Introduction

Psoriasis is a chronic, relapsing, inflammatory and hyper-proliferative skin disease ***(Barker, 2007)***.

It was originally thought of as an inflammatory disorder solely affecting the skin, but it is now recognized as a systemic inflammatory disease ***(Kremers et al., 2008).***

The pathological changes affecting the skin in psoriasis are mainly driven by abnormal differentiation secondary to activation of T- cells or antigen presenting cells, which in turn release various chemokines and cytokines that signal keratinocytes to hyperproliferate ***(Gudjonsson et al., 2004).***

Apoptosis or programmed cell death is critical for tissue homeostasis in multi-cellular organisms. It plays an important role in many physiological processes ***(Krammer, 2000).***

Normally in healthy skin the proliferation of cells in the basal cell layer is balanced and regulated by keratinocytes in the superficial layer of epidermis through the process of apoptosis ***(Polakowska et al., 1994).***

Keratinocytes may undergo apoptosis by loss of cell-cell contact after cross-linking of the FAS *(CD95)* molecule, or by ultraviolet radiation ***(Krueger et al., 2001).***

The epidermal hyperplasia characteristic of psoriasis has been implicated to be a result of epidermal expression of

apoptosis-related molecules representing suppression of the apoptotic process ***(Takahashi et al., 2002)***.

A key inhibitor of death receptor signaling is c-FLIP *(cellular FLICE inhibitory protein)*, which interacts with FADD *(FAS-associated death domain protein)* and procaspase-8, thereby inhibiting the initiation of the apoptotic cascade ***(Irmler et al., 1997)***.

Gene-expression analysis is increasingly important in many fields of biological research. Understanding the patterns of the expressed genes is expected to provide insight into complex regulatory networks and will most probably lead to the identification of genes relevant to new biological processes or implicated in diseases. Real-time RT-PCR provides the simultaneous measurement of gene expression in many different samples for a limited number of genes and is especially suitable when only a small number of cells are available ***(Heid et al., 1996).***

Real-time RT-PCR is based on the process of reverse transcription, which reverse transcribes RNA into DNA and was initially isolated from retroviruses ***(Prediger, 2001)***.

Aim of the Work

This thesis is designed to to analyze the expression levels of anti-apoptotic c-FLIP in the epidermis of patients diagnosed clinically with psoriasis and to determine whether it has a role in development of psoriasis or in its severity.

Real time quantitative RT-PCR is the method used to measure the level of mRNA of c- FLIP in skin biopsies.

Psoriasis

Psoriasis is a common chronic inflammatory disease of the skin and joints that is heterogeneous in presentation ***(Almutawa et al., 2013)***.

It can have a significant negative impact on the physical, emotional and psychosocial wellbeing of the affected patients. Approximately 80% to 85% of patients have limited skin involvement whereas 15% to 20% have more extensive skin involvement that may require systemic therapy ***(Gelfand, Wang et al., 2005).***

Although the cause of this disease remains unknown, the evolving evidence suggests that psoriasis is a complex disorder caused by interaction of multiple genes, immune system and environmental factors ***(Nakajima, 2012).***

Epidemiology

- **Prevalence**

Distribution of psoriasis in the world population varies according to ethnic groups and geographical locations with a peak prevalence of approximately 0.4 - 4.8% of the general population ***(Almutawa, 2013).***

In U.S.A it affects between 2% to 3% of the population ***(Papp et al., 2012).***

The common clinical variant of psoriasis is termed psoriasis vulgaris and it affects approximately 85 to 90% of all patients with the disease ***(Griffiths et al., 2007).***

- **Impact of age and gender on incidence of psoriasis**

The onset of psoriasis can occur anytime from birth to advanced ages. Many studies show that the age of onset of psoriasis is bimodal peaking in early adult life *(late teens to 20s)* and then again in later adult life *(50s and 60s)*. Prevalence data indicate that the frequency of psoriasis decreases in older age group (over 70), this may be due to remission of the disease, lack of seeking medical advice from the diseased individuals at this age or higher mortality rate from associated co-morbidities and behaviors ***(Langley et al., 2005).***

Most studies suggest that psoriasis may be slightly prevalent in males compared to females, however in young patients (<20 years) the prevalence of psoriasis is greater in females than in males, suggesting an earlier age of onset of psoriasis in females compared with males ***(Gelfand, Weinstein et al., 2005).***

