***(Fuchs, 2001).***

Hair follicle epithelial growth continues down into the mesoderm until the follicle has reached its full size. At this time, matrix cells begin dividing and pushing upward, eventually forming a hair shaft ***(Agaiby et al., 2000).***

Above the dermal papilla an epidermal plug "or peg" of cells develops and proliferates growing into the dermis towards the dermal papilla. The epidermal plug of cells does not grow

straight down into the dermis but at a slight angle. The mesoderm derived dermal papilla and the ectoderm derived epidermal plug "communicate" with the result of further proliferation of epidermal matrix cells and eventual differentiation into the various sheath and hair fiber structures ***(Paus et al., 2001).***

This gradual differentiation of the hair plug first begins with the development of three distinct buds of cells on the same side of the down growth one above the other. The cell bud closest to the epidermis may develop into an apocrine (sweat type) gland. However, this only happens in a minority of hair follicles, primarily those on the scalp, genitals and anus. Isolated apocrine glands may develop in connection to hair follicles elsewhere but they are usually few and far between. Most of these upper cell buds regress as the hair follicle matures ***(Muller-Rover, 1999).***

The cell bud in the middle will gradually develop into the oil producing sebaceous gland while the cell bud below it forms what is called the hair follicle bulge. The bulge is the area of the hair follicle to which a small muscle will attach. The "arrector pili" muscle develops separately from the hair follicle within the mesoderm/dermis. It grows up towards the epidermis and down towards the hair follicle bulge region. Eventually it will attach to both the epidermis at one end and the hair follicle at the other. The bulge is also important as this is the location

where the stem cells reside in adult hair follicles ***(Foitzik, 1998).***

As the epidermal plug of cells penetrates down into the dermis, mesodermal cells congregate around it and develop into a fibrous follicular sheath and/or collagen capsule to encase the epidermal cells. As the epidermal plug comes into contact with the dermal papilla, the growth is still downward. The epidermal plug seems to push the dermal papilla down into the dermis as it grows to its full size. It may penetrate up to 5mm below the skin surface (more in adults) ***(Holbrook and Minami, 1991).***

The dermal papilla develops into a more identifiable structure of rounded cells containing organelles vital for product synthesis, although the cells themselves at this later stage of development are non-proliferative. The dermal papilla cells communicate with the plug cells and these epidermal cells begin to arrange themselves into concentric layers above the dermal papilla. The layers eventually differentiate into the hair fiber and the inner and outer root sheaths encasing the fiber. These layers begin to keratinize higher up the hair follicle while the cells close to the dermal papilla remain undifferentiated and continue to multiply. The multiplying cells can't push out into the dermis because of the surrounding fibrous capsule. The only way out is up. The cells push up and away from the dermal papilla pushing other cells in front of them. As the cells mature they are incorporated into the layers of the hair follicle,

become keratinized, die and eventually are extruded from the surface of the skin ***(Foitzik, 1998).***

Hence, development of a hair follicle requires a continuum through induction, initiation, elongation and differentiation **"Fig.2"**. The basic hair follicle structures are complete throughout the skin of an embryo by 160 days gestational age ***(McElwee and Hoffmann, 2000).***

