Study of Inflammation, Oxidative stress and Cardiometabolic Markers in Psoriasis

Thesis submitted for the award of the

Degree of Doctor of Philosophy in Medical Biochemistry

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I hereby declare that thesis entitled **"Study of Inflammation, Oxidative stress and Cardiometabolic Markers in Psoriasis"** is bonafide and genuine **research** work carried out by me under the supervision of **Dr. Basavaraj Devaranavadagi** (Guide) Professor and Head Dept of Biochemistry Shri B.M.Patil Medical College, Hospital & Research Centre, BLDE (Deemed to be University), Vijayapur, Karnataka, India and **Dr. Balachandra S. Ankad**(Co-guide), Professor and Head Dept of Dermatology, S. Nijalingappa Medical College, Bagalkot, Karnataka, and No part of this thesis has been formed the basis for the award of any degree or fellowship previously.

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Dedication

This is dedicated to my late Father

'In loving memory of

Basavaraj S .Mannangi

Whose love and care

has been a pillar of support to me

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LIST OF ABBREVIATIONS

ABCG - ATP-binding cassette subfamily G member

ACS – Acute coronary syndrome

AHA –american heart association
Ang II - angiotensin II
ApoB - apolipoprotein B
Apo(a) – apolipoprotein (a)
ATP – adenosine triphosphate
ATP-III –adult treatment panel
BMI – body mass index
BSA – Body Surface Area
CAD – coronary artery disease
CAT - catalase
CHD – coronary heart disease
CIMT - Carotid intima-media thickness
Cm – centimeter
CRP- C-reactive protein
CVD – cardiovascular disease
DM – diabetes mellitus
EDGF – endothelial derived growth factor
EDTA – ethylene diamine tetra acetic acid
ELISA - Enzyme-linked immunosorbent assay
FFA – free fatty acid
GSH - glutathione peroxidase
HbA1c – glycosylated hemoglobin
hsCRP – high sensitivity C reactive protein
HDL-C – high density lipoprotein cholesterol
ICAM - Intercellular Adhesion Molecule
IDDM –insulin dependent diabetes mellitus

IFN-Interferon

IL- Interleukin

LDL - low density lipoprotein

Lp(a) – lipoprotein(a)

LDL-C- low density lipoprotein cholesterol

mg/dL – milligram per decilitre

µIU/L – micro International units per litre

mL – milli liter

MetS – Metabolic Syndrome

mm of Hg – milli metre of mercury

n = number of sample

NADP – nicotinamide adenine dinucleotide phosphate

NAPSI - Nail Psoriasis Severity Index

NCEP – national cholesterol education programme

NF-κB – Nuclear factor kappa B

NK cell – Natural killer cell

NO – nitric oxide

NIDDM – non insulin dependent diabetes mellitus

Ox Lp(a) – oxidised lipoprotein (a)

OD - optical density

PAI - plasminogen activator inhibitor

PASI – Psoriasis Assessment Severity Index

PGA - Physician's Global Assessment

PKC – protein kinase C

Plg – plasminogen

ROS –reactive oxygen species

SD – standard deviation

SOD - superoxide dismutase

SPSS – statistical package social system

SMC – smooth muscle cell

SNP – single nucleotide polymorphism

TAOC – Total Anti-Oxidant Capacity

TC – total cholesterol

TG – triglyceride

TNF – tumor necrosis factor

TGF – transforming growth factor

tPA – tissue plasmionogen activator

TF – transcription factor

TOS – Total Oxidative Stress

URAT - urate transporter

VLDL – very low density lipoprotein

VSMC – vascular smooth muscle cell

WHO – world health organisation

ABSTRACT

Aim and Objectives:

The aim of this hospital-based case-control study was to assess the association between psoriasis and cardiovascular comorbidities in India. The objectives were to compare inflammatory markers (serum TNF-α, IFN-γ, IL-2) and total oxidative stress with cardiometabolic risk markers (lipid profile, uric acid, Lp(a), hs-CRP) in psoriasis patients. The study also aimed to explore the relationship between serum uric acid levels and the severity of psoriasis using the PASI score.

Materials and Methods:

The study was conducted in the department of Biochemistry and included a total of 220 participants, with 110 individuals in both the case and control groups. Psoriasis patients were further categorized into mild, moderate, and severe groups based on their PASI score. Fasting venous blood samples were collected to estimate the various markers, including serum TNF-α, IFN-γ, IL-2, total oxidative stress, total antioxidant capacity, lipid profile, Lp(a), hs-CRP, and uric acid.

Results:

In psoriasis patients, there were significant increases in serum TNF-α, IFN-γ, IL-2, total oxidative stress, triglycerides, total cholesterol, Lp(a), hs-CRP, and uric acid compared to healthy controls. Conversely, serum total antioxidant capacity and HDL-cholesterol were significantly decreased in psoriasis patients compared to controls. Notably, serum TNF-α and uric acid levels showed a significant proportionate increase with the severity of psoriasis, as measured by the PASI score. Additionally, there were significant positive correlations between serum triglyceride levels and TNF-α, as well as significant negative correlations between hs-CRP and IFN-γ.

Discussion:

The results of this study highlight the presence of systemic inflammation and dyslipidemia in psoriasis patients, which may contribute to an increased risk of developing cardiovascular diseases. The findings support the importance of monitoring inflammatory markers in psoriasis,

alongside routine assessments of cardiometabolic risk markers such as lipid profile, hs-CRP, and uric acid. Moreover, the significant correlation between uric acid levels and the severity of psoriasis suggests the potential utility of uric acid as a marker for assessing disease severity in these patients.

Conclusion:

In conclusion, this study reveals an association between psoriasis and cardiovascular comorbidities, emphasizing the need for comprehensive care to address the increased cardiovascular risk in psoriasis patients, especially in the early stages of the disease. The findings underscore the importance of considering comorbidities, such as gout development, in the management of psoriasis. Healthcare professionals in the field of dermatology should be vigilant in monitoring inflammatory and cardiometabolic markers to provide effective management and reduce the risk of cardiovascular complications in psoriasis patients.

Key Words: TNF-α, IFN-γ, IL-2, hs-CRP, uric acid, PASI score, Psoriasis.

Chapter 1

INTRODUCTION

Psoriasis is a chronic inflammatory disease of the skin. The common sites affected are nails and joints. Robert Willan, who is considered as the father of modern dermatology, mentioned the first detailed clinical presentation. Hence this disorder of skin is known as Willan's lepra [1]. The prevalence of psoriasis in the world is around 2–3% [2]. India is a diverse country; therefore, the prevalence of psoriasis varies from region to region. This difference in epidemiology is due to environmental and genetic factors [3].

In Greek, the word psoriasis means "the state of having the itch". The characteristic feature presented by psoriasis patients is the color change associated with the plaque formation. Patients complaint lot of itch which varies from none at all to severe. The other symptoms mentioned by psoriasis patients are troublesome sensations, burning, hurting and stinging [4].

Recently psoriasis is defined as a papulosquamous disorder of the skin, which is characterized by impaired keratinization, because of impaired immune system of T cells [5]. The epidemiological survey on psoriasis mentions that males are more affected than the females (2.46:1). The age group of 20-39 years are most commonly affected [6]. The exact etiology is not known. But, some studies have shown that genetic involvement has been observed on familial clustering in psoriasis. These studies have concluded that childhood psoriasis is more common than adults [7]. In India, there are very few studies based on familial predisposition to psoriasis.

There is altered immune function because of genetic and epigenetic changes. This leads to abnormal activation and excessive proliferation of keratinocytes in psoriasis[8]. The

environmental factors responsible for psoriasis are stress, smoking, alcohol consumption, free radicals etc. Patients with genetic predisposition, there is abnormal proliferation and maturation of keratinocytes, dermal blood vessels and mast cells [9].

The clinching feature of psoriasis is involvement of immune system. There is excessive secretion of cytokines such as interleukins (IL): IL-2, IL-6, IL-8, IL-17, IL-18, IL-22, IL-23, IL-24, interferon-γ (INF-γ) and tumor necrosis factor-α (TNF-α) [10]. The cells involved for secretion of cytokines are T-lymphocytes, keratinocytes and dermal macrophages, CD11+ dendritic cells and mastocytes [11]. All these cells produce TNF-α. The TNF-α cytokine enhances the release of IL-6 and Intercellular Adhesion Molecule (ICAM-1) expression. This production of IL-6 by TNF-α leads to stimulation of liver cells and there by release of acute phase reactants such as C-reactive protein (CRP) and fibrinogen [12].

CRP is an acute phase protein associated with the pathogenesis of psoriasis, highlighting its pro-inflammatory role in cytokine activity. Rocha-Pereira et al., conducted a study that demonstrated a link between inflammation and psoriasis. They observed elevated levels of CRP, fibrinogen, haptoglobin, C3, C4 which correlated with the severity of the disease. The study also suggested that assessing elastase, CRP, elastase/α-macroglobulin and elastase/neutrophils in blood vessels could serve as valuable indicators for predicting psoriasis outcomes [13].

The development of comorbidities is a frequent outcome following any-immune mediated inflammatory disease. Psoriasis, in particular, is associated with several

comorbidities, including rheumatoid arthritis, cardiometabolic disease, inflammatory bowel disease and multiple sclerosis[14]. Studies have highlighted the significance of cardiometabolic conditions, kidney disease and mood disorders as important comorbidities in psoriasis patients [15]. Among these comorbidities, cardiovascular diseases are of paramount importance as they directly impact the patients mortality[16]. Research on psoriasis has indicated that cardiovascular risk factors act as independent risk factors, leading to the formation of atherosclerosis through inflammation-mediated endothelial dysfunction[17].

Environmental factors contribute to the exacerbation of psoriasis by promoting the production of Reactive Oxygen Species (ROS). ROS are molecules with unpaired electrons that are generated in normal cells through processes like Fenton reaction, non-Fenton reaction and myeloperoxidase activity[18]. These molecules can adversely affect various biological components of cells, including proteins, carbohydrates, lipids and DNA, contributing to both physiological and pathological processes in the body[19]. Psoriasis patients are particularly susceptible to experiencing increased oxidative stress due to the generation of ROS. Oxidative stress is characterized by an imbalance between ROS production and the concentration of antioxidants in the human body [20]. This imbalance can have significant implications for the pathogenesis of psoriasis.

An imbalance between the production of Reactive Oxygen Species and the body's ability to generate antioxidants leads to the development of various diseases in the body[21]. Antioxidants play a crucial role in maintaining this balance, and they can be both enzymatic and non-enzymatic molecules. Enzymes such as catalase (CAT), superoxide

dismutase (SOD), and glutathione peroxidase (GSH) are part of the body's defense mechanism against oxidative stress[22]. However, studies have indicated that psoriasis is associated with alterations in antioxidant enzyme activity, resulting in increased levels of oxidative compounds like nitric oxide (NO), hydrogen peroxide (H_2O_2), and superoxide anion (O_2) in the skin of affected patients[23]. This imbalance of antioxidants and ROS may contribute to the pathogenesis of psoriasis and potentially other diseases.

Numerous studies have indicated a higher susceptibility of psoriasis patients to develop atherosclerosis and various cardiovascular diseases (CVD), including coronary artery disease, ischemic heart disease, and myocardial infarction [24, 25, 26]. The findings from literature reviews have consistently shown that the risk of developing CVD remains elevated in psoriasis patients, particularly those with severe PASI (Psoriasis Assessment Severity Index) scores [27]. This increased risk persists even after accounting for conventional cardiovascular risk factors, such as obesity, hypertension, diabetes, and smoking[28].

The process of atherosclerosis formation is influenced by inflammation. However, it remains unclear whether psoriasis itself is the primary cause of cardiovascular diseases (CVDs) in psoriasis patients or if other factors like genetic and lifestyle elements play a significant role[29]. The link between psoriasis and the development of CVD is exacerbated by the presence of additional risk factors such as hypertension, obesity, diabetes, and dyslipidemia, as well as the use of certain anti-psoriatic treatments like acitretin, cyclosporine, and corticosteroids[30]. Epidemiological studies have highlighted that the presence of comorbidities, particularly CVD, significantly impacts the life expectancy of

psoriasis patients, especially those with severe PASI scores. These patients tend to have a lifespan that is 3 to 5 years shorter compared to individuals without psoriasis[31].

The current study underscores the importance of developing improved laboratory markers to gain a deeper understanding of psoriasis pathogenesis. Having such markers would facilitate early detection of cardiovascular complications and enhance management during patient follow-up. These markers should ideally correlate with the severity of the disease, as measured by the PASI score, enabling healthcare providers to intervene early in the onset of cardiovascular complications. By utilizing these markers, healthcare professionals can provide timely and targeted care, leading to more effective management of the condition and its associated cardiovascular risks.

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Chapter 2

AIM & OBJECTIVES

2.1 AIM:

The study aims to provide insights into the underlying inflammatory and oxidative stress mechanisms linking psoriasis to cardiometabolic comorbidities and to identify potential targets for prevention and management strategies.

2.2 THE STUDY OBJECTIVES:

1. To measure the levels of serum Th1 cytokines (TNF-α, IFN-γ, and IL-2) in patients with psoriasis and healthy controls.
2. To measure the levels of serum total oxidative status (TOS) and total antioxidant capacity (TAOC) in patients with psoriasis and healthy controls.
3. To measure the levels of serum cardiometabolic risk markers (blood pressure, lipid profile, Lp(a), uric acid, and hs-CRP) in patients with psoriasis and healthy controls.
4. To correlate the levels of serum inflammatory markers, oxidative burden, and cardiometabolic risk markers with the severity of the disease (PASI) in patients with psoriasis.
5. To correlate the inflammatory parameters, oxidative burden with cardiometabolic risk markers in patients with psoriasis.

2.3 HYPOTHESIS FOR RESEARCH

Null hypothesis (H_0):

There is no significant association between serum inflammatory markers and oxidative stress burden with both cardiometabolic risk markers and PASI score in psoriasis patients.

Alternate Hypothesis (H_1) :

There is a significant association between serum inflammatory markers, oxidative stress burden and cardiometabolic risk markers with PASI score in psoriasis patients.

CHAPTER 3

REVIEW OF LITERATURE

3.1 PSORIASIS:

3.1.1 HISTORY:

In the beginning of medicine in the Corpus Hippocraticum, “psoriasis” was described as inflammatory disease of the skin. According to Hippocrates (460 377 BCE) coined the term psora, means “to itch.” The exact etiology of psoriasis is unknown. It is believed due to combination of *genetic and environmental* factors such as infection or stress [1].

3.1.2 EPIDEMIOLOGY

The worldwide prevalence of psoriasis is around 2–3% [2]. The disease is having higher prevalence in the polar regions of the world. But its burden in developing countries like India cannot be underestimated. Ours is a diverse country. So, the prevalence in India varies from region to region. This variation can be due to both environmental and genetic factors. Studies have reported that males have higher prevalence than females during the third and fourth decade of life [3]. It can present at any age. Studies have also observed that psoriasis is having bimodal distribution. The mean age of onset for first presentation in children is between 15 and 20 years, followed by second peak in adults between 55-60 yrs. Based on its bimodal distribution it is classified into two types. The first type or Type I (early onset psoriasis) is more common accounting for more than 75% of cases. The age of onset is <40 years. There is strong evidence of family history. The second type or Type II psoriasis (late onset) which is less common accounts to overall 35% with peak onset at the age of >40 years [4]

3.1.3 CLASSIFICATION:

Since psoriasis is a very broad condition with varied signs and symptoms among different patients, it is useful to classify the condition to help guide the appropriate treatment decisions. Psoriasis can be classified by type, evidence of pustules, pathogenetic cause, affected body area severity and histological characteristics.

The different types of psoriasis are:-

a) Plaque psoriasis :

It is the most common form of psoriasis, which is characterized by clearly circumscribed, round-oval, or coin-sized (nummular) plaques. The lesions start with erythematous papules which then spread peripherally, and later club together to form characteristic plaques, with a white blanching ring surrounding it called Woronoff‘s ring. Based on the structures of plaques, this is further sub-classified into :

i) N psoriasis gyrate - in which curved linear patterns are predominantly seen

ii) N annular psoriasis - ring like fashion secondary to central clearing

iii) N psoriasis follicularis - in which small scaly papules at the openings of pilosebaceous follicles [5].

b) Psoriasis vulgaris:

It is chronic plaque type of psoriasis characterized by sharply demarcated erythematous plaques covered with silvery scales. This is the clinching feature of this type. It accounts for 90% of the cases. The common sites involved are extensor surfaces on elbows and

knees, peri-umbilical, peri-anal, and retro-auricular regions. Around 75-90% of patients have scalp involvement. The two important signs elicited here are Koebner Phenomenon[6] (tendency of skin injuries to trigger psoriasis lesions) and Auspitz Sign (pinpoint bleeding that occurs when psoriasis scales are removed) [7] .

c) Erythrodermic psoriasis:

It is very rare type and involves more than 90% of the body surface area. It is potentially life-threatening due to widespread erythema and exudative exfoliation [8].

d) **Inverse psoriasis**:

Occurs in flexural and intertriginous areas. The important sign seen here is lesions are devoid of scales [9].

e) Pustular psoriasis :

They exist in different forms.

i)The ***generalised pustular psoriasis***, is characterized by disseminated, dark erythematous patches having multiple sterile pustules [10].

ii) The ***localized forms of pustular psoriasis*** include pustulosis palmoplantaris in palms and soles, and acrodermatitis continua suppurativa (of Hallopeau) affecting the tips of fingers and toes. This is potentially life-threatening [11].

f) **Guttate (droplet) psoriasis :**

This is more common in children and young adults. Is occurs following streptococcal throat infection. The possible cause for lesion is antigenic similarity between keratinocytes and streptococcal proteins. Children with guttate psoriasis are prone to develop plaque psoriasis later in their life [12].

g) Psoriatic arthritis :

Inflammation of joints following psoriasis, which leads to joint deformations, bone erosion resulting seronegative arthritis. Around 20-30% of psoriasis patients develop joint inflammation later in their life. The common clinical features are peripheral arthritis, dactylitis (profuse swelling of the fingers or toes), spondylitis and enthesitis (inflammation of the sites where tendons insert into the bone [13].

h) Nail psoriasis :

Involvement of nails following skin lesions seen in 90%. The nail changes include pitting, yellow/brown discoloration, thickening and in severe cases disabling dystrophy [14].

3.1.4 SEVERITY OF THE DISEASE:

The severity of the disease is assessed by Psoriasis Area and Severity Index (PASI). This is a validated tool which combines includes the severity of lesions and the area affected and ending with a single score. This score ranges from 0 to 72 (maximal disease). Scores with 0-7 are considered as mild, 7-12 as moderate, more than 12 as severe [15]. The body is

divided into four areas with head accounting to 10%, arms 20%, trunk 30%, and legs 40% of an individual's skin. The signs seen in each area are erythema (redness), induration (thickness) and desquamation (scaling). The sum of these three severity parameters is calculated for each section and multiplied with area score for that region [16].

Like PASI score another tool which is used called Dermatological Life Quality Index (DLQI) is studied in these patients. This consists of 10 questions related to the quality of life following the impact of skin diseases in individuals. Each question is then rated on a four-point scale (0-3). The sum of all questions ranges from 0-30. The higher the score, the more quality of life is impaired [17].

Other tools to assess disease severity are Body surface area (BSA), Physician's Global Assessment (PGA) and Nail Psoriasis Severity Index (NAPSI) [18].

3.1.5 ETIOLOGY :

The exact etiology in Psoriasis till today is not yet known. It is believed that both genetic and environmental factors are the cause for disease progression in psoriasis.

A) Environmental Factors :

Both exogenous and endogenous environmental factors which includes seasonal variation, skin trauma, hormonal and psychological stress, infection, drugs play a major role in the initiation and progression of the disease [19]. Skin injury, like mild trauma, sunburn and chemical irritants also produces psoriasis through Koebner phenomenon [6]. Following streptococcal infection of throat can ehance the lesions

in psoriasis. Studies have mentioned that patients undergoing tonsillectomy due to recurrent tonsillitis can improve the course of disease.this is due to activation of T-cells following infection and cross-react with keratin self-antigens [20]. Intake of certain drugs such as β blockers, lithium, antimalarials and non-steroidal anti-inflammatory agents are known to exacerbate psoriasis [21]. The other causes are mental stress, chewing tobacco, cigarette smoking and obesity are also responsible for psoriasis [22].

B) Genetics:

Patients with genetic predisposition, psoriasis is high in case of first degree relatives of patients. As per the literature, the risk of getting psoriasis in a child is about 40% when both parents are affected, 15% if one parent is affected and 5% if a sibling is affected by psoriasis. Studies on twins with psoriasis is about 60% in monozygotic and 20% in dizygotic twins. The major genetic determinant of psoriasis is the PSORS1 (Psoriasis Susceptibility gene 1) which is located on chromosome 6p accounting for 35% to 50% of heriditary of the disease [23]. The three important genes associated are HLA- C (HLACw 0602-allele) encoding a class I MHC protein, CCHCR1 (WWCC) encoding the x-helical rod protein 1 and corneodesmosin (allele 5) encoding the protein corneodesmosin. The genes involved in cytokine responses can have either gain or loss of function through NF- κB pathway [24].

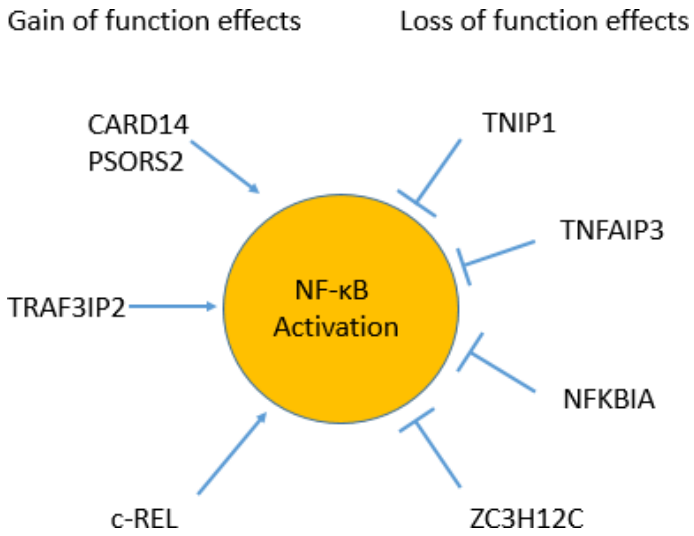

Figure 3.1: Genetic associations in psoriasis through NF-*κ*B pathway.

Source: Reprint from Journal of Autoimmunity, volume 64, Harden J.L. et al, The immunogenetics of Psoriasis :

3.1.6 PATHOGENESIS :

3.1.6.1 Immunology :

Psoriasis is a chronic inflammatory disease of skin. In individuals with genetic predisposition there is involvement of both innate and adaptive immune system due to autoantigen stimulation resulting in "*amplifying inflammatory loops"* [25]. The autoantigens of epidermis which includes LL37 (cathelicidin), keratin 17 and melanocyte-derived antigen ADAMTS-like protein 5 are enhanced in psoriasis. In predisposed individuals, these autoantigens lead to activation of T-cells, mediated through MHC (Major Histocompatibility Complex) class I and KCs (Keratinocytes) [26]. The other pro-inflammatory antimicrobial peptides and

proteins (AMPs) like **S100A7** (*psoriasin*), **S100A15** (*koebnerisin*) and **defensins** which are overexpressed in psoriasis. These act as chemoattractant factors for cells like leukocytes and prime immune cells for extensive secretion of proinflammatory markers [27]. Thus, there is link between KCs and Dendritic cells (DC's) is important for beginning of psoriasis (Figure 2).

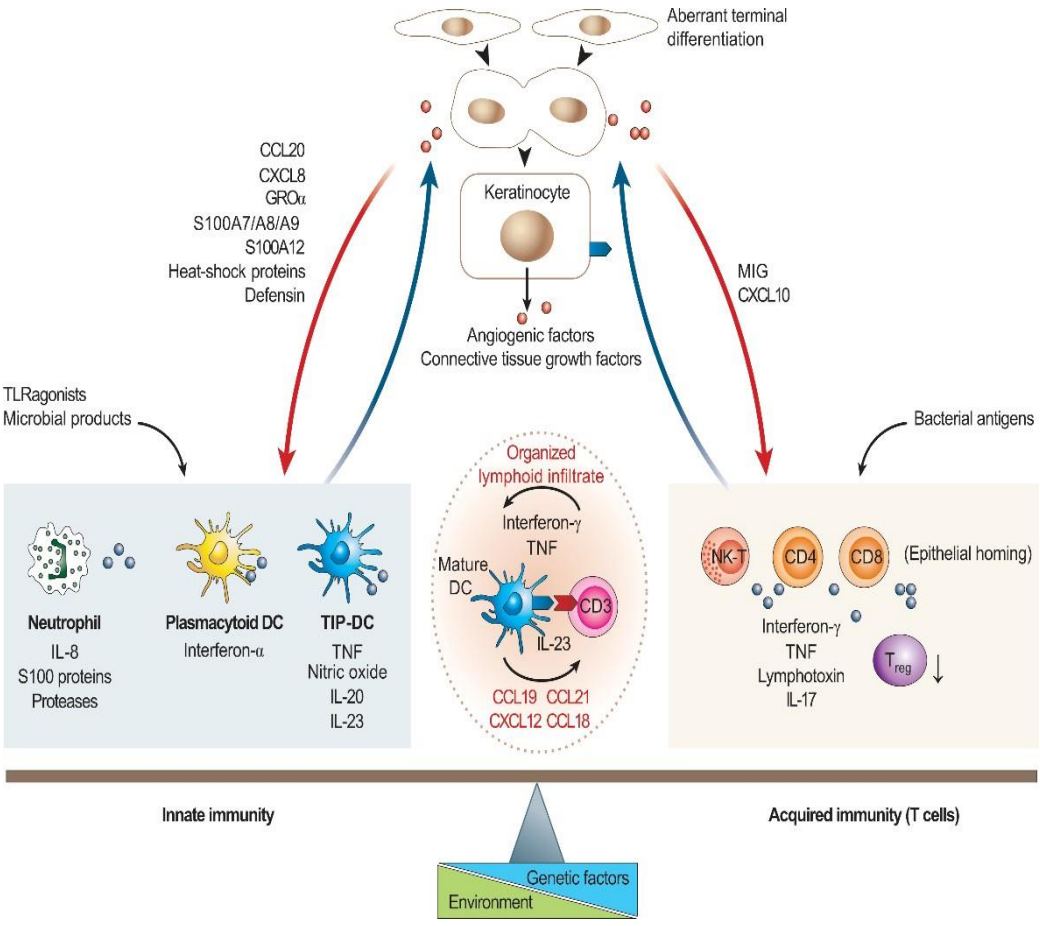

Figure 3.2: Balance between Innate and Adaptive Immune Systems.

Source: Reprinted by permission from Springer Nature, Nature, Pathogenesis and therapy of psoriasis, Lowes M.A. et al, . © 2007.

3.1.6.2 Immunopathogenesis in Psoriasis Plaque Formation:

Formation of plaques due to keratinocytes proliferation is important in Psoriasis. The formation of plaques is due to immune response by cytokines. In psoriasis, the LL37 gets released from activated keratinocytes combines with either DNA and RNA released from neutrophil extracellular traps (NETs) [28].

The LL37 carries positive charge and DNA, RNA carries negative charge, therefore they combine together [29]. This DNA-LL37 complex stimulates plasmacytoid DCs (pDCs) via toll like receptor (TLR) 9 or TLR7 releasing IFN-α. The immature pDCs proliferate into epidermis and identify the keratinocyte expressed autoantigens resulting in hyperproliferation, differentiation and activation of KCs. This is in acute forms of psoriasis.

When RNA is bound to LL37, this complex stimulates myeloid DCs (mDCs) to secrete TNF-α, IL-23 and IL-22 [30]. The mDCs also activates native T-cells into Th1, Th17 and Th22 in the lymphnode [31] (figure 3). The Th1 cells are stimulated by IL-12 and IFN-γ via pSTAT1/4 and T-be [32]. The Th 17 cells are stimulated by IL-1β, IL-6 and IL-23 via pSTAT3 and RORγT [33]. The Th22 cells are stimulated by IL-6 and TNF-α via pp38 and pNF-κB [34].

Table 3.1: Summary of cytokines involved in the pathogenesis of psoriasis

Cells	Stimulated by	Cytokines produced
Th1 cells	Intracellular bacteria, viruses IL-12, STAT4, T-bet	IFN-γ, TNF-α Cellular Immunity
Th2 cells	Extracellular pathogens IL-4, STAT6, GATA3	IL-4, IL-5, IL-13 Hummoral Immunity
Th17 cells	Extracellular pathogens, fungi, autoimmune diseases	IL-17A, IL-17F, IL-22 Cell mediated inflammation
Treg (Regulatory T cells)	TGFβ, FoxP3	TGFβ, IL-10 Immune regulation, peripheral tolerance
Others	Various factors	Other T-cell lines (Th-22)

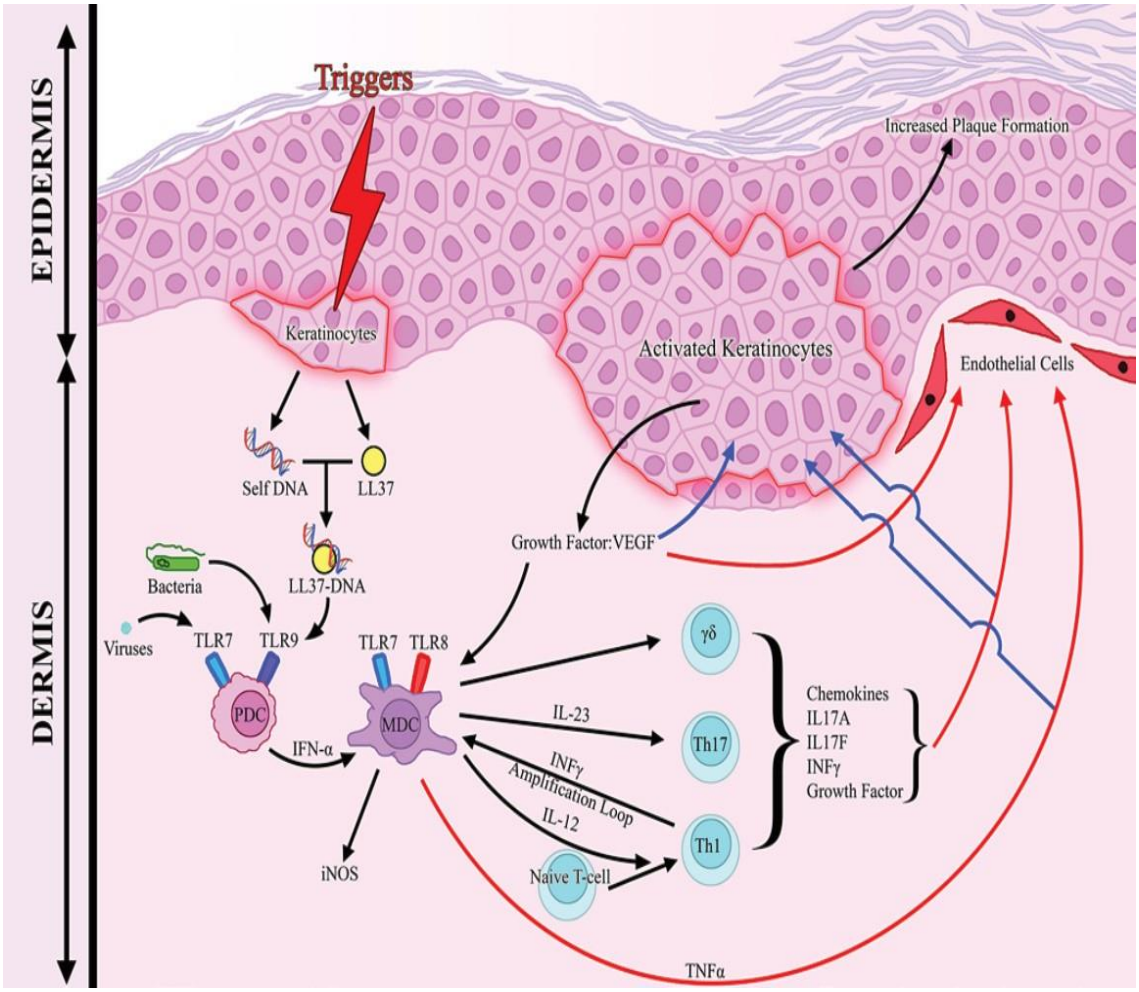

Figure 3.3: Psoriasis plaque formation.

Source: S. Karger AG, Basel, Dermatology, Psoriasis: Keratinocytes or Immune Cells – Which Is the Trigger?, Benhadou F. *et al*, © 2018

3.1.6.3 Recirculation of T-cells from the skin – " The Psoriatic March"

Patients with moderate and severe PASI score have LL37-specific Th/Tc cells in their blood. These T-cells and antibodies against LL37 circulate in the peripheral blood vessels causing systemic inflammation in psoriasis patients. This leads to spread of inflammation into distant sites [35]. This concept of recirculation of T-cells causing systemic inflammation is termed as *" The Psoriatic March"*. The events are as follows :

- Severe psoriasis is a chronic systemic inflammatory disorder
- This state of systemic inflammation induces insulin resistance
- Which in turn causes endothelial dysfunction
- Endothelial cell dysfunction drives atherosclerosis

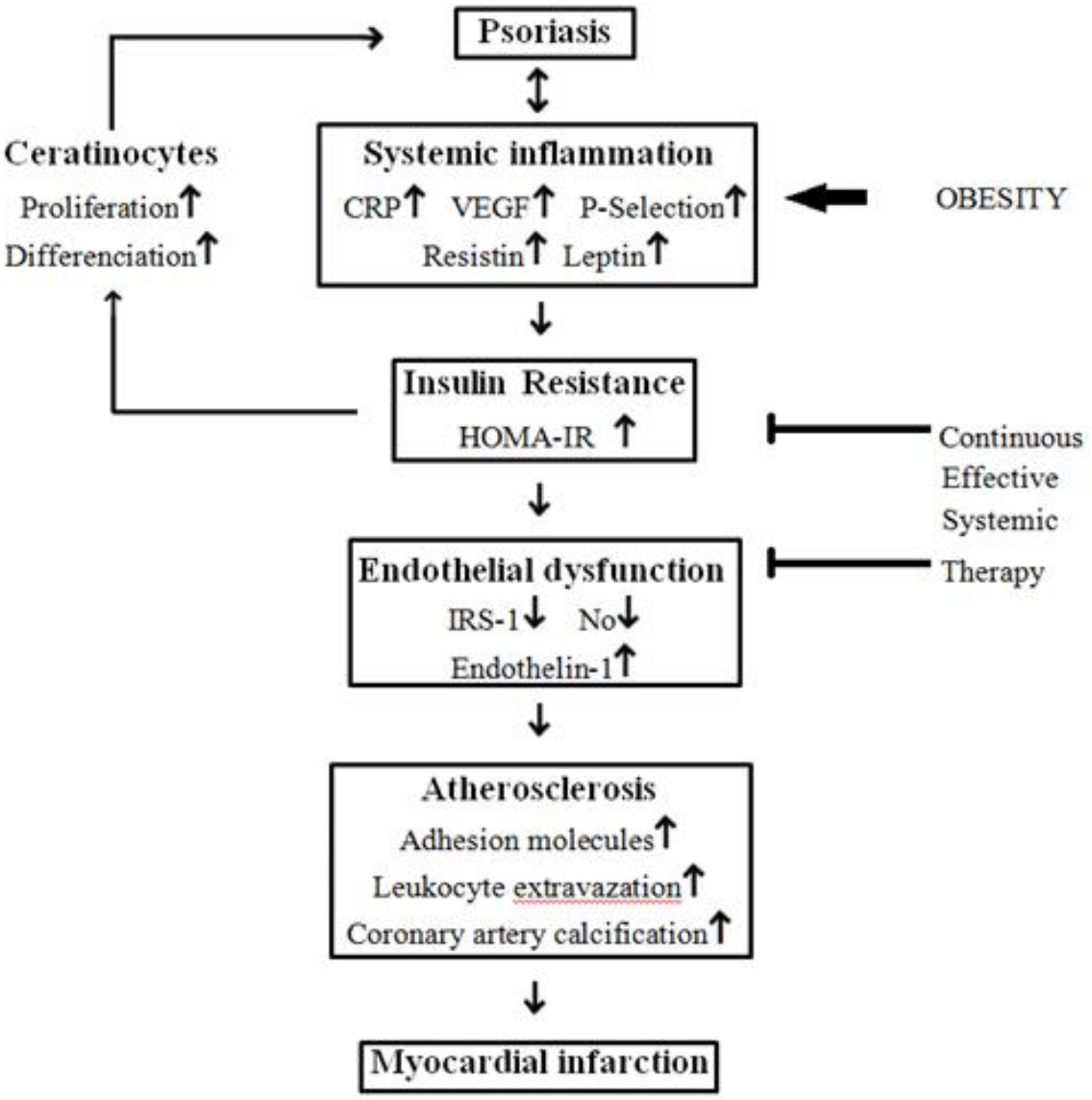

Figure 3.4: The concept of " Psoriatic March"

Source: Boehncke WH, Boehncke S, Tobin AM, Kirby B. The *'psoriatic march'*: a concept of how severe psoriasis may drive cardiovascular comorbidity. Exp dermatol 2011;20:303-7.

3.2 Oxidative Stress in Psoriasis:

The oxidative metabolic pathways are affected in psoriasis. This may lead to development of systemic complications like atherosclerosis and myocardial infarction. The inflamed psoriatic skin liberates unstable free radicals, reactive oxygen species (ROS) and results in production of superoxide anion ($O_2^{\bullet}$) [36]. Many studies have observed, psoriasis patients following immune response are prone for oxidative stress and leading to finally atherogenesis [37]. Reactive oxygen species mediate pathogenesis of atherosclerosis formation, starting from the formation of fatty streak to plaque formation and ending with plaque rupture [38].

This oxidative stress leads to lipid peroxidation (ox-LDL) and decreased levels of antioxidants and drug-metabolizing enzymes in psoriasis patients. This oxidative burden in psoriasis patients aggravate the pre-existing risk of cardiovascular disease [39]. The Reactive Oxygen Species (ROS) are short lived produced from oxygen metabolism in human body. The name itself tells that they are highly reactive creating havoc in the body. The high reactivity is due to presence of unpaired electrons in their atomic orbit [40]. The ROS are *superoxide radical* $O_2^{\bullet-}$, *hydoxyl radical* ($OH^{\bullet}$) and $H2O2^{\bullet}$, and the reactive nitrogen species, are *nitric oxide* ($NO^{\bullet}$) and the *peroxy nitride radical* ($ONOO^{\bullet}$). They become stable by binding with electrons present in nucleic acids, lipids, proteins, carbohydrates. This leads to series of chain reaction in cell leading to death of cell and damage to tissue [41].

Under normal circumstances, our body is protected against ROS by the action of anti-oxidants. There are two types of anti-oxidant system in our body. They are

enzymatic and non-enzymatic. The enzymatic antioxidant includes *superoxide dismutase* (SOD) and *catalase* (CAT), *glutathione reductase* (GSH) and *sulfhydryl* (-SH) groups [42]. The non-enzymatic anti-oxidants are vitamin, vitamin, vitamin E and vitamin. When ROS overrides anti-oxidants, then it leads to exacerbation of numerous cellular process contributing to the development of psoriasis [43].

The exact role of oxidative stress in psoriasis development is not known, however generation of ROS from polymorpho neutrophils (PMNs), keratinocytes, and fibroblasts inturn leads to proliferation of neutrophils and keratinocytes. This is the main pathogenesis involved in alteration of dermal vasculature, and leading to overall a chronical and recurrent inflammatory state fn the skin [44].

It has been observed that some cellular signalling pathways such as *mitogen-activated protein kinase* (MAPK), *activator protein 1*, *nuclear factor-kappa B* (NF-kB), and *Janus kinase* (JAK) which are signal transducers and activators of transcription in psoriasis are said to be redox-sensitive and hence they are involved in the development of psoriasis [45]. Thus there is increased production of *interleukins* (IL2, IL6, IL8, IL17, IL22, IL23), *TNF-alfa, interferon gamma (IFNγ)*, and *vascular endothelial growth factor (VEGF)* in oxidative stress mediated psoriasis . This leads to further a self-amplifying process in psoriatic skin causing keratinocyte over proliferation, hyper vascular hyperplasia, and tissue inflammation appearance [46].

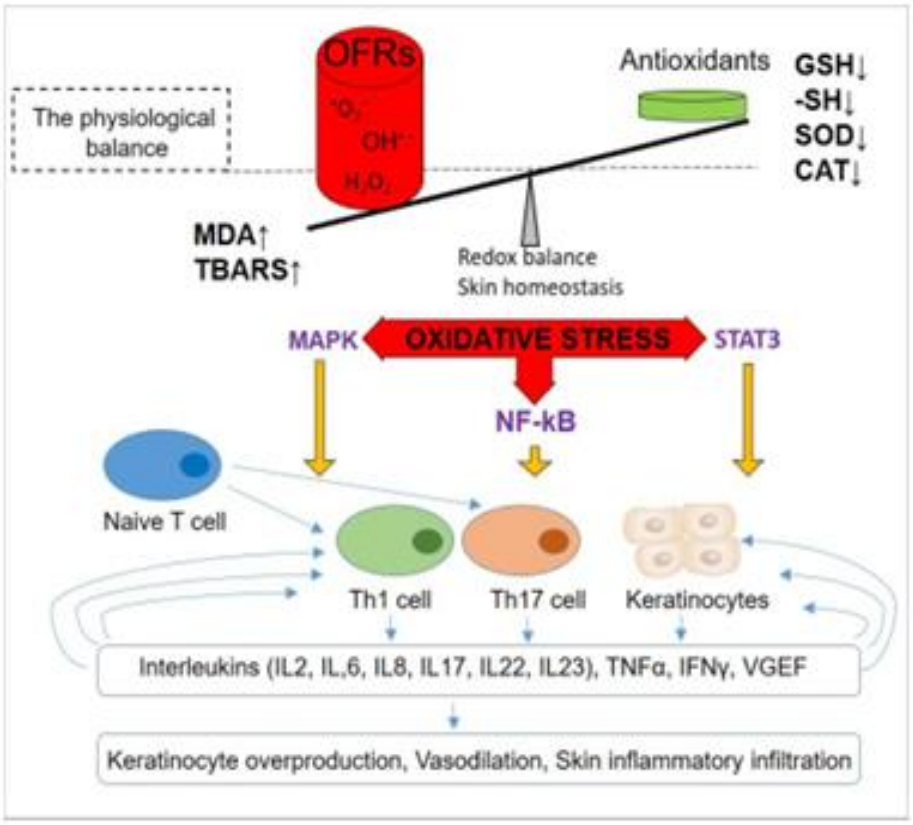

Figure 3.5: Role of oxidative stress in Psoriasis

Source: Zhou Q, Mrowietz U, Rostami-Yazdi M. Oxidative stress in the pathogenesis of psoriasis. Free Rad Biol Med 2009;47:891-5.