- **RISK FACTORS**

It is widely accepted that genetic–environmental interaction plays a role in the development of psoriasis. Although the genetic influence on psoriasis is well established, the role of environmental factors is less precisely defined. Smoking habits, alcohol consumption, diet, body mass index *(BMI)*, stressful life events and infections have been repeatedly considered as potentially important causative factors ***(Fig.1) (Naldi, 2004).***

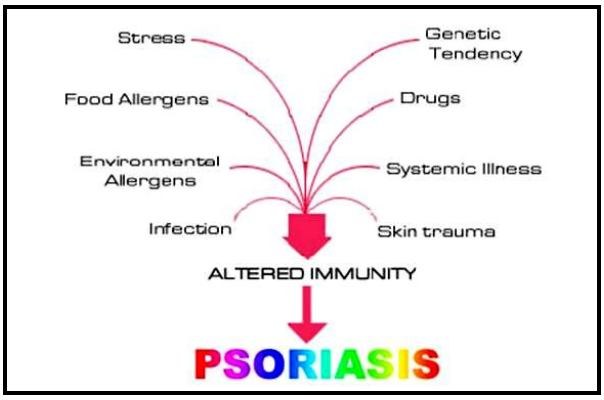

Figure (1): Psoriasis: Multi-factorial causes ***(Home-cure.net)***.

- **Family history and genetics**

Psoriasis is a genetic-based (inherited) disease that affects the body's immune system ***(Torpy, 2011)***.

Certain efforts have been made to study the genetic basis of psoriasis. Genome-wide linkage analyses have identified nine susceptibility loci (PSORS1–9) ***(Table 1)***. A meta-analysis of multiple genome wide scans reveals genetic linkage to the major histocompatibility complex *(MHC)* on chromosome 6p21.3 that includes the PSORS1 locus. The PSORS1 locus is likely to account for about 30% to 50% of the heritability of the disease and has been believed to be the major genetic determinant of psoriasis ***(Kun-Ju et al., 2011)***.

The disease has a strong but complex genetic background with an incidence of approximately 60% in monozygotic twins ***(Shankarkumar, 2012).***

Table (1): Summary of susceptibility loci linked to psoriasis vulgaris in genome scan studies ***(Campalani, 2005).***

Locus name	Chromosomal location
PSORS 1	6p21.3
PSORS 2	17q24-25
PSORS 3	4q34
PSORS 4	1q21
PSORS 5	3q21
PSORS 6	19p13-q13
PSORS 7	1p35-p34
PSORS 8	16q
PSORS 9	4q31

- **Trauma**

Many injurious stimuli have been recognized to elicit psoriatic lesions at the sites of injured skin e.g. physical, chemical, electrical, surgical, infective and inflammatory insults. Psoriasis at the site of injury is well known as Köbner phenomenon ***(Griffiths et al., 2004).***

Mechanical trauma can activate keratinocytes that begin to release cytokines *(e.g. IL-1 and TNF-α)* and proteins of thermic shock. These substances activate the dendritic cells *(DCs)* in the epidermis and dermis ***(Guttman et al., 2007).***

- **Infections**

Bacterial and viral infections may be linked to psoriasis. Case reports have raised the hypothesis that upper respiratory tract infections especially with Streptococcus pyogenes are strongly

associated with the onset and flaring of guttate psoriasis and exacerbation of chronic psoriasis ***(Naldi et al., 2001).***

Subclinical Streptococcal infection and local skin infections with Staphylococcus aureus, Malassezia furfur and Candida albicans may play a role in exacerbation of chronic plaque psoriasis ***(Tagami, 1997)***.

- **Smoking**

Previous cross-sectional and case-control studies have suggested a link between cigarette smoking and psoriasis, also the intensity and duration of smoking and the clinical severity of psoriasis, but no prospective data are available ***(Herron et al., 2005).***

Cigarette smoke contains many potentially toxic materials that may affect the immune-pathogenesis of psoriasis including T cell activation and overproduction of pro-inflammatory cytokines *(e.g. TNF-α, IL-2, IL-6, IL-8 and γ-interferon)* ***(Orosz et al., 2007).***

Nicotine itself alters a wide range of immunological functions including innate and adaptive immune responses, it can modulate the functional capacity of DC and can increase the secretion of pro-inflammatory Th1 cytokines by DC, additionally nicotinic cholinergic receptors have been demonstrated on keratinocytes that stimulate calcium influx and accelerate cell differentiation, also they can control keratinocyte adhesion and upward migration in the epidermis ***(El-Darouti et al., 2010).***