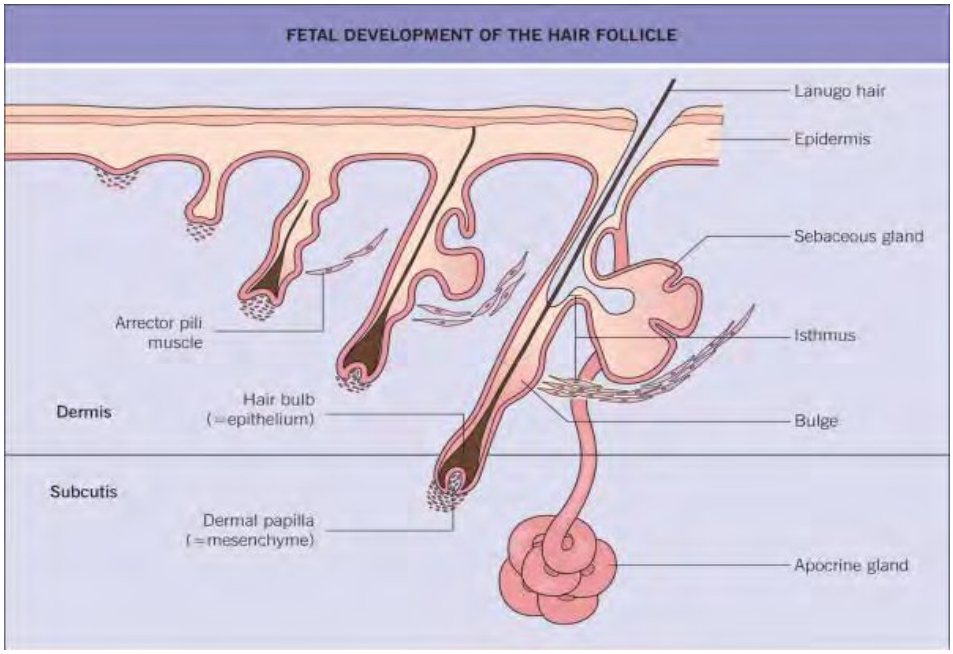

Fig. (2): Fetal development of the hair follicle ***(Paus et al., 2008).***

Types of hair produced in embryogenesis

Follicles first produce soft, short and fine hairs, which are called **lanugo hairs**. Lanugo hairs are shed between the 32nd and 36th week of gestation, but in one third of babies, lanugo

hairs can be retained until after birth. The newborns can have non-pigmented lanugo hairs covering all over the body and this may cause sometimes a shock to the parents, it is nothing to worry about and does not indicate any problem If lanugo hairs are still present at birth as they will be shed within the first month or so after birth ***(McElwee and Hoffmann, 2000).***

Lanugo hairs are replaced by small and almost imperceptible hairs called **vellus hairs** over most of the body. Vellus hairs increase tactile perception of the skin surface by acting as sensitive pressure detectors. Larger follicles on the scalp generate into **terminal hairs**, although up to 10 percent of scalp follicles can remain at a vellus state. The growth of scalp hairs in newborns can be quite variable. While some are born with pigmented terminal hairs covering the head completely, others have relatively little hairs. For these babies, the hair follicles are growing terminal hairs, but it has not yet become visible above the skin. Within a few months from birth, the scalp and body hair follicles should have sorted themselves out and will be producing vellus or terminal hairs as appropriate. No hair follicles form naturally after birth in humans, though other animals can produce new hair follicles. For example, red deer grow new hair follicles each year as a part of the velvet covering their antlers ***(Muller-Rover, 1999).***

Chapter 2

MORPHOLOGY OF THE NORMAL HAIR FOLLICLE

Hair follicles vary considerably in size and shape, depending on their location, but they all have the same basic structure. Rapidly proliferating matrix cells" that arise from the epidermis" in the hair bulb produce the hair shaft, whose bulk "the cortex" is composed of hair-specific intermediate filaments and associated proteins. Pigment in the hair shaft is produced by melanocytes interspersed among the matrix cells. As the matrix cells differentiate and move upward, they are compressed and funneled into their final shape by the rigid inner-root sheath, whose dimensions and curvature largely determine the shape of the hair. The dermal papilla, which is composed of specialized fibroblasts located at the base of the follicle, is thought to control the number of matrix cells and thus the size of hair ***(David, 2005).***

Hair follicle anatomy

Transverse or horizontal sections of the scalp show that hair follicles are organized into follicular units. Each unit contains one to four terminal hairs, one or two vellus hairs, nine sebaceous glands and arrector pili muscle insertions and a perifollicular vascular plexus, neural net and connective tissue **"Fig. 3"**. These units are arranged in a regular mosaic pattern

and likely function as distinct physiologic entities. The density of hair follicles is approximately 1135/cm^2 at birth and quickly decreases to 795/cm^2 by 1 year of age. A gradual decrease is then seen so that 20-30 year olds average 615 follicles/cm^2 , 30-50 year old average 485/cm^2 and by age 80-90 average only 435/cm^2 ***(Capaldi et al., 1983).***