3.3 CARDIOMETABOLIC RISK MARKERS IN PSORIASIS :

3.3.1 Cardiovascular Risk in Psoriasis :

Psoriasis being a chronic inflammatory disease of the skin is associated with many co-morbidities and systemic diseases. Among these, relationship with cardiovascular disease and Psoriasis is very important. Study done by Ahlehoff et al., have observed the risk of cardiovascular disease (CVD) and its consequences in psoriasis [47]. What is the cause for this relation between cardiovascular disease and psoriasis is not clearly known. However, factors like obesity, metabolic syndrome, altered lipid metabolism, diabetes mellitus, hypertension, smoking index, alcohol consumption, drug history [48]. Drugs like retinoids, cyclosporins which are used routinely for the treatment of psoriasis are known to cause hyperlipidemia which results in the development of CVD in these patients. Study done by Cohen et al., have observed higher prevalence of subclinical atherosclerosis analysed through Carotid intima-media thickness (CIMT) was more compared to healthy controls in psoriatic arthritis patients [49]. The above-mentioned factors and their role in pathogenesis of psoriasis is explained as follows:

3.3.2 Role of lipids in Psoriasis:

i) **Lipid profile (Triglyceride, Total cholesterol, HDL-cholesterol, LDL-cholesterol)**

Psoriasis is a chronic inflammatory disease of the skin. Study done by Gelfand et al., have mentioned that the plasma lipid levels of total cholesterol(TC), triglyceride (TG)

and high-density lipoprotein cholesterol (HDL-C) and Low density lipoprotein cholesterol (LDL-C) are altered in psoriasis [50]. Few studies have also observed that there are no significant changes in lipid concentrations in plasma in psoriasis patients [51].

The major lipids present in human plasma are total cholesterol, triglycerides, esterified cholesterol and phospholipids. Lipids are non-polar compounds; therefore they are insoluble in the plasma. To solubilize in the plasma, they are complexed with proteins to form Lipoproteins. The lipoproteins are made up of inner coat triglycerides, cholesteryl ester and outer coat made up of cholesterol unesterified, phospholipids and apo-proteins[52]. The apolipoproteins play an important role in maintaining structural configuration of lipoprotein molecule and in regulation of lipoprotein metabolism. These lipoproteins are classified based on density, lipid composition and presence of type of apoprotein on the lipid molecule (Table 2) [53].

Table 3.2: Classification of lipoprotein on the basis of density, lipid composition and type of apolipoprotein on the surface of the molecule.

Lipoprotein	**Source**	**Major Lipid Component**	**Major Apolipoprotein**
Chylormicrons	Intestine	Triglyceride	ApoA I, A C II, C II, A-IV; ApoC III; ApoB-- I, 48: ApoE
Very low-density lipoprotein (VLDL)	Liver	Triglyceride	ApoB 100, ApoC ApoE I, C II, C III;
Intermediate density lipoprotein (IDL)	Catabolism of VLDL	Cholesterol ester	ApoB 100; ApoE, ApoC
Low density lipoprotein (LDL)	Catabolism of IDL	Cholesterol ester	ApoB 100
High density lipoprotein (HDL)	Liver, intestine	Cholesterol ester, Phospholipid	ApoA C I. A II, C-- II, A IV; ApoC III; ApoE

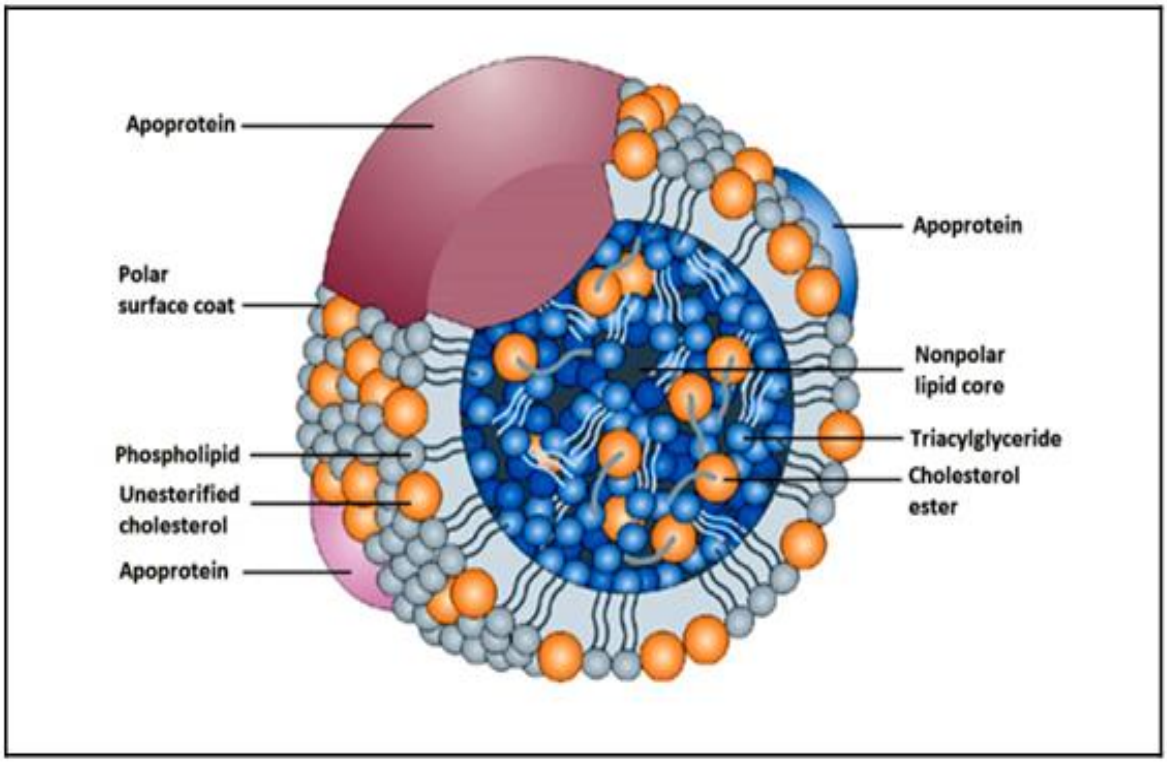

Figure3. 6: Structure of Typical Lipoprotein.

Source : Wasan, K., Brocks, D., Lee, S. *et al.* Impact of lipoproteins on the biological activity and disposition of hydrophobic drugs: implications for drug discovery. *Nat Rev Drug Discov***7**, 84–99 (2008). https://doi.org/10.1038.

Following inflammation in psoriasis, there is acute – phase response (APR) in the body. This includes lipid abnormalities like increased cholesterol, triglycerides and decreased LDL-cholesterol levels. These changes favor atherosclerosis formation. The important mediators responsible for these changes are cytokines TNF-α and IL-1, which are involved in infection and inflammation [54].

The important consequence observed in lipid abnormality following inflammation is hypercholesterolemia. This is due to changes in enzymes involved in lipid metabolism, ATP binding cassette A1 (ABCA-1)- dependent flux, anti-oxidant capacity, impairment in reverse cholesterol transport (HDL-Cholesterol). This is counter balanced by immune response normally. However, due to chronic inflammation in psoriasis, the body is not able to repair this injury, resulting in adverse reactions and thus lipid changes continue to produce abnormalities and thus leading to atherosclerosis formation [55]. Many studies have shown that deposition of lipid abnormalities in epidermis, adipose tissue, erythrocytes (RBCs) is a common observation in psoriasis [56].

The serum LDL-cholesterol is produced in the liver and the main function is to transport cholesterol from liver to peripheral tissues [57]. In oxidative stress condition, the LDL-cholesterol undergoes modification to form oxidized LDL (ox-LDL) [58]. The ox-LDL helps in foam cell formation and leads to endothelial damage [59]. The deposition of ox-LDL in the lumen of arteries along with esterified cholesterol, calcium ions and proliferated smooth muscle cells results in narrowing of lumen and thus stiffens the arterial wall and progressing to atherosclerosis formation [60]. Studies have observed that oxidized LDL leads to activation of cytokine mediated VCAM-1 gene expression in human endothelial cells [61]. The ox- LDL leads to plaque instability by means of apoptosis of vascular smooth muscle cells and decreasing collagen production which are required for plaque stability. This mechanism of ox-LDL for atherosclerosis is increased in psoriasis [62].

3.3.3 Lp(a) in Psoriasis:

Lipoprotein(a)[Lp(a)] is LDL-like particle with apoprotein apo B-100. The apo B-100 is attached through single disulphide link between apo B-100 Cys 3734 and apo(a)kringle (K) IV type 9 Cys67 [63]. Lp(a) is produced in the liver. It is considered as genetic variant of LDL. Around 90% Lp(a) is genetically pre-determined. Lp(a) is having structural similarity to plasminogen and causes impaired fibrinolysis [64]. Studies have observed that Lp(a) levels more than 15mg/dl increases risk of atherosclerosis in individuals. The lipoprotein (a) is considered as an atherogenic oxidative marker. Recent studies have shown that that lipoprotein (a) and apolipoprotein B levels were significantly higher in patients with psoriasis compared with controls [65].

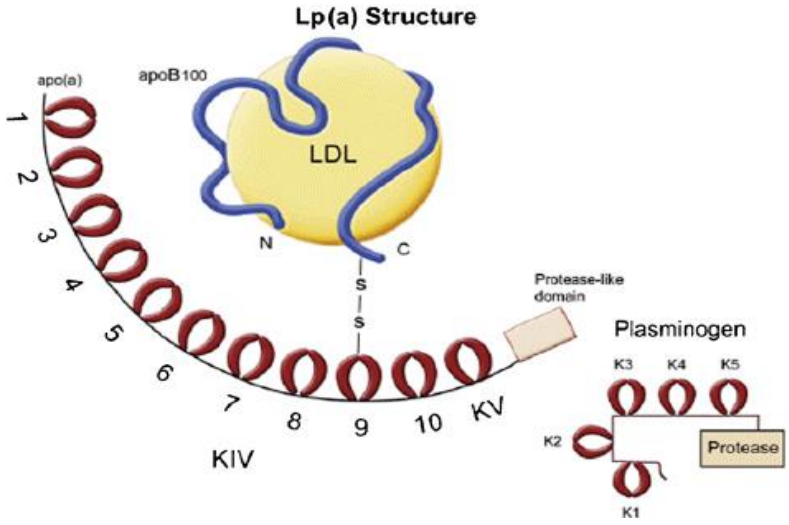

Figure 3.7: Structure of Lipoprotein (a)

Source: Hoover-Plow J, Huang M. Lipoprotein(a) metabolism: Potential sites for therapeutic targets. Metabolism 2013;62(4):479-91.

Lp(a) lacks standard methods of estimation, therefore it is not used routinely to make clinical significance. It is difficult to mention precise cutoff values. This is overcome by establishing reference interval and reported in terms of percentile values. The recent guidelines suggest that value of less than 30mg/dl is used as normal. Lp(a) levels above this are considered as risk for CAD [66]. Since Lp(a) is genetically predetermined, higher concentrations in plasma and lack to environmental triggers make Lp(a) as a risk factor for the disease [67].

3.3.4 Dyslipidemia in Psoriasis:

Studies on lipid metabolism in psoriasis started in the beginning of 20th century. Several studies have proved dyslipidemia is observed in psoriasis patients. Study by Grutz and Burger *et al.,* observed increases levels of total lipids in serum in psoriasis patients and also found improvement in psoriasis after making patients to consume low or less fat diet. They hypothesized that the intestinal epithelial cells similar to psoriatic keratinocytes, the accumulated excessive lipids is known to hinder or delay the absorption of lipids through the intestinal wall [68]. Study by Versari (1936), Incedayi and Ottenstein *et al.,* (1939) found a correlation between psoriasis and abnormal lipid metabolism [69]. Melczer *et al.,* also observed changes in the composition of phospholipids in psoriasis patients and concluded that inflammation, congestion, and parakeratosis seen in psoriatic lesions are due to lipid deposition in the reticular-endothelial system [69]. Some studies have also suggested that removal of psoriatic scales leads to the permanent loss of lipids which could affect serum lipid levels [70].

Study by Rosen et al., observed only hypercholesterolemia in a study on "Lipid Partitions in Psoriasis" in 130 patients [71]. Whereas, study done by Madden et al., in 1939 did not find any significant changes in serum total cholesterol levels in psoriatic individuals and controls [72]. There are many studies which have observed even altered triglyceride, HDL-cholesterol, LDL-cholesterol, Lp(a) levels. With altered lipid metabolism in psoriasis, these patients are prone for CVD risk in future [73].

3.3.5 Hypertension in Psoriasis:

Many studies have shown that there is association between psoriasis and hypertension [74]. A prospective study on evaluation of the frequency of concomitant diseases, using data base analysis. It was found that around 40,000 dermatologic patients had a greater association of concomitant hypertension in psoriasis compared to patients with other dermatologic conditions [75]. Study done by Qureshi *et al.,* found that females with psoriasis had an increased risk for hypertension compared to males in their study [76]. Amstrong *et al.,* showed that psoriatic patients with hypertension on mono anti-hypertensive therapy were lesser in number compared to dual antihypertensive therapy, triple antihypertensive treatment and quadruple therapy [77].

The exact mechanism underlying between psoriasis and hypertension is not known. Research studies have proposed that adipose tissue in psoriasis patients serves as a major source for angiotensinogen production, which gets converted into angiotensin II later. This Angiotensin II promotes salt retention in kidneys [78]. Studies have observed that in psoriasis, this Angiotensin II along with retention of salt in kidney also stimulates

proliferation of T-cells. The T-cells promotes inflammation and there by leads to development of atherosclerosis An association between psoriasis and hypertension may also be due to the increased oxidative stress in patients with psoriasis [79]. Some researchers have mentioned that increased the visceral adipose tissue in psoriasis patients may contribute for hypertension development. Increased visceral adipose tissue leads to accumulation of perivascular fat, which stimulates the effector T cells and promotes dysfunction in hypertension as well as in psoriasis. However, these findings do not illustrate completely about the persistent, significant association between both psoriasis and hypertension even after adjusting for body mass index (BMI) [80]. Other studies have hypothesized that endothelin-1 plays an important role in the development of hypertension in psoriasis patients. Endothelin-1 is a protein which constricts blood vessels and thereby increases blood pressure. This is produced by several different cells in the body including keratinocytes[81]. In psoriasis the expression of gene for endothelin-1 seems to be altered [82]. Studies have found that the levels of serum endothelin-1 were increased in psoriasis patients when compared to controls and also showed correlation with the severity of disease. Increased endothelin-1 levels are known to exert a greater vasoconstrictive effect on blood vessels which leads to the development of hypertension [83].

3.3.6 Metabolic Syndrome (MetS):

In 1988 Gerald Reaven *et al.,* an endocrinologist from Stanford University, USA described the metabolic syndrome (MetS)[84]. It is cluster of four risk factors, which includes dyslipidemia, hypertension, glucose intolerance, central obesity [85]. Mallbris *et*

al., in his study discussed about the metabolic disorders of glucose, lipids in patients with psoriasis and psoriatic arthritis [86]. Sommer *et al*., in his study reported higher occurrence of metabolic syndrome in psoriasis compared to controls [87].

Different studies have different criteria to diagnose metabolic syndrome. However, in the year 2009 a joint statement by the *IDF Task Force on Epidemiology and Prevention, National Heart, Lung, and Blood, American Heart Association, World Health Federation, International Atherosclerosis Society, and International Association for the Study of Obesity* stated that, obesity and insulin resistance are not pre-requisites for MetS. Any three out of the five components would be necessary for a diagnosis of MetS (**Table 3**). However, waist circumference (WC) is important since it measures ethnic and nation specificity [88].

Table 3.3: Criteria for Definition and Diagnosis of Metabolic Syndrome by various Associations

S. No	WHO (1998)	EGIR (1999)	NCEP-ATP III (2005 revision)	IDF(2005)	AACE (2003)
1	Insulin resistance or diabetes + Two of the following	Hyperinsulinemia (plasma insulin > 75th percentile) + *Two of the following*	Any three of the Following	Central obesity = WC (ethnicity and gender specific) + *Two of the following*	IGT or IFG plus any of the following based on the clinical judgment
2	Abdominal obesity Waist/hip ratio: > 0.90 (male), >0.85 (female); or BMI > 30 kg/ m2	WC ≥ 94 cm (male) ≥80 cm(female)	WC ≥ 40 inches (male); ≥ 35 inches (female)	Triglycerides >150 mg/dL	BMI > 25 kg/ m2
3	Triglycerides 150 mg/Dl	Triglycerides >177 mg/dL Or HDL C	Triglycerides >150 mg/dL	HDL-C <40mg/dl(male) <50mg/dl(female)	TGs ≥150 mg/dL or on TGs Rx. HDL-C < 40 mg/dL (male)
4	HDL-C 35 mg/ dL (male) 39 mg/dL (female)		HDL-C <40mg/dl(male) <50mg/dl(female)	BP > 130/85mm Hg	
5	BP>140/90 mmHg	BP >140/90mm Hg	BP >130/85mm Hg	IGT or IFG (but not diabetes)	BP >130/85 mmHg
6	Microalbuminuria 30 mg/g		FBS >100 mg/dL	Other features of insulin resistance.	FBS >100 mg/dL

The pathogenesis of MetS is very complicated and is still clearly not known. The important causative factors are central obesity and IR [89]. The major causes for developing MetS are decreased physical inactivity, a high fat and carbohydrate intake in diet, leading to central obesity and IR. The defining components in MeS are obesity, IR, hypertension and dyslipidemia [90].

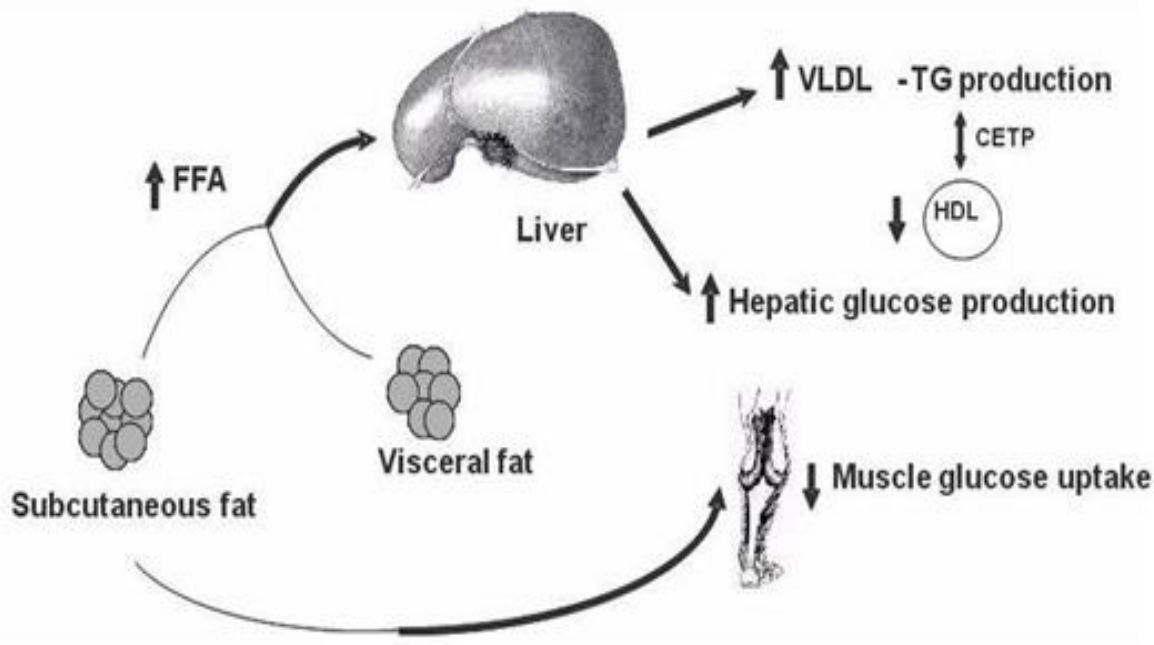

Figure 3.8: Inter-relationship between features of Metabolic Syndrome

Source : Kirk EP, Klein S. Pathogenesis and pathophysiology of the cardiometabolic syndrome. J Clin Hypertens (Greenwich). 2009; 11(12):761-5.

3.3.7 hs-CRP in Psoriaisis:

In 1930 Tillett and Francis described C-reactive protein (CRP) is a substance present in the serum of patients with acute inflammation which has reacted with the C polysaccharide capsule of Pneumococcus [91]. CRP is synthesized only in the liver following stimulation by proinflammatory cytokines particularly IL-6, which plays an important role in the synthesis of all major acute phase proteins [92]. It belongs to a family member of pentraxin proteins, because of its disc-like (annular) pentameric structure with five identical sub-units (Figure 8). The pentraxins are calcium and phospholipid binding proteins having immune defence function [93].

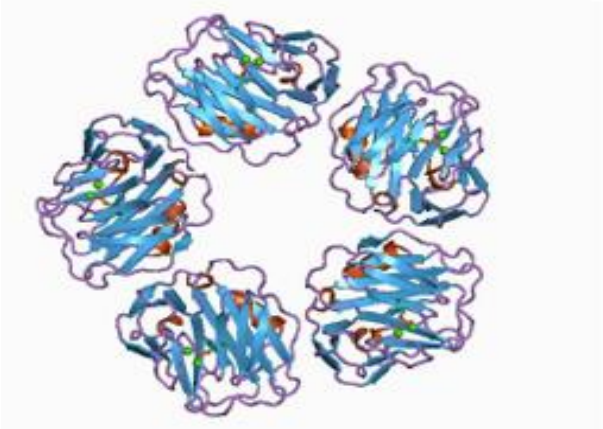

Figure 3.9: Three-dimensional structure of CRP

Source: Mantovani A, Garlanda C, Doni A, Bottazzi B (January 2008). "Pentraxins in innate immunity: from C-reactive protein to the long pentraxin PTX3". *Journal of Clinical Immunology*. 28 (1): 1–13. doi:10.1007/s10875-007-9126-7

Highly sensitive C-reactive protein (hs-CRP)is a subunit of CRP. Standard measurements for serum CRP lacks sensitivity which is require to determine the cardiovascular risk clinically.to overcome this hs-CRP is used which detects smaller amounts in the sample. hsCRP is called Lower detection limit using highly sensitive assay [94]. Hs-CRP is considered an independent risk marker for cardiovascular disease. When cholesterol concentrations are low in serum hs-CRP concentration can be used to predict vascular risk [95]. When patients are having low LDL-Cholesterol and high hs-CRP levels in serum, they are known to have higher risk of development of coronary events in future [96]. C-reactive protein has special importance for psoriasis due to its relation with cytokines which are responsible for skin inflammation.

Studies have shown association between CRP and psoriasis. Isha *et al*., studied on comparison on levels of serum CRP and uric acid in psoriasis patients before and after 12 weeks of treatment and in group of patients with other skin diseases other than psoriasis. They observed that serum CRP levels were found to be increased by 20 times more in patients with psoriasis before 12 weeks treatment and decreased around 50% in patients after 12 weeks of treatment. They suggested that both CRP and uric acid levels should be monitored while treating the patients with psoriasis [97]. Studies have shown that CRP can be a used as a better marker for disease severity in psoriasis [98]. Study done by Pereira *et al*., observed the role to of CRP and comparison with the severity of psoriasis and found that severe psoriasis patients had higher CRP values than those patients with mild or moderate psoriasis [99].

C-reactive protein marker is considered as predictor for cardiovascular diseases like myocardial infarction, ischemic stroke and sudden cardiac death in patients with and without coronary heart disease (CHD) [100]. CRP plays a key role in atherosclerosis formation by means of activation of complement pathway, uptake of fats by macrophages (foam cells), release of proinflammatory cytokines. It also activates the expression of tissue factor in monocytes cells, stimulates the endothelial cells dysfunction and inhibits NO release [101].

3.3.8 Uric acid in Psoriasis

Serum uric acid (SUA) is a metabolic end product of purine nucleotides by the enzyme xanthine oxidoreductase [102]. Uric acid is a heterocyclic compound composed of carbon, nitrogen, oxygen, and hydrogen derived from the both exogenous and endogenous purine metabolism. The main sites of uric acid production in the body are in liver, gut, muscles, lungs, kidneys and the vascular endothelium. The normal levels of SUA is from 1.5 to 6.0 mg/dL in females and 2.5 to 7.0 mg/dL in adult males [103]. The SUA homeostasis in the body is maintained by a balance between its production in liver and excretion in kidney and intestines. Kidneys and intestine excrete uric acid via the presence of urate transporters on the cell surface.

The primary urate transporters are *urate transporter 1* (URAT1), located on the apical surface of proximal tubular cells, *glucose transporter 9* (GLUT9), located on the basolateral membrane of the proximal tubule, and *ATP-binding cassette subfamily G member 2* (ABCG2), located on both intestinal and renal cells surface [104]. Whereas

organic anion transporters (OAT1,OAT2,andOAT3) present at the basolateral membrane, *sodium dependent phosphate cotransporters* (NPT1 and NPT4) and *multidrug resistance protein-4* (MRP4) at the apical membrane, mediates urate secretion [105]. URAT 1, GLUT 9 and OAT4 transporters are also responsible for the tubular urate reabsorption [106].

Uric acid is formed via series of biochemical reactions which lead to the degradation of adenosine and guanosine. The key enzyme xanthine-oxidase will finally convert xanthine to uric acid molecule [107]. Earlier it was known as the causative agent for gout, but now it has gained much because of its double-faced nature as a risk and protective marker in conditions.[108]

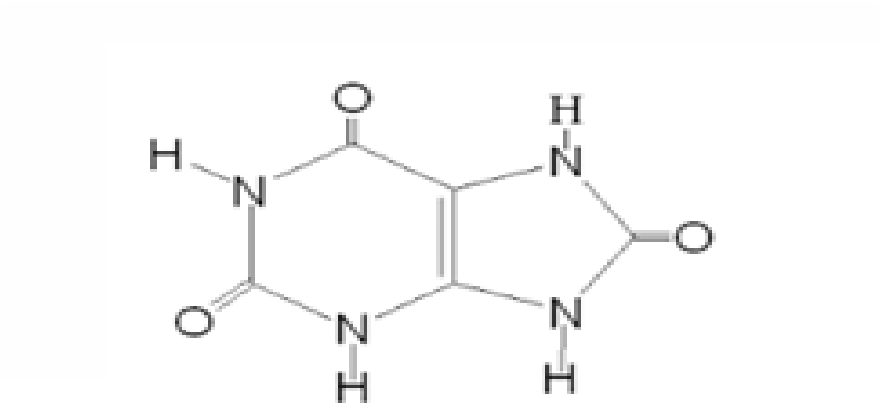

Figure 3.10: Structure of Uric Acid

Molecular formula for uric acid $C_5H_4N_4O_3$.

Chemical structure of uric acid is 7, 9-dihydro-1H-purine 2,6,8(3H)-trione (2,6,8-trioxypurine).

Source : Busch A, Stief, Thomas. Taurine strongly enhances the ROS generation of blood neutrophils . Thrombin and Singlet Oxygen (1ΔO2*) Main Factors of Hemostasis 2013;1:217-228 .

Earlier hyperuricaemia was known to cause gout in a individual with lavish lifestyle having rich foods and alcohol consumption. In recent years it is also associated with major chronic diseases like CVD, DM and MetS [109]. Studies have shown that the prevalence of CVD is associated with higher levels of serum uric acid [110]. Hyperuricemia is associated more commonly in patients with psoriasis. Many studies have shown correlation between serum uric acid levels with psoriasis and psoriatic arthritis [111]. However, some observational studies have reported conflicting results with respect to serum uric acid levels and psoriasis [112]. Studies in western countries have observed serum uric acid levels higher in patients with psoriasis but no significant variations were found from East Asia, India and the Middle East regions. The studies showed that the correlation between psoriasis and hyperuricemia was related to either ethnicity- or region-dependent [113]. The relationship between uric acid, metabolic disorders and cardiovascular events in patients with psoriasis is not yet clear.

Serum uric acid (SUA) is involved in inflammatory pathways by stimulating secretion of proinflammatory chemokines [114] (figure 10). Not only as inflammatory marker but also acts as antioxidant in psoriasis [115]. Kwon et al. observed that in psoriasis proliferation of keratinocytes induces an increased metabolism of purine, which is responsible for increased levels of SUA in patients with psoriasis [116]. In the year 2016, studies reported that there is a relationship between psoriasis and SUA levels [117]. Gisondi *et al*., observed

that psoriasis is the most important causative factor for hyperuricemia along with other risk factors like obesity / metabolic syndrome. Elevated SUA levels are associated with metabolic syndrome outcomes such as obesity, cardiovascular diseases, and hypertension [118].

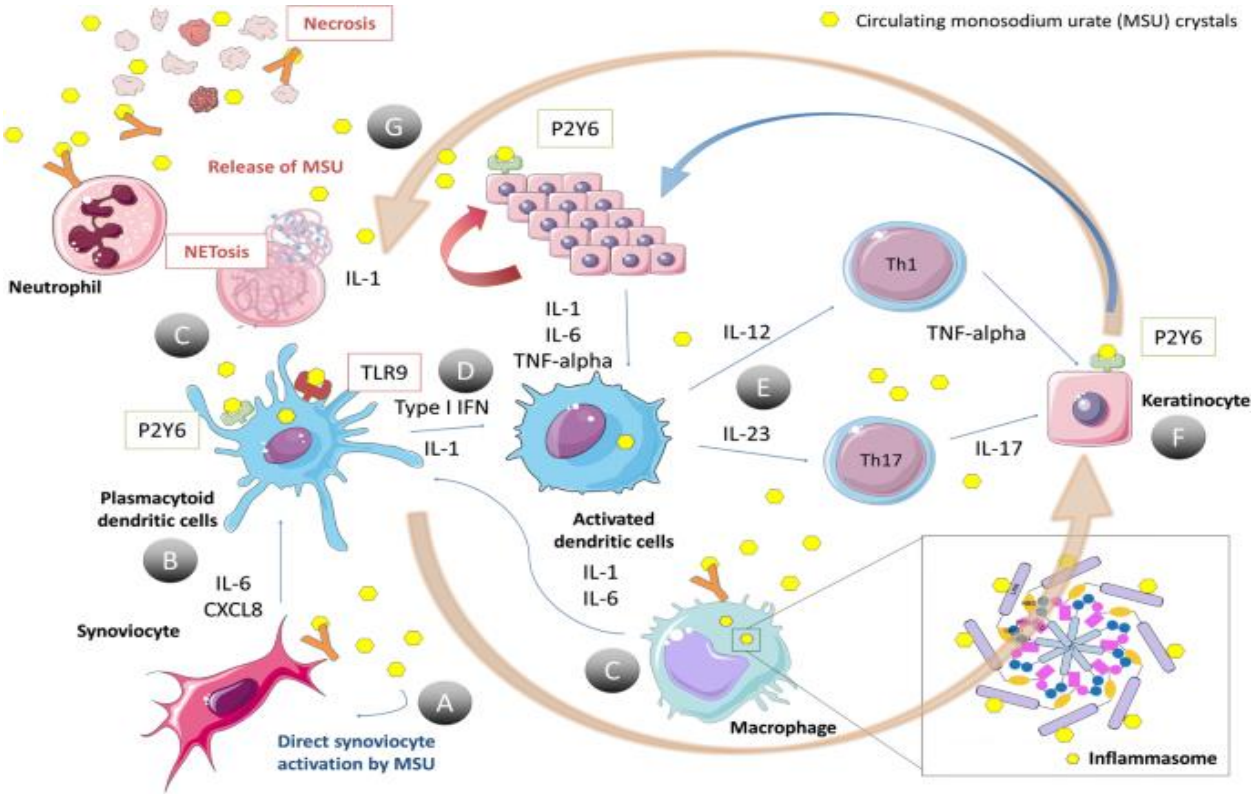

Figure 3.11: Interplay between Uric acid and Psoriasis

Source : Felten, R., Duret, PM., Gottenberg, JE. *et al.* At the crossroads of gout and psoriatic arthritis:"psout". *Clin Rheumatol* **39**, 1405–1413 (2020). https://doi.org/10.1007/s10067-020-04981-0.

3.3.9 Other risk markers

a) Leptin :

Leptin is known to induce in obese individuals other proinflammatory mediators which includes C-reactive protein (CRP), plasminogen activator inhibitor-1 (PAI-1), transforming growth factor β, interleukins (IL-1, IL-6) and tumor necrosis factor-alpha (TNF-α) [119]. It stimulates cholesterol uptake by macrophages, angiogenesis, platelet aggregation, stimulates the oxidative stress in endothelial cells and proliferation of vascular smooth muscle cells increasing the risk of atherosclerosis in psoriasis [120].

b) Insulin Resistance:

Systemic inflammation induces insulin resistance. This pathway involves activation of *phosphoinositide-3-kinase* (PI3K) and leads to activation of endothelial nitric oxide synthase (eNOS) by means of phosphorylation. Insulin may also activate the pro-atherogenic *mitogen activated protein kinase* (MAPK) pathway in endothelial cells. Insulin resistance is by pro-inflammatory cytokines and adipokines [121].

c) Adipokines :

Adipokines are cytokines secreted by adipose tissue which play in fatty liver development is involved in the pathogenesis of psoriasis. The different adipokines like adiponectin, IL-6, resistin, TNF-α are involved in the pathogenesis of psoriasis by means of increased keratinocytes proliferation. These adipokines increase pro-inflammatory cytokine production by T- lymphocytes and macrophages resulting increased expression

of vascular endothelial cell adhesion molecules (e.g. ICAM-1) that results in angiogenesis formation in future in psoriasis patients [122].

3.4 Comorbidities Development in Psoriasis :

Psoriasis is associated with development of several co-morbidities in patients. They are decreased quality of life, depression, increased risk of cardiovascular disease, type 2 diabetes mellitus, hypertension, metabolic syndrome and Crohn's disease. Psoriasis is considered as a chronic inflammatory disease of the skin. The characteristic Th-1 chronic inflammation of the psoriatic plaque is linked to the chronic systemic inflammatory process such as development of insulin resistance, atherosclerosis and plaque rupture via various inflammatory cells and mediators [123].

a) Cardiovascular Disease (CVD):

The most important comorbidity observed is link between psoriasis and cardiovascular disease. Literature mentions that psoriasis is an independent risk factor for development of cardiovascular disease. The causative factors for development of CVD in psoriasis are dyslipidemia, obesity, diabetes mellitus, hypertension, coronary calcification, increased highly sensitive C-reactive protein (hs-CRP), decreased foliate and hyperhomocysteinemia [124]. The inflammatory response is the common mechanism for both psoriasis and systemic disease, characterized by the presence of proinflammatory cytokines and endothelial activation [125]. The cardiovascular risk factors seen in psoriasis are found to be associated with atherosclerosis formation leading to development of coronary artery, cerebrovascular and peripheral vascular diseases [126]. A cross sectional

study by Kimball *et al.*, showed an increase prevalence of cardiovascular diseases patients with psoriasis compared with controls [127]. A population based retrospective cohort study by Gelfand *et al.*, suggested an independent risk for development of myocardial infarction (MI) in patients with psoriasis [128]. The incidence of MI was higher in patients with psoriasis than in the control group and also showed relationship with the severity of the disease. Shapiro et al., observed strong association between psoriasis and development of atherosclerosis, heart failure and diabetes mellitus in the population of Israel [129].

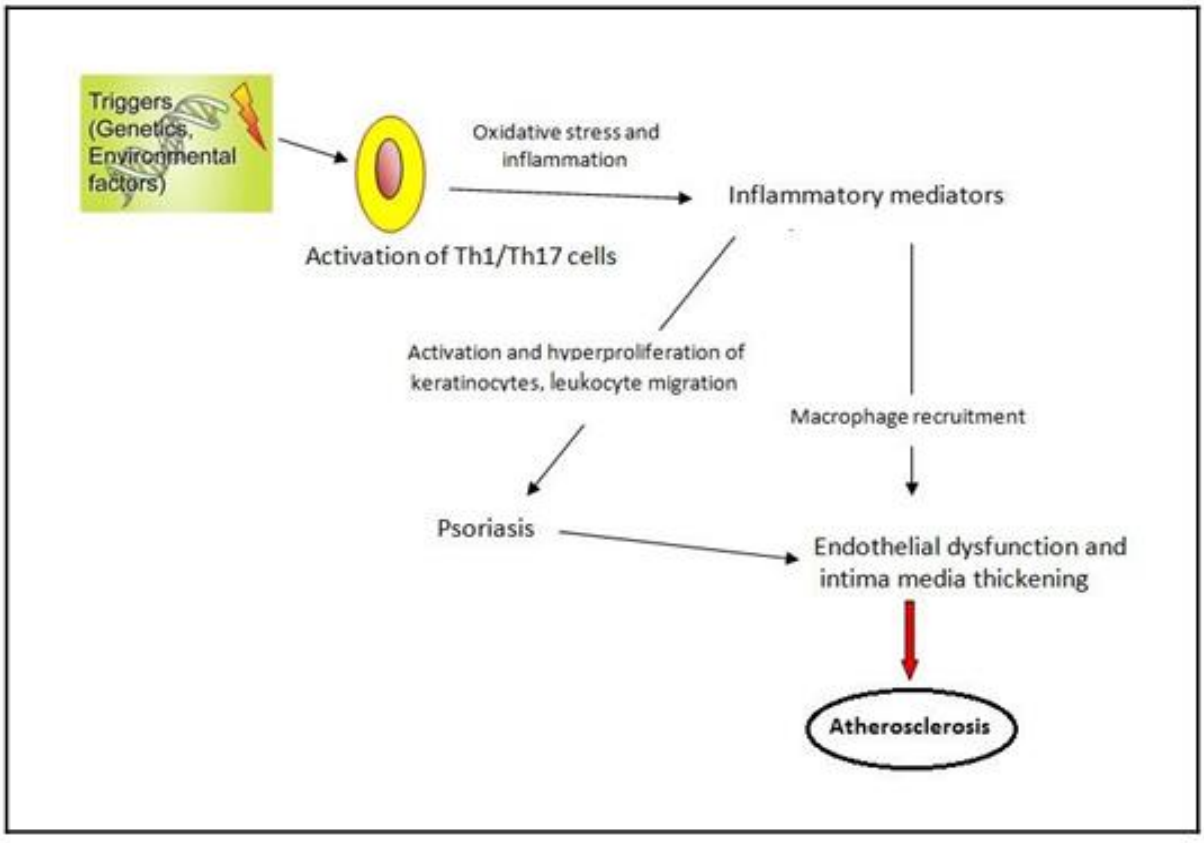

Figure 3.12: Progression of Psoriasis to Atherosclerosis formation.

Source : Andreas J et al., Psoriasis and atherosclerosis: two plaques, one syndrome? European Heart Journal.2012; 33:1989–1991 doi:10.1093/.

b) Autoimmune Diseases :

Earlier psoriasis was thought to be a disease of skin primarily, however the incidence of Crohn's disease and ulcerative colitis is 3.8 to 7.5 times greater in patients with psoriasis than in the general population [130]. These three diseases have similar multiple genetic loci present on chromosome16. Studies have observed possible link between multiple sclerosis (MS) and psoriasis [131].

c) Obesity:

Several studies have shown association between psoriasis and obesity. This obesity itself has serious health consequences such as hypertension, vascular disease, and type 2 diabetes mellitus. All these risk factors make obese individual with psoriasis for development of cardiovascular disease [132]. Studies from the United States have shown that an elevated BMI in patients is associated with psoriasis [133]. Obesity is due to the interaction between genetic and environmental factors [134]. Obesity is due to imbalance between intake of food and calorie burnt [135]. In other words weight gain would be due combined factors such as low calorie burn associated with sedentary life style, decreased physical activityand high respiratory coefficient termed as *carbohydrate-to fat oxidation ratio* [136].

3.4.4 Metabolic Syndrome:

Metabolic syndrome is defined as the combination of obesity, impaired glucose tolerance, hypertriglyceridemia, reduced high- density lipoprotein, and hypertension. Patients with metabolic syndrome are at an increased risk for developing cardiovascular morbidity and mortality in psoriasis [137].

d) Quality of Life (QOL):

Griffiths *et al.,* suggested that psoriasis causes development of psychosocial morbidity and decrement in occupational function [138]. In a large Health survey study with more than 300 psoriasis patients the experienced physical and mental disability compared to patients suffering from chronic illnesses such as cancer, arthritis, hypertension, heart disease, diabetes, and depression [139]. QOL is an important measurement adjunct tool to skin lesion in assessing complete effect on health illness in psoriasis. Dermatology specific assay tool, such as the Dermatology Life Quality Index or SKINDEX are very useful to assess the QOL in psoriasis [140].

e) Depression/Suicide:

Studies have observed that psoriasis patients are associated with lack of self- decision making and increased prevalence of mood disorders including depression. The prevalence of depression in patients with psoriasis may be as high as 60% compared to other non-psoriatic causes . Depression sometimes be very severe enough that, some patients compel to commit suicide. In a study of 217 psoriasis patients , around10% reported a wish to be dead and 5% reported active suicide [141].

Treatment in Psoriasis:

Treatment for psoriasis is individual based which includes such as the nature / type of the disease, sites involved, response to previous therapy, severity and duration of disease. In addition to these, specific parameters like age, sex, general health, quality of life and their knowledge in understanding about the disease need to be taken into consideration [142].