- **Alcohol**

Alcohol may alter the expression of psoriasis and its clinical course. It stimulates the release of histamine, which can aggravate skin lesions as a consequence. Moreover, a high alcohol intake may be accompanied by an excessive intake of high-fat foods and saturated fats and a low intake of vegetables and fresh fruit ***(El-Darouti et al., 2010).***

- **Diet (Energy intake)**

The prevalence and severity of psoriasis have been reported to be improved by low-calorie diets with decrease in the epidermal cell proliferation rate. The most important reason is probably the lack of arachidonic acid *(AA)* intake resulting in lower leukotriene *(LT)* production. During fasting CD4+T cell *(T- helper cell)* activation is reduced and anti-inflammatory cytokines such as IL-4 increase. Another reason may be a reduction of oxidative stress due to calorie restriction because psoriasis appears to be associated with oxidative stress ***(El-Darouti, 2010).***

- **Obesity**

There is a strong association between increased body mass index *(BMI)* and psoriasis suggesting that psoriasis patients are more frequently overweight or obese than the general population also the severity of psoriasis may be correlated to the BMI ***(Prey et al., 2010)***.

Adipose tissue has been shown to produce pro-inflammatory cytokines *(e.g. TNF-α)* contributing in the severity of the disease ***(Johnston et al., 2008).***

- **Stress**

Stressful psychosomatic factors may play a great role in development and exacerbation of diffuse plaque psoriasis ***(Picardi, 2003).***

- **Drugs**

Several drugs such as lithium salts, diuretics, beta blockers, non steroidal anti-inflammatory drugs, calcium channel blockers, anti-malarial, interferon and rapid tapering of high doses of corticosteroids have been associated with the onset or exacerbation of psoriasis but data that estimates the relative risks are scanty ***(Dika et al., 2006)***.

- **QUALITY OF LIFE AND PSYCHOLOGICAL ASPECTS OF PSORIASIS**

Although psoriasis generally does not affect survival, it certainly has a number of major negative effects on the quality of life of the patients ***(Krueger et al., 2000).***

The patient's quality of life is influenced by the areas of the body which are affected. When psoriasis occurs in visible sites such as the face, scalp and hands, it can have a large impact on quality of life this itself contributes to everyday disability leading to depression and suicidal ideation in more than 5% of patients ***(Murphy et al., 2011).***

- **COMORBIDITIES**

Several epidemiological studies have confirmed the associations of chronic plaque psoriasis and psoriatic arthritis with cardio-metabolic disorders including myocardial infarction, stroke, diabetes, obesity, dyslipidemia and non-alcoholic fatty liver disease *(NAFLD),* that may confer a higher mortality rate ***(Targher et al., 2010).***

There is also marked elevation of the risk of coronary artery sclerosis among patients with psoriasis depending on their age and the severity of the disease ***(Fig.2) (Gelfand et al., 2006).***

The direct promotion of cardiovascular diseases is due to endothelial dysfunction caused by permanently elevated levels of mediators such as vascular endothelial growth factor *(VEGF)* leading to the increased frequency of atherosclerosis even among patients who have none of the classical risk factors ***(Ludwig et al., 2007)***.

The immunological nature of psoriasis suggests that these patients are more likely to develop other immune-related diseases. The strongest link so far is to inflammatory bowel disease specifically Crohn's disease ***(Gupta et al., 2005).***

Case series have reported a wide range of psychological characteristics in psoriasis patients including depression, anxiety, obsessive behavior and difficulty expressing emotions such as anger ***(Richards et al., 2004).***