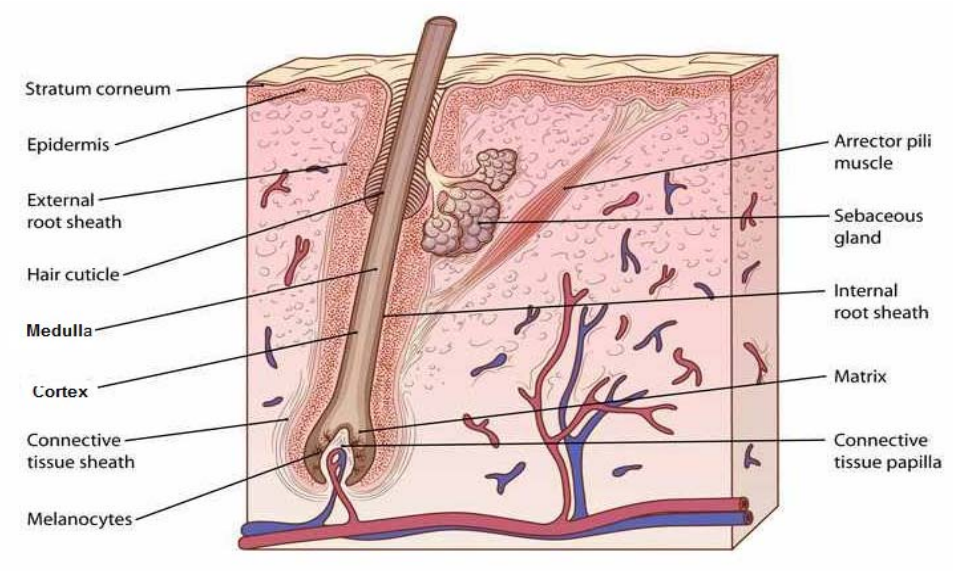

Fig. (3): Hair follicle anatomy**.** Adapted from http://illuminationstudios.com/archives/146/anatomy-of-a-hair-follicle

The hair follicles are formed as epithelial fingers. Under the influence of the dermal papilla, epidermal cells differentiation "during anagen" produces a keratinized hair fiber and associated products ***(Hutchinson and Thompson, 1997).***

The matrix cells "the epithelium derived cells"" which are a living and active proliferating group of cells that lie in the

immediate vicinity of the dermal papilla" differentiate and push upwards, forming three enclosed cylinders **"Fig 4"**. The outermost cylinder separates the hair structure from the rest of the dermis and it is called the Outer Root Sheath (ORS). As the matrix cells differentiate upward, they are given a shape by the rigid middle cylinder or the Inner Root Sheath (IRS). The dimensions and curvature of the inner root sheath play a role in determining the final shape of the hair shaft (the innermost cylinder). The IRS also guides the shaft upwards as the IRS and the hair fiber move outwards together ***(Sundberg et al., 2005).***

The active matrix cells differentiate and become keratinized to form the hair shaft cortex (Co), the surrounding hair cuticle (Hc) and the medulla (M) which is situated at the center of it. Cells around the hair shaft comprise the inner root sheath (IRS) which can be divided into three layers the cuticle layer(Cu), Huxley layer (Hu) and Henle layer (He) based on structure, patterns of keratinization and incorporation of a product called trichohyaline . The IRS breaks down at the level of the sebaceous gland to leave only the hair cortex and surrounding cuticle to protrude above the epidermis **"Fig.4"** ***(Hutchinson and Thompson, 1997).***