The treatment includes:

- Topical treatment
- Systemic treatment
- Phototherapy
- Biologic treatment

Conclusion of the Literature:

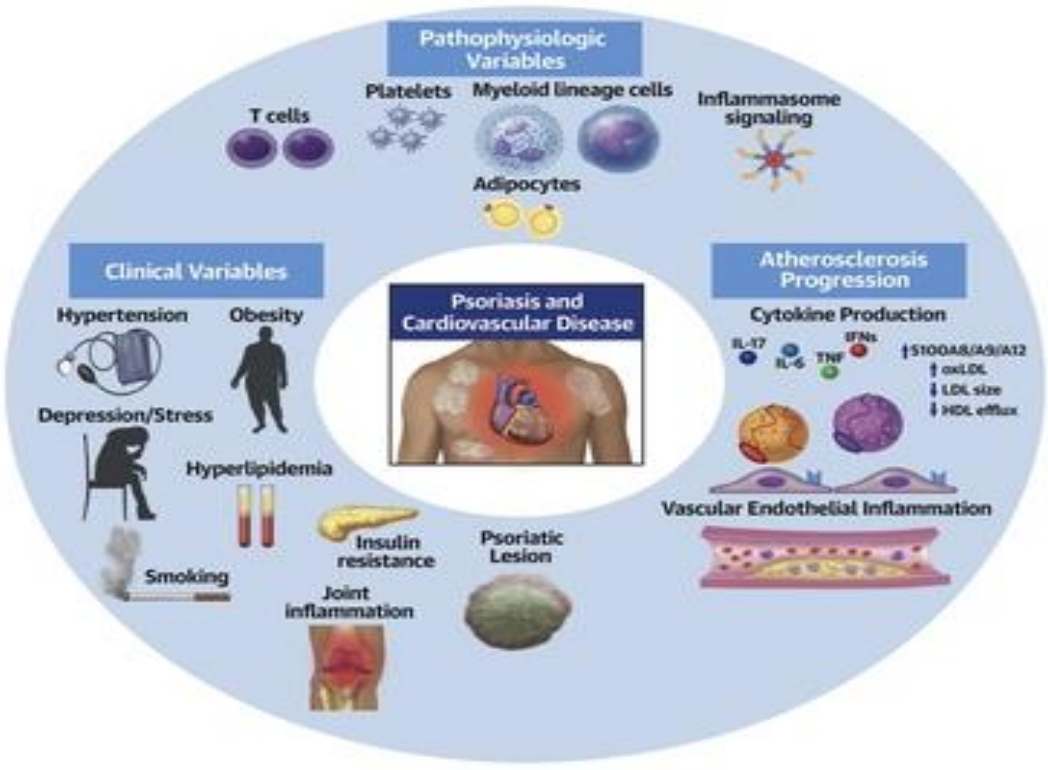

Figure 3.13: Psoriasis and Cardiovascular Disease

Source : Garshick MS et al., J Am Coll Cardiol 2021;77(13):1670-80.

Psoriasis is recognized as a chronic, systemic, immune-mediated inflammatory skin disease. Population-based epidemiological studies have shown that patients with moderate to severe psoriasis have an increased risk for various cardiovascular comorbidities including hypertension, diabetes, hyperlipidaemia, obesity, metabolic syndrome and cardiovascular diseases. Associated cardiometabolic risk factors, lifestyle issues, pro-atherogenic medications, and the underlying chronic systemic inflammation of psoriasis will all contribute to the increased cardiovascular risk.

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CHAPTER 4

MATERIALS AND METHODS

4.1 Study design:

This was a case-control study conducted in a tertiary care centre SNMC and HSK, RC Bagalkot of North Karnataka, India.

4.2 Study Period :

The study was conducted from 2019 to 2021 for a period of three years.

4.3 Sampling Technique :

Simple Randomisation method.

4.4 Sample size :

According to study done by Sandhya M et al, (2002)[1] sample size was calculated using Open Epi software using glutathione peroxidase (GPX) as parameter, the study included 110 as cases and 110 as controls.

Mean ± S. D of GPX in psoriasis was 51.0 ± 9.6 IU/g of Hb

Mean ± S.D of GPX in controls was 47.6 ± 8.2 IU/g of Hb

Confidence level: 95%

Power of the study : 80%.

The Formula used :

Z : Standard variance

α : Significance level

β : power

σ^2 : variance

d : precision

$$\frac{2\,(Z\alpha + Z\beta)^2\,\sigma^2}{d^2}$$

4.5 Selection Criteria of Study Population :

Patients with psoriasis (n=110) and age and sex matched controls (n=110) without psoriasis attending the outpatient department of Dermatology, S. Nijalingappa Medical College and HSK Hospital, RC Bagalkot, India were allowed to participate in the study. An informed consent was taken from the patients and controls willing to participate in the study. The age group was between 20-60 years both for cases and controls.

4.6 Inclusion and Exclusion Criteria:

4.6.1 Inclusion Criteria :

Cases: Newly diagnosed patients attending out patient department of Dermatology, aged between 20-60 years with clinical features of erythema, itching, thickening and scaling of the skin were included for the study. The cases were further classified into mild, moderate and severe based on PASI score.

Controls: Healthy people aged between 20-60 years not having psoriasis and any other skin diseases accompanying patients, attending HSK Hospital were included as controls.

4.6.2 Exclusion Criteria :

Patients having other chronic inflammatory diseases, diabetes mellitus, renal disorders, IHD, hypothyroidism, hyperthyroidism, nephritic syndrome, obstructive liver disease, any other skin disorder were excluded from the study.

Patients receiving systemic drug therapy like beta blockers, thiazides, retinoids, cyclosporine and lipid lowering agents in the recent 6 months were also excluded from the study.

4.7 Ethical Clearance :

4.7.1 Ethical Approval : Institutional Ethics committee clearance was obtained from SNMC and HSK, RC Bagalkot (**SNMC/IECHSR/2018-19/A-86/1.1**) and BLDE Deemed to be University (**BLDE(DU)/IEC/2018-19/334**)

Informed written consent (English and Kannanda): was obtained from the participants in the study.

4.7.2 Declaration of Helsinki : We followed the declaration of Helsinki during the entire study.

4.8 Study Protocol:

4.8.1 History and Clinical Examination -

Detailed history was taken.

Clinical examination - general, systemic and dermatological was carried out. Relevant data including age, gender, weight, height, diet, waist circumference, blood pressure (BP), age of onset, history of smoking and alcoholism were taken.

Duration of psoriasis, type and severity of psoriasis was recorded.

4.8.2 Waist circumference -

The waist circumference was measured by placing the measuring tape snugly around the abdomen at the level of the iliac crest (Fig). A waist circumference of more than 90 cm and 80 cm for men and women, respectively, was considered as abdominal obesity [2].

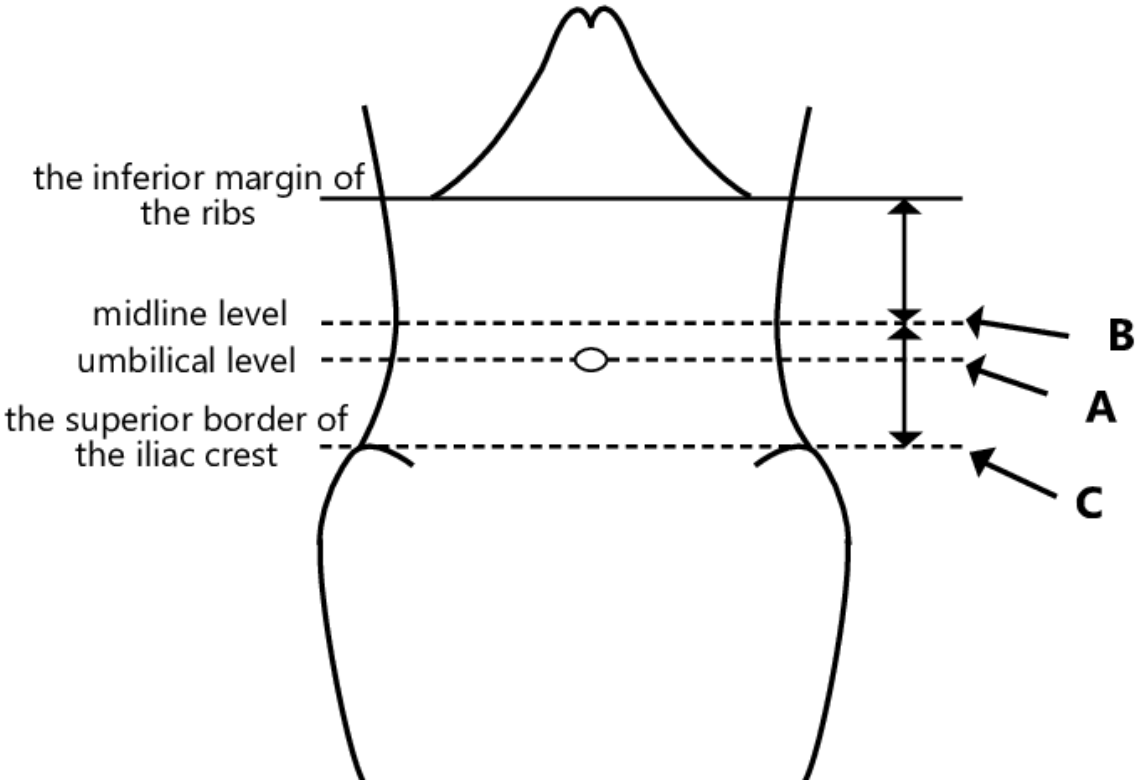


Figure 4.1: Measurement of Waist Circumference

Measured at the mid-point between the lower margin of the least palpable rib and top of the iliac crest.

Source: Lemoncito MV, Pacheco EP , Abrahan MA, Jasul G, Tan IT, Sison CM. Impact of Waist Circumference Measurement Variation on the Diagnosis of Metabolic Syndrome. Philippine Journal of Internal Medicine. 201;48(3):1-12.

4.8.3 Blood Pressure

Blood pressure (BP) was measured on a single visit, using a standard mercury sphygmomanometer and appropriate cuff sizes with the subject in a seated position, and having rested for at least 10 minutes. Three separate readings were taken per subject, after two minutes intervals and the average of the second and third readings recorded. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were taken at the 1st and 5th Korotkoff sounds respectively. The same trained personnel took all blood pressure measurements [3].

4.8.4 Assessment of Psoriasis Severity using PASI score :

To assess the severity of psoriasis clinically, Dermatologists use PASI (Psoriasis area and Severity Index) score. It is a quantitative rating score based on area coverage and plaque appearance. According to the British Association of Dermatology[3], PASI score is calculated as follows:

A score : Four affected sites on the body viz., head (h), upper limbs (u), Trunk (t), and lower limbs (l) were separately scored (Figure). The three factors were considered for morphologic scoring of psoriasis, viz., erythema, induration and desquamation, each of which was graded on a severity scale of 0 to 4 where 0 = nil, 1 = mild, 2 = moderate, 3 = severe and 4 = very severe.

B score : The addition of these scores for each site is multiplied by the grading for area-wise percentage involvement of that particular site in the following manner: 1 = less than

10% area, 2 = 10%–29%, 3 = 30%–49%, 4 = 50%–69%, 5 = 70%–89% and 6 = 90% or more are involved by psoriasis.

C score : Multiply score A with score B to each body region to get separate four C sores.

D score : Multiply each C score with the body surface area of that region. For head 0.1, upper limb 0.2, trunk 0.3 and lower limb 0.4 respectively. This gives four separate D scores.

PASI score = Add all four D scores.

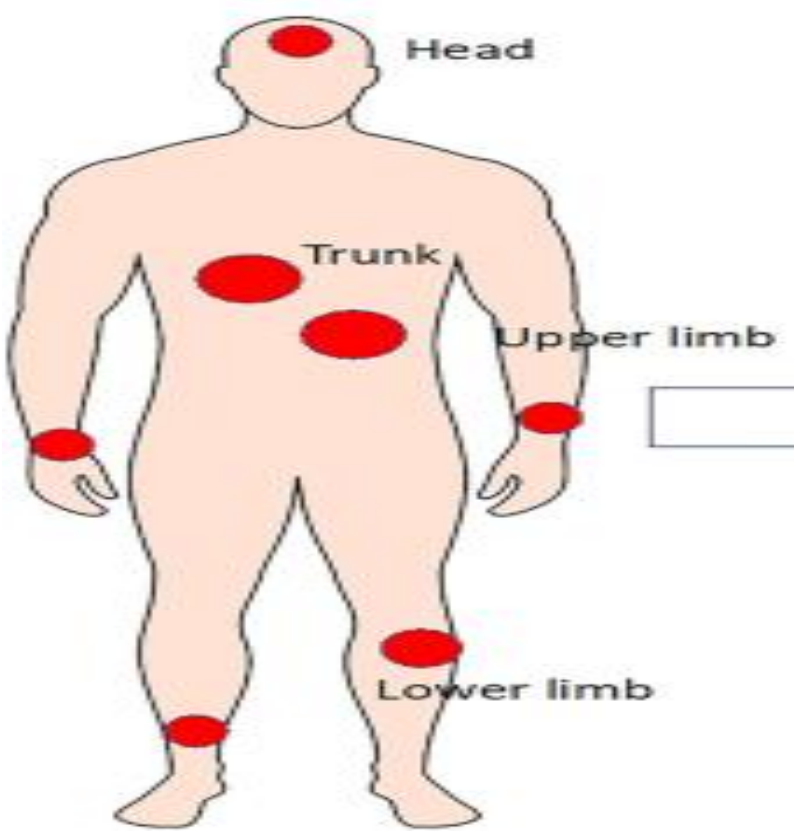


Figure 4.2: Psoriasis plaques in four (head, trunk, upper limb, lower limb) sites.

Source: Burden, A.D., Choon, S.E., Gottlieb, A.B. *et al.* Clinical Disease Measures in Generalized Pustular Psoriasis. *Am J Clin Dermatol* **23.** 2022; (1):39–50. https://doi.org/10.1007/s40257-021-00653-0.

Table 4.1: Calculation of PASI score

Plaque characteristic	Lesion score	Head	Upper Limbs	Trunk	Lower Limbs
Erythema	0 = None 1 = Slight 2 = Moderate 3 = Severe 4 = Very severe				
Induration/Thickness					
Scaling					
Add together each of the 3 scores for each body region to give 4 separate sums (A).					
Lesion Score Sum (A)					

Percentage area affected	Area score	Head	Upper Limbs	Trunk	Lower Limbs
Area Score (B) *Degree of involvement as a percentage for each body region affected (score each region with score between 0-6)*	0 = 0% 1 = 1% - 9% 2 = 10% - 29% 3 = 30% - 49% 4 = 50% - 69% 5 = 70% - 89% 6 = 90% - 100%				
Multiply Lesion Score Sum (A) by Area Score (B), for each body region, to give 4 individual subtotals (C).					
Subtotals (C)					
Multiply each of the Subtotals (C) by amount of body surface area represented by that region, i.e. x 0.1 for head, x 0.2 for upper body, x 0.3 for trunk, and x 0.4 for lower limbs.					
Body Surface Area		**x 0.1**	**x 0.2**	**x 0.3**	**x 0.4**
Totals (D)					
Add together each of the scores for each body region to give the final PASI Score.					

The PASI score ranges from: 0-72 [4].

Based on the severity, patients were divided into three groups as

mild (PASI score 0 – 7)

moderate (PASI score 7 – 12) and

severe (PASI score > 12)

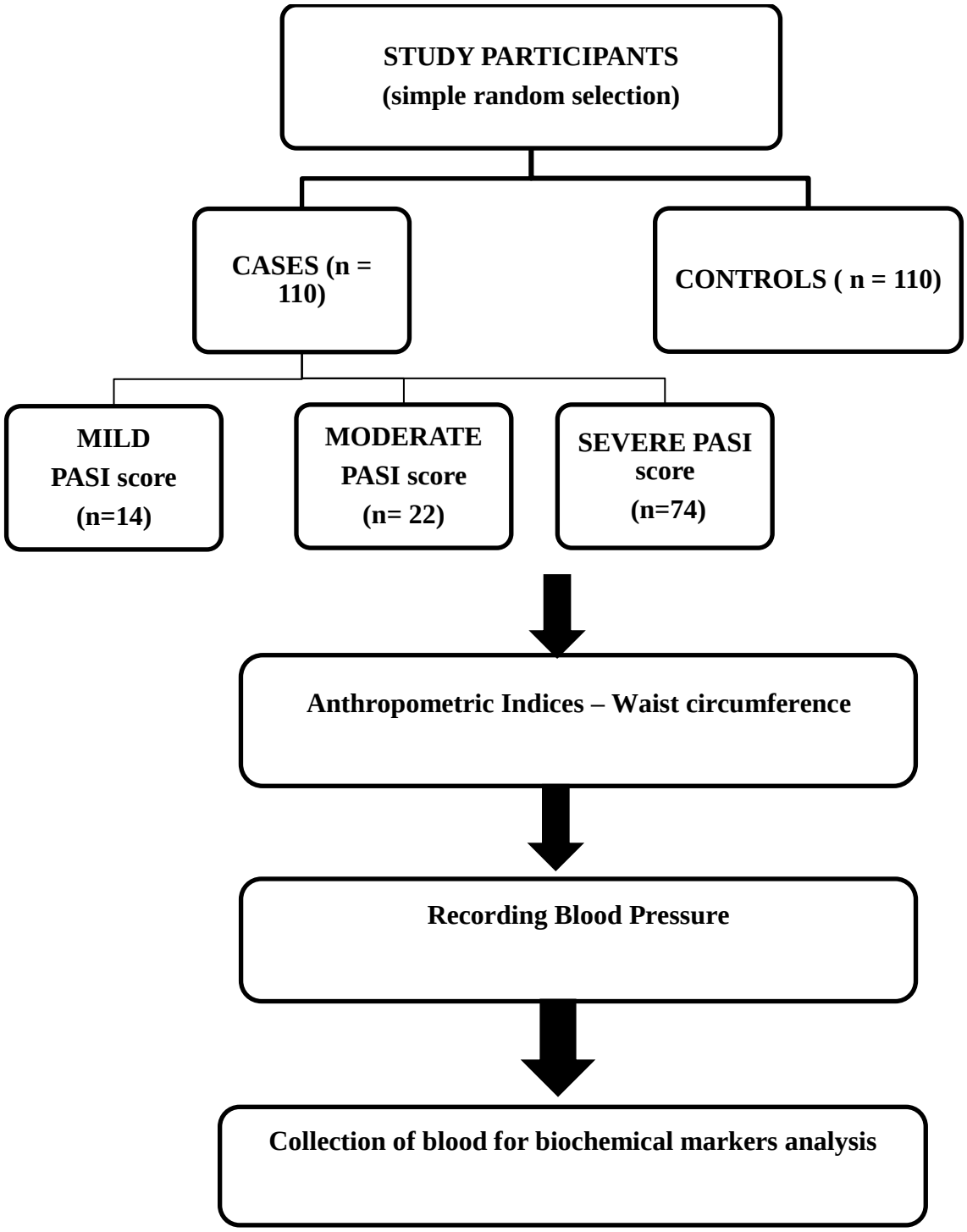


Figure 4.3: Study Protocol

4.9 Collection of Blood:

Under aseptic precautions around 5-6 ml venous blood was collected from antecubital vein in plain tube and was allowed to clot for few minutes. It was then subjected to centrifugation at 3500 revolutions per minute for 10 minutes. Around 1.5ml serum was used to estimate serum lipid profile and uric acid on the same day, whereas around 3-4ml serum was separated in aliquot vials to avoid freeze- thaw cycles and was stored at -20^{o}C . The stored serum was used for biochemical parameters analysis i.e inflammatory markers, oxidant stress and anti-oxidant markers, and cardiometabolic risk markers In the above collected serum the inflammatory markers were TNF-α, IFN-γ, IL-2, Total Oxidant Stress (TOS), Total Anti-Oxidant capacity TAOC), cardiometabolic risk markers like lipid profile, Lp(a), hs-CRP, were estimated by semi-automated ELISA reader Chromate Awareness Technology INC and lipid profile in fully automated analyser BA-400.

4.9.1 Estimation of serum TNF-α:

The estimation of serum TNF-α was done using Diaclone kit in semi –automated ELISA analyser.

PRINCIPLE OF THE TEST [5]:

- A monoclonal antibody specific for TNF-α has been coated onto the wells of the microtiter strips provided.
- During the first incubation, TNF-α present in the sample or standard and a monoclonal anti TNF-α antibody conjugated to biotin are simultaneously

incubated.

- Following incubation unbound biotinylated anti-TNF-α is removed during a wash step.
- Streptavidin-HRP is added and binds to the biotinylated anti TNF-α. After incubation and a wash step a substrate solution reactive with HRP is added to the wells.
- A coloured product is formed in proportion to the amount of TNF-α present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm.
- **PREPARATION OF REAGENTS**

1. Washing Buffer

- Dilute the Washing Buffer Concentrate (200X) in a clean graduated cylinder. Mix gently to avoid foaming.
- Pour entire contents (10 ml) of the Washing Buffer Concentrate into a clean 2,000 ml graduated cylinder.
- Bring final volume to 2,000 ml with glass-distilled or deionized water. Mix gently to avoid foaming.

Transfer to a clean wash bottle and store at 2° to 25°C. Washing Buffer may be prepared as needed according to the following table:

Table 4.2: Preparation of Washing buffer for TNF-α

Number of Strips	Washing Buffer Concentrate (ml)	Distilled Water (ml)
1 – 6	5	995
1 – 12	10	1,990

2. Preparation of Standard Diluent Buffer –

Add the content of the vial (10X) to 225 ml distilled water before use.

3. Preparation of TNF-α Standards -

Depending on the type of samples we are assaying, the kit includes two standard diluents. Because biologicals fluids might contain proteases or cytokine-binding proteins that could modify the recognition of the cytokine we want to measure. We should reconstitute standard vials with the most appropriate Standard Diluent.

For serum and plasma samples: use Standard Diluent : human serum

For cells culture supernatants: use Standard Diluent Buffer

Reconstitute TNF-α Standard by addition of appropriate Standard Diluent. Reconstitute volume is stated on the label of the standard vial.

This reconstitution produces a stock solution of 800 pg/ml TNF-α. Allow standard to stand for 5 minutes with gentle swirling prior to making dilutions.

Serial dilutions of standard must be made before each assay and cannot be stored.

4. Preparation of Controls

Freeze-dried control vials should also be reconstituted with the most appropriate Standard Diluent to your samples.

For serum and plasma samples: use Standard Diluent : human serum

For cells culture supernatants: use Standard Diluent Buffer.

Control have to be reconstituted with the volume of Standard Diluent indicated on the vial. Reconstitution of the freeze-dried material with the indicated volume, will give a solution for which the TNF-α concentration is stated on the vial. Allow control to stand for 5 minutes with gentle swirling prior to distribute in control wells. Do not store after use.

5. Preparation of biotinylated anti TNF-α

Preparation immediately before use is recommended. Dilute the biotinylated anti-TNF-α with the biotinylated antibody diluent in a clean glass vial. Biotinylated anti TNF-α may be prepared as needed according the following table.

Table 4.3 : Preparation of biotinylated anti TNF-α

Number of Strips	Biotinylated Antibody Concentrate (µl)	Biotinylated Antibody Diluent (µl)
2	40	1,060
3	60	1,590
4	80	2,120

6	120	3,180
12	240	6,360

6. Preparation of Streptavidin-HRP -

Dilute the Streptavidin –HRP 1:100 just prior to use by adding 0.5 ml of HRP diluent to the vial containing Streptavidin-HRP concentrate.

Make a further dilution with HRP-Diluent in a clean glass vial as needed according to the following table:

Table 4.4: Preparation of Streptavidin-HRP for TNF-α

Number of Strips	Pre-diluted Streptavidin-HRP (µl)	HRP Diluent (ml)
2	30	2
3	45	3
4	60	4
6	75	5
12	150	10

TEST PROTOCOL:

a. Mix all reagents thoroughly without foaming before use.

b. Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank, and control samples should be assayed in duplicate. Remove sufficient Microwell Strips coated with Antibody to human TNF-α from the aluminum pouch immediately prior to use. Return any wells not required for this assay with desiccant to the pouch. Seal tightly.

c. Add 100µl of appropriate Standard Diluent (see preparation of reagents) to standard wells B1, B2, C1, C2, D1, D2, E1, E2, F1, F2.

Pipette 200 µl of standard into wells A1 and A2. Transfer 100 µl from A1 and A2 to B1 and B2 wells. Mix the contents by repeated aspirations and ejections. Take care not to scratch the inner surface of microwells. Repeat this procedure from the wells B1, B2 to wells C1, C2 and from wells C1, C2 to D1, D2 and so on creating two parallel rows of TNF-α standard dilutions ranging from 800 to 25 pg/ml. Discard 100 µl from the content of the last microwells used.

Alternatively, these dilutions can be done in separate tube and diluted standard pipetted directly into wells.

Preparation of standard dilutions:

d. Add 100 µl of appropriate Standard Diluent in duplicate, to the blank wells (G1, G2).

e. Add 100 µl of Sample to sample wells, in duplicate, to the designated wells and 100 µl of reconstituted control vial, in duplicate, to control wells (H1, H2).

f. Prepare biotinylated anti (refer to Preparation of reagents 5.).

g. Add 50 µl of diluted biotinylated anti TNF-α to all wells.

h. Cover with a Plate Cover and incubate at room temperature (18° to 25°C) for 3 hours.

i. Remove the cover and wash the plate as follows:

1. Aspirate the liquid from each well;
2. Dispense 0.3 ml of washing solution into each well;

3. Aspirate again the content of each well;
4. Repeat step 2. and 3. two times

j. Prepare Streptavidin-HRP solution just before use: (refer to Preparation of reagents 6.)

k. Distribute 100 µl of Streptavidin-HRP solution to all wells, including blanks.

l. Cover the plate and incubate the plate at room temperature (18□C to 25□C) for 30 min.

m. Remove the cover and empty wells. Wash microwell strips according to step i. Proceed immediately to the next step.

n. Pipette 100 µl of ready-to-use TMB Substrate Solution to all wells, including the blank wells and incubate in the dark for about 12-15 minutes at room temperature. Avoid direct exposure to light by wrapping the plate in aluminium foil.

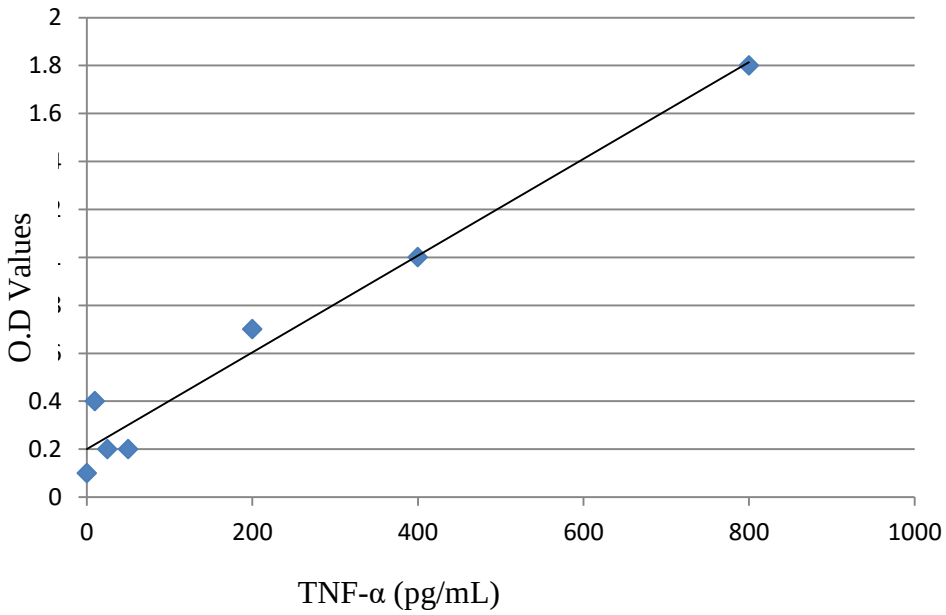
o. Incubation time of the substrate solution is usually determined by the ELISA reader performances. Many ELISA readers record absorbance only up to 2.0 O.D. Therefore the colour development within individual microwells must be watched by the person running the assay, and the substrate reaction stopped before positive wells are no longer properly recordable.

p. The enzyme-substrate reaction is stopped by quickly pipetting 100 µl of H2SO4 : Stop Reagent into each well, including the blank wells to completely and uniformly inactivate the enzyme. Results must be read immediately after the H2SO4 : Stop Reagent is added.

q. Read absorbance of each microwell on a spectro-photometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both, the samples, controls and the TNF-α standards.

CALCULATION OF RESULTS:

- Calculate the average absorbance values for each set of duplicate standards, samples and controls. Duplicates should be within 20 per cent of the mean.
- Create a linear standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the TNF-α standard concentration on the abscissa.
- To determine the concentration of TNF-α in each sample, first find the mean OD value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding TNF-α concentration.



4.9.2 Estimation of IFN-γ :

The serum IFN-γ was estimated using Diaclone kit in semi-automated ELISA analyser.

PRINCIPLES OF THE TEST [6]:

- A monoclonal antibody specific for IFN-γ has been coated onto the wells of the microtiter strips provided.
- During the first incubation, IFN-γ present in the sample or standard and a monoclonal anti IFN-γ antibody conjugated to biotin are simultaneously incubated.
- Following incubation unbound biotinylated anti-IFN γ is removed during a wash step.
- Streptavidin-HRP is added and binds to the biotinylated anti IFN γ. After

incubation and a wash step a substrate solution reactive with HRP is added to the wells.

- A coloured product is formed in proportion to the amount of IFN-γ present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm.

PREPARATION OF REAGENTS:

1. Washing Buffer

Dilute the Washing Buffer Concentrate (200X) in a clean graduated cylinder. Mix gently to avoid foaming.

Pour entire contents (10 ml) of the Washing Buffer Concentrate into a clean 2,000 ml graduated cylinder. Bring final volume to 2,000 ml with glass-distilled or deionized water. Mix gently to avoid foaming.

Transfer to a clean wash bottle and store at 2° to 25°C. Washing Buffer may be prepared as needed according to the following table:

Table 4.5: Preparation of Washing buffer for IFN-γ

Number of Strips	Washing Buffer Concentrate (ml)	Distilled Water (ml)
1 – 6	5	995
1 – 12	10	1,990

2. Preparation of Standard Diluent Buffer

Add the content of the vial (10X) to 225 ml distilled water before use.

3. Preparation of IFN-γ Standards:

Depending on the type of samples you are assaying, the kit includes two standard diluents. Because biologicals fluids might contain proteases or cytokine-binding proteins that could modify the recognition of the cytokine you want to measure. We should reconstitute standard vials with the most appropriate Standard Diluent.

For serum and plasma samples: use Standard Diluent : human serum

For cells culture supernatants: use Standard Diluent Buffer

Reconstitute IFN-γ Standard by addition of appropriate Standard Diluent. Reconstitute volume is stated on the label of the standard vial.

This reconstitution produces a stock solution of 400 pg/ml IFN-γ. Serial dilutions of standard must be made before each assays and cannot be stored.

4. Preparation of Controls:

Freeze-dried control vials should also be reconstituted with the most appropriate Standard Diluent to the samples.

For serum and plasma samples: use Standard Diluent : human serum

For cells culture supernatants: use Standard Diluent Buffer.

Control have to be reconstituted with the volume of Standard Diluent indicated on the vial. Reconstitution of the freeze-dried material with the indicated volume, will give a solution for which the IFN-γ concentration is stated on the vial.

5. Preparation of biotinylated anti IFN-γ

Dilute the biotinylated anti-IFN-γ with the biotinylated antibody diluent in a clean glass vial. Biotinylated anti IFN-γ may be prepared as needed according the following table.

Table 4.6: Preparation of biotinylated anti IFN-γ.

Number of Strips	Biotinylated Antibody Concentrate (µl)	Biotinylated Antibody Diluent (µl)
2	40	1,060
3	60	1,590
4	80	2,120
6	120	3,180
12	240	6,360

6. Preparation of Streptavidin-HR

It is recommended to centrifuge vial for a few seconds in a microcentrifuge to collect all the volume at the bottom.

Dilute the Streptavidin-HRP 1:100 just prior to use by adding 0.5 ml of HRP diluent to the vial containing Streptavidin-HRP concentrate

Make a further dilution with HRP-Diluent in a clean glass vial as needed according to the following table:

Table 4.7: Preparation of Streptavidin-HR for IFN-γ

Number of Strips	Pre-diluted Streptavidin HRP (µl)	HRP Diluent (ml)
2	30	2
3	45	3
4	60	4
6	75	5
12	150	10

TEST PROTOCOL :

a. Mix all reagents thoroughly without foaming before use.

b. Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank, and control samples should be assayed in duplicate. Remove sufficient Microwell Strips coated with Antibody to human IFN-γ from the aluminium pouch immediately prior to use. Return any wells not required for this assay with desiccant to the pouch. Seal tightly.

c. Add 100µl of of appropriate Standard Diluent to standard wells B1, B2, C1, C2, D1, D2, E1, E2, F1, F2. Reconstitute standard vial with the appropriate volume as described in the chapter preparation of reagents. Pipette 200 µl of standard into wells A1 and A2. Transfer 100 µl from A1 and A2 to B1 and B2 wells. Mix the contents by repeated aspirations and ejections. Take care not to scratch the inner surface of microwells. Repeat this procedure from the wells B1, B2 to wells C1, C2 and from wells C1, C2 to D1, D2 and so on creating two parallel rows of IFN-γ standard dilutions ranging from 400 to 12.5 pg/ml. Discard 100 µl from the content of the last microwells used (F1, F2). Alternatively these dilutions can be done in separate tube and diluted standard pipetted directly into wells.

d. Add 100 µl of appropriate Standard Diluent in duplicate, to the blank wells (G1, G2).

e. Add 100 µl of Sample to sample wells, in duplicate, to the designated wells and 100 µl of reconstituted control vial, in duplicate, to control wells (H1, H2).

f. Prepare biotinylated anti IFN Γ: (refer to Preparation of reagents 5.).

g. Add 50 µl of diluted biotinylated anti IFN Γ to all wells.

h. Cover with a Plate Cover and incubate at room temperature (18° to 25°C) for 2 hours.

i. Remove the cover and wash the plate as follows:

1. Aspirate the liquid from each well;

2. Dispense 0.3 ml of washing solution into each well;

3. Aspirate again the content of each well;

4. Repeat step 2. and 3. two times

j. Prepare Streptavidin-HRP solution just before use: (refer to Preparation of reagents 6.)

k. Distribute 100 µl of Streptavidin-HRP solution to all wells, including blanks.

l. Cover the plate and incubate the plate at room temperature (18^0C to 25^0C) for 30 min.

m. Remove the cover and empty wells. Wash microwell strips according to step i. Proceed immediately to the next step.

n. Pipette 100 µl of ready-to-use TMB Substrate Solution to all wells, including the blank wells and incubate in the dark for about 15-20 minutes at room temperature. Avoid direct exposure to light by wrapping the plate in aluminium foil.

o. Incubation time of the substrate solution is usually determined by the ELISA reader performances. Many ELISA readers record absorbance only up to 2.0 O.D. Therefore the colour development within individual microwells must be watched by the

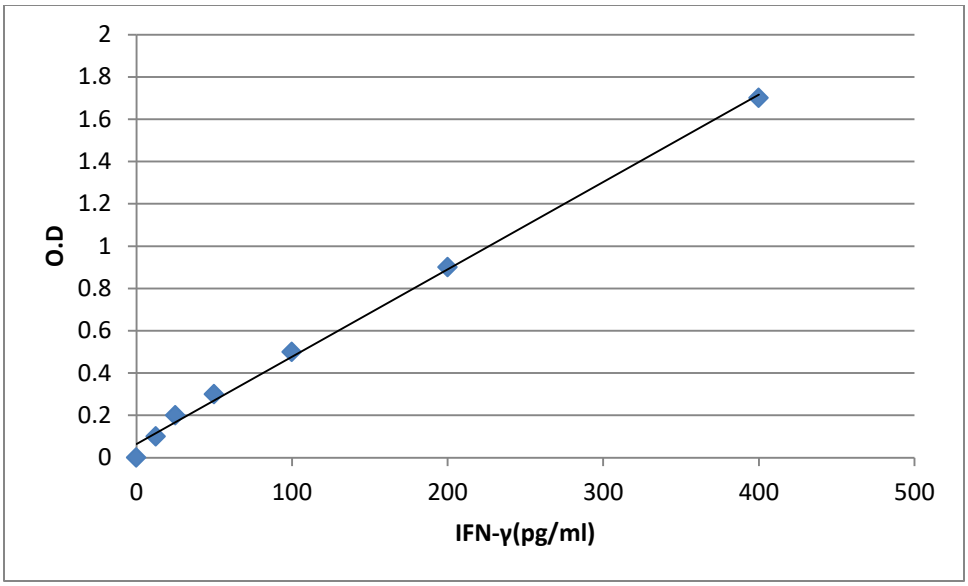
person running the assay, and the substrate reaction stopped before positive wells are no longer properly recordable.

p. The enzyme-substrate reaction is stopped by quickly pipetting 100 µl of H_2SO_4 : Stop Reagent into each well, including the blank wells to completely and uniformly inactivate the enzyme. Results must be read immediately after the H_2SO_4 : Stop Reagent is added.

q. Read absorbance of each microwell on a spectro-photometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Blank the plate reader according to the manufacturer's instructions by using the blank wells. Determine the absorbance of both, the samples, controls and the IFN-γ standards.

CALCULATION OF RESULTS :

- Calculate the average absorbance values for each set of duplicate standards, samples and controls. Duplicates should be within 20 per cent of the mean.
- Create a linear standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the IFN -γ standard concentration on the abscissa.
- To determine the concentration of IFN -γ in each sample, first find the mean OD value on the ordinate and extend a horizontal line to the standard curve.
- At the point of intersection, extend a vertical line to the abscissa and read the corresponding IFN-γ concentration.



4.9.3 Estimation of serum IL-2 :

The serum IL-2 was estimated by using Diaclone kit using semi-automated ELISA analyser.

PRINCIPLE OF THE TEST [7] :

- A polyclonal antibody specific for IL-2 has been coated onto the wells of the microtiter strips provided.
- During the first incubation, IL-2 present in the sample or standard and a monoclonal anti IL-2 antibody conjugated to biotin are simultaneously incubated.
- Following incubation unbound biotinylated anti-IL-2 is removed during a wash step.
- Streptavidin-HRP is added and binds to the biotinylated anti IL-2. After incubation and a wash step a substrate solution reactive with HRP is added to the wells.

- A coloured product is formed in proportion to the amount of IL-2 present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm.

PREPARATION OF REAGENTS :

1. Washing Buffer

Dilute the Washing Buffer Concentrate (200X) in a clean graduated cylinder. Mix gently to avoid foaming. Pour entire contents (10 ml) of the Washing Buffer Concentrate into a clean 2,000 ml graduated cylinder. Bring final volume to 2,000 ml with glass-distilled or deionized water. Mix gently to avoid foaming.

Transfer to a clean wash bottle and store at 2° to 25°C. Washing Buffer may be prepared as needed according to the following table:

Table 4.8: Preparation of Washing buffer for IL-2

Number of Strips	Washing Buffer Concentrate (ml)	Distilled Water (ml)
1 – 6	5	995
1 – 12	10	1,990

2. Preparation of Standard Diluent Buffer -

Add the content of the vial (10X) to 225 ml distilled water before use.

3. Preparation of IL-2 Standards :

Depending on the type of samples you are assaying, the kit includes two standard diluents. Because biologicals fluids might contain proteases or cytokine-binding proteins that could modify the recognition of the cytokine you want to measure. We should reconstitute standard vials with the most appropriate Standard Diluent.

For serum and plasma samples: use Standard Diluent : human serum

For cells culture supernatants: use Standard Diluent Buffer

Reconstitute IL-2 Standard by addition of appropriate diluent. Reconstitute volume is stated on the label of the standard vial. This reconstitution produces a stock solution of 1000 pg/ml IL-2.. Allow standard to stand for 5 minutes with gentle swirling prior to making dilutions. Serial dilutions of standard must be made before each assays and cannot be stored.

4. Preparation of Controls -

Freeze-dried control vials should also be reconstituted with the most appropriate Standard Diluent to your samples.

For serum and plasma samples: use Standard Diluent : human serum

For cells culture supernatants: use Standard Diluent Buffer.

Controls have to be reconstituted with the volume indicated on the vial. Reconstitution of the freeze-dried material with the indicated volume, will give a solution for which the IL-2 concentration is stated on the vial. Allow control to stand for 5 minutes with gentle swirling prior to distribute in control wells. Do not store after use.

5. Preparation of biotinylated anti IL-2.

Preparation immediately before use is recommended. Dilute the biotinylated anti-IL-2 with the biotinylated antibody diluent in a clean glass vial. Biotinylated anti IL-2 may be prepared as needed according the following table. Extemporaneous preparations are recommended.

Table 4.9: Preparation of biotinylated anti IL-2.

Number of Strips	Biotinylated Antibody Concentrate (µl)	Biotinylated Antibody Diluent (µl)
2	40	1060
3	60	1590
4	80	2120
6	120	3180
12	240	6360

6. Preparation of Streptavidin-HRP

Dilute the Streptavidin-HRP 1:100 just prior to use by adding 0.5 ml of HRP diluent to the vial containing Streptavidin-HRP concentrate. **Table 4.10:** Preparation of Streptavidin-HRP for IL-2

Make a further dilution with HRP-Diluent in a clean glass vial as needed according to the following table:

Number of Strips	Pre-diluted Streptavidin-HRP (µl)	HRP Diluent (ml)
2	30	2
3	45	3
4	60	4
6	75	5
12	150	10

TEST PROTOCOL :

a. Mix all reagents thoroughly without foaming before use.

b. Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Each sample, standard, blank, and control samples should be assayed in duplicate. Remove sufficient Microwell Strips coated with Antibody to human IL-2 from the aluminium pouch immediately prior to use. Return any wells not required for this assay with desiccant to the pouch. Seal tightly.

c. Add 100µl of of appropriate Standard Diluent (see preparation of reagents) to standard wells B1, B2, C1, C2, D1, D2, E1, E2, F1, F2.