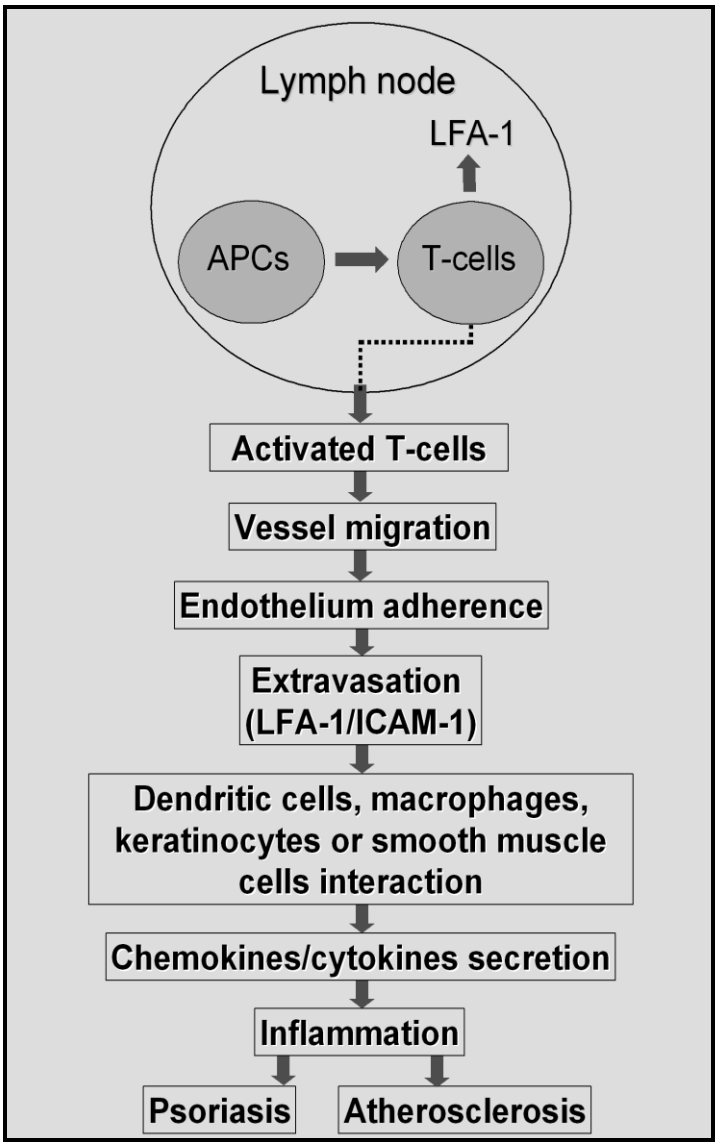

Figure (2): Schematic representation of stepwise developmental process shared between psoriatic and atherosclerotic lesions ***(Ghazizadeh et al., 2010).***

In the lymph node, antigen-presenting cells *(APCs)* activate naive T-cells to increase expression of leukocyte-function-associated antigen-1 *(LFA-1)*. Activated T-cells migrate to blood vessel and adhere to endothelium (and macrophages in case of atherosclerosis). After extravasation mediated by LFA-1 and intercellular adhesion molecule-1 *(ICAM-1)* or CD2 and LFA-3, they interact with dendritic cells, macrophages and keratinocytes in psoriasis but smooth muscle cells in atherosclerosis. These re-activated T-cells and macrophages secrete chemokines and cytokines that contribute to the inflammatory environment, resulting in the formation of psoriatic plaque or atherosclerotic plaque.

Pathogenesis

The 3 main histopathological features of psoriasis are epidermal hyperplasia, dilatation and proliferation of dermal blood vessels and accumulation of inflammatory cellular infilterate ***(Lowes et al., 2007).***

- **Hyperproliferation of keratinocytes**

The cell cycle time of hyperproliferating psoriatic keratinocytes is short. Normally maturation and shedding of keratinocytes in epidermis takes twenty-six days, while in psoriatic epidermis it occurs in four days. Growth factors coming from various cell types are believed to control the increased proliferation ***(Traub et al., 2007).***

The epidermis is thickened and the stratum corneum is poorly organized, inflammatory cells are seen within both the epidermis and the dermis. Both activated T cells and keratinocytes release cytokines and chemokines that stimulate additional inflammation epidermal and vascular proliferation ***(Fig.3) (Kupper, 2003).***

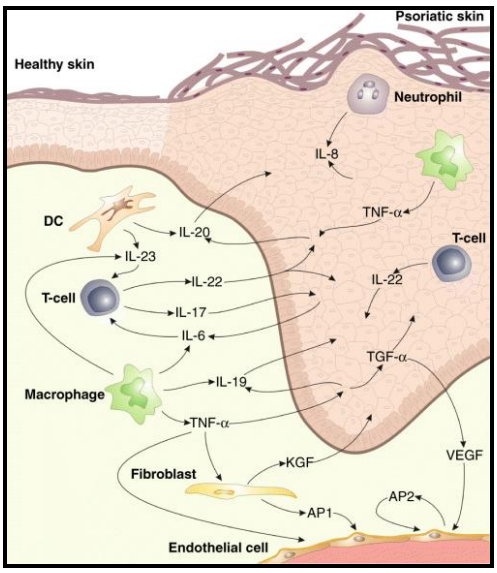

Figure (3): Various cell populations and their mediators are responsible for the 'keratinocyte response' stage of our model of psoriasis pathogenesis ***(Sabat et al., 2007).***

Only the cell types and mediators which are most important for the induction of hyper-proliferation and altered maturation of keratinocytes are presented.