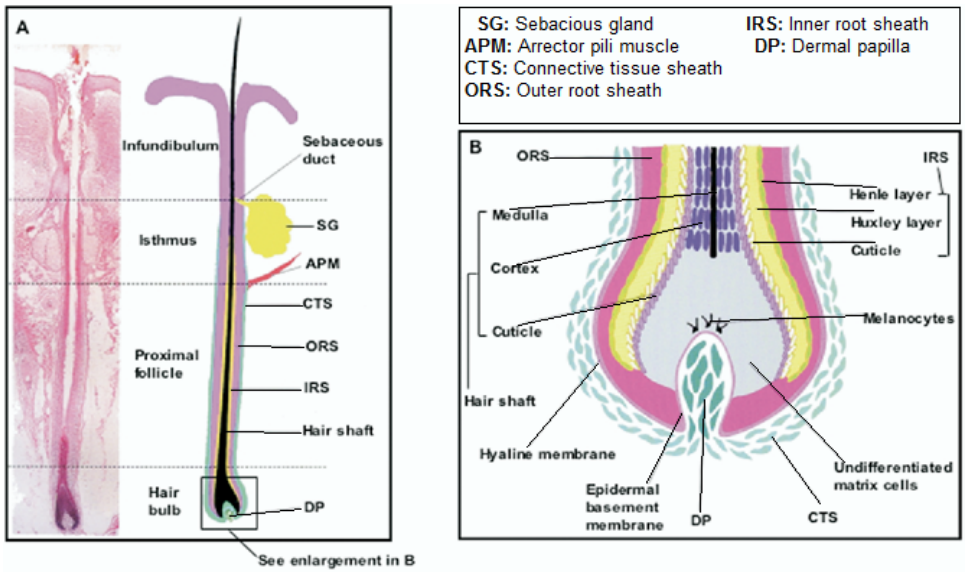

Fig. (4): A: Anagen VI hair follicle. Histologic longitudinal section on the left hand side. Schematic drawing of an anagen VI follicle with anatomical details on the right hand side. B: Anagen VI hair bulb in detail (enlargement of schematic drawing in A) ***(Krause and Foitzik, 2006)***

On the outer root sheath, we also find the "bulge" "**Fig. 5**". This is a cluster of cells biochemically different from the others, having properties of epithelial stem cells. The bulge region is also rich in nerve endings and contains neuropeptides that control the proliferation of follicles ***(Stenn and Cotsarelis, 2005).***

Dermal papilla of the hair follicle

It is the dermal papilla (DP), which directs and dictates the embryonic generation of a hair follicle and it also retains this instructive ability throughout the life of the hair follicle. The DP presents as a healthy "pear" shape in normal hair follicles "**Fig. 5**". The DP consists of a highly active group of cells

shown to be capable of inducing follicle development from the epidermis and production of hair. It is comprised of a small group of spindle-shaped cells called fibroblasts "derived from the mesoderm" in addition to collagen bundles, stroma, nerve fibers and a single capillary loop. DP is in a continuum with a perifollicular sheath of connective tissue (the dermal sheath), which envelops the lower follicle ***(Peters et al., 2006).***

Those fibroblasts are held close to the base of the matrix cells that produce the hair fiber and root sheaths but there is a thin layer, called the basement membrane (or basement lamina, or glassy membrane) that separates the DP cells from the hair fiber sheath cells. In other words, the basement membrane provides a physical dividing line between cells separating between embryonic ectoderm (epidermis) and embryonic mesoderm (dermis). This physical barrier has a role to play in our immunological protection. Holding the DP cells in place is a capsule that surrounds the DP cells in a cup and extends up the sides of the hair follicle to the epidermis. The whole follicle structure sits on a pad of fibrous tissue called the Arao-Perkins body. Nerve fibers and blood vessels penetrate through small gaps in the base of the hair capsule and invade into the DP area ***(Pacini and Bonelli, 1991).***

The bigger the DP, the more cells it has, then the thicker the hair fiber that the hair follicle produces. The DP cells are very active with lots of cytoplasm when the hair follicle is producing a hair fiber although the DP cells do not multiply and