Reconstitute standard vial with the appropriate volume as described in the chapter preparation of reagents. Pipette 200 µl of standard into wells A1 and A2 (see Figure 1 and 2). Transfer 100 µl from A1 and A2 to B1 and B2 wells. Mix the contents by

repeated aspirations and ejections. Take care not to scratch the inner surface of microwells. Repeat this procedure from the wells B1, B2 to wells C1, C2 and from wells C1, C2 to D1, D2 and so on creating two parallel rows of IL-2. standard dilutions ranging from 1000 to 31.25 pg/ml. Discard 100 µl from the content of the last microwells used (F1, F2).

Alternatively these dilutions can be done in separate tube and diluted standard pipetted directly into wells.

d. Add 100 µl of appropriate Standard Diluent in duplicate, to the blank wells (G1, G2).

e. Add 100 µl of Sample to sample wells, in duplicate, to the designated wells and 100 µl of reconstituted control vial, in duplicate, to control wells (H1, H2).

f. Prepare biotinylated anti IL-2 (refer to Preparation of reagents 5.).

g. Add 50 µl of diluted biotinylated anti IL-2 to all wells.

h. Cover with a Plate Cover and incubate at room temperature (18° to 25°C) for 1 hour.

i. Remove the cover and wash the plate as follows:

1. Aspirate the liquid from each well.
2. Dispense 0.3 ml of washing solution into each well;
3. Aspirate again the content of each well;
4. Repeat step 2. and 3. two times

j. Prepare Streptavidin-HRP solution just before use: (refer to Preparation of reagents 6.)

k. Distribute 100 µl of Streptavidin-HRP solution to all wells, including blanks.

l. Cover the plate and incubate the plate at room temperature (18 ^{0}C to 25 ^{0}C) for 30 min.

m. Remove the cover and empty wells. Wash microwell strips according to step i. Proceed immediately to the next step.

n. Pipette 100 µl of ready-to-use TMB Substrate Solution to all wells, including the blank wells and incubate in the dark for 12-15 minutes at room temperature. Avoid direct exposure to light by wrapping the plate in aluminium foil.

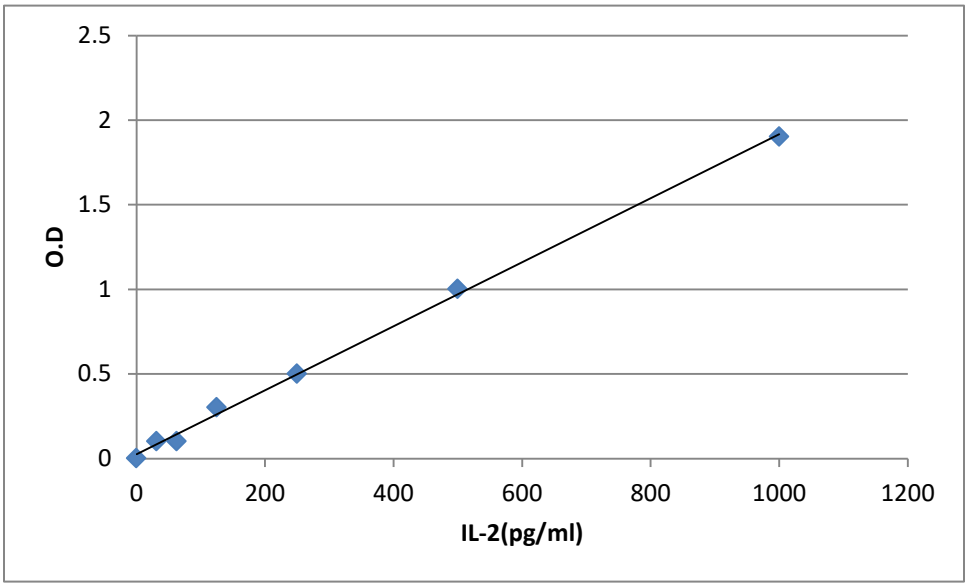
o. Incubation time of the substrate solution is usually determined by the ELISA reader performances. Many ELISA readers record absorbance only up to 2.0 O.D. Therefore the colour development within individual microwells must be watched by the person running the assay, and the substrate reaction stopped before positive wells are no longer properly recordable.

p. The enzyme-substrate reaction is stopped by quickly pipetting 100 µl of H2SO4 : Stop Reagent into each well, including the blank wells to completely and uniformly inactivate the enzyme. Results must be read immediately after the H2SO4 : Stop Reagent is added.

q. Read absorbance of each microwell on a spectro-photometer using 450 nm as the primary wave length (optionally 620 nm as the reference wave length; 610 nm to 650 nm is acceptable). Determine the absorbance of both, the samples, controls and the IL-2 standards.

CALCULATION OF RESULTS–

- Calculate the average absorbance values for each set of duplicate standards, samples and controls.
- Duplicates should be within 20 per cent of the mean. Create a linear standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the IL-2 standard concentration on the abscissa.
- To determine the concentration of IL-2 in each sample, first find the mean OD value on the ordinate and extend a horizontal line to the standard curve.
- At the point of intersection, extend a vertical line to the abscissa and read the corresponding IL-2 concentration.



4.9.4 Estimation of serum Total Oxidant Stress (TOS) :

The serum total oxidant stress was estimated using Bioassay Technology Laboratory kit in semi-automated ELISA analyser.

Principle [8] :

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with human TOS antibody. TOS present in the sample is added and binds to antibodies coated on the wells. And then biotinylated human TOS Antibody is added and binds to TOS in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated TOS antibody. After incubation unbound, Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of human TOS. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Reagent Preparation :

All reagents should be brought to room temperature before use.

Standard Reconstitute the 120µl of the standard (32U/ml) with 120µl of standard diluent to generate a 16U/ml standard stock solution.

Allow the standard to sit for 15 minutes with gentle agitation prior to making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (16U/ml) 1:2 with standard diluent to produce 8U/ml, 4U/ml, 2U/ml and 1U/ml solutions. Standard diluent serves as the zero standard(0 U/ml). Any remaining solution should be frozen at -20°C and used within one month.

Dilution of standard solutions suggested are as follows:

Table No 4.11: **Dilution of standard solutions for Total Oxidative Stress.**

Standard Concentration	Standard No.	Standard Dilution
16U/ml	Standard No.5	120µl Original Standard + 120µl Standard Diluent
8U/ml	Standard No.4	120µl Standard No.5 + 120µl Standard Diluent
4U/ml	Standard No.3	120µl Standard No.4 + 120µl Standard Diluent
2U/ml	Standard No.2	120µl Standard No.3 + 120µl Standard Diluent
1U/ml	Standard No.1	120µl Standard No.2 + 120µl Standard Diluent

- Wash Buffer Dilute 20ml of Wash Buffer Concentrate 30x into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

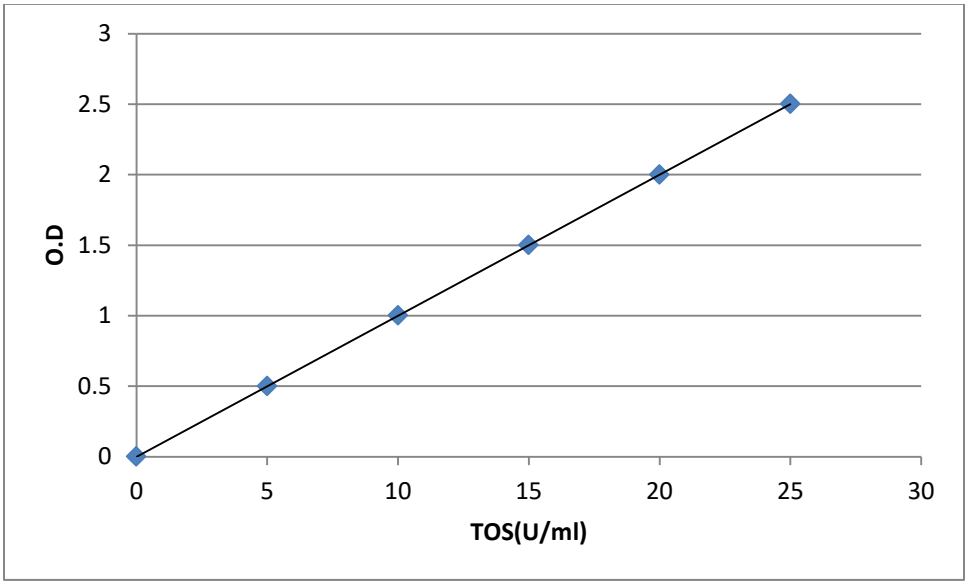
Procedure:

1. Prepare all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature.
2. Determine the number of strips required for the assay. Insert the strips in the frames for use. The unused strips should be stored at 2-8°C.

3. Add 50µl standard to standard well. Note: Don't add antibody to standard well because the standard solution contains biotinylated antibody.
4. Add 40µl sample to sample wells and then add 10µl anti-TOS antibody to sample wells, then add 50µl streptavidin-HRP to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a sealer. Incubate 60 minutes at 37°C.
5. Remove the sealer and wash the plate 5 times with wash buffer. Soak wells with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate all wells and wash 5 times with wash buffer, overfilling wells with wash buffer. Blot the plate onto paper towels or other absorbent material.
6. Add 50µl substrate solution A to each well and then add 50µl substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.
7. Add 50µl Stop Solution to each well, the blue color will change into yellow immediately.
8. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minuets after adding the stop solution.

Calculation of Result :

Construct a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve through the points on the graph. These calculations can be best performed with computer-based curve-fitting software and the best fit line can be determined by regression analysis.



4.9.5 Estimation of serum Total Anti-oxidant Capacity [9]:

The serum total anti-oxidant capacity is estimated using Qayee-Bio kit in semi-automated analyser.

Principle :

The kit uses a double-antibody sandwich enzyme-linked immunosorbent one-step process assay (ELISA) to assay the level of Total antioxidant capacity(T-AOC) in samples.

Add standard, test sample and HRP-labeled Total antioxidant capacity(T-AOC) antibodies to enzyme wells which are Pre-coated with Total antioxidant capacity(T-AOC) antibody, then carry out incubation and wash to remove the uncombined enzyme. Upon adding Chromogen Solution A and B, the color of the liquid will change into blue, and the reaction with the acid will cause the color to become yellow. The depth of color and the concentration of the Total antioxidant capacity (T-AOC) sample are positively correlated.

Reagent Preparation :

20 × dilution of washing buffer: distilled water, diluted by 1:20, or 1 copy of the 20 × washing buffer plus 19 copies of the distilled water.

Washing Method :

Manually washing method: Empty the plate by inverting it and shaking the content out , and tap it on the absorbent papers to dry. Add at least 0.35ml washing solution into each well, and soak the plate for 1~2 minutes. Repeat this process 5 times. Pay attention to

avoid spillover. Automatic washing method: If there is an automatic washing machine, it should only be used in the test when you are quite familiar with its function and performance.

Procedure :

1. The quantity of the strips depends on the quantity to-be-tested samples and the standards. It is suggested to duplicate each standard and blank well. Every sample should be made according to your required quantity, and try to use the duplicated wells for samples as well.
2. Set blank wells, standard wells, and test sample wells respectively:
    1. Blank well: do not add samples and horseradish peroxidase (HRP), other operations are the same.
    2. Standard wells: Add standard 50µl to Standard wells.
    3. Test sample wells: Add 40µl of Special diluent and then add 10µl of sample. (The final sample dilution is five times and the final result calculation should be multiplied by five times).
    4. Add 50µl of horseradish peroxidase (HRP) into each well, except blank well. Then seal the plate, and gently shake, then incubate 60 minutes at 37 ℃.
3. Discard Liquid excess, drying, fill each well with diluted washing liquid, mix and shake for 30 seconds, discard the washing liquid and tap the plate into absorbent papers to dry. Repeat five times, and then pat dry.

4. Add 50µl of chromogen solution A to each well, and then add 50µl of chromogen solution B to each well. Gently shake and incubate for 10 minutes at 37℃ away from light.
5. Stop: Add Stop Solution 50µl into each well to stop the reaction (the blue changes into yellow immediately).
6. Final measurement: Set blank well zero, measure the optical density (OD) at 450 nm wavelength which should be carried out within 15 minutes after adding the stop solution.
7. According to standards' concentration and the corresponding OD values, calculate out the standard curve linear regression equation, and then apply the OD values of the sample on the regression equation to calculate the corresponding sample's concentration. It is acceptable to use a variety of software to make calculations.

Prepare reagents, samples and standard

↓

Add prepared sample and standard and HRP-Conjugate Reagent, incubate for 60 minutes at 37 ℃

↓

Wash plate five times and add Chromogen Solution A and B, incubate for15 minutes at 37 ℃

↓

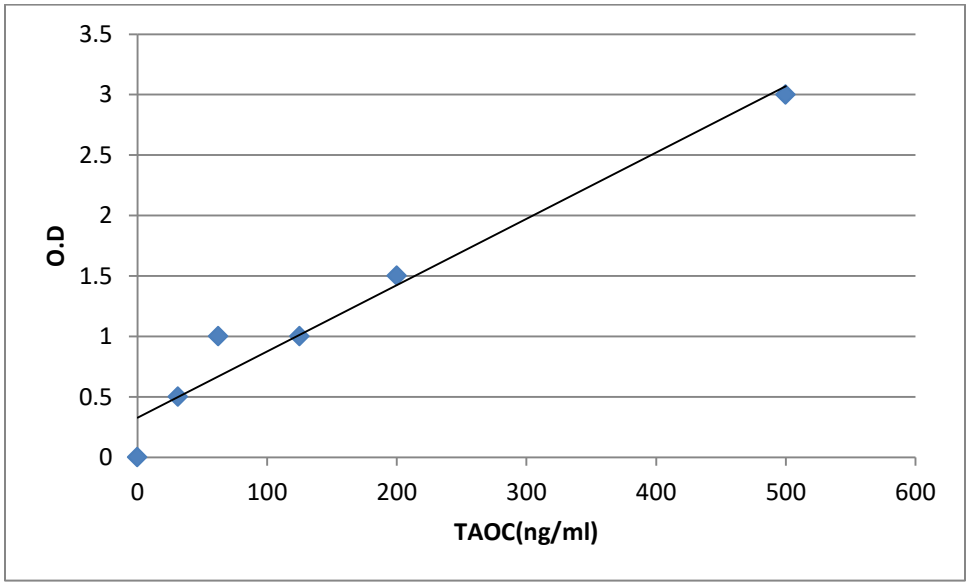
Add stop solution

↓

Measure within 15minutes

↓

Calculation



4.9.6 Estimation of serum Triglycerides :

The serum Triglycerides are estimated by using Biosystem kit in fully automated analyser BA400.

Principle [10] :

Triglycerides in the sample originates, by means of the coupled reactions described below, a coloured complex that can be measured by spectrophotometry.

$$\text{Triglycerides} + H_2O \xrightarrow{\text{lipase}} \text{Glycerol} + \text{FFA}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol kinase}} \text{glycerol 3-phosphate} + \text{ADP}$$

$$\text{Glycerol 3-phosphate} + O_2 \xrightarrow{\text{Glycerol phosphate oxidase}} \text{DAP} + H_2O_2$$

$$H_2O_2 + \text{4AAP} + \text{4-Cholorophenol} \xrightarrow{\text{peroxidase}} \text{quinoneimine} + 2H_2O$$

DAP – Dihydroxyacetone phosphate

4AAP - 4 Aminoantipyrine

Reagents:

Triglyceride mono reagent:

Pipes buffer	- 45 mmol/L
4-chlorophenol	- 6 mmol/L
Magnesium chloride	- 5 mmol/L
ATP	- 1 mmol/L
Lipase	> 100 U/mL
Peroxiadse	≥ 0.8 U/mL
Glycerol kinase	≥ 1.5 U/mL

4 AAP - 0.75mmol/L

Glycerol 3 phosphate oxidase ≥ 4 U/mL

2. Triglyceride standard:

Triglyceride - 200 mg/dL

Assay:

Mode : End point

Wavelength : 500nm

Optical path length : 1 cm

Procedure:

	Blank	Standard	Test
Serum	-	-	10µL
Standard	-	10µL	-
Reagent	1000µL	1000µL	1000µL

Mix well. Incubate at tubes for 15 minutes at room temperature for 5 minutes.

Calculation:

$$\text{Triglyceride concentration (mg/dL)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 200$$

Reference range: (as per NCEP ATP III guidelines)

Normal : < 150 mg/dL

Borderline high : 150-199 mg/dL

High : 200-499 mg/dL

Very high : ≥ 500 mg/dL

4.9.7 ESTIMATION OF TOTAL CHOLESTEROL:

Lipid profile includes total cholesterol, triglyceride and high density lipoprotein. These were measured in fully automated analyser Biosystem A-25 using commercially available kits from the company Biosysatem. VLDL-C and LDL-C was calculated.

Method: Cholesterol oxidase and peroxidase

Principle [11] :

The free and esterified cholesterol in the given sample is determined by enzymatic method

Cholesterol ester + H_2O $\xrightarrow{\text{cholesterol esterase}}$ cholesterol + Fatty Acid

Cholesterol + $1/2O_2$ + H_2O $\xrightarrow{\text{cholesterol oxidase}}$ cholesteone +H_2O_2

$2H_2O_2$ + 4AAP + phenol $\xrightarrow{\text{Peroxidase}}$ $4H_2O$ + quinoneimine

Absorbance of quinoneimine formed is directly proportional to the concentration of cholesterol in the given sample and absorbance measured at 480 to 520 nm of wavelength.

Reagents:

1. Cholesterol reagent contains:

Sodium cholate - 0.5mmol/L

Cholesterol esterase ≥ 0.2U/mL

Cholesterol oxidase ≥ 0.1U/mL

Peroxidase ≥ 0.8U/mL

4AAP - 0.5mmol/L

pH - 7

2. **Cholesterol standard:**

Cholesterol - 200 mg/dL

Assay:

Mode : End point method

Wavelength : 500nm (480-520nm)

Optical path length : 1cm

Procedure:

Reagents	Blank	Standard	Test
Serum	-	-	10µL
Standard	-	10µL	-
Cholesterol reagent	1000µL	1000µL	1000µL

Mix well. Incubate all the tubes for 10 minutes at room temperature or at 37^0C for 5 minutes.

Calculation:

$$\text{Cholesterol concentration} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 200$$

Reference range: (As per NCEP ATP III guidelines)

For total cholesterol

Desirable : < 200mg/dL

Borderline high : 200-239mg/dL

High : ≥ 240mg/dL

4.9.8 ESTIMATION OF HDL CHOLESTEROL:

Principle [12]:

The cholesterol from LDL-C, VLDL-C and chylomicrons, is broken down by the cholesterol oxidase in an enzymatic reaction. The detergent in reagent B, will solubalizes cholesterol from HDL-C in the given sample. The HDL-C is measured at 600 to 700 nm. Absorbance of quinoneimine formed is directly proportional to amount of HDL-C present in the given sample.

$$\text{Cholesterol ester} + H_2O \xrightarrow{\text{cholesterol esterase}} \text{cholesterol} + \text{Fatty Acid}$$

$$\text{Cholesterol} + 1/2O_2 + H_2O \xrightarrow{\text{cholesterol oxidase}} \text{cholestenone} + H_2O_2$$

$2H_2O_2$ + 4AAP + DSBmT $\xrightarrow{\text{peroxidase}}$ $4H_2O$ + quinoneimine**Reagents:**

1. Reagent:

Cholesterol oxidase < 1U/mL

Peroxidase < 1U/mL

N,N-bis(4-sulfobutyl)-m-toluidine (DSBmT) - 1mmol/L

2. Accelerator - 1mmol/LReagent:

Cholesterol esterase < 1.5 U/mL

4-aminoantipyrine - 1 mmol/L

Ascorbate oxidase < 3KU/L

Assay:

Mode : End point

Wavelength : 600-700nm

Optical path length : 1cm

Procedure:

Pipette into tube marked	Test
Serum	7 µL
Reagent A	750 µL
Mix well and insert the cuvette in the photometer. After 5 minutes read the absorbance (A1) at 600/700 nm against distilled water	
Reagent B	250µL
Mix well. Incubate at 37^0C for 5 minutes and read the absorbance (A2) at 600/700 nm.	

Calculation:

Concentration of HDL = $\frac{\text{Absorbance of (A2-A1) sample}}{\text{Absorbance of calibrator (A2-A1)}}$ X Calibrator

Reference range: (As per NCEP ATP III guidelines)

For HDL cholesterol

Low risk : ≥ 60 mg/dL

High risk :< 35 mg/dL

4.9.9 ESTIMATION OF LDL CHOLESTEROL:

Low density lipoprotein was calculated using "Friedwald's equation" and was applied for those TG levels which were less than 400 mg/dL [13].

$$\boldsymbol{LDL - C = Total\ Cholesterol - \frac{Triglycerides}{5} - HDL - C}$$

Reference range: (As per NCEP ATP III guidelines)

Optimal < 100mg/dL

Near optimal : 100-129mg/dL

Borderline high : 130-159mg/dL

High : 160-189mg/dL

Very high ≥190mg/dL

4.9.10 Estimation of serum Lp(a):

The serum Lp(a) was estimated using Bioassay Technology Laboratory kit by ELISA method.

Principle [14]:

The plate has been pre-coated with human Lp(a) antibody. Lp(a) present in the sample is added and binds to antibodies coated on the wells. And then biotinylated human Lp(a) Antibody is added and binds to Lp-A in the sample. Then Streptavidin-HRP is added and binds to the Biotinylated Lp-Aantibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of human Lp(a). The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

Reagents Preparation :

All reagents should be brought to room temperature before use.

Standard Reconstitute the 120µl of the standard (240ng/ml) with 120µl of standard diluent to generate a 120ng/ml standard stock solution. Allow the standard to sit for 15 mins with gentle agitation prior to making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (120ng/ml) 1:2 with standard diluent to produce 60ng/ml, 30ng/ml, 15ng/ml and 7.5ng/ml solutions. Standard diluent serves as the zero standard(0 ng/ml). Any remaining solution should be frozen at -20°C and used within one month. Dilution of standard solutions suggested are as follows:

Table 4.12: Dilution Standards for Lp(a)

Standard Concentration	Standard No.	Standard Dilutions
120ng/ml	5	120µl Original Standard + 120µl Standard Diluent
60ng/ml	4	120µl Standard No.5 + 120µl Standard Diluent
30ng/ml	3	120µl Standard No.4 + 120µl Standard Diluent
15ng/ml	2	120µl Standard No.3 + 120µl Standard Diluent
7.5ng/ml	1	120µl Standard No.2 + 120µl Standard Diluent

Wash Buffer Dilute 20ml of Wash Buffer Concentrate 30x into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

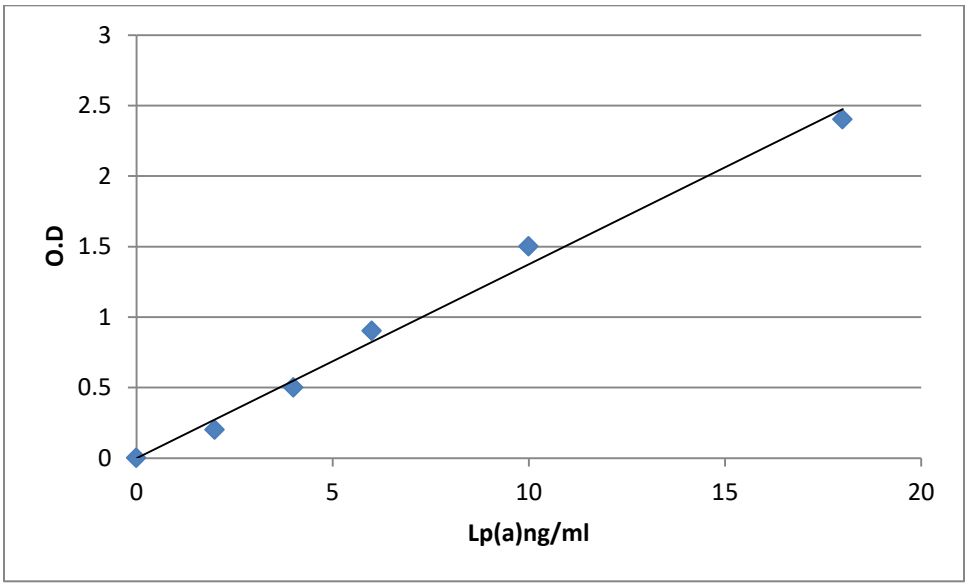
Procedure :

1. Prepare all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature.
2. Determine the number of strips required for the assay. Insert the strips in the frames for use. The unused strips should be stored at 2-8°C.
3. Add 50µl standard to standard well. Note: Don't add antibody to standard well because the standard solution contains biotinylated antibody.
4. Add 40µl sample to sample wells and then add 10µl anti-Lp-A antibody to sample wells, then add 50µl streptavidin-HRP to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a sealer. Incubate 60 minutes at 37°C.
5. Remove the sealer and wash the plate 5 times with wash buffer. Soak wells with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate all wells and wash 5 times with wash buffer, overfilling wells with wash buffer. Blot the plate onto paper towels or other absorbent material.
6. Add 50µl substrate solution A to each well and then add 50µl substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.
7. Add 50µl Stop Solution to each well, the blue color will change into yellow immediately.

8. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minuets after adding the stop solution.

Calculation of Result :

Construct a standard curve by plotting the average OD for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis and draw a best fit curve.



4.9.11 Estimation of serum hs-CRP:

The serum hs-CRP was estimated using Diayme kit in semi-automated ELISA analyser.

Principle [15]:

Diazyme's hsCRP Assay is based on a latex enhanced immunoturbidimetric assay. When an antigen-antibody reaction occurs between CRP in a sample and anti-CRP which has been sensitized to latex particles, agglutination results. This agglutination is detected as

an absorbance change (570 nm), with the magnitude of the change being proportional to the quantity of CRP in the sample. The actual concentration is then determined by the interpolation from a calibration curve prepared from calibrators of known concentration.

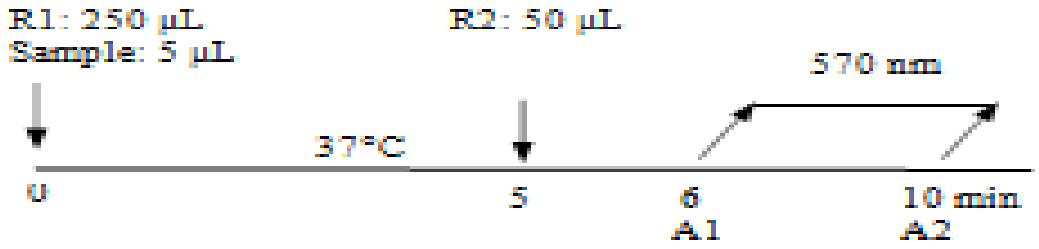
Preparation of Reagents :

REAGENT 1: 100 mM Tris-buffer solution with 0.09% sodium azide, ready to use

REAGENT 2: Suspension of latex particles (≤0.5%) coated with goat anti-human CRP with 0.09% sodium azide, ready to use.

Procedure :

CRP should be measured according to the specific application parameters for each specific chemistry analyzer. Below is a general example of the assay test scheme and the specific application parameters for the Hitachi 917 analyzer. Calculate CRP value with the read absorbance change from a calibration curve prepared with calibrators of known concentrations.



Reference Range:

The assay reference interval was determined using serum specimens from 103 apparently healthy adults with age of 18-62 according to CLSI C28-A3 guideline. The serum specimens were tested in duplicate by the Diazyme hsCRP method. EP Evaluator 8 Software was used to veri- fy the reference interval. The results showed that < 5.0 mg/L CRP was obtained in 95% of the population tested.

Linearity :

CRP linearity set was prepared by diluting a specimens containing 40.0 mg/L CRP with saline according to CLSI EP6-A. Assay linearity was tested on the Hitachi 917. Data analysis using EP Evaluator 8 showed that the Diazyme hsCRP assay was linear through a measured range of 0.20 to 20.0 mg/L with an allowable systematic error of 4.5%.

4.9.12 Estimation of Uric acid:

The serum uric acid was estimated by using Biosystem kit in fully auto-mated analyser BA-400.

Principle [16]:

Uric acid in the sample originates, by means of the coupled reactions described below, a coloured complex that can be measured by spectrophotometry

$$\text{Uric acid} + O_2 + 2H_2O \xrightarrow{\text{uricase}} \text{Allantoin} + CO_2 + H_2O_2$$

$$2H_2O_2 + 4 - \text{Aminoantipyrine} + \text{DCFS} \xrightarrow{\text{peroxidase}} \text{Quinonemine} + 4H_2O$$

Reagents :

Phosphate 100 mmol/L, detergent 1.5 g/L, dichlorophenolsulfonate 4 mmol/L, uricase > 0.12 U/mL, ascorbate oxidase > 5 U/mL, peroxidase > 1 U/mL, 4-aminoantipyrine 0.5 mmol/L, pH 7.8.

Procedure :

The reagents are ready to use.

1. Bring the Reagent to room temperature.
2. Pipette into labeled test tubes

	Blank	**Standard**	**Sample**
Distilled water	25µl	-	-
Uric acid Standard	-	25 µl	-
Sample	-	-	25µl
Reagent (A)	1.0ml	1.0ml	1.0ml

3. Mix thoroughly and incubate the tubes for 10 minutes at room temperature (16-25°C) or for 5 minutes at 37°C.
4. Measure the absorbance (A) of the Standard and the Sample at 520 m against the Blank. The colour is stable for at least 30 minutes.

Calculation Results :

The uric acid is calculated using the following formula:

$$\frac{\text{A sample}}{\text{A standard}} \text{ x C x Sample dilution factor } = \text{C sample}$$

Reference values :

Serum and plasma :

Men : 3.5 – 7.2 mg/dl

Women : 2.6 – 6.0 mg/dl

Urine :

250 – 750 mg/24 hr

Statistical Analysis :

- Data obtained from the study was summarized in microsoft excel sheet and analysis was done. The excel and SPSS (SPSS Inc, Chicago version v.23.0) software packages were used for data entry and analysis. Results were expressed as mean ± standard deviation (SD).
- Unpaired 't' test was used to compare the parameters between psoriasis patients and controls. Correlation coefficients was used to correlate between serum inflammatory markers, oxidative stress and anti-oxidants along with PASI (Psoriasis Area and Severity Index) score in psoriasis patients.
- ANOVA was used to compare among the 3 groups in patients with respect to PASI score (mild, moderate & severe).
- $p < 0.05$ was considered to be significant.

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Chapter - 5

Results

In the present study there were totally 220 subjects involved in the study. Out of which 110 were cases and 110 healthy controls. The study was carried out for 2 years (2019 to 2021). The cases were appropriately matched for age and gender distribution with controls. The age group was between 20 to 60years. The cases were further classified into mild, moderate and severe based on PASI score.

5.1 Study subjects:

Healthy controls = 110 subjects

Cases: Totally 110 subjects, out of which

- Subjects with Mild PASI score = 14
- Subjects with Mild PASI score = 22
- Subjects with Mild PASI score = 74

Demographic Characteristics

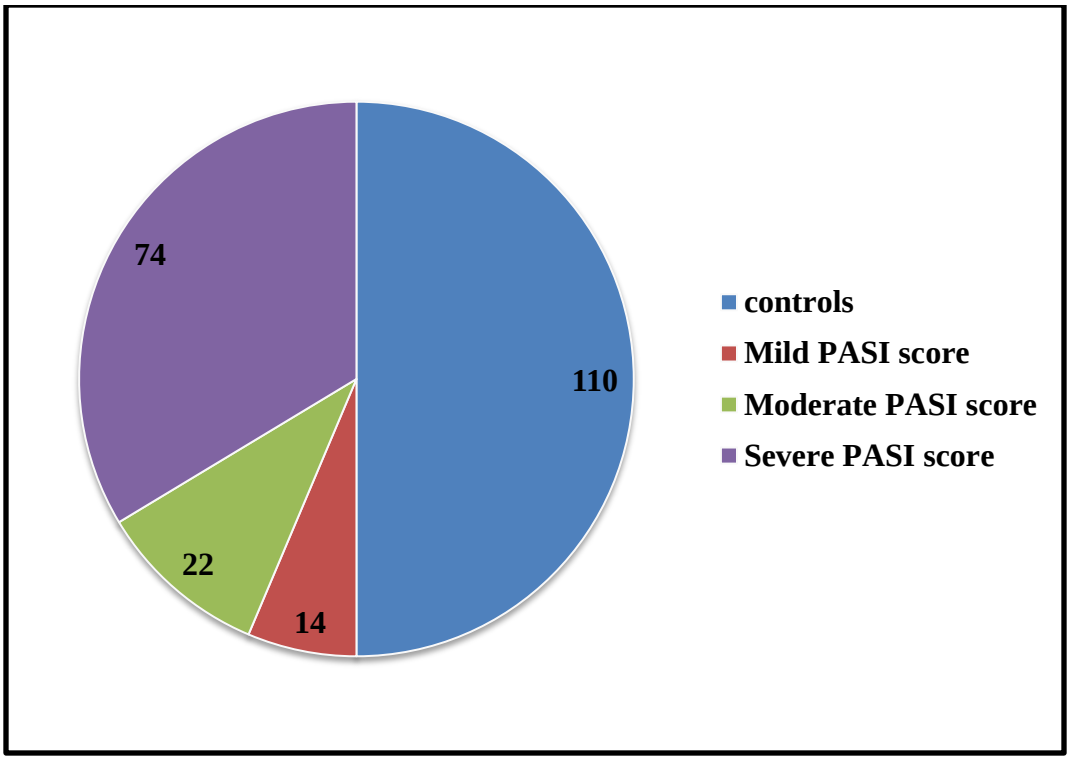


Figure 5.1: Pie chart showing number of participants involved in the study.

In the above figure maximum subjects in cases belonged to severe PASI score, followed by moderate PASI score and mild PASI score. 110 were appropriately matched healthy controls representing half of the circle.

5.2: Gender distribution of participants:

In gender distribution maximum subjects were males both in cases (55.5%) and controls (57.3%) compared to females (44.50%) and (42.7%) which is shown in **Figure 5.2**. The total number of males and females in cases were 61 and 49 respectively, whereas in controls 63 and 47 respectively.

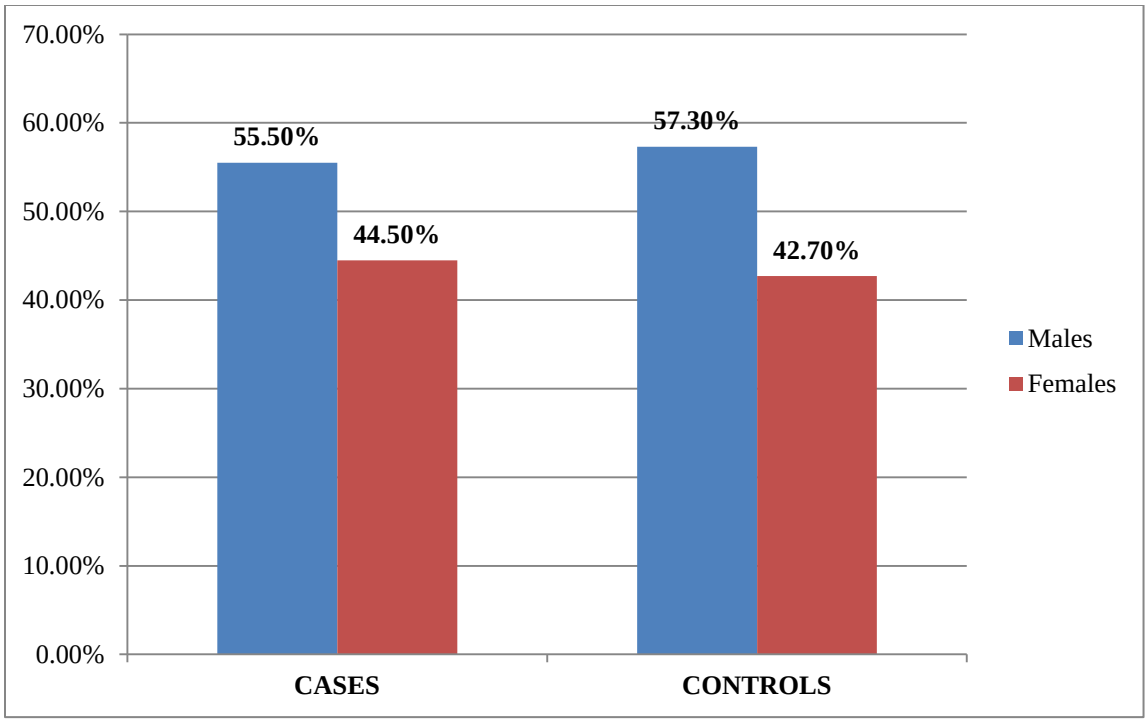


Figure 5.2: Percentage of male and female participants among the study

Among cases the percentage of male patients with mild PASI score were 57.1%, moderate PASI score were 50.0% and severe PASI score were 56.8% respectively. The distribution of females in mild PASI score is 42.9%, moderate PASI score is 50.0% and severe PASI score is 43.2%. This distribution of gender with respect to PASI score between cases and controls was not significant with 'p' value 0.939, as depicted in **Table 5.1.**

Table 5.1: Distribution of gender in mild, moderate and severe PASI score in cases and controls.

Gender	Cases with PASI Score						Controls		'p' value
	Mild		Moderate		Severe				
	N	**%**	**N**	**%**	**N**	**%**	**N**	**%**	
Male	8	**57.1%**	11	**50.0%**	42	**56.8%**	63	57.3%	0.939
Female	6	42.9%	11	50.0%	32	43.2%	47	42.7%	
Total	14	100.0%	22	100.0%	74	100.0%	110	100.0%	

5.3 Age Distribution :

The subjects both in cases and controls were between the age group of 20to 60yrs. In the present study maximum subjects belonged to age group of 31 to 40 years, then followed by 41 to 50yrs. The percentage of subjects of age group 31 to 40 years both in cases and controls was 34.5% and 35.5% respectively, where as in the age group 41-50years was 22.7 and 20.9 respectively. The age distribution between cases and controls was not statistically significant with 'p' value 0.904. For comparison Chi square test was used with value 1.039, depicted in **Table 5.2**.

Table 5.2 : Distribution of age among cases and controls

Age(yrs)	Total patients	Cases		Controls		chi square value	p value
		N	%	N	%		
≤30	53	24	21.8	29	26.4	1.039	0.904
31-40	77	38	**34.5**	39	**35.5**		
41-50	48	25	**22.7**	23	**20.9**		
51-60	34	19	17.3	15	13.6		
>60	8	4	3.6	4	3.6		
Total	220	110	100.0	110	100.0		

The age distribution in cases with mild, moderate and severe PASI score compared to controls was plotted in box plot graph as shown in **Figure 5.3**. The middle line in the box represents median, either side of median are $1^{st,}$ 3^{rd} quartiles. The threads on either side are called whiskers. The circles are outliers with numerical data lying outside box plot graph distribution. In mild PASI score patients maximum number of patients are above median in 3^{rd} quartile with Mean ± S.D 38.8 ± 8.5. There is 1 outlier with numerical value which is not related to the distribution of data in the box plot graph, which doesn't make much impact on the overall score. In moderate PASI score patients the median is in the middle with equal distribution of patients in both 1^{st} and 3^{rd} quartile. The Mean ± S.D

of patients in moderate PASI score 39.9 ± 12.7 respectively. In severe PASI score more patients are distributed above the median falling in 3rd quartile with Mean ± S.D of 41.4±11.5. The whiskers are slightly negatively skewed, but still the median is in mid-range. The distribution of patients with respect to PASI and controls was significant with 'p' <0.05.

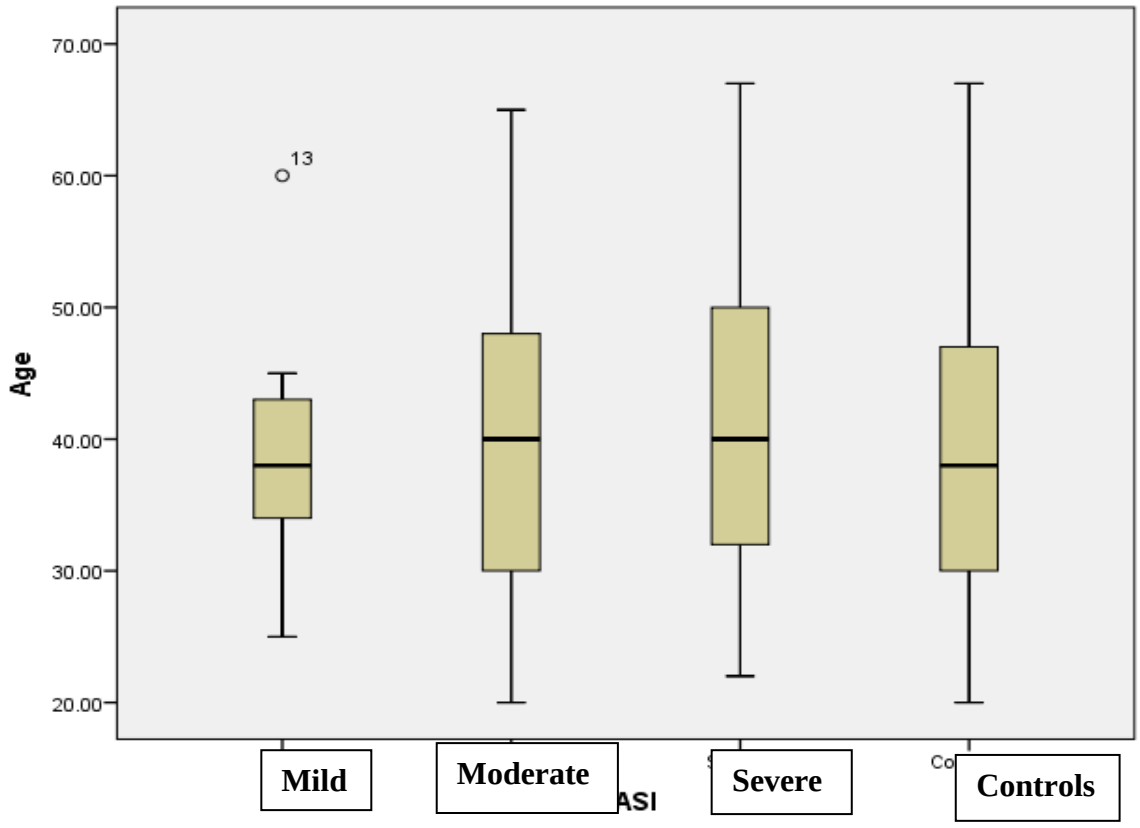


Figure 5.3: Distribution of Age between mild, moderate, severe PASI score patients and controls.