TGF-α (transforming growth factor α); KGF-α (keratinocyte growth factor α); AP1 (activator protein 1).

- **Vascular Changes (Angiogenesis)**

Evidence of the role of endothelial cells in psoriasis includes the increased expression of VEGF. In contrast to the microvasculature of normal skin, the psoriatic microvasculature is characterized by tortuous and leaky blood vessels that facilitate leukocyte migration into inflamed skin. VEGF and angiopoietins are some of the factors believed to be responsible for these vascular changes in psoriasis ***(Young et al., 2006).***

- **Inflammatory Infilterate**

Perivascular cell infiltration composed of T cells, DC, monocytes and macrophages ***(Fig.4)***, later the density of infiltrates increases and CD8+ T *(T- cytotoxic)* cells and neutrophilic granulocytes are found particularly in the epidermis. Neutrophilic granulocytes form the characteristic Munro's micro-abscesses in the epidermis ***(Sabat et al., 2007).***

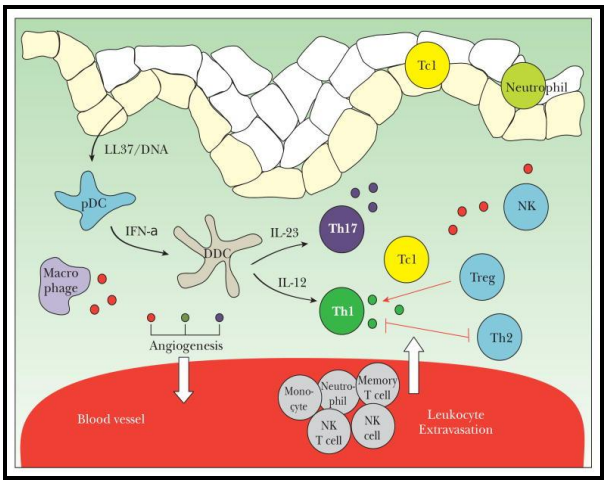

Figure (4): Immune cell types and interactions implicated in psoriasis ***(Rose, 2009)***

Green dot denotes Th1 cytokines including IFN-γ and TNF-α. Purple dot denotes cytokines produced by Th17 including IL-17A, IL-17F and IL-22. Red dot denotes other inflammatory mediators such as IL-2 and IL-6. Low levels of anti-inflammatory cytokines released by Th2 and Treg cells potentially counteract but cannot balance the effects of Th1/Th17 cytokines. Th1 and Th2 cells have a mutually inhibitory effect as denoted by the red line. Treg inhibits Th1 actions as denoted by the red arrow.

HISTOPATHOLOGY

The disease is usually manifested as raised, well-demarcated, erythematous plaques with adherent silvery scales. Histopathologically there is ***(Fig.5)***:

- Hyperproliferation of the epidermis with premature maturation of keratinocytes and incomplete cornification with retention of nuclei in the stratum corneum (parakeratosis) ***(Nickoloff et al., 2004)***.
- The stratum granulosum is thinned or absent ***(Sindhu, 2009).***
- Acanthosis with regular elongation of rete ridges due to the increase of the mitotic rate of the basal keratinocytes ***(Nickoloff et al., 2004)***.
- A dense inflammatory infiltrate composed of clusters of CD4+ Th cells and antigen presenting dendritic cells in the dermis while in the epidermis it is composed of CD8+ T cells and neutrophils ***(Nakajima, 2012)***.
- The redness of the lesions is due to increased number of tortuous capillaries that reach the skin surface through a markedly thinned supra-papillary epidermis ***(Christensen et al., 2006).***
- A sub-corneal aggregation of neutrophils forms Munro's micro-abscesses**.** Presence of spongiform pustules of Kogoj and Munro's micro-abscesses are important for the histopathological diagnosis of psoriasis ***(Mobini et al., 2005).***