• 13 - outlier with numerical data value 13 years which is lying outside boxplot in mild PASI score doesn't impact on overall score in cases.

Anthropometric Indices & Blood Pressure

5.4 Blood Pressure and Anthropometric Indices :

5.4.1 Blood Pressure:

To assess the cardiovascular risk both in cases and controls, the blood pressure was recorded. The mean systolic blood pressure was 136.1 ± 14.0 and diastolic blood pressure 81.4 ± 8.3 in cases respectively. The mean systolic blood pressure in controls was 125.0±8.9 and diastolic blood pressure 76.2±5.5 respectively. The blood pressure was statistically significant in cases compared to controls with 'p' value <0.001 (<0.05) is depicted in **Table 5.3.**

5.4.2 Waist Circumference:

The mean waist circumference in cases was 85.4±15.3 and in controls 44.7±11.9 respectively. It shows that waist circumference is significantly increased in cases compared to controls ($p<0.05$).

Table 5.3: Recorded Blood Pressure (Mean ± S.D) and Waist Circumference (Mean ± S.D) in cases and controls.

Parameters	Cases	Controls	t value	p value	95% CI	
	Mean±S.D	Mean±S.D			Lower	Upper
Systolic-BP(mm of Hg)	**136.1±14.0**	125.0±8.9	7.0	<0.001*	8.0	14.2
Diastolic-BP(mm of Hg)	**81.4±8.3**	76.2±5.5	5.5	<0.001*	3.3	7.1
Waist circumference(cm)	85.4±15.3	44.7±11.9	22.1	<0.001*	37.1	44.3

Note: p value* significant at 5% level of significance ($p<0.05$)

The recorded blood pressure was compared among the groups shown by box plot graph **(Figure 5.4 and Figure 5.5)**. In **Figure 5.4** the systolic blood pressure in cases with mild PASI sore with mean value 135.7±14.3, moderate PASI score with mean value 133.5±11.9 , severe PASI score with mean value 136.9±14.6 respectively. The diastolic blood pressure in cases with mild PASI with mean value 79.4±8.8, in moderate PASI score the mean value 81.1±7.8 and with severe PASI score the mean value 81.8±8.4 respectively. The data is negatively skewed as it is dispersed towards the 3rd quartile i.e above the median. There were 2 outliers with numerical data value 100, 101 mm of Hg for systolic Blood pressure and significant outliers for diastolic Blood pressure in patients with mild PASI score. However, the outliers doesn't make much impact on overall score in patients with severe PASI score. In mild PASI score patients with diastolic Blood pressure the lower quartile is only having lower whisker, but the 3rd quartile and median score are of minimum value. In other words patients with mild PASI score are having same dispersion as median and 3rd quartile.

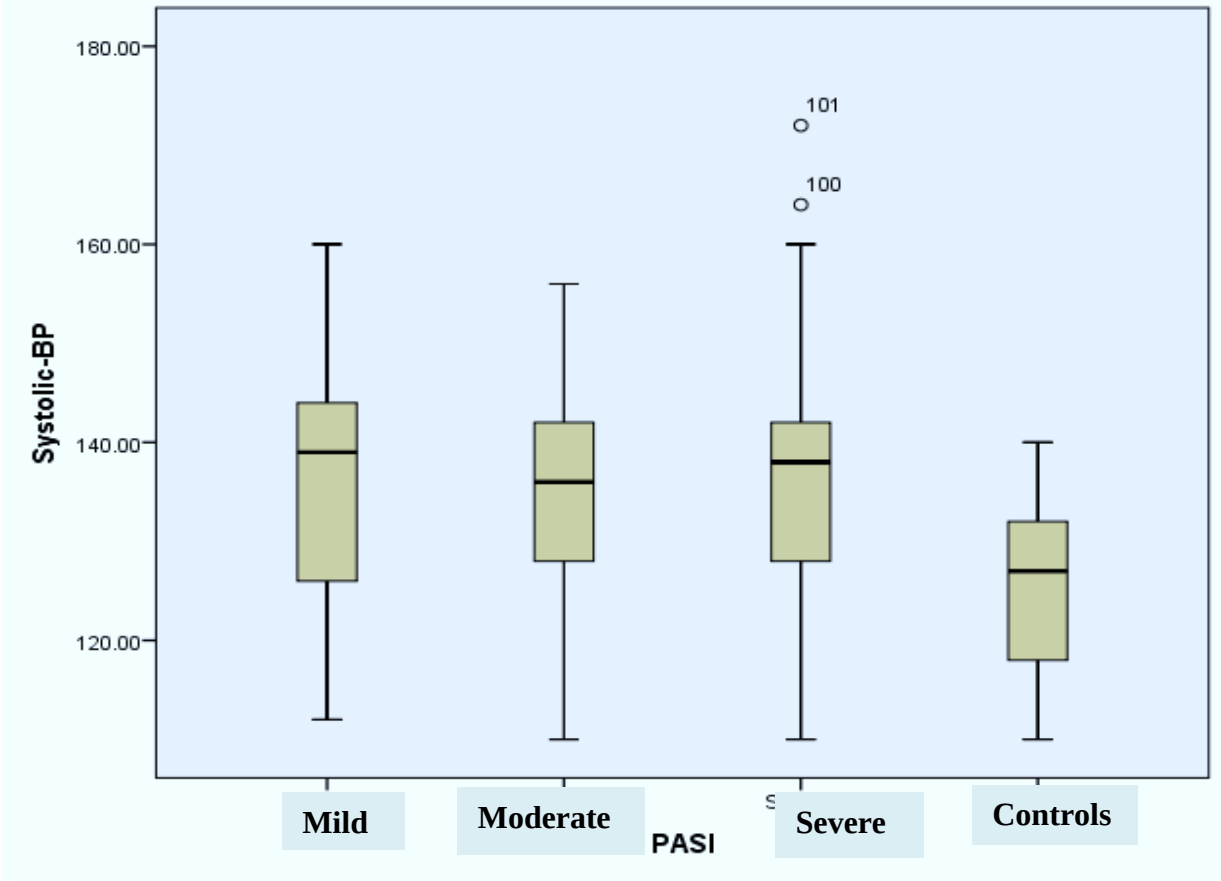


Figure 5.4: Recorded Systolic Blood Pressure in mild, moderate and severe PASI score

- : 101, 100 – outliers with data value 101, 100 mm of Hg systolic Blood pressure.

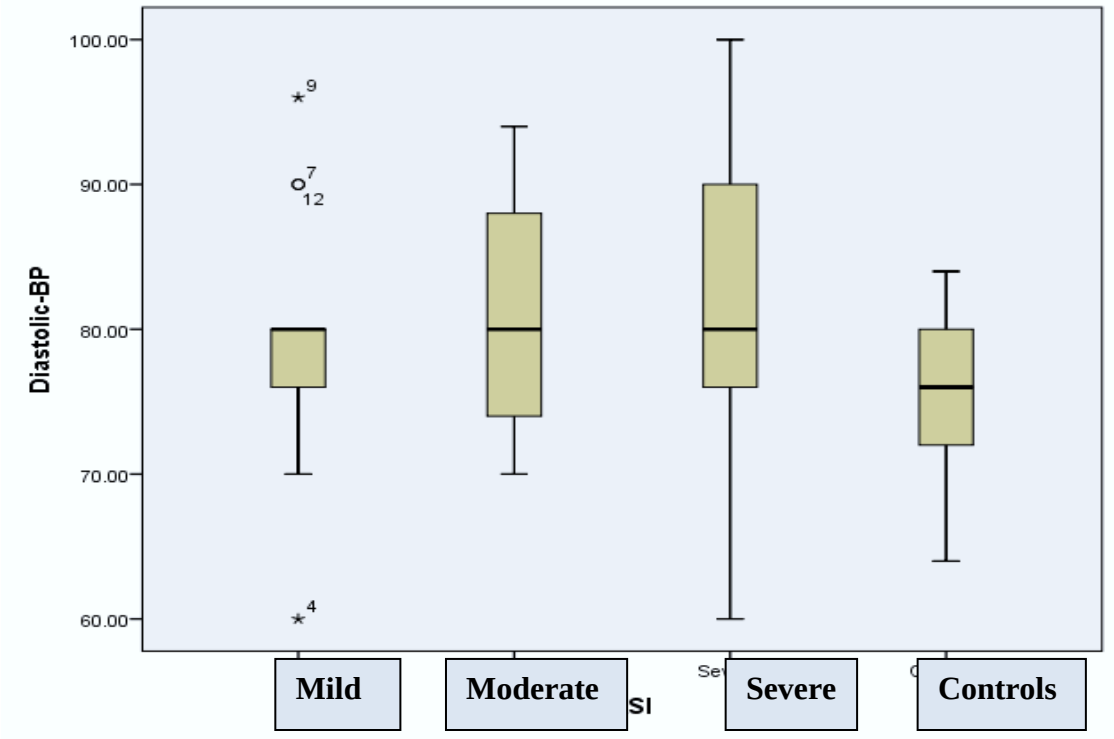


Figure 5.5: Recorded Diastolic Blood Pressure in mild, moderate and severe PASI score and controls

'*': p< 0.05(significant)

- - outliers with numerical data value7 7, 12,4 diastolic Blood pressure

The recorded Blood pressure (Systolic and Diastolic – Blood pressure) was compared among the groups using ANOVA as shown in **Table 5.4**. Post-hoc ANOVA reveals variance of systolic blood pressure and waist circumference is significant (p <0.05).

Table 5.4: Comparison of variance Blood pressure and waist circumference among the groups using ANOVA.

Parameters	Severity of PASI Score in cases and controls (p value)					
	Mild & Moderate	**Mild & Severe**	**Moderate & Severe**	**Mild & Control**	**Moderate & Control**	**Severe & Control**
Systolic-BP(mm of Hg)	0.949	0.985	0.638	0.008*	0.011*	<0.001*
Diastolic-BP(mm of Hg)	0.902	0.648	0.972	0.371	0.017*	<0.001*
Waist circumference (cm)	0.984	0.998	0.890	<0.001*	<0.001*	<0.001*

Note: p value* significant at 5% level of significance ($p<0.05$)

The waist circumference was compared with mild, moderate and severe PASI score and controls as depicted by box plot graph in **Figure 5.6**. In this graph, patients with mild PASI score with mean value 85.3±13.2, moderate PASI score mean value 83.6±20.1, severe PASI score mean value 86±14.2 and in controls 44.7±11.9 respectively. Patients with mild PASI score showed with negative skewed dispersion with median near to 3rd quartile value. In moderate and severe PASI score patients showed symmetric distribution with median value lying in the middle of 2nd and 3rd quartile. In controls the waist circumference variable showed positive skewed dispersion with median close to 2nd quartile. The outliers were not significant in mild PASI score patients whereas in moderate and severe PASI score patients the outliers were significant but however doesn't make much impact on overall score.

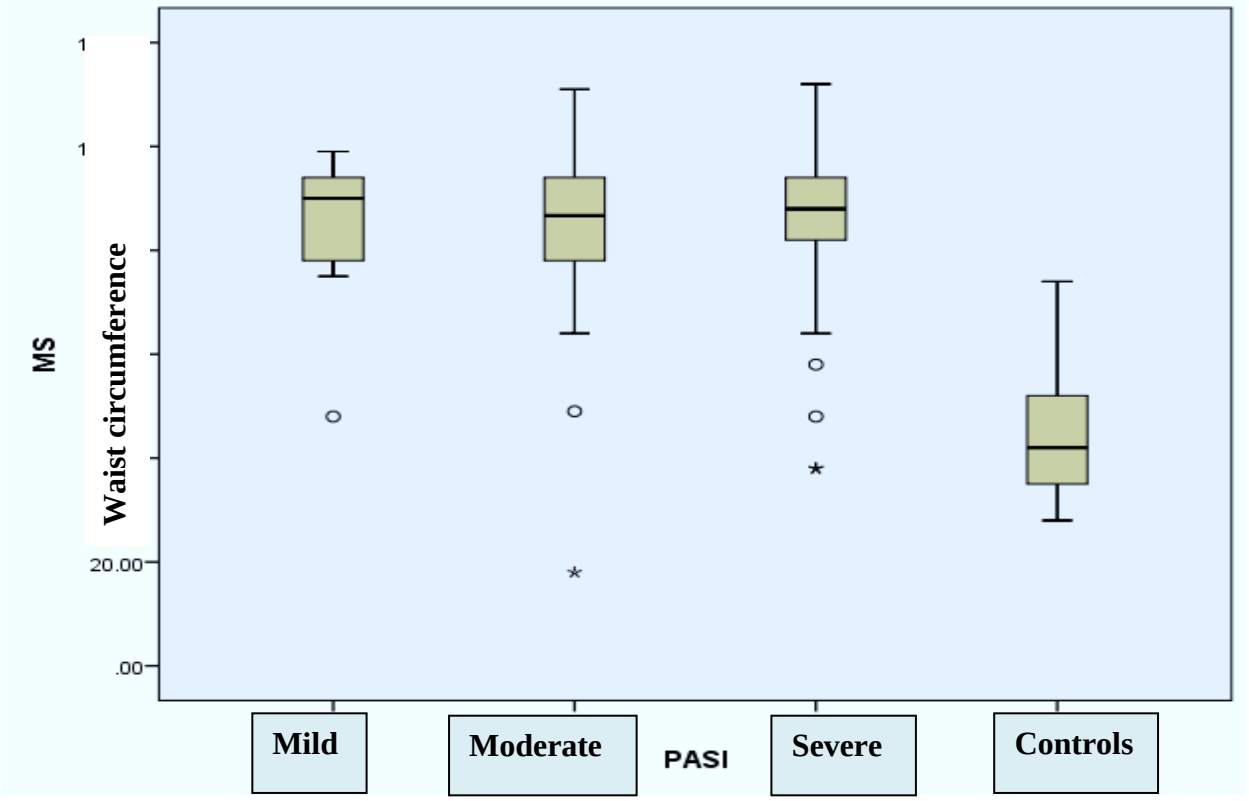


Figure 5.6: Waist circumference in mild, moderate and severe PASI score and controls.

- ● - outliers
- * - significant ($p<0.05$)

Inflammatory Markers

5.5 Serum Inflammatory Markers:

Psoriasis is a chronic inflammatory disease. The cytokine cascade network is disturbed in psoriasis patients. In the present study the serum inflammatory markers TNF-α, IFN-γ, IL-2 were estimated. The Mean±S.D for TNF-α in cases and controls was 30.8 ± 25.3 and 3.9 ± 2.2 respectively. The Mean±S.D for IFN-γ in cases and controls was 31.1 ± 21.7 and 8.0 ± 3.0 respectively. The Mean±S.D for IL-2 in cases and controls was 25.7 ± 10.3 and 11.7 ± 4.1 respectively. The inflammatory markers showed statistical significant ($p<0.05$) in cases compared to controls which is shown in **Table 5.5.**

Table 5.5: Mean±S.D for serum TNF-α, IFN-γ, IL-2 levels in cases and controls.

Parameters	**Cases**	**Controls**	**t value**	**p value**
	Mean ± S.D	**Mean ± S.D**		
TNF-α(pg/ml)	30.8 ± 25.3	3.9 ± 2.2	11.098	<0.001*
γ – Interfereon (pg/ml)	31.1 ± 21.7	8.0 ± 3.0	11.065	<0.001*
IL- 2(pg/ml)	25.7 ± 10.3	11.7 ± 4.1	13.144	<0.001*

Note: p value* significant at 5% level of significance ($p<0.05$)

The serum inflammatory markers were compared among the patients with mild, moderate, severe PASI score and controls using ANOVA post-hoc analysis. The distribution of inflammatory markers among mild, moderate, severe PASI score patients and controls was depicted using box plot graph. The serum TNF- α in mild PASI score patients showed negative skewed dispersion with median value slightly close to 3^{rd} quartile. The lower limit whisker is near to 2^{nd} quartile indicating both 1^{st} and 2^{nd} quartile have same value dispersion.in moderate and severe PASI score there is positive skewed dispersion with median value near to 2^{nd} quartile. Here also the lower limit whisker is near to 2^{nd} quartile which implies same value dispersion. The outliers are present in both moderate and severe PASI score, however the outlier is significant in moderate PASI score patients which doesn't impact on overall score. In controls the median value is dispersed nearly equal to 2^{nd} and 3^{rd} quartile implying a single value for 2^{nd}, 3^{rd} quartiles and median range. The length of upper limit whisker is same as lower limit whisker. There is outlier which is significantly related, however doesn't have any impact on overall value of TNF-α shown in **Figure 5.7**.

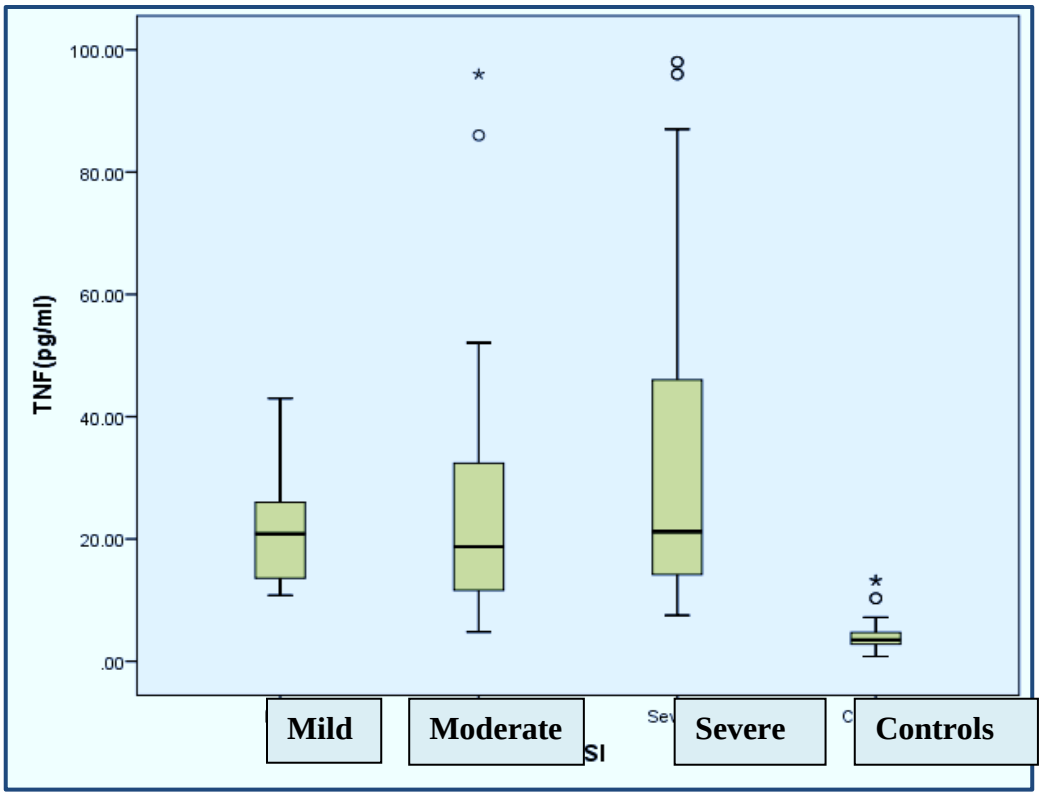


Figure 5.7: Serum TNF-α in mild, moderate, severe PASI score cases and controls.

*- p< 0.05(significant)
• - outlier

The serum IFN-γ with mean value in mild PASI score was 23.5±12.4, in moderate PASI score was 35.6±19.9, in severe PASI score was 31.3±23.4 and in controls was 8±3 respectively. The serum IFN-γ was compared among the groups using ANOVA. The dispersion of values for IFN-γ with respect to PASI score was plotted using box plot graph shown in **Figure 5.8.** In mild PASI score the median value is at 2nd quartile and the lower limit whisker at 1st quartile and the values were positively skewed. In moderate PASI sore the values were dispersed symmetrically with median in the middle at 2nd quartile. In severe PASI score the values were positively skewed showing median value

near to 1st quartile. Here the lower limit whisker is near to 1st quartile dispersion. In controls the values were dispersed near to 1st, 2nd, and 3rd quartile with single outlier lying outside the box near to lower limit whisker which is not significant.

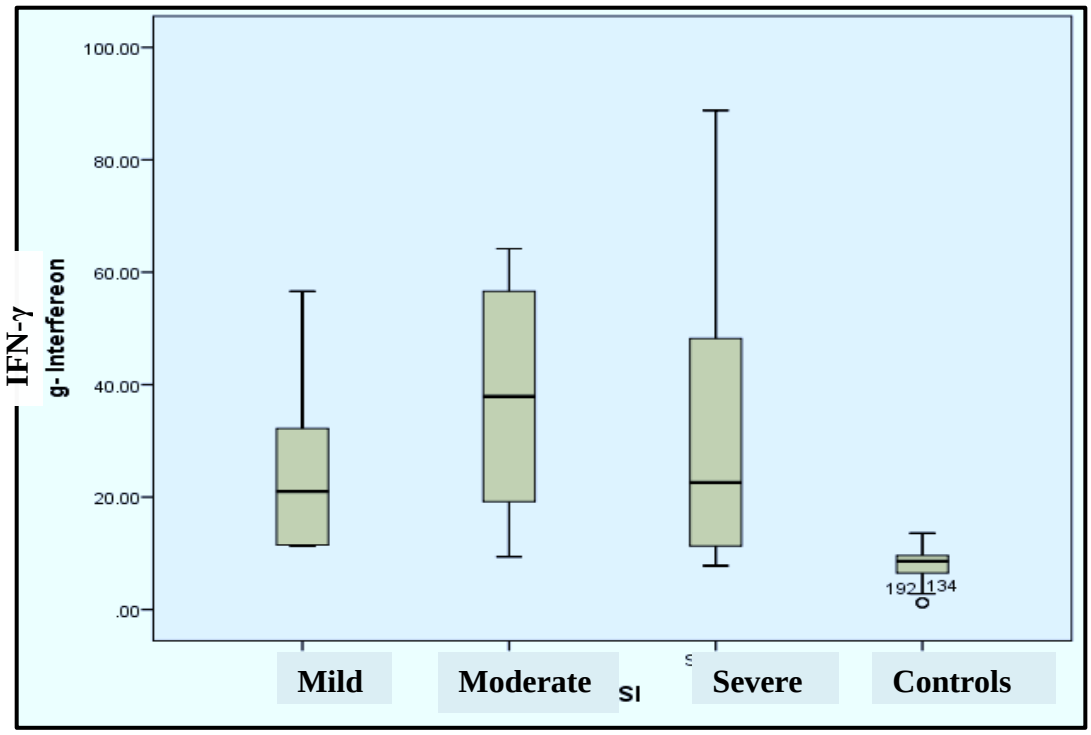


Figure 5.8: Serum inflammatory IFN-γ marker in mild, moderate, severe PASI score

- - outlier with numerical data value 192 , 134 ols.

The serum IL-2 with mean value in mild PASI score was 19.1±6, moderate PASI score was 22.7±8.8, in severe PASI score was 27.8±10.8 and in controls 11.7±4.1 respectively. The serum IL-2 values were compared using ANOVA. The dispersion of values in mild,

moderate and severe PASI score was analysed using box plot graph as shown in **Figure 5.9.** In mild PASI score there was negative skewed dispersion with median value slightly near to 3rd quartile. In moderate PASI score the values were positively skewed implying the median at near to 1st quartile. In severe PASI score the values were positively skewed with median slightly near to 1st quartile. In controls the dispersion of values was symmetrical with median at 2nd quartile and the lower limit whisker near to 1st quartile. The outliers were present in moderate PASI score patients and in controls.

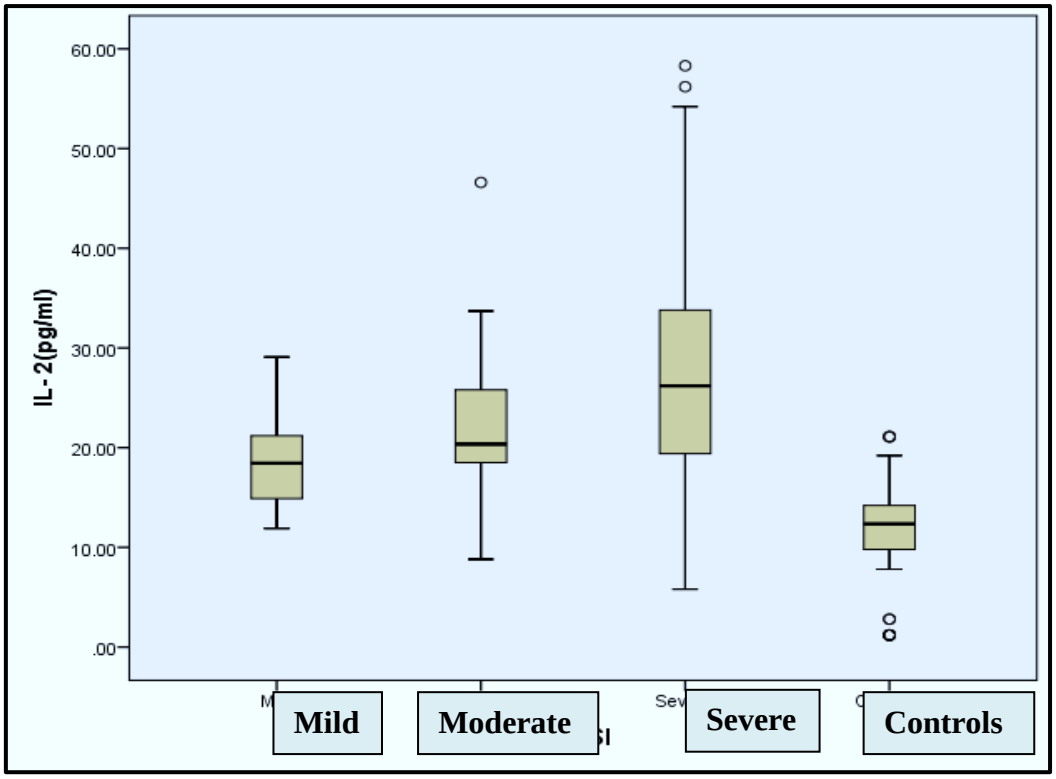


Figure 5.9: Serum IL-2 in mild, moderate, severe PASI score and controls.

- -outlier

The serum inflammatory markers were compared among the groups using ANOVA shown in **Table 5.6. Post-hoc ANOVA** reveals variance of serum inflammatory markers is significant ($p < 0.05$). Serum IL-2 showed significant ($p<0.05$) among mild and severe with p value 0.001, moderate and severe with p value 0.031, mild and control p value 0.004, moderate and control with p value <0.001and severe and control with p value <0.001 respectively.

Table 5.6: Comparison of Serum TNF-α, IFN-γ, IL-2 variances among groups using ANOVA.

Inflammatory Markers	**Cases (PASI) Score (p value)**					
	Mild & Moderate	**Mild & Severe**	**Moderate & Severe**	**Mild & Control**	**Moderate & Control**	**Severe & Control**
TNF(pg/ml)	0.844	0.157	0.526	0.002*	<0.001*	<0.001*
Interfereon- γ (pg/ml)	0.102	0.308	0.659	0.003*	<0.001*	<0.001*
IL- 2(pg/ml)	0.521	0.001*	0.031*	0.004*	<0.001*	<0.001*

Note: p value* significant at 5% level of significance ($p<0.05$)

Oxidative Burden

5.6 Oxidative Burden :

In psoriasis there is development of oxidative burden with decreased anti-oxidant capacity. In the present study, total oxidative stress and total anti-oxidant capacity was estimated in cases and controls. The mean value for total oxidant stress was 21.5 ± 7.6 in cases and 9.4 ± 3.7 in controls respectively as shown in **Table 5.7.** The values were statistically significant ($p<0.05$) in cases compared to controls. The mean value for Total Anti-oxidant capacity in cases was 12.0 ± 4.2 and 21.7 ± 8.4 in controls respectively. There was statistical significant ($p<0.05$) increase in Total Anti-oxidant capacity in controls compared to cases.

Table 5.7: Showing Total Oxidative Stress (TOS) and Total Anti-Oxidant Capacity (TAOC) in cases and controls.

Oxidative Burden	**Cases**	**Controls**	**t value**	**p value**	**95% CI**	
	Mean ± S.D	**Mean ± S.D**			**Lower**	**Upper**
TOC(pg/ml)	21.5 ± 7.6	9.4 ± 3.7	15.1	<0.001*	10.6	13.7
TAOC(ng/ml)	12.0 ± 4.2	21.7 ± 8.4	-10.9	<0.001*	-11.5	-8.0

Note: p value* significant at 5% level of significance ($p<0.05$)

The serum Total oxidant stress and Total Anti-oxidant capacity was compared among mild, moderate, severe PASI score and controls using ANOVA. The mean value for Total oxidant stress in mild PASI score was 21.1±10.4, moderate PASI score was 22.5±8.2, in severe PASI score was 21.3±6.9 respectively. The mean value for Total Anti-oxidant

capacity in mild PASI score was 11.1±4.6, in moderate PASI score was 12.3±4.4, in severe PASI score was 21.3±6.9 respectively. The dispersion of values for Total oxidant stress and Total Anti-oxidant capacity was plotted using box plot graph (**Figure 5.10** and **Figure 5.11**). In mild PASI score the values were symmetrically dispersed on either side of median and lower limit whisker slightly near to 1st quartile. In moderate and severe PASI score the values were positively skewed which means the values were more dispersed near the 1st quartile. In severe PASI score there was single outlier with numerical data value corresponding to 67 which is not impacting on overall PASI score. in controls the values were dispersed symmetrically on either side of median similar to mild PASI score group, however there was outlier with numerical data value 172, 132 respectively. The dispersion of values for TAOC in mild, moderate PASI score was near to symmetrical with median at 2nd quartile. In severe PASI score the values were positively skewed with median value dispersed near to 1st quartile. There were 2 outliers with numerical data value 49, 83 respectively not impacting on overall PASI score. In controls the values were positively skewed with median value dispersed near to 1st quartile.

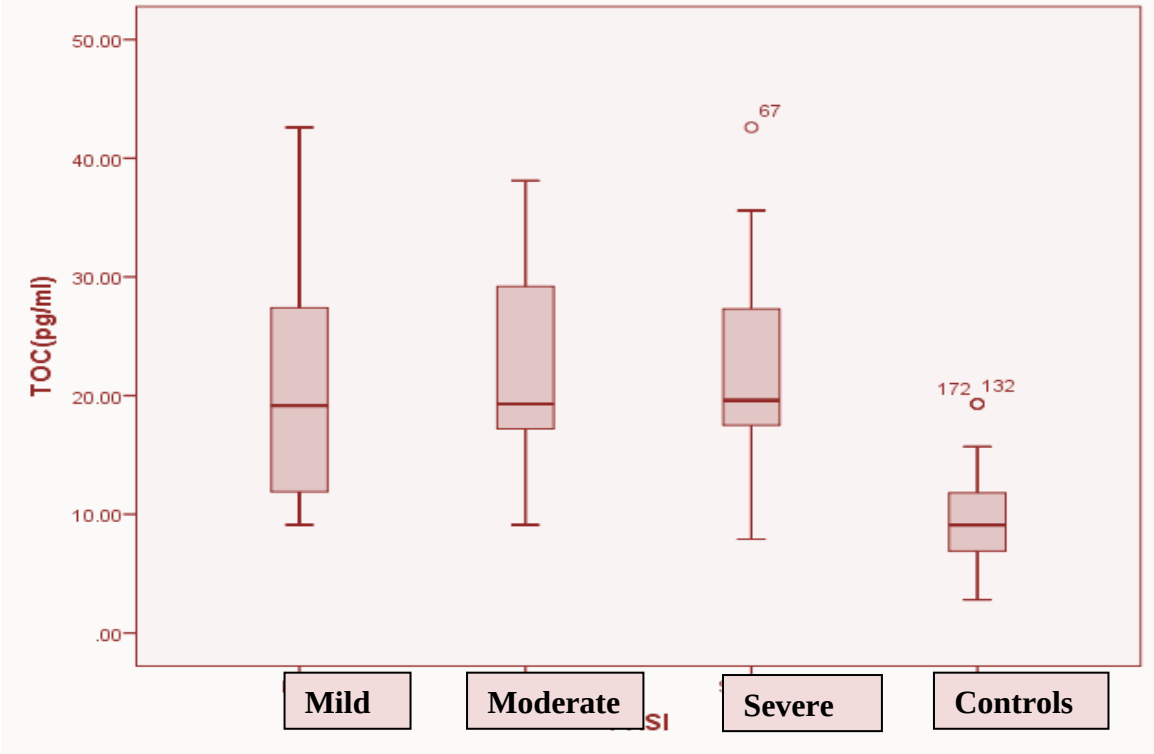


Figure 5.10: Total Oxidative Stress in mild, moderate, severe PASI score cases and controls.

- - outliers with numerical data 67 in severe and 172,132 in controls

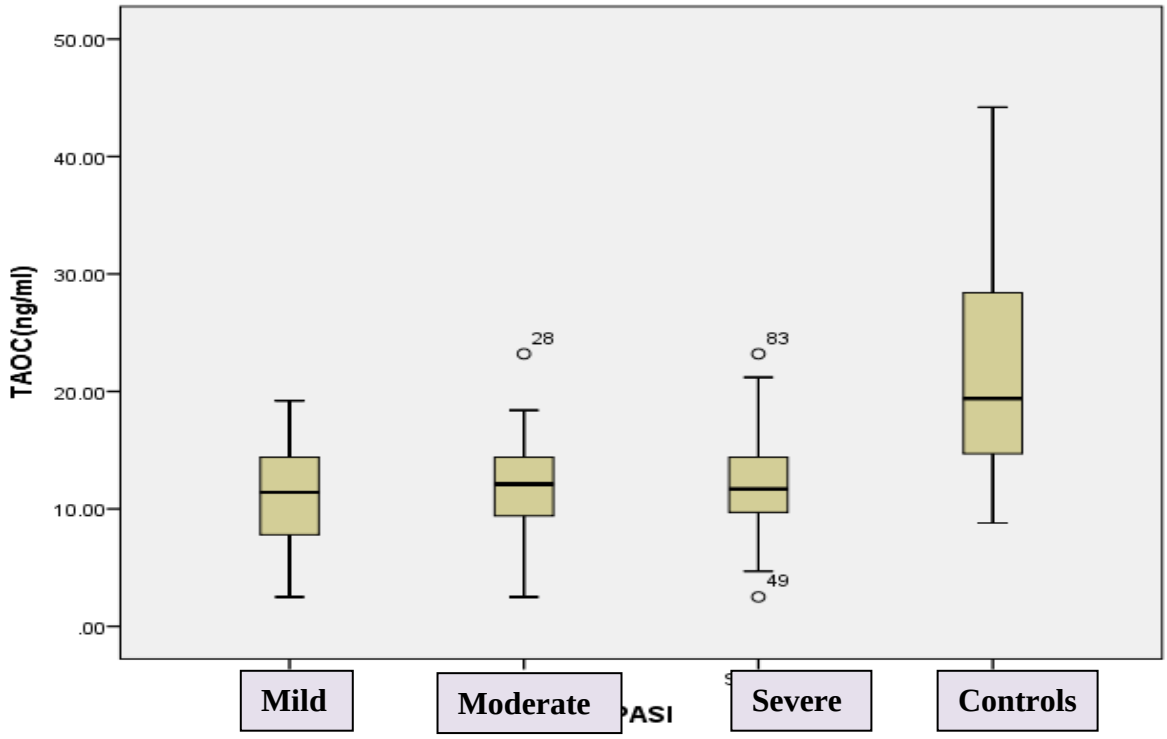


Figure 5.11: Total Anti-Oxidant Capacity (TAOC) in mild, moderate, severe PASI score cases and controls.

- - outliers with numerical data value 28, 49, 83

The serum Total oxidant stress and Total Anti-oxidant capacity was compared among mild, moderate, severe PASI score and controls using ANOVA post-hoc shown in **Table 5.8.** The serum Total oxidative stress was significant with 'p' value <0.001 and serum Total Anti-oxidant capacity was significant with 'p' value <0.001 .

Table 5.8: Comparison of serum TOS and TAOC variances in among groups in cases and controls using ANOVA.

Oxidative Burden	Cases (PASI) Score ('p' value <0.05)					
	Mild & Moderate	**Mild & Severe**	**Moderate & Severe**	**Mild & Control**	**Moderate & Control**	**Severe & Control**
TAOC(ng/ml)	0.953	0.954	1.000	<0.001*	<0.001*	<0.001*
TOC(pg/ml)	0.907	0.999	0.869	<0.001*	<0.001*	<0.001*

Note: p value* significant at 5% level of significance (p<0.05).

Cardiometabolic Risk Markers

5.7 Cardiometabolic Risk Markers :

In the present study, the serum inflammatory markers along with total oxidative stress was increased in cases compared to controls. It suggests that, psoriasis being inflammatory skin disease these patients are prone for development of other systemic diseases and co-morbidities. Since cardiovascular disease also shares common systemic inflammation, we estimated cardiometabolic risk markers like lipid profile, Lp(a), uric acid, hs-CRP. The lipid profile included serum triglycerides, total cholesterol, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL). The mean value for serum triglycerides in cases and controls was 212.3 ± 39.5 and 122.9 ± 14.6 respectively. The mean value for serum total cholesterol in cases and controls was 241.0 ± 60.4 and 155.0 ± 43.2 respectively. The mean value for serum LDL-cholesterol in cases and controls was 196.8 ± 43.1and 132.0 ± 10.0 respectively. The mean value for serum HDL-cholesterol in cases and controls was 34.9 ± 6.8 and 43.5 ± 9.8 respectively. The mean value for serum Lp(a) in cases and controls was 41.3 ± 8.7 and 25.1 ± 7.9 respectively. The mean value for serum hs-CRP in cases and controls was 16.2 ± 7.3 and 2.8 ± 1.3 respectively. The mean value for serum uric acid in cases and controls was 17.1 ± 7.2 respectively. The serum lipid profile, Lp(a), hs-CRP and uric acid were significantly ($p<0.05$) increased in cases compared to controls in **Table 5.9**.

Table 5.9: Serum lipid profile, Lp(a), uric acid, hs-CRP in cases and controls.

Cardiometabolic risk markers	Cases	Controls	t value	p value
	Mean ± S.D	Mean ± S.D		
Triglycerides (TG)(mg/dl)	212.3 ± 39.5	122.9 ± 14.6	22.257	<0.001*
Total Cholesterol(mg/dl)	241.0 ± 60.4	155.0 ± 43.2	12.76	<0.001*
High Density Lipoprotein(HDL) (mg/dl)	34.9 ± 6.8	43.5 ± 9.8	-7.568	<0.001*
Low Density Lipoprotein (LDL)(mg/dl)	196.8 ± 43.1	132.0 ± 10.0	15.368	<0.001*
Uric acid(mg/dl)	17.1 ± 7.2	4.5 ± 1.0	18.2	<0.001*
Lipoprotein(a) [p(a)] (mg/dl)	41.3 ± 8.7	25.1 ± 7.9	14.515	<0.001*
hs-CRP(mg/L)	16.2 ± 7.3	2.8 ± 1.3	18.952	<0.001*

Note: p value* significant at 5% level of significance ($p<0.05$).

The serum triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol was compared among mild, moderate, severe PASI score and controls was analysed using box plot graph (**Figure 5.12**) . The mean value of serum triglycerides in mild, moderate and severe PASI score in cases was 210±36.2, 206±43.6, 214.6±39.1 was respectively. The serum triglycerides in mild, moderate and severe PASI score were positively skewed with values being dispersed in 3rd quartile and median lying close to 1st quartile. In moderate PASI score the outliers were present in the upper limit with numerical data value 36, 26, 19 and data value 17 was significant which doesn't impact on overall score. In controls the values were negatively skewed with median near to 3rd quartile.

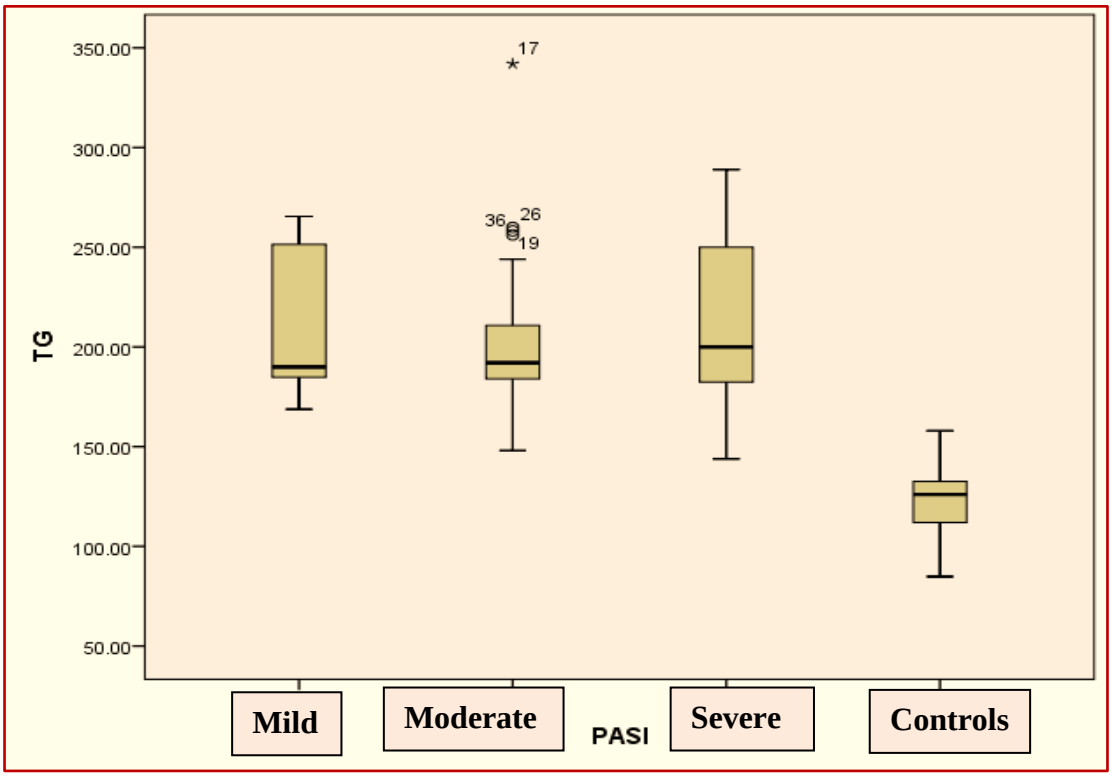


Figure 5.12: Serum Triglycerides levels in mild, moderate, severe PASI score with

- ● - outlier with numerical data value 36, 26, 19 and 17
- * - $p<0.05$

The mean value for serum total cholesterol in mild, moderate and severe PASI score was 259.4 ± 62.4, 229.8 ± 50.4, 240.8 ± 62.7 respectively. The serum total cholesterol in mild, moderate and severe PASI score were positively skewed with values being dispersed in 3rd quartile and median lying close to 1st quartile. In moderate PASI score the upper limit and lower limit whiskers were near to 1st and 3rd quartile. In controls the dispersion of values were symmetric, with outlier lying outside box with numerical data value 207, 127 respectively shown in **Figure 5.13**.

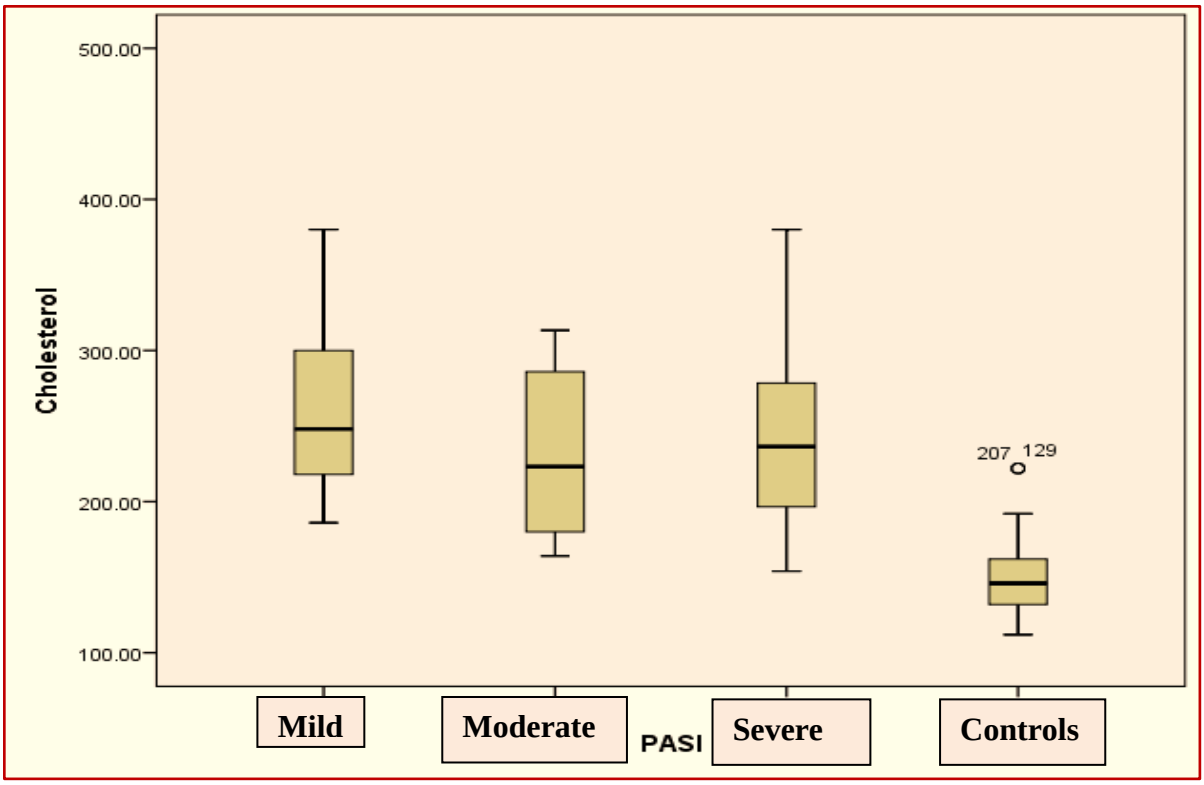


Figure 5.13 : Serum Total Cholesterol levels in mild, moderate, severe PASI score with controls.

- - outlier with numerical data value 207, 129

The mean value for serum HDL-cholesterol in mild, moderate and severe PASI score was was 34.6±6.5, 35.5±7.5, 34.8±6.8 respectively. The serum HDL-cholesterol values were compared with mild, moderate, severe PASI score and controls using ANOVA. The dispersion of values were interpreted using box plot graph **Figure 5.14.** The dispersion of values in mild, severe PASI score were symmetrically distributed on either side of median, where as in moderate PASI score the values were positively skewed with median near to 1st quartile. In mild PASI score the lower limit whisker was almost near to 1st

quartile. The mean value for LDL-cholesterol in mild, moderate, severe PASI score was 192.7±33.8, 190.3±36.9 and 199.5±46.4 respectively. The dispersion of serum LDL cholesterol values were interpreted using box plot graph **Figure 5.15** The dispersion of values in mild, moderate and severe PASI score were positively skewed with median lying near t 1st quartile. The values in controls were also positively skewed and having outlier with numerical data value 135, 162, 191, 214 respectively.

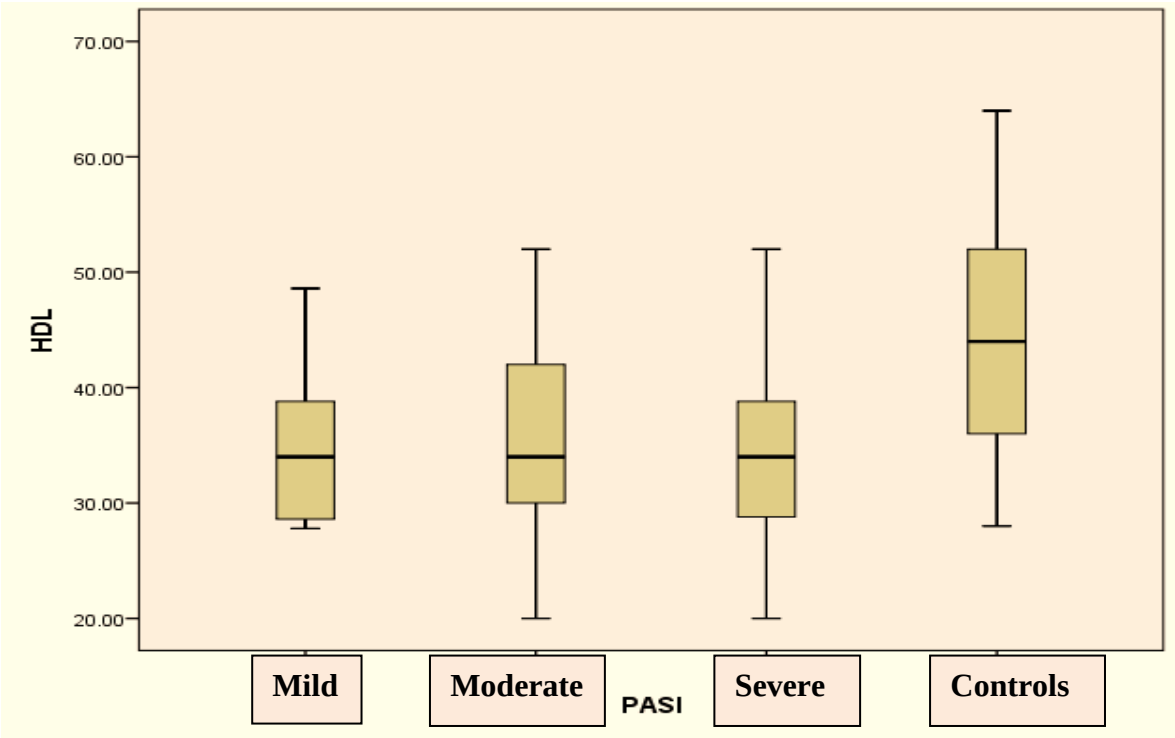


Figure 5.14: Serum HDL- cholesterol levels in mild, moderate, severe PASI score with controls.

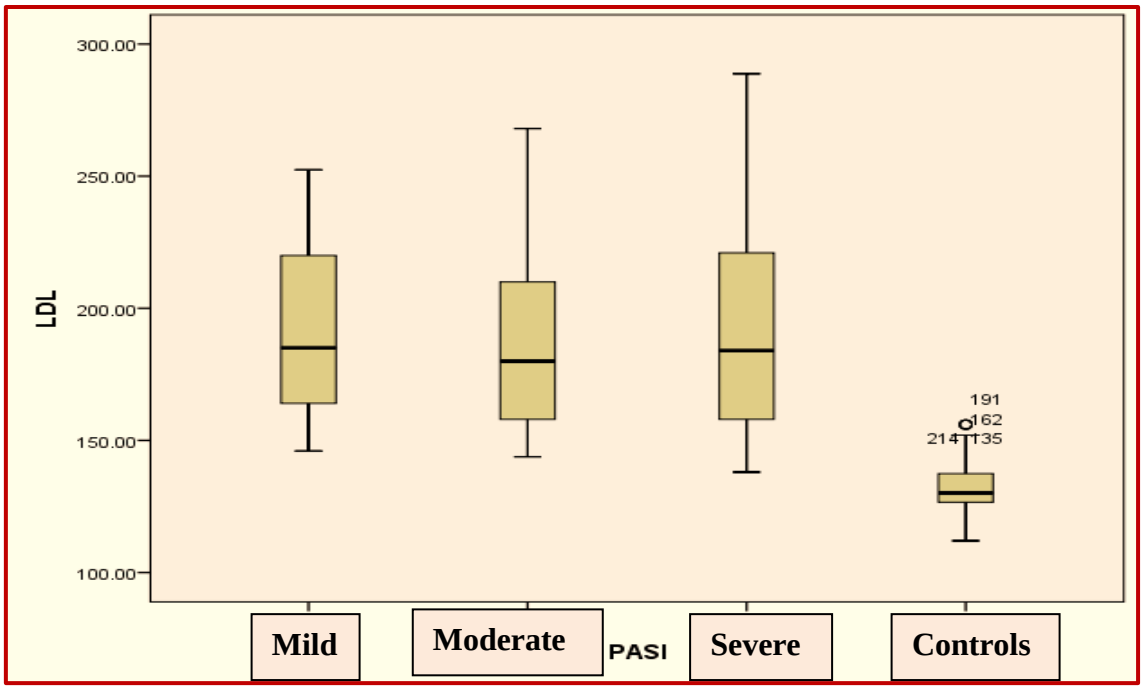


Figure 5.15: Serum LDL- cholesterol levels in mild, moderate, severe PASI score with

- **-** outlier with numerical data value 135, 162, 191,214

The mean value for Lp(a) in mild, moderate, severe PASI score was 42.4±8.4, 38.4±8.9, 41.9±8.6 respectively. The dispersion of values in mild, moderate, severe PASI score was shown in box plot graph (**Figure 5.16)**. In mild PASI score the values were positively skewed with median lying near to 1st quartile. In moderate, severe PASI score the values were negatively skewed with median near to 3rd quartile. There was 1 outlier with numerical data value 51, 100 which doesn't impact on overall score. In controls the dispersion of values was near to symmetrical with median near to 2nd quartile.

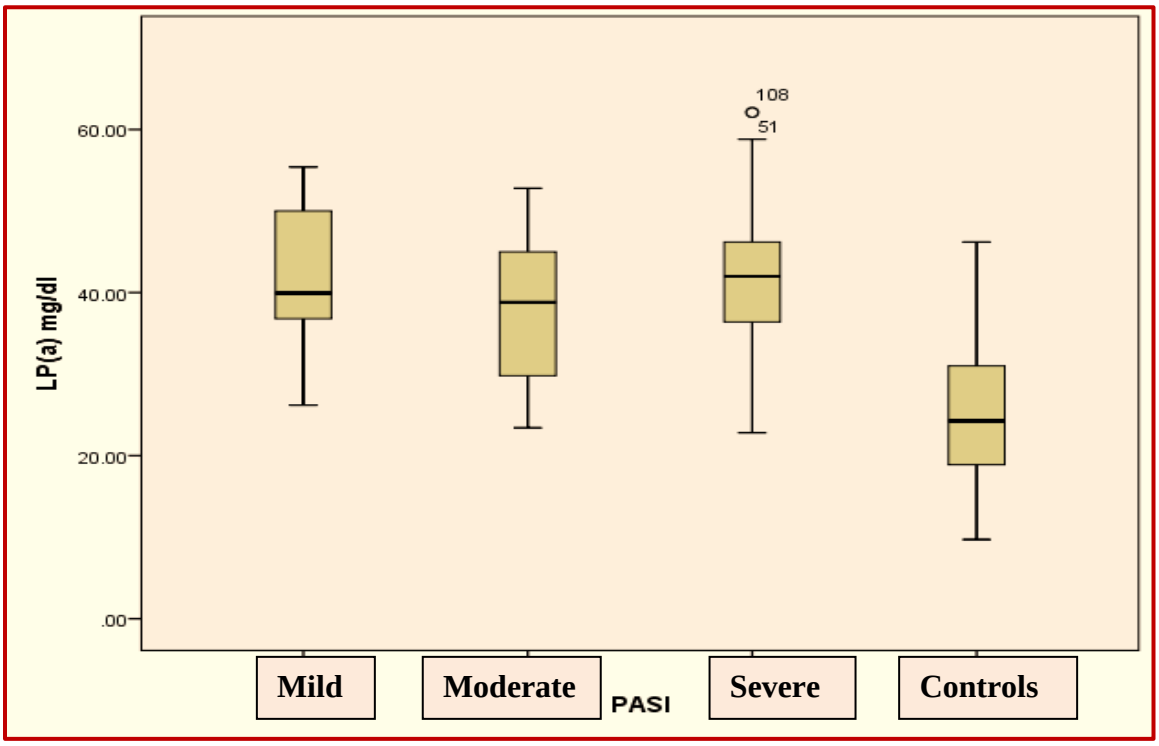


Figure 5.16: Serum Lp(a) levels in mild, moderate, severe PASI score with controls.

- - outlier with numerical data value 51, 108

The mean values of uric acid in mild, moderate and severe PASI score were 16.3±9.2, 16.7±6.2 and 17.4±7.2 respectively. The dispersion of values in mild, moderate, severe PASI score and controls were interpreted using box plot graph **Figure 5.17.** The values in mild PASI score were positively skewed with median near to 1st quartile. In moderate PASI score the values were negatively skewed with median near to 3rd quartile. In severe PASI score the values were positively skewed with median near to 1st quartile. In controls

the values were dispersed symmetrically on either side of median with both upper and lower limit whiskers near to 1st and 3rd quartile.

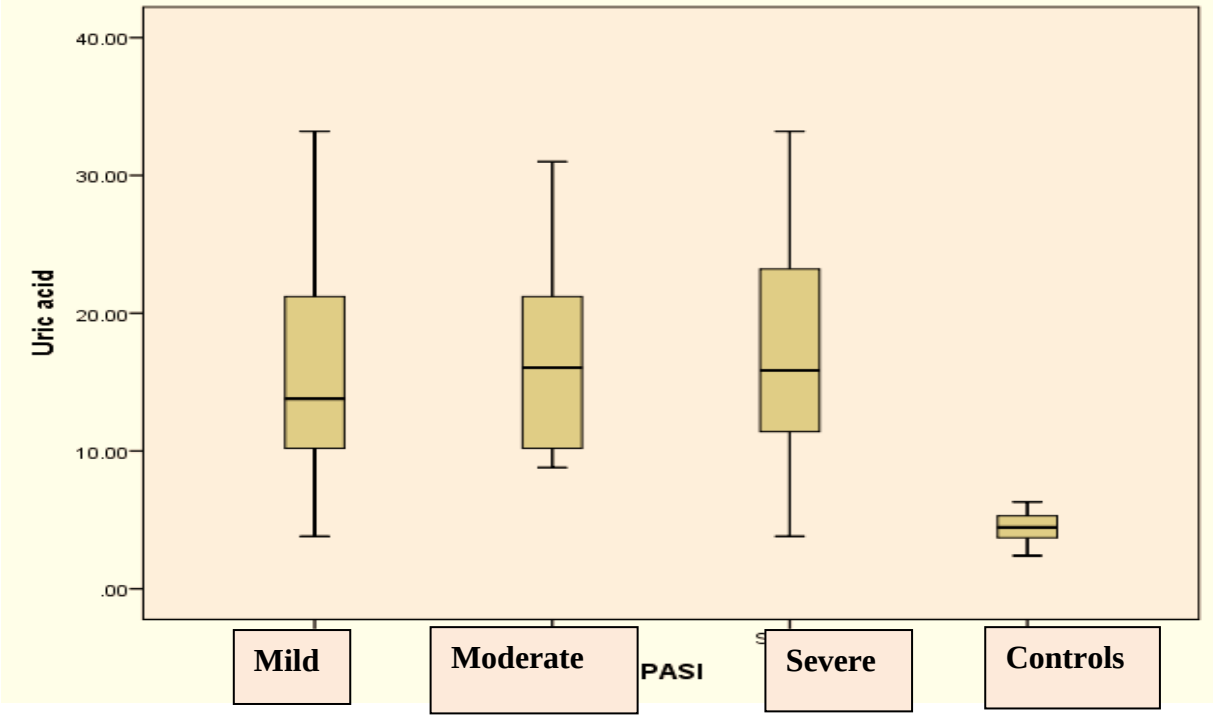


Figure 5.17: Serum Uric Acid levels in mild, moderate, severe PASI score with controls.

The mean value of hs-CRP in mild, moderate and severe was 16±6.6, 15.8±6.4 and 16.3±7.7 respectively. The dispersion of values in mild, moderate, severe PASI score and cntrols is interpreted using box plot graph **Figure 5.18.** The dispersion of values in mild PASI score were symmetrical, equal on either side of median range. In moderate and severe PASI score the values were negatively skewed with median near to 3rd quartile. The outliers were lying outside the box in both moderate and severe PASI score which doesn't impact on overall score. The lower limit whisker in mild moderate PASI score was almost near to 1st quartile. In controls the values were dispersed equally on

either side of median wirth 1 outlier lying outside which is not having any impact on the present value.

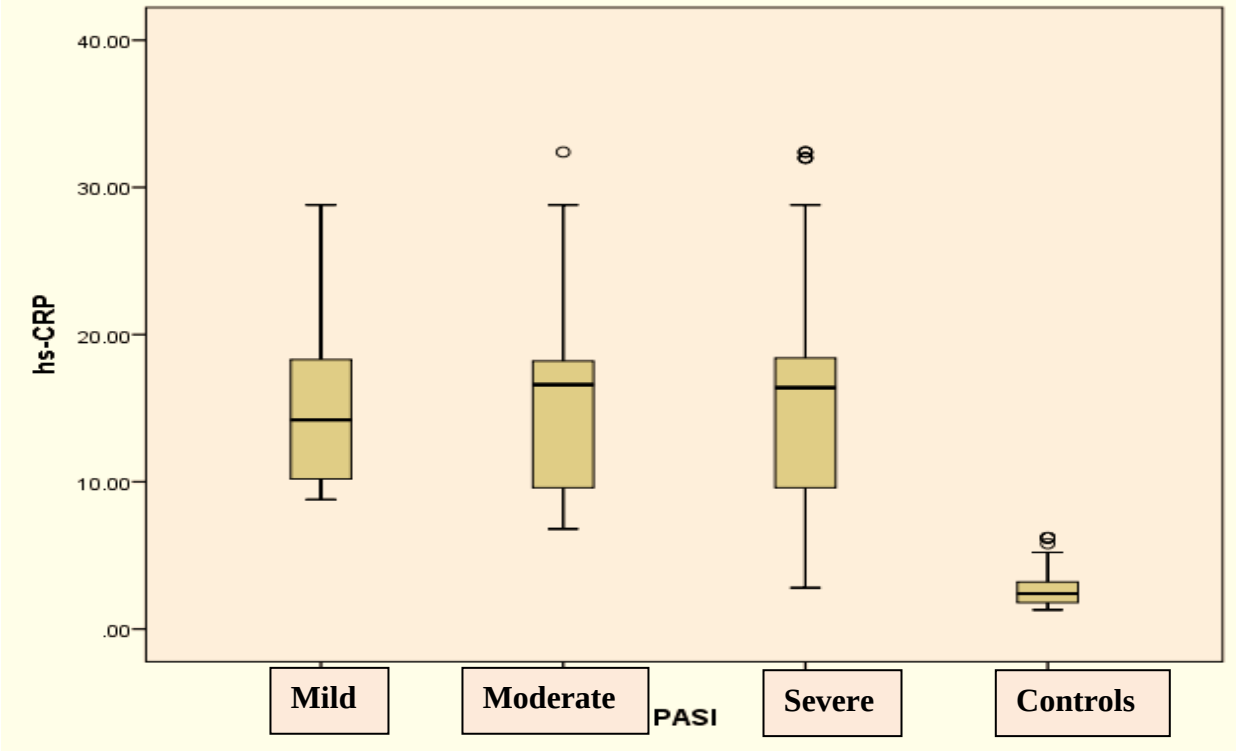


Figure 5.18: Serum hs-CRP levels in mild, moderate, severe PASI score with controls.

- - outliers

The cardiometabolic risk markers were compared among mild, moderate, severe PASI score and controls using ANOVA. Post-hoc analysis showed significant triglycerdes, HDL-cholesterol, LDL-cholesterol, Lp(a), uric acid, hs-CRP with 'p' value < 0.001 among mild and control,

moderate and control, severe and control, depicted in **Table 5.10.**

Table 5.10: Comparision of cardiometabolic risk markers variance among groups using ANOVA.

Cardiometabolic risk markers	**Cases (PASI Score) and controls and 'p' value**					
	Mild & Moderate	**Mild & Severe**	**Moderate & Severe**	**Mild & Control**	**Moderate & Control**	**Severe & Control**
TG	0.979	0.954	0.640	<0.001*	<0.001*	<0.001*
Cholesterol	**0.999**	**1.000**	**1.000**	**0.993**	**0.958**	**0.904**
HDL	0.987	1.000	0.981	0.001*	<0.001*	<0.001*
LDL	0.996	0.879	0.621	<0.001*	<0.001*	<0.001*
Uric acid	0.995	0.876	0.942	<0.001*	<0.001*	<0.001*
LP(a) mg/dl	0.489	0.996	0.316	<0.001*	<0.001*	<0.001*
hs-CRP	1.000	0.995	0.978	<0.001*	<0.001*	<0.001*

Note: p value* significant at 5% level of significance ($p<0.05$).

CORRELATION OF INFLAMMATORY MARKERS WITH OXIDATIVE BURDEN & CARDIOMETABOLIC MARKERS

5.8 Correlation of Inflammatory markers with oxidative burden and cardiometabolic risk markers :

The serum cardiometabolic risk markers were co-related with inflammatory markers in cases to assess the future development of co-morbidities in these patients using student unpaird 't' test **(Table 5.9).** The serum HDL, hs-CRP with TNF -α, with 'r' value -0.125 and -0.063 rsepectively. But, they were statistically not significant. The serum triglyceride showed positive correlation with TNF-α with 'r' value 0.184 and was statistically significant 'p' value 0.045. The serum Lp(a) and uric acid showed negative correlation with 'r' value -0.098 and -0.091 and were not statistically significant. The serum hs-CRP showed negative correlation with 'r' value -0.228 and was statistically significant with 'p' value 0.017. The serum LP(a), HDL, LDL, Uric acid, TAOC were negatively correlated with IL-2 with 'r' value -0.023, -0.016, -0.025, -0.057, and -0.068 respectively. They were statistically not significant shown in **Table 5.11**.

Table 5.11: Correlation of inflammatory markers (TNF-α, IFN-γ, IL- 2) with cardiometabolic markers oxidative burden in cases.

Cardiometabolic markers and oxidative burden	**TNF-α(pg/ml)**		**IFN-γ**		**IL- 2(pg/ml)**	
	r value	**p value**	**r value**	**p value**	**r value**	**p value**
LP(a) mg/dl	0.068	0.483	**-0.098**	0.306	**-0.023**	0.811
TG	0.184	**0.045***	0.171	0.073	0.024	0.803
Cholesterol	0.066	0.494	0.056	0.559	0.011	0.908
HDL	**-0.125**	0.193	0.03	0.760	**-0.016**	0.867
LDL	0.035	0.716	0.057	0.552	**-0.025**	0.792
Uric acid	0.027	0.782	**-0.091**	0.342	**-0.057**	0.555
TAOC(ng/ml)	0.028	0.768	0.041	0.668	**-0.068**	0.480
TOC(pg/ml)	0.003	0.975	0.005	0.955	0.092	0.337
hs-CRP	**-0.063**	0.513	**-0.228**	**0.017***	0.051	0.600

Note: p value* significant at 5% level of significance ($p<0.05$).

Chapter 6

DISCUSSION

6.1 Demographic characteristics in cases and controls:

In the present case control study, the cases were 110 and appropriately with 110 healthy controls. The cases were further categorized into mild, moderate and severe PASI score. PASI score is a clinical tool to categorize them into mild, moderate and severe. The cases were appropriately matched for age and gender distribution with controls. The age was between 20-60yrs. The study was conducted for a duration of 2years. Psoriasis being chronic inflammatory skin disease, in the present study serum TNF-α, IFN-γ and IL-2 were estimated. Following inflammation there was imbalance in oxidant and anti-oxidant capacity. This lead further to assess cardiometabolic risk factors in cases compared with controls.

In the present study, 14 patients were with mild PASI score, 22 with moderate and 74 were with severe PASI score in **Figure 5.1**. These patients were appropriately matched for age and gender with 110 healthy controls. In both subjects maximum were males with 55.5% in cases and 57.3% in controls. The mean age for cases was 40.8 ± 11.4, and for controls 39.4 ± 11 in **Figure 5.3**. In the study maximum number of subjects belonged to middle age group, where cases 38 and controls 39 belonged to age group 31-40 years followed by 25 cases and 23 controls for age 41-50years in **Table 5.2**. Various studies have shown the prevalence of the psoriasis in human population with different regional and ethnic groups [1]. The survey done by global psoriasis epidemiology revealed that prevalence is different in different countries of the world with different age group. The prevalence of psoriasis in children indifferent countries is 0% in Taiwan, 0.71% in Germany and 2.1% in Italy [2]. Higher values were reported in the North-East and South Europe than the UK, specifically 3.73% in Denmark, 4.82-8.50% in Norway respectively [3].

However, the prevalence of psoriasis in India is not so much as in the western countries, though some cases have been reported [4]. This data on prevalence is mainly from hospital-based studies. Very few studies are available on well-defined large population to get the prevalence in our country [5]. Okhandiar *et al.,*[6] to understand this prevalence collected a comprehensive data from different medical colleges located in Dibrugarh, Calcutta, Patna, Darbhanga, Lucknow, New Delhi and Amritsar. According to their research, the incidence of psoriasis ranged between 0.44 and 2.2%, with overall incidence of 1.02%. They also noted that the incidence in Amritsar (2.2%) was higher as compared to other centers in Eastern India. This variation in incidence may be related to various environmental conditions (temperature, climate), dietary habits, and genetic differences [7]. The ratio of male to female (2.46:1) was very high in their study population [8]. The highest incidence was observed in the age group between 20-39 years and the mean age of onset was appropriated for both in males and females [9]. These observations were in accordance to our study findings. In the present study it was observed the maximum incidence was in the age group between 31-40 years in **Table 5.2**. The percentage of patients and controls in this age group was 34.5% and 35.5% respectively.

Among gender distribution in the present study, maximum patients were males both in cases and controls. The age and gender for cases were appropriately matched with healthy controls. In cases and controls the percentage of males was around 55.5% and controls 57.3% respectively . The percentage of females in cases and controls was around 44.50% and 42.7% respectively. Study done by Bedi *et al.*, showed in their study male to female ratio was around 2.5:1. The study also found out that females had lower mean age group compared to males [10]. Another study done by Bedi *et al* , which included larger subjects, found 2.8% of prevalence of

psoriasis and gender distribution was equal both in cases and controls [11]. However, in our study it was observed males were proportionately more compared to controls in **Figure 5.2**. In a study from tertiary health care center from North India, the prevalence of psoriasis patients accounted for 2.3% of the total dermatology out patients [12]. Of these total psoriasis patients, 67% were men and 33% were women, male to female ratio being 2.03:1 similar to our study . The age distribution of patients ranged from infancy to eighth decade, with mean age being 33.6 years. The percentage of children accounted for 4.4% of total psoriasis patients [13].

It shows that, in India the prevalence of psoriasis varies from 0.44 to 2.8%, and it is twice more common in males compared to females. Most of the patients are in their third or fourth decade during the time of presentation. In our country these studies are limited by the absence of commonly accepted and validated diagnostic criteria. There is still no reliable information on time trends of this disease with respect to age and gender distribution.

6.2: Blood Pressure and Anthropometric indices

In the present study the mean value for systolic blood pressure in cases was 136.1±14.0 and in controls 125.0±8.9 respectively in **Table 5.3**. The mean value for diastolic blood pressure in cases was 81.4±8.3 and in controls was 76.2±5.5 respectively in **Table 5.3.** The blood pressure was compared among mild, moderate and severe PASI score using ANOVA in **Figure 4 and Figure 5**. The blood pressure was statistically high in cases compared to controls. The results reflect that the cases were hypertensive compared to controls and prone for cardiovascular diseases. The mean waist circumference in cases 85.4±15.3 and in controls was 44.7±11.9 respectively in **Table 5.3**. In addition, hypertension, where activation of the renin–angiotensin system plays a prominent role, and atherosclerosis have been more frequently observed in patients with psoriasis [14]. Studies have mentioned that the rate of hypertension was twice as high in psoriatic patients compared with controls. A recent study by Gisondi *et a .*, observed waist circumference higher in cases compared to controls similar to our study [15]. The waist circumference was significantly high in cases compared to controls. The waist circumference was compared among the mild, moderate and severe PASI score groups using ANOVA in **Table 5.4**.

6.3: Serum Inflammatory Markers

(TNF-α, IFN-γ, IL-2)

Psoriasis is defined as chronic inflammatory skin disease. This disease is now considered as a major global health problem in our country. The disease is characterized by well-defined, red, scaly plaques. The appearance of these lesions is on and off, with a chronic-recurrent course involving preferential sites such as elbows, knees, and scalp [16]. Earlier psoriasis was described as a primary disorder of keratinocytes with abnormal proliferation and differentiation. But recent studies on genetic and immunological techniques have led to better understanding of psoriasis. Therefore it is now widely recognized as a complex and multi-factorial, immune-mediated inflammatory disease of the skin [17].

There is dysregulated interaction between keratinocytes and the immune system in this underlying disease. The inflammatory nature of psoriasis is characterized by a systemic and dermal secretion of cytokines such as interleukins (IL): IL-2, IL-6, IL-8, IL-17, IL-18, IL-22, IL-23, IL-24, interferon-γ (INF-γ) and tumor necrosis factor-α (TNF-α) [18]. In the present study we estimated serum cytokines such as TNF-α, IFN-γ and IL-2. It was observed that the Mean ± S.D of serum TNF-α was 30.8 ± 25.3 in cases and 3.9 ± 2.2 in controls respectively. The Mean ± S.D of serum IFN-γ was 31.1± 21.7 in cases and 8.0 ± 3.0 in controls respectively. The Mean ± S.D of serum IL-2 was 25.7 ± 10.3 in cases and 11.7 ± 4.1 in controls respectively. It showed that the serum inflammatory markers were increased in cases compared to controls and was statistically significant ($p<0.05$) shown in **Table 5.5**. Study done by Aikaterini Kyriakou *et al* ., observed in their study that the serum TNF- α in were significantly elevated in psoriasis patients compared to controls. Along with TNF-α, they also estimated IL-12, IL-17 and their correlation with severity of the disease PASI score in cases. However, they couldn't find any significant changes with IL-12, IL-17 levels between cases and controls. They also observed serum TNF-α showed significant

correlation with PASI score, similar findings to our study. As we know psoriasis is an immune-mediated inflammatory disease which affects the skin, joints, and nails. The exact etiology is not known. In genetically pre disposed individuals, the elements of epidermis and the dermis which are involved in the maintenance of the barrier integrity are deregulated in response due to environmental or self-antigenic stimulation. The Th1 cytokines subsets play a determine role in pathogenesis of the disease [19]. Study done by Rakesh Pandey *et al.,* observed that there is a network of cytokines involved in the pathogenesis of psoriasis. According to their study, psoriasis which is characterized by hyperproliferation of keratinocytes is mainly due to release of cytokines TNF-α, IL-23, IL-17 and IL-15. These cytokines exhibit different mechanisms for the development of characteristic lesions in psoriasis [20]. Though many studies showed increased levels of serum TNF-α in these patients, but study done by Tigalonova *et al.,* didn't find significant serum TNF- α changes between cases and controls. According to this study, compared to other studies authors have mentioned that this controversy may be due to the heterogeneity of the inclusion criteria, demographic characters in study populations [21].

In the present study along with serum TNF- α, there was also increase in serum IFN-γ, IL-2 in cases compared to controls and was statistically significant as shown in **Table 5.4**. A Study done by Nam Kyung Roh *et al,* on stimation of both serum and tissue different inflammatory cytokines in psoriasis patients. In their study they estimated different cytokines mainly Th1 and Th17 subsets (IL-1RA, IL-2, IL-12p40, IL-17A, IL-22 and IFN-γ) in cases and controls [22]. In their study all these cytokines were elevated in cases compared to controls and was statistically significant similar to our study with increased serum IL-2 levels in cases compared to controls. They mentioned that not only Th1 but Th17 cytokines were also involved in

pathogenesis of psoriasis. Previous studies have mentioned that T-helper (Th)-1 cells play a dominant role in the initiation and maintenance of psoriasis. The inflammatory mediated eruption in psoriasis is due to the initial secretion of serum IFN-γ from plasmacytoid dendritic cells (DCs). The produced IFN-γ further stimulates DCs to produce tumor necrosis factor (TNF)-α, inducible nitric oxide synthase, IL-23. The IL-23 stimulates Th-17 cells to produce IL-17A, IL-17F, IL-6, and IL-22 [23]. Similar findings were observed by Camila Cataldi *et al.,* with increased in TNF-α, IFN-γ and IL-2 in cases compared to controls and was statistically significant. . Under the influence of IL-12, the Th1 cells produce and secrete IL-2 cytokine. The major function of IL-2 is induction of the Th1 phenotype thereby producing IFN-γ, TNF and activating Natural Killers (NK) cells [24]. But study done by Takahashi *et al.,* showed no significant differences in the levels of IL-2 between psoriasis patients and healthy controls in a Japanese population. They have explained that probably the genetic differences in study populations could be the reason for finding no difference in IL-2 levels in cases and controls [25].

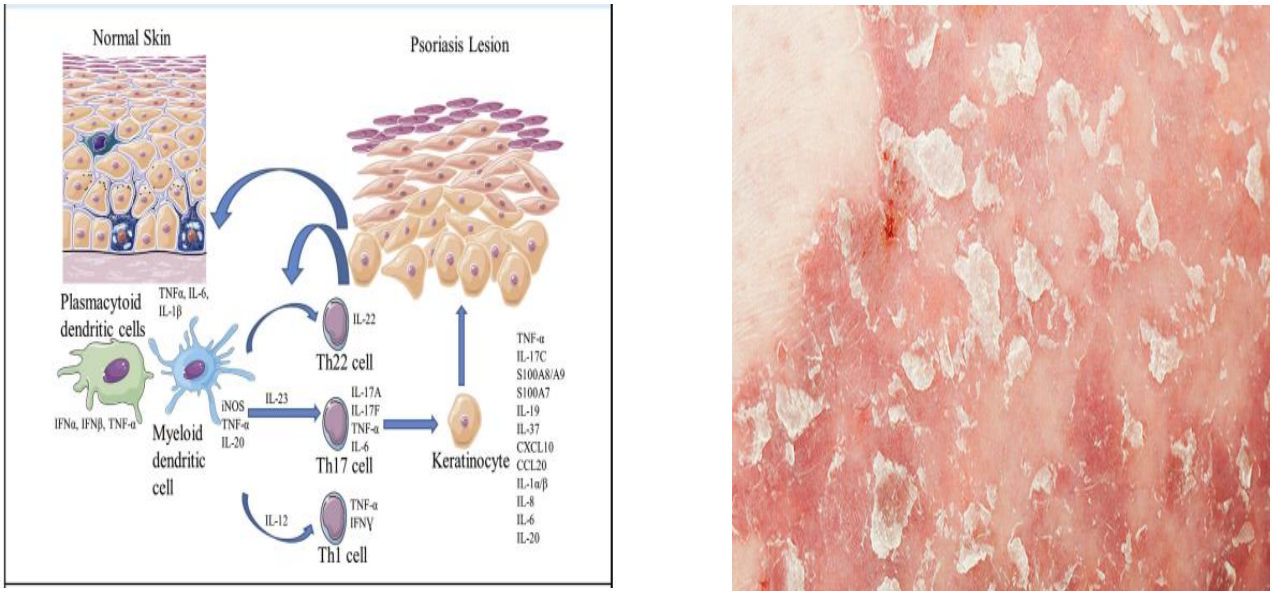
The serum inflammatory markers were further compared with mild, moderate and severe PASI score. The mean value for serum TNF-α in mild, moderate and severe PASI score was 22.4±10.3, 27.4±24.9, 33.3±27.1 respectively in **Figure 5.7**. The mean value serum IFN-γ in mild, moderate and severe PASI score was 23.5±12.4, 35.6±19.9, 31.3±23.4 respectively in **Figure 5.8**. The mean value for serum IL-2 in mild, moderate and severe PASI score was 19.1±6, 22.7±8.8, 27.8±10.8 respectively in **Figure 5.9**. These serum inflammatory markers were compared with PASI score using ANOVA and were interpreted in box plot graph as shown in figure 5.7, 5.8 and 5.9 and in **Table 5.6**. This shows that serum TNF- α showed proportionate increase in its levels from mild to severe PASI score as mentioned in **Figure 5.7**. There has been

no consistent conclusion in the literature on the correlation between the serum levels of TNF-α and disease severity.

Study done by Ozer Arican *et al.,* in his study also observed proportionate increase in serum TNF-α levels with respect to PASI score, which was similar to our findings in the present study. The primary defect in psoriasis patients was due to abnormal proliferation of epidermal cells. It was proposed that, normally there is an interaction between the epidermis and circulating T-cells in the skin. Cytokines are nothing but small, biologically highly active proteins that regulate the growth, function, and differentiation of cells. These cytokines directs the immune response and inflammation [26]. The exact role of TNF-α in the pathogenesis of psoriasis is still unclear, however literature mentions that anti-TNF-α therapy is highly effective in reducing the lesions in psoriasis. This implies that along with IFN-γ, play a central role in the pathogenesis of this disease [27]. Both IFN-γ and TNF-α induce secretion of IL-6,IL-8,IL-12, and IL-18 which constitute an important link in the cytokine network in the pathogenesis of psoriasis [28].

Study done by Jacob *et al.,* couldn't find any co-relation between serum inflammatory markers and severity of the disease PASI score. In the present study also, we couldn't get the significant results when the inflammatory markers were compared among the groups. However, they were significant ($p<0.05$) when compared with controls. This controversy may be due to the heterogeneity of the inclusion criteria and study populations [29, 30]. As we know cytokines play an important role in the pathogenesis of psoriasis, these cytokines levels when measured in serum, doesn't reflex the origin of the sites. Many factors are involved in affecting the levels of cytokine concentrations such as production, tissue/cellular deposition, degradation, elimination, and also other tissue sources of cytokine production might exist beside the circulating T cells [31].

They also found that high levels of IFN-γ, IL-12, and IL-18 correlated with PASI score. These data confirm that psoriasis can be considered as a true systemic disease with particular immunologic pathways [32]. The controversial findings from the above studies mentions that, the cytokine assay results may vary due to the methods used for cytokines detection and their sensitivities, interferences due to different drugs used, and the effect of concomitant pathologies [33]. Thus, the exact role of serum cytokines needs to be clarified and further studies are required in the pathogenesis of psoriasis. It appears likely that these changes are not the cause, but the consequence of, the dermatological diseases.



(A) **(B)**

Figure 6.1: (A)Cytokine cascade & (B) Scaly lesions in Psoriasis

Source: Lloyd-Jones DM, Lewis CE, Schreiner PJ, Shikany JM, Sidney S, Reis JP. The Coronary Artery Risk Development In Young Adults (CARDIA) .J Am Coll Cardiol. 2021;78(3):260-277. doi: 10.1016/j.jacc.2021.05.022.

6.3 : Total Oxidative Stress (TOS) and Total Anti-Oxidant Capacity (TAOC)

In the present study we estimated serum total oxidative stress (TOS) and total anti-oxidant capacity (TAOC). The mean values of serum TOS in cases and controls were 21.5 ± 7.6 and 9.4 ± 3.7 respectively. The mean values of serum TAOC in cases and controls were 12.0 ± 4.2 and 21.7 ± 8.4 respectively. These findings suggest that in the present study, there was increased oxidative stress with compensated decrease in anti-oxidant capacity in cases compared to controls and was statistically significant as shown in **Table 5.7**. Study done by Karababa *et al.,* also got the same findings in their study. According to authors oxidative stress is involved in the pathogenesis of psoriasis [34]. The association between oxidative stress biomarkers and psoriasis in humans remains unclear. Rocha-Pereira et al., based on a case-control study, reported that the TAOC levels were significantly decreased in patients with psoriasis compared to controls similar to our study [35]. However, contrasting results were noticed by Gavan et al, with significantly higher TAOC levels in psoriasis patients than in healthy controls [36]. Study done by Wacewicz et al. suggested that the levels of TAOC were not significantly different between psoriasis patients and controls. They proposed that the controversial reports were due to heterogeneity in the outcomes, population study, different measures and units employed [37].

Skin is the main target organ of oxidative stress. The long-term existence of reactive oxidative stress (ROS) in the microenvironment and the skin's metabolism together destroy the ROS defense mechanism and resulting in various skin diseases. The exact mechanism of oxidative stress on psoriasis is not fully understood, but researches have mentioned that oxidative stress can change cell signaling pathways, which promotes the pathogenesis and progression of psoriasis [38]. In psoriasis, the abnormal activation of tumor necrosis factor (TNF)- α induces keratinocytes and fibroblasts to release ROS[39,40]. Neutrophils infiltrate into the epidermis and

form munro microabscesses, which produces large amount of ROS. The imbalance between abnormal release and accumulation of ROS in these cells lead to oxidative stress. Because of continuous over production of ROS, dendritic cells get stimulated to release antigens to T cells, which leads to the imbalance of T helper cells, resulting in abnormal proliferation of keratinocytes and abnormal angiogenesis [41]. Thus proinflammatory and oxidative stress are involved in the development of comorbidities in psoriasis.

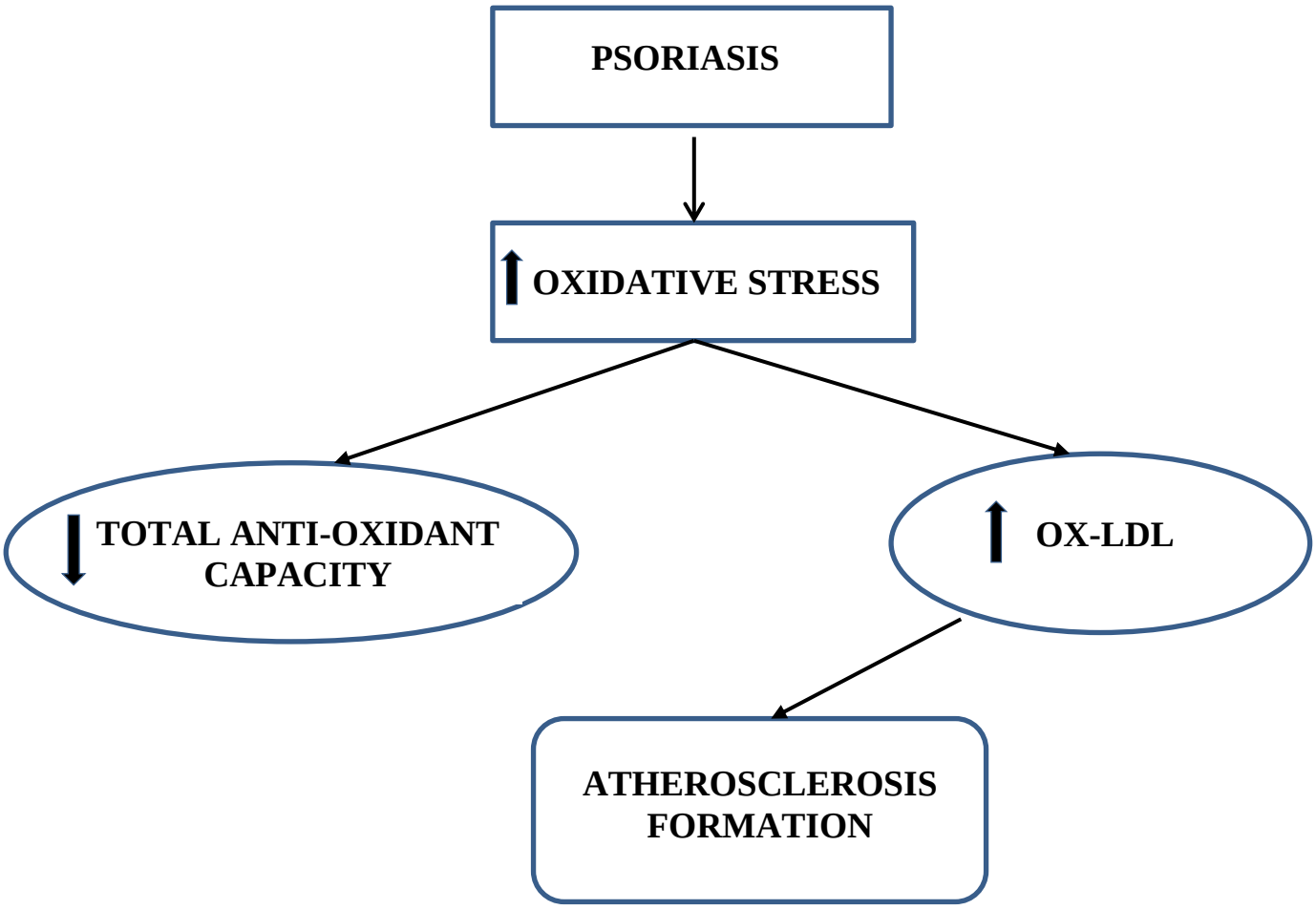


Figure 6.2 : Psoriasis & Oxidative Stress

6.4 Cardiometabolic Risk markers :

6.4.1 Lipid profile

Psoriasis is a chronic disease affecting more than 2% of the population. The exact etiology is not known. There are many factors like lipid and lipoprotein profiles, increase oxidative stress, decreased anti-oxidant capacity and other established risk factors such as hypertension, obesity and diabetes mellitus associated with psoriasis [42].

As we know, psoriasis is characterized by sharply demarcated, red and slightly raised lesions with silver-whitish scales. The loss of these scales from the surface is mainly due to lipid disorders in epidermis and in serum[43].

In the present study the mean values of serum triglycerides in cases and controls was 212.3 ± 39.5 and 122.9 ± 14.6 respectively, serum total cholesterol was 241.0 ± 60.4 and 155.0 ± 43.2 respectively, serum low density lipoprotein cholesterol was 196.8 ± 43.1 and 132.0 ± 10.0 respectively, serum high density lipoprotein cholesterol was 34.9 ± 6.8 and 43.5 ± 9.8 respectively shown in **Table 5.9**. These lipid parameters were statistically significant ($p<0.05$) in cases compared to controls. Study by Vanizor Kural *et al.*, on 35 psoriatic patients have shown that total cholesterol, triglycerides, LDL-cholesterol levels in patients with psoriasis are significantly higher than those of healthy subjects [44] which was in accordance to our study. Piskin in his study on 100 psoriasis cases showed serum to total cholesterol and LDL-C levels to be significantly higher than that of control group [45]. Rocha-Preira reported rise in total cholesterol, triglycerides, LDL-cholesterol, VLDL and a reduction in HDL in psoriatic patients. But the difference was not statistically significant in their study [46]. Dreiher in his study on 10,669 psoriasis patients and 22,996 subjects without psoriasis observed that triglyceride levels were higher in psoriasis patients and high-density lipoprotein cholesterol levels were lower [47]. It is

reported that macrophages get activated by engulfing low density lipoprotein (LDL),and release large quantities of tumor necrosis factor (TNF) -alpha and IL-1β. This cytokine driven inflammation and tissue destruction is a common theme for chronic inflammatory diseases such as psoriasis and atherosclerosis [48]. Genetic studies have demonstrated that psoriasis and cardiovascular disease both share common pathogenic features, for like inflammatory cytokines like TNF-α and IL-1which play an important role in the pathogenesis [49].

In the present study the lipid profile was affected in cases compared to controls in **Figures 5.12, 5.13, 5.14, 5.15**. These lipid parameters were compared with mild, moderate and severe PASI score shown in figures 6.1, 6.2, 6.3, 6.4. Study done by Chetana Shenoy *et al.*, observed that serum total cholesterol and LDL-cholesterol were found to be significantly higher in the severe psoriasis group than in the mild to moderate group, however no statistically significant differences were found in HDL-cholesterol and triglyceride levels between the two groups [50] which was partially in accordance to our study. Study done by Rocha-Pereira *et al* ., found significant differences between total cholesterol, TG, HDL-C and LDL-C in severe psoriasis and mild psoriasis [51]. Literature mentions that psoriasis could be associated with lipid abnormalities at the beginning of the disease and these lipid abnormalities may be due to genetic pre-disposition [52]. However, psoriasis is associated with multiple acquired precipitation factors, lipid abnormalities can also be influenced by multiple acquired factors.

Study done by Melek Aslan Kayıran *et al.,* found no statistically significant difference in total cholesterol, triglyceride, and HDL, LDL, and VLDL cholesterol levels between the patient and control groups [53]. McDonald *et al.,* have shown that patients with psoriasis are predisposed to atherosclerotic cardiovascular diseases and thromboembolic events [45]. This may be due to

abnormal lipid metabolism, and its possible relationship with oxidative stress in the development of this chronic disease [54].

6.4.2 Lp(a) levels in study groups

As mentioned earlier, macrophages are activated by engulfing low density lipoprotein(LDL) and releasing large quantities of TNF-α, and IL-1β [55]. Numerous studies have demonstrated that lipoprotein (a) [Lp(a)], a genetically determined lipoprotein, is most powerful and most prevalent independent risk factor for coronary heart disease. Lp (a) is a heterogeneous lipoprotein that incorporates a low-density lipoprotein (LDL) particle and highly polymorphic apolipoprotein(a) [Apo(a)] [56].

In the present study the mean Lp(a) values in cases and controls was 41.3 ± 8.7 and 25.1 ± 7.9 respectively as shown in table 5.8. It shows that Lp(a) levels were significantly increased in cases compared to controls in **Table 5.9**. Study done by Latha KP *et al.,* [57] also found similar findings in their study. In a study by Uyanik *et al.,* Lp (a) level were significantly higher in patients with psoriasis than in controls [58] . Lp (a) may be one among the many factors contributing to an increased cardiovascular risk in patients with psoriasis. A pathogenetic link exists between this lipoprotein and psoriatic pathophysiology [59]. Since lipids and Lp(a) are involved in the pathogenesis of immuno-inflammatory and oxidative stress process in psoriasis, the present study has been taken the possible of usefulness of these parameters as markers of risk factor for development of cardiovascular disease in psoriasis.

Stinson *et al.,* reported that serum cholesterol and Lp(a) levels in patients with psoriasis are not different from the control group [60]. This was not in accordance to our study. Seçkin *et al.,* [61] investigated serum lipids and lipoproteins including Lp(a) in 32 male patients with psoriasis

and reported that serum Lp(a) levels are higher in patients with psoriasis than in the control group, but there was no statistically significant intergroup difference in. This was partially in accordance to our study. According to the authors statistically higher levels of Lp(a) contributed to the development of atherosclerosis in the psoriasis group relative to the control group. Thus measurement of Lp(a) in patients with psoriasis who are prone to cardiovascular diseases may be an appropriate diagnostic tool in the evaluation of atherosclerotic and other vaso-occlusive pathologies [62].

In the present study these elevated Lp(a) levels were compared with mild, moderate and severe PASI score. The mean values in mild, moderate and severe PASI score were 42.4±8.4, 38.4±8.9, 41.9±8.6 respectively and was interpreted using box plot graph **Figure5.16**. However when Lp(a) levels were compared among the groups using ANOVA the 'p' value was statistically significant with 'p' value 0.001.

6.4.3 Uric acid in study subjects

Serum uric acid (SUA) mediates inflammatory pathways via the secretion of proinflammatory chemokines [63]. Kwon *et al.,* suggested that increased keratinocyte cell production induces an increase in purine metabolism, which elevates SUA levels in patients with psoriasis [64]. In the present study the mean uric acid levels in cases and controls were 17.1 ± 7.2 and 4.5 ± 1.0 respectively **Table 5.9**. Gisondi *et al.,* got similar findings and identified psoriasis as the most important risk factor for hyperuricemia compared with other known risk factors such as obesity or metabolic syndrome [65]. In another study, Li *et al.,* and Chen *et al.,* examined 136 unique associations between SUA level, SUA-lowering treatment and health outcomes. The studies suggested that few samples had high uric acid levels and were associated with heart

failure, hypertension, impaired fasting glucose or diabetes, chronic kidney disease, and coronary heart disease [66,]. Cassano *et al.,* indicated a trend toward a correlation between SUA level and cardiovascular risk profile in psoriasis patients. They concluded from their study that serum uric acid can be a new marker for atherosclerosis and other cardiovascular events in psoriasis patients [67].

The elevated serum uric acid levels were further correlated with mild, moderate and severe PASI score. The mean values of uric acid were 16.3±9.2, 16.7±6.2, 17.4±7.2 respectively and were interpreted using box plot graph **Figure 5.17**. It shows that the uric acid levels were proportionately increased from mild to severe and was statistically significant when compared to controls. Study done by Maryam Ghiasi Amir Houshang *et al.,* suggested that mean serum uric acid levels were in normal range but value significantly higher in patient with more severe form of psoriasis and uric acid level exacerbate the severity and duration of psoriasis[68] which was similar to findings of our study. However study done by Ashishkumar M *et al.,* although found more uric acid levels in cases compared to controls but did not get significant correlation with mild, moderate and severe PASI score [69]. Similar study by Brenner W *et al.,* in their study couldn't get correlation with uric acid levels and PASI score, hence they suggested that there is no relationship between the frequency of hyperuricemia and the extent of psoriatic skin involvement, indicating that the increased epidermal turn over may not play a role in psoriatic hyperuricemia [70]. They also mentioned that the most reasonable explanation for elevated uric acid in psoriasis seems to be a combination of genetic predisposition and hyperalimentation.

6.4.4 hs-CRP

The ongoing inflammatory process in psoriasis affects the arterial wall, promoting the atherosclerotic process and increasing the risk of cardiovascular disease. Inflammation plays a fundamental link between psoriasis and atherosclerosis. C-reactive protein (CRP) plays an important role in the defense mechanism against infection and is also considered a pivotal marker for acute inflammation, infection, and tissue injury [71]. Highly sensitive C-reactive protein is a brand of CRP and refers to the detection of small amounts in C reactive protein concentrations that occur below the 'normal' cut-off values. Hs-CRP is considered an independent risk marker of cardiovascular disease [72]. In the present study the mean hs-CRP levels in cases and controls was 16.2 ± 7.3 and 2.8 ± 1.3 respectively. It was statistically significant in cases compared to controls as shown in **Table 5.9**. A study was done by Yiu *et al.,* in China, and another one by Lucy Piper in 2009 has shown that patients with psoriasis have significantly high baseline levels of hs-CRP compared with healthy controls [73, 74]. Another two studies done by Agravatt et al 23 and Jagannath et al., also showed the same results [75]. Studies were conducted to define the inflammatory process of psoriasis by measuring multiple proinflammatory cytokines such as IL-1 and hepatic acute phase reactants as CRP. CRP testing and to be particular, hs-CRP, is especially important as it has been proved to be a risk predictor for many CVDs [76].

This hs-CRP was compared with mild, moderate and severe PASI score and interpreted using box plot graph figure 6.7. The mean values in cases with mild, moderate and severe PASI score were 16±6.6, 15.8±6.4, 16.3±7.7 respectively **Figure5.18**. Study done by Siham Taher Amedi *et al.,* observed significant association between disease activity (represented by PASI) and elevated hs-CRP levels [77]. Psoriatic patients with PASI more than 10 had significantly higher hs-

CRP levels than those with PASI less than 10, and the correlation was statistically positive. Several other studies have also reported a correlation between high hs-CRP and PASI [78]. Therefore hs-CRP can be considered as a helpful marker to detect disease severity, as well as to monitor the disease course and its treatment [79] . hs-CRP could be used as a strong and sensitive biomarker to evaluate psoriasis disease activity, as it is not based on visual assessment of the skin lesion. hs-CRP can be considered as a helpful marker to detect disease severity, as well as to monitor the disease course and its treatment [80]. Recent study in Japanese plaque type psoriasis found significant higher hs-CRP level over control group, but no significant correlation with PASI score, similar to the study of Sergeant *et al.*, which included all types of psoriasis including psoriasis arthritis with various kinds of treatment [81]. The possible explanation for correlation between hs-CRP level and psoriasis severity is, keratinocytes in psoriasis lesion secreted interleukin-1 and tumor necrosis factor-α, which can stimulate hepatocytes to produce hs-CRP into the circulation, so mild psoriasis will have lower level of this marker than severe disease [82].

6.5 Correlation of inflammatory markers (TNF-α, IFN-γ, IL- 2) with cardiometabolic markers and oxidative burden in cases (psoriasis).

In the present study, there was positive correlation between triglycrides and TNF-α with r value 0.184, and statistically significant with ‘ p’ value 0.045. The serum hs-CRP also showed negative correlation IFN-γ with r value -0.228 and was statistically significant with ‘p’ value 0.017 as shown in **Table 5.11**. Patients with psoriasis are at a high risk for the incidence of cardiovascular disease and myocardial infarction [83]. The risk factors for development cardiovascular diseases in patients with psoriasis compared with the general population have a

higher incidence which includes diabetes, hypertension, obesity, and hyperlipidemia [84]. Psoriasis is highly correlated with cardiovascular diseases in particular with hyperlipidemia-induced atherosclerosis is the predominant one.

Pathogenetic mechanisms of CVD in psoriasis patients is not clear understood due to complex nature and still remains unclear. The development of atherosclerosis and its increased prevalence may be partially explained by the presence of atherosclerotic risk factors - hypertension, obesity, and hyperlipidemia as well as by the chronic inflammatory processes that are commonly observed in psoriasis [85].

Thus from the present study it suggests that psoriasis patients are associated with increased production of Th1 cytokines (TNF-α, IFN-γ IL-2). Along with disturbed cytokines there was increased oxidative burden and increased cardiometabolic risk markers(lipid profile, Lp(a), hs-CRP, uric acid). These results mention us that patients with psoriasis are at increased risk of developing severe vascular events (including myocardial infarction and stroke) in future. The presence of common inflammatory pathways may provide an explanation for the association between psoriasis and cardiovascular comorbidities. It helps physicians should be more aware of the cardiovascular risk when assessing patients with psoriasis. In particular, adequate treatment of psoriasis may not only ameliorate the skin condition, but also decrease the risk and severity of cardiovascular and metabolic disorders.

Further investigations are required to clarify the mechanisms underlying the association between psoriasis and cardiovascular comorbidities, and define optimal treatment regimens to reduce the risk of cardiovascular events in patients with psoriasis.

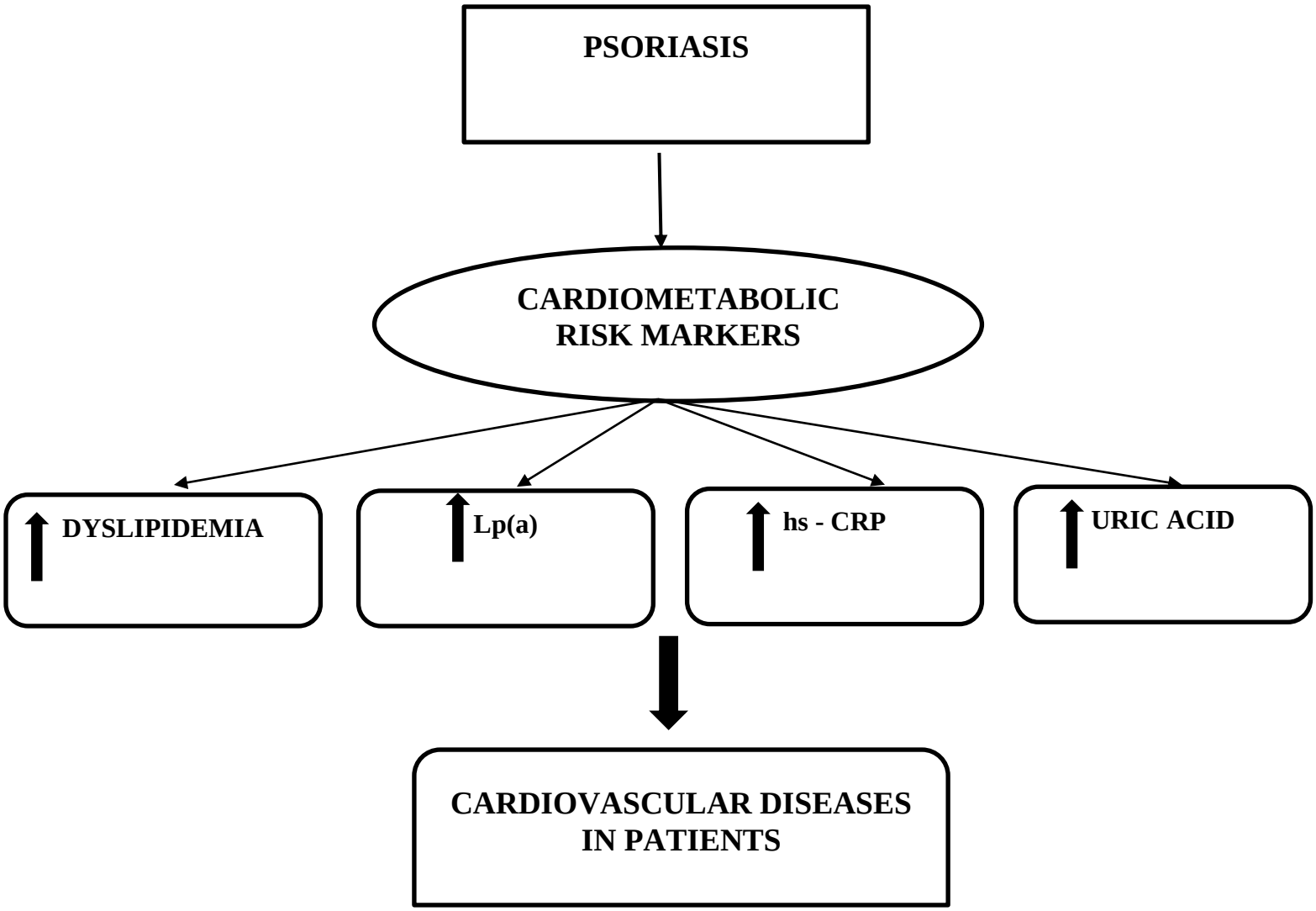


Figure 6.3 Psoriasis and Cardiometabolic Risk Markers

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CHAPTER 7

SUMMARY and

CONCLUSION

A study was conducted to investigate the relationship between psoriasis and cardiometabolic risk factors. The study included 220 participants, with 110 individuals in both the cases and controls group. The cases had psoriasis, while the controls did not. The study found that patients with psoriasis had increased levels of serum inflammatory markers, total oxidative stress, and cardiometabolic risk markers compared to the control group. Additionally, the cases had decreased levels of total antioxidant capacity and HDL-cholesterol compared to the controls.

The severity of psoriasis, as measured by the PASI score, also played a role in determining the levels of certain markers. Specifically, serum TNF-α and uric acid levels increased proportionately with the severity of the disease, from mild to moderate and moderate to severe PASI scores.

The study also found a positive correlation between serum triglyceride levels and TNF-α, as well as a negative correlation between hs-CRP and IFN-γ. These correlations suggest potential links between the inflammatory response and lipid metabolism in psoriasis patients.

The results of this study indicate that psoriasis is associated with a systemic inflammatory state and cardiometabolic alterations, which may contribute to an increased risk of developing cardiovascular disease (CVD) later in life for these patients.

Overall, the study provides valuable insights into the interplay between skin disease, inflammation, and systemic health in psoriasis patients. The findings underscore the importance of monitoring cardiometabolic risk factors and inflammatory markers in these

individuals to help identify those at higher risk for CVD and to implement appropriate preventive measures and management strategies. Further research in this area could potentially lead to better management and improved outcomes for patients with psoriasis and related comorbidities.

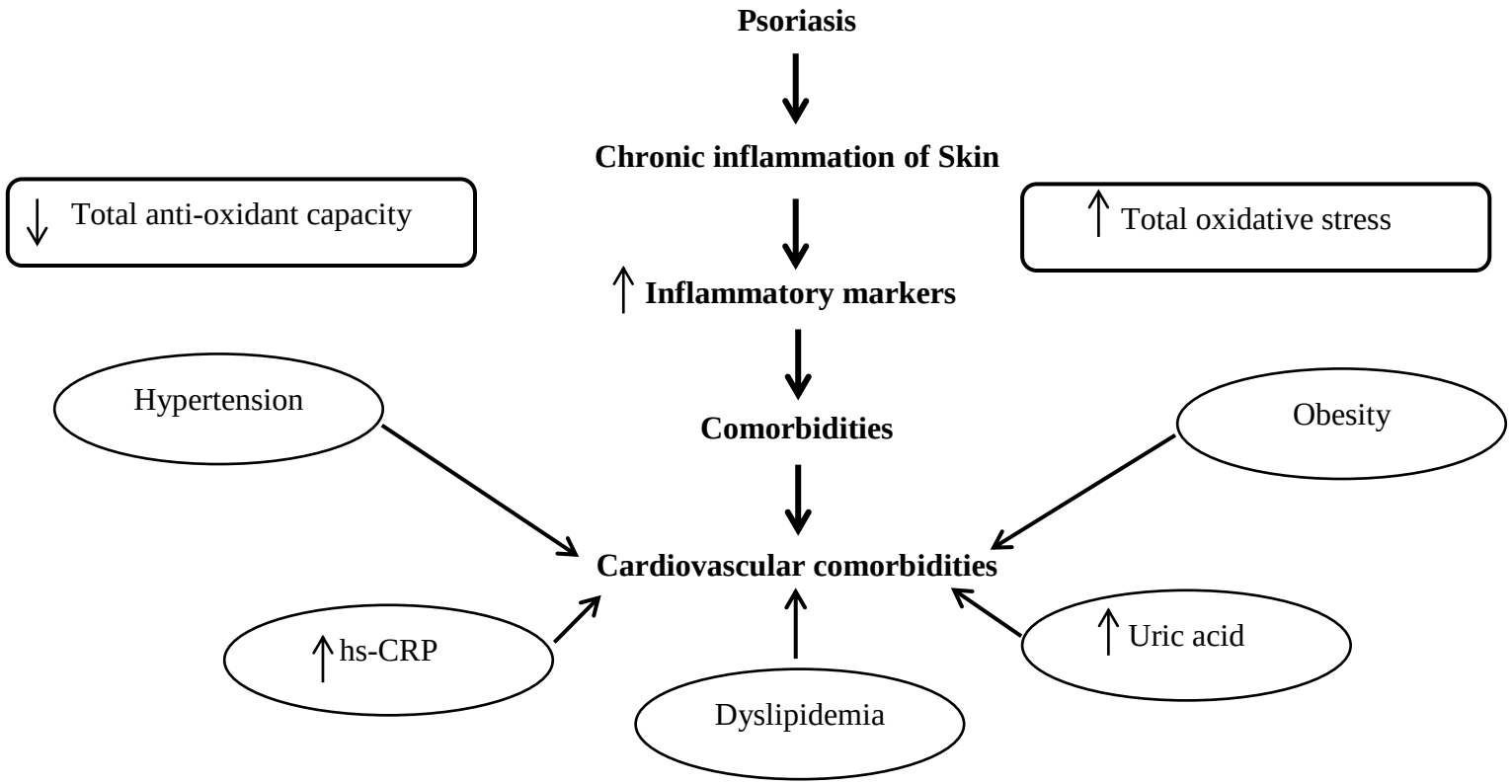

Figure 7.1 Graphical representation of Inflammation in Psoriasis leading to Cardiovascular co

LIMITATIONS &

FUTURE PERSPECTIVE

- Inclusion of other main cytokines Th17 and Th22.
- Measurement of ox-LDL
- Assessment of Atherosclerosis tool by Carotid Intima Media Thickness.
- Assessment of quality of life by Quality of Life Index Score

Future research on psoriasis and cardiovascular disease should focus on the following:

- ✓ Conducting longitudinal studies to establish causality between psoriasis, inflammatory markers, and cardiovascular diseases.
- ✓ Understanding the underlying pathways linking psoriasis, inflammation, and dyslipidemia.
- ✓ Exploring the efficacy of interventions, lifestyle modifications, and biologic therapies in reducing cardiovascular risk in psoriasis patients.
- ✓ Taking a multi-disciplinary approach involving dermatologists and cardiologists to optimize patient outcomes.
- ✓ Validating uric acid as a reliable biomarker for disease severity and developing composite biomarker panels to aid risk stratification.
- ✓ Conducting population-based studies to identify high-risk subgroups and inform public health strategies.
- ✓ Educating patients about the cardiovascular risks associated with psoriasis and promoting self-management and healthier lifestyles.
- ✓ These efforts will lead to improved patient care, risk management, and potentially novel treatments.

CHAPTER 8

ANNEXURES

ANNEXURE II

INSTITUTIONAL ETHICAL CLEARANCE

BLDE (DEEMED TO BE UNIVERSITY)
[Declared as Deemed-to-be-University u/s 3 of UGC Act, 1956 vide Government of India Notification No.F.9-37/2007-U.3(A)]
The Constituent College
SHRI. B. M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

BLDE (DU)/IEC/334/2018-19 21-12-2018

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The ethical Committee of this University met on21st December 2018 at 11 a.m.to scrutinizes the Synopsis/ Research Projects of Post Graduate Student / Under Graduate Student Faculty members of this University / College from ethical clearance point of view. After scrutiny, the following original/ corrected and revised version Synopsys of the thesis/ research projects has been accorded ethical clearance.

Title. Study of Systemic inflammation, Oxidative stress and Cardiometabolic markers in Psoriasis.

Name of the Faculty member /PhD/PG/UG student. Dr. Neela B. Mannangi.

Name of the Guide; Dr. Basavaraj Devaranavadagi, Professor and HOD Dept of Biochemistry

Dr. Sharada Metgud
Chair person
IEC, BLDE (DU),
VIJAYAPURA

Dr.G.V.Kulkarni
Member Secretary
IEC, BLDE (DU),
VIJAYAPURA

MEMBER SECRETARY
Institutional Ethics Committee
BLDE (Deemed to be University)
Vijayapura-586103, Karnataka

Note:-Kindly send Quarterly progress report to the Member Secretary

Following documents were placed before ethical committee for Scrutinization.

- Copy of Synopsis/Research Projects
- Copy of inform consent form
  Any other relevant documents

Smt. Bangaramma Sajjan Campus, B. M. Patil Road (Sholapur Road), Vijayapura - 586103, Karnataka, India.
BLDE (DU): Phone: +918352-262770, Fax: +918352-263303 . Website: www.bldeuniversity.ac.in. E-mail:office@bldeuniversity.ac.in
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B.V.V. Sangha's
S. Nijalingappa Medical College & Hanagal Shri Kumareshwar Hospital & Research Centre
Navanagar, Bagalkot-587102, Karnataka State, India.
(Recognized by Medical Council of India and Affiliated to Rajiv Gandhi University of Health Sciences, Karnataka.)
SNMC-INSTITUTIONAL ETHICS COMMITTEE ON HUMAN SUBJECTS RESEARCH
☎**08354-235340 Fax: 08354-235360** **Website: www.snmcbgk.in**
Email: iechsrsnmcbgk@gmail.com

Office of the Institutional Ethics Committee

The Ethical Committee of SNMC reviewed the following documents:

1. Research Protocol entitled **Study of systemic inflammation, oxidative stress and cardiometabolic markers in Psoriasis.**
2. Information sheet for participants of the study (Consent Form –I) and (Consent Form –II) of) **Study of systemic inflammation, oxidative stress and cardiometabolic markers in Psoriasis.**
3. **NOTE:** It is to be noted that neither PI nor any of the proposed study team members were present during the decision-making procedures of the Ethics Committee, and members who are independent of the Investigator, have voted/ provided opinion on the trial.

Discussion points:
After reviewing the documents submitted by the Principal Investigator, the Committee has decided to grant approval for conducting the above mentioned study.

You are requested to report to the Ethics Committee the Following:
1. Progress of the study at the end of 4 months.
2. Provide a report to the Ethics Committee on completion of the study.

The Ethics Committee of SNMC follows procedures that are in compliance with the requirements of ICH (International Conference on Harmonization) related to GCP (Good Clinical Practice), schedule Y and all other applicable Indian regulations.

If you have any Questions concerning the above, please feel free to contact the undersigned.
Thanks & Regards,

(Dr. Vijayamahantesh SN)
Member Secretary p 2/2

Member Secretary,
IEC
S.N. Medical College
BAGALKOT

ANNEXURE III

PRESENTATIONS

Silver Jubilee Year

ASSOCIATION OF MEDICAL BIOCHEMISTS OF INDIA

AMBICON 2017

25th Annual Conference of Association of Medical Biochemists of India

"Unfolding New Facets of Medical Biochemistry:The Bridging of Academia and Clinics"

17th - 19th November 2017 @ Lalitha Mahal Palace Hotel, Mysuru

CERTIFICATE

This is to Certify that

Dr. Neela Mannangi bearing MCI/State Reg.No. 65901.

Address SNMC & HSK, Bagalkot has participated as

Delegate/~~Speaker~~/Chairperson and has presented an **Oral** presentation in the **"25th Annual Conference of Association of Medical Biochemists of India"** held from 17th to 19th November 2017. The Karnataka Medical Council has granted 5 Credit hours for delegates with Vide letter No.K.M.C./C.M.E./547/2017 dated 28-10-2017.

Dr. V. Govindaraju
Organizing Chairman

Chairman/Director
K.M.C CME Accreditation Committee

Dr. Pragna B Dolia
President, AMBI

Dr. Shanthi K Naidu
Hon. Secretary, AMBI



B L D E (Deemed to be University)

SHRI B. M. PATIL MEDICAL COLLEGE,HOSPITAL AND RESEARCH CENTRE, VIJAYAPUR

International Hypoxia Symposia 2019

(Celebration of Nobel Prize for Physiology or Medicine 2019 to Hypoxia Biologists)

Theme: Oxygen Cell Signalling: Mechanisms to therapeutics

Organized by: Laboratory of Vascular Physiology and Medicine, Department of Physiology

Certificate

This is to certify that Dr. Neela B. Mannangi

has participated as **Delegate/Organizer** in Symposia conducted on November 23rd 2019, at BLDE(DU) Shri. B. M. Patil Medical College, Hospital & Research Centre, Vijayapura, Karnataka, India.

Dr. Aravind Patil Dean Faculty of Medicine & Principal	Dr. Sumangala Patil Organizing Chairperson	Dr. Lata M Mullur Convener

IABSCON 2020

9th ANNUAL INTERNATIONAL CONFERENCE of INDIAN ACADEMY OF BIOMEDICAL SCIENCES

27th, 28th, 29th February 2020

Organized By

DEPARTMENT OF BIOCHEMISTRY

D. Y. PATIL MEDICAL COLLEGE, KOLHAPUR (MAHARASHTRA, INDIA)

A Constituent College of

D.Y. PATIL EDUCATION SOCIETY

Deemed To Be University, Kolhapur-Reaccredited by NAAC with 'A' Grade

CERTIFICATE

Type of Conference-Multi speciality

(CPD Code–MMC/MAC/2020/F–014720)

This is to certify that

Dr/Mr/Ms Neela Mannangi

has Participated as Delegate / Delivered an Oral / ~~Poster~~ presentation in the 9th Annual International Conference of Indian Academy of Biomedical Sciences.

He / She is granted 4 CPD credit points by Maharashtra Medical Council.

Prof. (MS) Syed Mudassar	Prof. Abbas Ali Mehadi	Prof. Bipin M. Tiwale	Dr.R.K.Sharma	Dr.Sandeep S. Kadam	Dr.Shailesh Kore
President, IABS	Secretary, IABS	Organizing Secretary	Organizing Chairman	MMC Observer (MMC/MAO-01898/2018)	MMC Observer (MMC/MAO-00603/2015)

WOCKHARDT | LIFE WINS

Certificate of Participation

This is to certify that

Dr. Neela B Mannangi

has participated as Delegate/Faculty/Resource Person in the Training Programme on

"Advance GCP Training & Recent Updates on Regulatory Requirements"

Organized by Dept. of Pharmacology, BLDE(DU) Shri.B.M.Patil Medical College Hospital & Research Centre, Vijayapur, in collaboration with Research & Development Unit , IQAC of BLDE(Deemed to be University), Vijayapur and Medical Affairs Department, Wockhardt Ltd. on 30th August 2019.

PRINCIPAL
BLDE (Deemed to be University)
Shri B. M. Patil Medical College
Hospital & Research Centre
Vijayapur-586103

Dr. Arvind V. Patil
Principal, Dean Faculty of Medicine
BLDE(DU) Shri.B.M.Patil Medical College Hospital & Research Centre, Vijayapur

Dr. Akram A Naikawadi
Prof. & HOD, Pharmacology
Dean,(R&D)BLDE(DU)

Dr. Rishi Jain
VP. Medical Affairs. Wockhardt Ltd

ACBICON
December 12-15, 2021

47th National Conference of Association of Clinicial Biochemists of India

12th -15th December 2021 | Hybrid Mode

Theme: Towards a Better Tomorrow

Certificate of Participation

This is to certify that

Prof./Dr. Neela B Mannangi

has presented the Scientific poster titled

Grading of Psoriasis by Inflammatory markers

during "ACBICON 2021" held virtually on 12th - 15th December 2021

Prof LM Srivastava	*Prof Rajiv R Sinha*	*Prof Subir Kr Das*
President, ACBI	*General Secretary, ACBI*	*Organizing Secretary*

ANNEXURE IV

PUBLICATIONS

Research article

A study of inflammatory markers and their correlation with PASI score in psoriasis – A case control study

Neela B. Mannangi[1], Basavaraj Devaranavadagi[2], Shubha Jayaram[3], Balachandra S. Ankad[4], Shankarprasad D.S.[1]

[1]Department of Biochemistry, [4]Department of Dermatology, SNMC & HSK, RC, Bagalkot, Karnataka, India
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(Received: December 2021 Revised: March 2022 Accepted: April 2022)

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ABSTRACT

Introduction and Aim: Psoriasis is one of the salient dermatological disorder and a chronic recurrent cutaneous disease. Research shows that immunological cells and cytokines play a principal causative role for skin lesions and comorbid systemic effects in these patients. Like other autoimmune diseases, psoriasis is also a multifactorial disorder which is triggered either by injury, trauma, infections, medications, and psychological stress. Among the cytokines, hs-CRP acts as both inflammatory and cardiovascular marker. It is markedly increased in psoriasis patients, there by leading to subsequent co-morbidities in these patients. The aim of present study was to estimate inflammatory markers viz-TNF-α, IL-2, IFN-γ and hs-CRP in psoriasis patients and controls and correlate these inflammatory markers with PASI score in psoriasis patients. Further to correlate hs-CRP with TNF-α, IL-2, IFN-γ in these patients.

Materials and Methods: The study is conducted at a tertiary level hospital and is designed as a Case Control study design conducted from January 2019 to January 2021. 110 subjects having diagnosed as psoriasis were included as cases and 110 controls. Inflammatory markers were estimated by ELISA method. Statistical analysis was done.

Results: The study showed that inflammatory markers in cases with psoriasis were significantly elevated when compared with controls. The inflammatory markers were proportionately altered from mild to severe in psoriasis patients but were not statistically significant. The hs-CRP showed negative significant correlation with IFN –γ.

Conclusion: The present study concludes that the inflammatory markers are significantly increased in psoriasis patients, and this is correlated with PASI score. These simple biomarkers are of utmost importance in the clinical scenario for better treatment and prognosis and thereby reducing comorbidities in these patients.

Keywords: TNF α; IFN –γ; IL-2; hs-CRP; psoriasis.

INTRODUCTION

Psoriasis is the commonest and chronic recurrent cutaneous disorder, which is distinguished by the excessive proliferation and extremely increased rate of epidermal turnover along with infiltration of activated mononuclear cells in the underlying dermis (1). The prevalence rate of psoriasis is 2–3% which signifies the epidemiological burden. The disease is predominant in the polar regions, however, tropical/ subtropical countries like India, also show significant burden of Psoriasis. India being a diverse country, there is a regional variation in the prevalence of Psoriasis with variable environmental and genetic factors (2). Based on previous studies it is inferred that in India there is a natural regional variation in the prevalence of psoriasis from 0.44 to 2.8%, and males are affected twice more than females. At the time of presentation, most of the patients are in their thirties or forties (3).

Psoriasis exhibits altered immune response with cutaneous involvement which induces a chronic inflammation of the skin. The immunological cells and their cytokines play an important causative role for skin lesions and comorbid systemic effects (4). The cutaneous tissue suffers a major influence on the disease outcome. In this cutaneous disease T cells and cytokines play an important role for the pathogenesis of the disease. Several studies have confirmed that immune system is impaired in psoriasis. Some studies have also mentioned that psoriasis is an immune mediated disorder with abnormal proliferation of keratinocytes which is further aggravated and mediated by T-lymphocytes. Psoriasis is being associated with an over expression of proinflammatory cytokines released by T helper1(Th1) cells and relative presence of pro-inflammatory cells. Their cytokines create a damaging environment leading to the development and further aggravation of psoriatic lesions (5).

In psoriasis the pattern is significantly complex. Th -1 cells are known to produce tumor necrosis factor-alpha (TNF-α), interleukin 2 (IL-2) and interferon-gamma (IFN-γ) under the effect of IL-12. In the same way, IL-1β and IL-6 are responsible for Th17 cell differentiation, which secretes IL-6, IL-17, IL-21 and IL-22. The recruitment and activation of Th1 and Th17 lymphocytes, thus drive the pathogenesis of psoriasis. On the other hand, neutrophils, antigen presentation cells (APCs), macrophages and keratinocytes contribute for the synthesis and secretion of cytokines (6)

TNF-α is a cytokine secreted by T lymphocyte, keratinocytes, and dermal macrophages, CD11+ dendritic cells and mastocytes. TNF-α effects the synthesis of IL-6 and ICAM-1 expression, which in turn leads to hepatic stimulation with increased production of acute phase reactants mainly C-reactive protein (CRP) and fibrinogen (7). In Psoriasis one of the principal events in the pathogenesis of inflammatory outbreak is the secretion of IFN-γ from plasmacytoid dendritic cells (DCs) .The Th-17-derived cytokines include IL-17A, IL-17F, IL-6, TNF-α, and IL-22. The hallmark of IL-22 activity is abnormal differentiation of keratinocytes along with increased hyperplasia resulting in plaque formation (8).

Psoriasis is shown to be characterized by increased levels of hs-CRP with increase in subclinical atherosclerosis (9). C-reactive protein is produced from hepatocyte within hours after being stimulated from inflammation, infection, tissue damage, and decreases rapidly when the stimulating factor has been eliminated or responded to treatment. This marker is over expressed in psoriasis (10).

Like other autoimmune diseases, psoriasis is also a multifactorial disorder which is triggered either by injury, trauma, infections, medications, and psychological stress (3). The etio-pathogenesis of psoriasis is less well understood. Nevertheless it has been shown that genetic and epigenetic factors play a strong role in this disease. In this cutaneous disease T-cells and cytokines overtly contribute to the pathogenesis of the disease (1).

The diagnosis of psoriasis done by the dermatologists is made by the clinical findings and Psoriasis Area and Severity Index (PASI) Score which is a tool for assessing the disease severity in these patients. This PASI score is assessed by the affected area and lesional characteristics. In these patients, the PASI score is directly proportional to the severity of the disease. A score of more than 12 is considered severe whereas a score of $\leq$12 is considered mild to moderate (11). It is very important to monitor and control inflammation to control the evolution of the disease and its comorbidities. It has been proved that IL-6 induces Type-2 DM (Diabetes mellitus) and significant cardiovascular adverse effects and that TNF-α and hs-CRP could be involved in causing atherosclerosis (12).

Among the cytokines, hs-CRP acts as both inflammatory and cardiovascular marker. It is markedly increased in psoriasis patients, there by leading to co-morbidities in these patients. Several studies have shown the importance of cytokines in the clinico-pathogenesis of dermatological disorders. With this complexity role of cytokines, the present study is focused on estimation of these inflammatory markers in psoriasis patients compared to controls. Further hs-CRP is correlated with other inflammatory markers to assess cardiovascular comorbidities in future. There is paucity of literature concerning inflammatory markers profile and their correlation with PASI score in South Indian population. Hence the present study is undertaken.

MATERIALS AND METHODS

It is a case control study conducted in a tertiary care hospital attached to a medical college from January 2019 to August 2020. Total sample size was 220 in which 110 were cases and 110 controls. Sample size was determined according to a study done by Sandhya *et al.,* (13) using Open Epi software Version 2.3.1 with confidence level: 95% and power of the study: 80%. Calculation results were 100 in each group.

Inclusion criteria

The newly diagnosed patients with Psoriasis were included for the study. The severity of the disease was assessed and graded by the PASI score with Mild <7, Moderate 7-12, Severe >12 (11).

Exclusion criteria

Patients with any chronic inflammatory disease, diabetes mellitus, renal disorders, IHD (Ischemic Heart Disease), hypothyroidism, hyperthyroidism, nephritic syndrome, obstructive liver disease, and other skin disorders were excluded from the study. All the patients receiving systemic drug therapy like beta blockers, thiazides, retinoids, cyclosporine and lipid lowering agents in the recent 6 months were excluded from the study.

Approval was obtained from the institutional ethics committee. After taking informed consent, detailed history and clinical examination was done.

Under aseptic precautions around 5ml of blood was drawn in plain vacutainers and subjected to centrifugation at 3000 rpm for 20 minutes to separate the serum. Separated serum was used for analysing different biochemical parameters such as TNF-α, IL-2, IFN- γ and hs-CRP by ELISA method as per kit instructions supplied by Diaclone Technologies Laboratory.

Statistical analysis was done using SPSS software. The number and percentage were used for categorical data and significant difference between two categorical variables was tested by the Chi-square (χ^2) test. Unpaired 't' test was used to compare between two independent variables as a test for difference of mean. The ANOVA and 'F' test were used if there were more than two independent groups for testing of equality of variance.

RESULTS

The present study had 110 cases (psoriasis) and 110 controls. Table 1 shows distribution of age in cases according to PASI score and controls. The gender distribution with respect to PASI score in cases and controls is depicted in Fig. 1. Males were more than females both in cases and controls. The Mean ± SD for inflammatory markers both in cases and controls are shown in Table 2. The Inflammatory markers were significantly increased in cases compared to controls.

Table 3 shows the distribution of inflammatory markers according to PASI score in cases and controls. There was no significant correlation between PASI score and inflammatory markers, however there was an increase trend of inflammatory markers in PASI score is seen.

Table 1: Distribution of age according to PASI score in cases and controls

Parameters (Mean± SD)	PASI score (Cases)			Control	'F' value	'p' value
	Mild	Moderate	Severe			
Age (yrs)	38.8±8.5	39.9±12.7	41.4±11.5	39.4±11.1	0.571	0.635

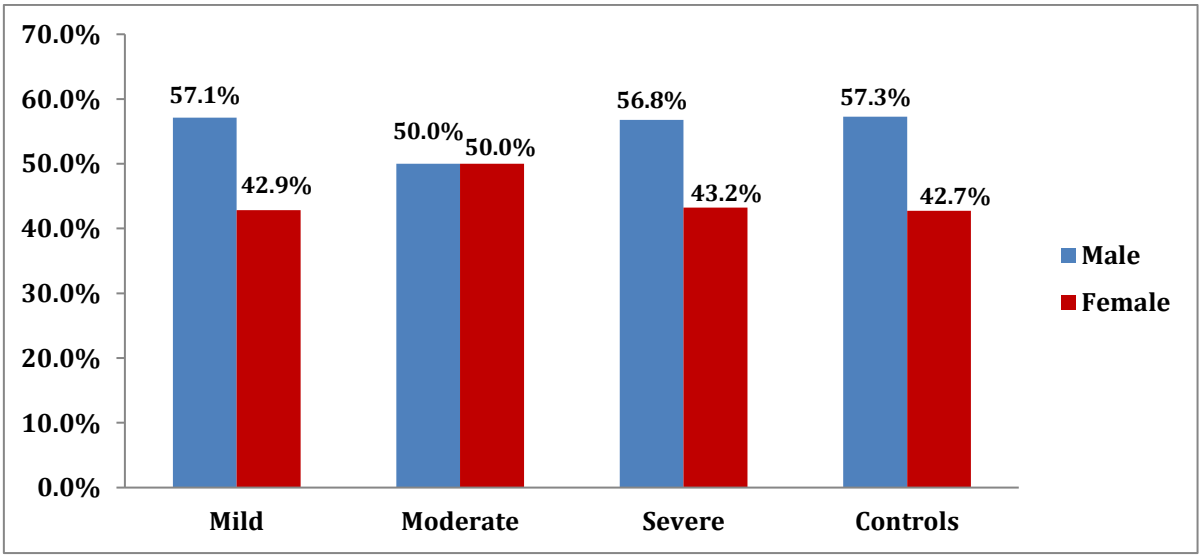

Fig. 1: Gender distribution with respect to PASI score in cases and controls

Table 2: Inflammatory markers in cases and controls (Mean± SD)

Inflammatory markers	Cases Mean± S.D	Controls Mean ± S.D	't'	'p'
TNF(pg/ml)	30.8 ± 25.3	3.9 ± 2.2	11.098	<0.001*
IFN-γ(pg/ml)	31.1 ± 21.7	8.0 ± 3.0	11.065	<0.001*
IL- 2(pg/ml)	25.7 ± 10.3	11.7 ± 4.1	13.144	<0.001*
hs-CRP	16.2 ± 7.3	2.8 ± 1.3	18.952	<0.001*

p value '*' significant at 5% level of significance (p<0.05)

Table 3: Distribution of inflammatory markers in PASI score and controls

Inflammatory Markers (Mean ± SD)	**Level of PASI Score**			**Control**	**F value**	**p value**
	Mild	**Moderate**	**Severe**			
TNF(pg/ml)	22.4±10.3	27.4±24.9	33.3±27.1	3.9±2.2	43.468	<0.001*
γ - Interferon	23.5±12.4	35.6±19.9	31.3±23.4	8.1±3.1	43.189	<0.001*
IL- 2(pg/ml)	19.1±6	22.7±8.8	27.8±10.8	11.7±4.1	68.755	<0.001*
hs-CRP	16±6.6	15.8±6.4	16.3±7.7	2.8±1.3	118.79	<0.001*

p value '*' significant at 5% level of significance (p<0.05)

Table 4: Correlation between hs-CRP and other inflammatory markers

Marker	**TNF-α**		**γ-IFN**		**IL-2**	
	'r'	'p'	'r'	'p'	'r'	'p'
hs-CRP	- 0.063	0.51	- 0.22	0.017*	0.051	0.60

p value '*' significant at 5% level of significance (p<0.05)

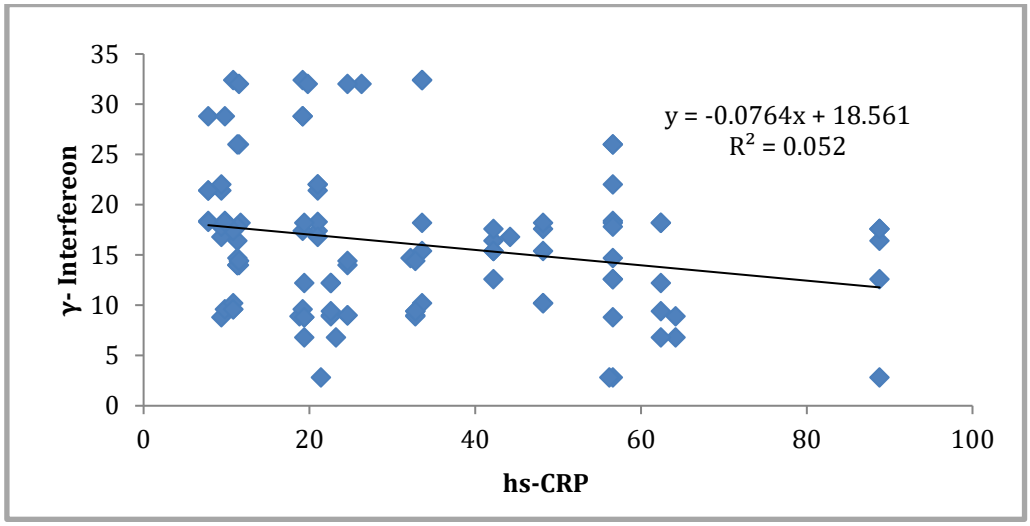

Fig. 2: Correlation between hs-CRP and γ-IFN

Hs-CRP being both inflammatory and cardiovascular marker was correlated with other inflammatory markers (TNF, γ – Interferon, IL- 2) as shown in Table 4. The hs-CRP showed negative correlation with TNF-α, γ-IFN and was statistically significant only with γ-IFN as shown in Fig.2 and positive correlation with IL-2 but was not statistically significant.

DISCUSSION

Psoriasis is one of the commonest and chronic recurrent cutaneous disease. In addition to cutaneous involvement it also involves nails and joints as in psoriatic arthritis (1). Based on previous studies it is inferred that in India there is a natural regional variation in the prevalence of Psoriasis from 0.44 to 2.8%, and males are affected twice more than females (3). It is manifested by the erythematous scaly plaques affecting all parts of the body, predominantly over elbows, knees, scalp, umbilical and perianal region. These lesions are the effects of inflammation, excessive proliferation and angiogenesis seen in psoriasis (3).

The inflammatory markers showed statistically significant increase in cases compared to controls. Cataldi *et al.*, also showed similar findings which is in accordance to our study (14). Macrophages and dendritic cells produce IL-12, which stimulates T effector cell differentiation into a pro-inflammatory Th1 response. Once activated, Th1 cells secrete cytokines which potentiates psoriasis pathophysiology.

Th1 stimulate release of cytokines such as IFN-γ, TNF-α and IL-2 (15). Th1 cells secrete and release IL-2, The major role of IL-2 is stimulation of the Th1 phenotype and thus producing IFN-γ, TNF-α and other pro-inflammatory cytokines, and activating Natural Killers (NK) cells (16). Some studies have shown no significant differences in the levels of IL-2 between psoriasis patients and healthy controls, however our study showed significant increase in IL-2 levels in psoriasis patients compared to controls. According to authors, the genetic differences in study populations could be the cause for discordant results (17). Studies have shown decreased serum IFN-γ levels in psoriasis patients which was not in accordance with the present study. According to authors IFN-γ signaling is multifaceted and complex, and the pathogenesis of which is not completely known. Recent studies have implicated a paradoxical role of IFN-γ in controlling of auto-inflammation in Psoriasis. (18). Therefore, it may behave as a pro-inflammatory molecule to regulate immune response.

The current study showed hs-CRP levels were significantly increased in cases compared to controls. However, there was no statistically significant severity with respect to PASI score in cases. hs-CRP being both inflammatory and cardiovascular marker it was correlated with other inflammatory markers. Current study showed significant negative correlation with IFN-γ. The possible explanation for correlation between hs-CRP level and psoriasis severity is, keratinocytes in psoriasis lesion secretes interleukin-1 and tumor necrosis factor-α, which influences hepatocytes to release hs-CRP into the circulation, so mild psoriasis will have lower level of this marker than severe disease (19). A study by Coimbra *et al.*, also showed a positive correlation between hs-CRP and IL-6 (21). Their study showed an increase in hs CRP, including insulin resistance and adiposity which are common in patients with psoriasis, whilst in certain studies there was no correlation observed (20). Following antipsoriatic therapy, there was a decrease in hs-CRP and IL-6 levels which can further decrease the systemic inflammation and thus decrease cardiovascular comorbidities in these patients. A Few studies have shown that after treating for 12-week phototherapy CRP levels in patients decreased significantly.

Some authors have shown low levels of inflammatory cytokines (IL-22, IL-17, IL-23, IL-8, TNF-α, and VEGF vascular endothelial growth factor) after 12-week phototherapy (21). Following treatment there was a decrease in the level of CRP which is evidence of decreased burden of systemic inflammation and atherosclerosis in these subjects. Cytokines are small, biologically highly active proteins that regulate the growth, function, and differentiation of cells which progresses the immune response and inflammation.

Keratinocytes secrete varied cytokines and chemokines which either induce or decrease the immune response. However, there is no clarity in their mechanism of action in the pathogenesis of Psoriasis. Under the influence of either local or systemic stimulus these keratinocytes produce more cytokines (22). In psoriasis, there is a cutaneous and systemic over expression of various inflammatory cytokines and these cytokines could impact each other. Once the cutaneous inflammation is stimulated by the antigen, the macrophages, keratinocytes, Th1 cells, T17 cells, Th22 cells and BDCA-1−inflammatory dendritic cells, which will produce TNF-α. This TNF-α plays an important role in the inflammatory process in psoriasis (23). It stimulates the movement of Langerhans cells by lowering the level of e-cadherin and is involved in the NF-Kβ-mediated inflammation pathway, which contributes to cell survival, proliferation, and transcription of antiapoptotic factors (24).

Th1 cells produce IFN-γ which stimulates the transduction of signal and activation of Transcription (STAT) thus regulating expression of genes in psoriatic cutaneous tissue.

In addition, the transcription of IFN-γ and TNF-α can be regulated by IL-2 and IL-12. IL-2 plays a role in the differentiation, proliferation, and maturation of T lymphocytes. There is also abnormal differentiation of keratinocytes along with increased hyperplasia resulting in plaque formation, which is the key feature of IL-22 activity (8). In addition, TNF- α, IL-1ß, IL-6, and INF-γ were known to increase the production of C3 from the liver and probably from adipose tissue in psoriasis patients.

Thus TNF-α and IFN-γ produce constellation of inflammatory cytokines like IL-6, IL-8, IL-12 and IL-18 and provide a major link in cytokine network in psoriasis (25). These data confirm the hypothesis that psoriasis with altered immunologic pathway leads to systemic disease burden in these subjects. This impaired pathway is worsened with progression of the disease. Further it aggravates in the development of comorbidities associated such as cardiovascular risk factors including altered lipid levels, impaired glucose tolerance in these patients. As a result, this leads to increased risk for cardiovascular diseases in psoriasis with respect to their severity.

CONCLUSION

The present study concludes that the inflammatory markers are increased in psoriasis patients. These cytokines are considered as prognostic markers in psoriatic patients and there by providing appropriate therapeutic strategies for monitoring these patients in routine medical practice. And also, hs-CRP can be used to assess the cardiovascular risk in these patients.

In fact, hs-CRP test is simple blood test and is inexpensive which can be routinely used.

The new ELISA methods are useful with their high specificity and good standardizability against the high costs. These markers can be used as surrogate indicators to assess cardiovascular risk in psoriasis, who are most susceptible to develop co-morbidities. Thus, these simple biomarkers have an important role in the clinics for better treatment and prognosis and thereby reducing comorbidities in these patients.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

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ORIGINAL ARTICLE

Study of Cardiometabolic Markers Along with Lipid Indices in Psoriasis

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Abstract

Background: Psoriasis is one of the most common chronic inflammatory skin diseases which affects 2-4% of the general population. The results of epidemiological studies have demonstrated that the risk to develop cardiovascular disease (CVD) is higher in patients with severe psoriasis. The pathogenesis of atherosclerosis followed by CVD is inflammation dependent in psoriasis. **Material and Methods:** It is a cross sectional observation study conducted in a tertiary care hospital from January 2019 to January 2021. 110 patients with psoriasis were included in cases and 110 controls. Anthropometric markers viz. blood pressure (BP), abdominal circumference and cardiometabolic markers lipid profile, hs-CRP, uric acid, Lp(a) were estimated in both groups. The data obtained was subjected to appropriate statistical analysis. **Results:** Anthropometric markers BP (136.1 ± 14.0/ 81.4 ± 8.3; 125.0 ± 8.9/76.2± 5.5) and abdominal circumference (85.4±15.3; 44.7± 11.9), BMI (40.9±8.3;22.3±3.2) were statistically high in cases than controls. The cardiometabolic markers as Triglycerides (TG) (212.3 ± 39.5; 122.9 ± 43.2), Total cholesterol (241.0 ± 60.4; 155.0 ± 43.2), High Density Lipoprotein-Cholesterol (HDL-C) (34.9 ± 6.8; 43.5 ± 9.8), Low Density Lipoprotein-Cholesterol (LDL-C) (196.8 ± 43.1; 132.0 ± 10.0), uric acid (17.1 ± 7.2; 4.5 ± 1.0), hs-CRP (16.2 ± 7.7; 2.8 ± 1.3), Lipoprotein (a) [Lp(a)](41.3 ± 8.7; 25.1 ± 7.9) and lipid indices AIP (0.79 ± 0.12, 0.46 ± 0.11), CI-I (7.20 ± 2.43; 3.70 ± 1.13), CI-II (5.85 ± 1.70; 3.21 ± 0.83), AC (239.97 ± 60.44; 152.25 ± 35.05), LTI (6654.34 ± 39207.47; 92.29 ± 38.92) were significantly high in cases compared to controls. **Conclusion:** The present study found significant increase in cardiometabolic markers in psoriasis patients compared to controls. So, this study emphasizes the importance of screening of cardiometabolic markers and other metabolic co-morbidities in psoriasis patients to help in early detection and treatment in order to reduce cardiovascular events.

Key Words

Psoriasis, Anthropometric Markers, Lipid Profile, Cardiometabolic Markers, Lipid Indices

Introduction

Psoriasis is one of the most common chronic inflammatory skin diseases which affects 2-4% of the general population. However, variations between and within countries and causes a significant social and pharmaco economic burden. [1]

In recent years, various population-based epidemiological studies have shown that patients with psoriasis have an increased risk for various cardiovascular comorbidities such as hypertension, hyperlipidaemia, obesity, metabolic syndrome and cardiovascular diseases. [2] The results of epidemiological studies have also demonstrated that the risk to develop CVD is higher in patients with severe psoriasis [3] and that this risk persists even after adjusting for conventional cardiovascular risk factors. [4] Psoriasis is known to be an independent risk factor for CV disease

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where as age, BMI, metabolic syndrome, and smoking status have been found to increase the risk of psoriasis. [5] The pathogenesis of atherosclerosis followed by CVD is inflammation dependent. [6] Central obesity, a component of metabolic syndrome (MS), is frequently encountered in patients with psoriasis. [7]

The PASI score is the most commonly used system to assess the severity. However, this system is subjective and difficult to interpret due to nonlinear scaling, time-consuming, and having high intra and inter-ratter variability and also other comorbidities are not evaluated. [8]

Previous studies have shown that mortality rates are increased in psoriasis patients and the life expectancy of patients with moderate to severe psoriasis is decreased by approximately 5 years, mainly due to cardiovascular comorbidities. [9]

Hence the present study is undertaken to assess cardiovascular risk in psoriasis patients. Therefore the objectives of the study were to assess anthropometric markers like BP, abdominal circumference and cardiometabolic markers like lipid profile, uric acid, hs-CRP, Lp(a) and atherogenic lipid indices in both cases and controls. Further to correlate these cardiometabolic markers with lipid indices in these patients.

Material & Methods

This is a cross-sectional observation study conducted in tertiary care hospital attached to a medical college from January 2019 to August 2020. Total sample size was 220 in which 110 cases and 110 controls. Sample size was calculated according to study done by Sandhya M *et.al.*, [10] using Open Epi software Version 2.3.1 with confidence level: 95% and power of the study: 80%. Calculation results were 100 in each group.

Inclusion Criteria: The newly diagnosed patients with clinical features of psoriasis like erythema, itching, thickening and scaling of the skin were included for the study. The clinical severity was determined according to the Psoriasis Area and Severity Index (PASI) score .[11]

Exclusion Criteria: Patients with any chronic inflammatory disease, diabetes mellitus, renal disorders, IHD, hypothyroidism, hyperthyroidism, nephritic syndrome, obstructive liver disease, and any other skin disorders were excluded from the study. All the patients receiving systemic drug therapy like beta blockers, thiazides, retinoids, cyclosporine and lipid lowering agents in the recent 6 months were excluded from the study.

Approval was obtained from the institutional ethical committee. After taking informed consent, detailed history and clinical examination was done and patients were classified as mild, moderate and severe according to PASI score. Mild <7, Moderate 7-12, Severe >12. [11]

Under aseptic precautions around 5mL of blood was drawn in plain tube and EDTA was added and subjected to centrifugation at 3000 rpm for 20 minutes to separate the serum.

Separated serum was used for analyzing different biochemical parameters. Lipid profile in fully automated analyser by enzymatic method, hsCRP by ELISA, Uric acid in fully automated analyser by enzymatic method as per kit instructions, BP by using sphygmomanometer, abdominal circumference by using measuring tape, Lipid indices were calculated using formulas.

Statistical analysis

was done using SPSS software. For categorical data, the number and percentage were used in the data summaries and diagrammatic presentation. Chi-square test was used for association between two categorical variables. The difference of the means of analysis variables between two independent groups was tested by unpaired't' test. The difference of the means of analysis variables between more than two independent groups was tested by ANOVA and 'F' test of testing of equality of Variance.

Results

In the present study there were totally 220 subjects out of which 110 were cases and 110 controls. The subjects in the age group were between 20-60yrs. Table 1 shows demographic and anthropometric markers of subjects who attended skin out-patient department at tertiary care hospital and Research centre. *Fig1* shows distribution of gender in both cases and controls. *Table 2* shows the mean levels and standard error of mean and comparision of cardiometabolic parameters between cases and controls. We found significant increase cardiometabolic parameters in cases compared to controls. *Table 3* shows distribution of uric acid according to level of PASI score and controls. There was significant increase in uric acid level from mild to severe cases. Table 4 shows distribution of lipid indices between cases and controls. The lipid indices were statistical significant in cases compared to controls. *Fig 2* shows comparison of different lipid indices between study groups.

Table 5 shows correlation between lipid indices and cardiometabolic parameters among cases and controls. Among cardiometabolic markers Lp(a),TG, Cholesterol, HDL-C, LDL, and anthropometric marker Abdominal circumference showed statistical significant correlation with some lipid indices.

Discussion

In the present study, anthropometric markers like blood pressure, abdominal circumference, BMI were statistically significant increased in psoriasis patients compared to

Table 1. Demographic and Anthropometric markers between Cases and Controls

Demographic & Anthropometric	Cases (Mean± S.D)	Controls (Mean± S.D)	't' value	'p' value
Age (yrs)	40.8± 11.4	39.4± 11.1	0.935	0.351
Systolic-BP(mm/Hg)	136.1 ± 14.0	125.0 ± 8.9	7.004	<0.001*
Diastolic-BP (mm/Hg)	81.4 ± 8.3	76.2± 5.5	5.456	<0.001*
Abdominal circumference(cm)	85.4±15.3	44.7± 11.9	22.055	<0.001*
BMI (wt in kg/ht in m^2)	40.9±8.3	22.3±3.2	12.6	<0.001*

Note: p value significant at 5% level of significance (p<0.05)*

Table 2. Cardiometabolic markers between Cases and Controls

Parameters	Cases Mean ± SD	Controls Mean ± SD	't' value	'p' value
TG	212.3 ± 39.5	122.9 ± 14.6	22.257	<0.001*
Cholesterol	241.0 ± 60.4	155.0 ± 43.2	12.76	<0.001*
HDL-Cholesterol	34.9 ± 6.8	43.5 ± 9.8	-7.568	<0.001*
LDL-Cholesterol	196.8 ± 43.1	132.0 ± 10.0	15.368	<0.001*
Uric acid	17.1± 7.2	4.5 ± 1.0	18.2	<0.001*
Lp(a) mg/dl	41.3 ± 8.7	25.1 ± 7.9	14.515	<0.001*
hs-CRP	16.2 ± 7.3	2.8 ± 1.3	18.952	<0.001*

Note: p value significant at 5% level of significance (p<0.05)*

Table 3. Distribution of uric acid serum levels according to level of PASI Score and Controls

Parameters (Mean±SD)	Level of PASI Score			Controls	F value	p value
	Mild	Moderate	Severe			
Uric acid	16.3±9.2	16.7±6.2	17.4±7.2	4.5±1	110.033	<0.001*

Note: p value significant at 5% level of significance (p<0.05)*

Table 4. Distribution of Lipid Indices between Cases and Controls

	Cases		Controls		t value	p value
	Mean	SD	Mean	SD		
AIP[log(TG/HDL)]	0.79	0.12	0.46	0.11	20.507	<0.001*
CI-I[TC/HDL]	7.20	2.43	3.70	1.13	13.687	<0.001*
CI-II[LDL/HDL]	5.85	1.70	3.21	0.83	14.582	<0.001*
AC[TC-HDL/HDL]	239.97	60.44	152.25	35.05	13.168	<0.001*
LTI[TCxTgxLp(a)/HDL]	66541.34	39207.47	92.29	38.92	17.775	<0.001*

AIP-Atherogenic Index of Plasma; CI- Castelli Index; AC- Atherogenic coefficient; LTI – Lipid tetrad index

Note: p value significant at 5% level of significance (p<0.05)*

controls as depicted in *Table 1*. Several studies have shown psoriasis patients had uncontrolled hypertension and this risk correlated with disease severity. A cross sectional study done in Germany have shown that central obesity or waist circumference was statistically more in psoriasis patients than controls. They also showed significant correlation between degree of obesity and severity of psoriasis. [12]

A study has shown that in patients with psoriasis who were obese, and weight loss has been shown to increase the efficacy of anti-TNF-alpha biologic therapy.[13] A study done by Dickison *et. al.*, showed women with psoriasis have a risk of hypercholesterolemia and hypertension, and overweight women are more susceptible to type 2 diabetes than are women with normal weight. [14] This was not in accordance to our study.

The molecular mechanisms underlying the association between psoriasis and obesity are currently not clearly understood. Various studies have shown that the disordered production of adipokines from fat tissue in obese patients with psoriasis may lead to chronic skin and systemic inflammation and increased cardiovascular risk. [12]

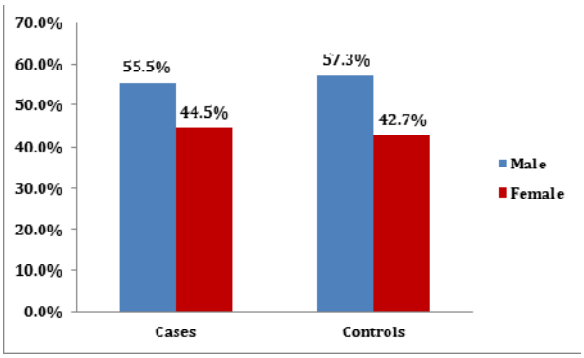
Among cardiometabolic markers uric acid was statistically significant from mild-moderate-severe (PASI score) in

Table 5. Correlation between lipid indices and cardiometabolic parameters among Controls / Cases

Parameter	AIP[log(TG/HDL)]		CI-I[TC/HDL]		CI-II[LDL/HDL]		AC[TC-HDL/HDL]		LTI[TCxTgxLp(a)/HDL]	
	r value	p value	r value	p value	r value	p value	r value	p value	r value	p value
LP(a) mg/dl	0.303	**0.044***	-0.037	0.899	0.011	0.971	-0.112	0.704	0.626	**0.017***
TG	0.72	**0.004***	-0.004	0.989	0.082	0.781	-0.038	0.897	0.587	**0.027***
Cholesterol	0.022	0.941	0.843	**<0.001***	0.23	0.429	1	**<0.001***	0.497	0.07
HDL	-0.793	**0.001***	-0.575	**0.032***	-0.7	**0.005***	-0.073	0.804	-0.455	0.102
LDL	0.144	0.624	0.242	0.405	0.755	**0.002***	0.246	0.397	0.14	0.632
Uric acid	-0.073	0.804	0.312	0.278	-0.076	0.795	0.219	0.451	0.003	0.991
Systolic-BP	-0.078	0.791	0.321	0.264	-0.082	0.78	0.195	0.503	-0.022	0.94
Diastolic-BP	0.059	0.84	-0.081	0.783	-0.447	0.109	0.008	0.978	-0.045	0.879
BMI	-0.034	0.908	-0.032	0.912	0.484	0.079	-0.276	0.34	-0.124	0.672
MS	-0.356	**0.044***	-0.045	0.88	-0.213	0.465	-0.007	0.981	-0.227	0.434
hs-CRP	-0.296	**0.044***	-0.084	0.775	-0.03	0.918	-0.164	0.576	-0.102	0.729

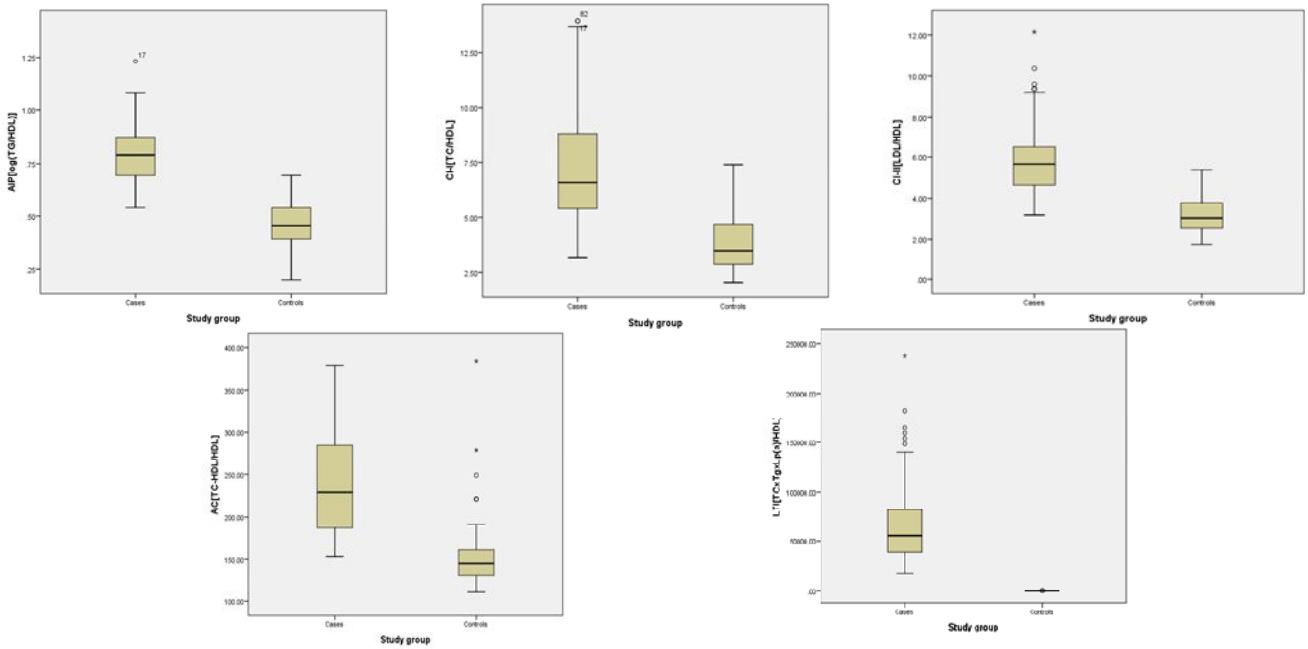
Note: p value significant at 5% level of significance (p<0.05)*

Fig 1. Distribution of Gender between Cases and Controls



cases shown in Table 3. Elevated uric acid levels are frequent finding in psoriasis. Study done by Ukonu and Ibekwe found a prevalence of 40.7% of hyperuricemia among psoriasis patients as compared to 7.0% of the control group (p=0.001) which showed strong association between serum uric acid level and psoriasis. [15] This study was in accordance to our study. The findings of our study have clinical implications as elevated serum uric acid levels causes gouty arthritis, which needs to be differentiated from psoriatic arthritis in clinical practice. The elevated serum uric acid is associated with increased carotid-artery intima-media thickness in patient with psoriatic arthritis, and independently predicts the development of cardiovascular events and mortality in

Fig 2. Showing comparison of different lipid indices between study groups



nonpsoriatic populations. [16] A study done by Xin-Yu Gu *et al.*, [17] showed increase in uric acid levels from mild to severe but with no statistical significance This was not in accordance to our study where along with increase uricd acid levels we got statistical significant correlation with severity.

All lipid indices showed statistical significant increase in cases compared to controls depicted in *Table 4*. These lipid indices were compared with cardiometabolic markers among cases and controls as depicted in *Table 5*. Among atherogenic markers Lp(a) and TG showed significant positive correlation with AIP and LTI where as cholesterol showed positive significant correlation with CI-I and AC lipid indices. A study done by Rocha-Pereira *et al* [18], reported increased serum total cholesterol, VLDL-cholesterol, LDL-cholesterol and a decrease HDL-cholesterol levels which was in accordance to our study. On the other hand, HDL good cholesterol showed negative significant correlation with AIP, CI-I, CI-II where as LDL being bad cholesterol showed positive correlation with CI-II. Both abdominal circumference and hsCRP showed negative correlation with AIP.

Along with lipid indices and anthropometric markers even hs-CRP, uric acid and Lp(a) also showed significant increase in psoriasis patients compared to controls. The increased levels of these markers in psoriasis patients have risk indicate that they are more susceptible for cardiovascular complications and require specific remedial steps which are necessary in considering the treatment. [12] A study by Ridker PM *et al* [19] have shown increased hs-CRP in psoriasis patients have been found to be associated with subclinical atherosclerosis and, therefore they have a predictive value for developing future cardiovascular events. These markers are over expressed in psoriasis, even in patients without overweight/obesity or other traditional CVD risk factors such as hypercholesterolemia, hypertension, and diabetes. [19]

A study generated from a German database of 42,461 dermatologic patients, in which 2,941 with psoriasis, reported that after controlling for age and sex, the rate of hypertension was twice as high in psoriatic patients compared with controls. However, two recent studies have failed to demonstrate a dose response relationship between hypertension and the psoriasis severity after controlling for confounders. [20]

Studies have shown that dyslipidaemia profile was present at the onset of psoriasis, suggesting that dyslipidaemia may precede the onset of psoriasis. [20] An increase in hs-CRP also reflects metabolic disorders, including insulin resistance and adiposity [21] , which are very common in patients with PS. As psoriasis is frequently associated with obesity, the excess adipose tissue might further contribute to atherogenic dyslipidemia. Hs-CRP, an acute phase reactant protein, is produced from hepatocyte within hours after being stimulated from inflammation, infection, tissue damage. [22] Kanelleas *et al*, found levels of hs-CRP was the only marker correlated with PASI score both before and after treating with etanercept and the more difference of hs-CRP level revealed the more treatment response. [23] Several studies have shown the common features of atherogenic dyslipidemia in psoriasis which include increased blood levels of total cholesterol, triglycerides, LDL, and apolipoprotein A and low HDL and apolipoprotein B levels in patients with psoriasis in patients with psoriasis, not only lipoprotein levels can be altered, but also their composition and function may be significantly different from controls. In the study done by, Mehta *et al*. showed that the HDL efflux capacity in psoriasis patients compared with controls was diminished beyond cardiovascular risk factors. [24]

Thus the pathogenetic mechanisms of cardiovascular disease in psoriasis patients appear to be of a complex nature. The pro-atherogenic lifestyle and cardiometabolic risk factor like diabetes, hypertension, hyperlipidaemia, obesity and metabolic syndrome, along with psychosocial and behavioral risk factors such as smoking, alcohol abuse, lack of exercise and depression will all increase the risk of cardiovascular disease in psoriasis patients. [25]

Limitations: The limitation of the present study is that psoriasis being inflammatory condition inflammatory markers could be included in the study. Further investigations are required to clarify the mechanisms underlying the association between psoriasis and cardiovascular comorbidities, and also to define optimal treatment regimens so as to reduce the risk of cardiovascular events in patients with psoriasis.

Conclusion

The present study found statistical significant increase in anthropometric markers BP, abdominal circumference & BMI in psoriasis patients compared to controls. Along with anthropometric markers, cardiometabolic markers also showed significant increase in psoriasis patients. Thus it is emphasized that the routine screening of cardiometabolic markers and other metabolic co-morbidities should be done which helps in early detection and treatment so as to reduce cardiovascular events in future. Physicians should be more aware of these cardiovascular risk while treating these patients.

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Nil.

Conflicts of Interest
There are no conflicts of interest.

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CONSENT FORM I
INFORMATION FOR PARTICIPANTS OF THE STUDY

Title of the project:
Study of Systemic inflammation, Oxidative stress and Cardiometabolic markers in Psoriasis.

1. Name, Designation, Address, Phone No. and Email ID of the Investigator:

Dr. Neela B. Mannangi.
Biochemistry PhD scholar (2017-18 batch), BLDE University
Asso. Professor of Biochemistry, S. Nijalingappa Medical College,
Bagalkot.
Phone: 9740174564
Email: nvs2579@gmail.com

2. Name of Guide with designation, Department, Phone No. and Email ID:

Dr. Basavaraj Devaranavadagi,
Professor and HOD of Biochemistry
Shri B. M. Patil Medical College, Vijayapura
Phone: 9448745957
Email: rohit1234@gmail.com

Name of Co-guide with designation, Department, Phone No. and Email ID:

Dr. Balachandra S. Ankad
Professor & HOD
Department of Dermatology
SNMC & HSK, RC. Bagalkot
Phone: 9980410056
Email: drbsankad@gmail.com

3. Purpose/ Objectives of this project /study:

1. To estimate Th1 cytokines - IFN-gamma, TNF -alpha, IL-2 in psoriasis patients.
2. To estimate serum oxidative stress, anti-oxidants levels in psoriasis.
3. To correlate the above parameters with the severity of the disease.
4. To compare the above parameters with caridometabolic markers like HTN, Lipid profile, Lp(a), BMI and DM(glucose) in psoriasis patients.

4. **Procedure/Methods of the study:** The required demographic information will be collected from the participants according to a pre-designed proforma. They will be examined clinically and the findings will be noted down. Severity will be assessed as mild, moderate and severe from Psoriasis score. Blood sample will be drawn under aseptic precautions from the antecubital vein. Serum inflammatory markers, oxidant & antioxidants will be estimated by ELISA method. Lipid profile by enzymatic method, Lp(a) by Immunoturbidimetry.

5. **Expected duration of the subject participation:** 25 minutes
6. **Expected benefits from the research to the participant:** The results of the present study will help us to understand the pathophysiology and appropriate management of psoriasis.
7. **Any risks expected from the study to the participant**: None
8. **Maintenance of confidentiality of records:**

   The study records will be kept confidential. Your personal identity will not be revealed in any publication or release of results. Study record will be kept indefinitely for analysis.
9. **Provision of free treatment for research related injury:**

   Although the study procedure itself carries minimal risk, treatment of any unforeseeable event will be provided free of cost by the Institute to you.
10. **Compensation of the participants for disability or death resulting from such injury:** Not applicable
11. **Freedom to withdraw from the study at any time during the study period without the loss of benefits that the participant would otherwise be entitled:**

    It is entirely your decision to participate in the study. If you want to discontinue from the study, you are free to leave without stating any reason. Your withdrawal would in no way result in SNMC withholding goodwill or normal medical care**.**
12. **Possible current and future uses of the biological material and of the data to be generated from the research and if the material is likely to be used for secondary purposes or would be shared with others, this should be mentioned**

    All the data and materials obtained from you will be used only for research purposes. It will not be used for secondary purposes nor will it be shared with others**.**

13. **Address and telephone number of the Investigator and Co-Investigator/Guide:**

Dr. Neela B. Mannangi
Biochemistry PhD scholar (2016-17 batch), BLDE University
Asso. Professor of Biochemistry, S. Nijalingappa Medical College,
Bagalkot.
Phone: 9740174564
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Name of Guide with designation, Department, Phone No. and Email ID:
Dr. Basavaraj Devaranavadagi,
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Name of Co-guide with designation, Department, Phone No. and Email ID:

Dr. Balachandra S. Ankad
Professor & HOD
Department of Dermatology,
SNMC & HSK, RC. Bagalkot
Phone: 9980410056
Email: drbsankad@gmail.com

14. **Contact details of Chairman of the IEC, BLD deemed to be university for appeal against violation of rights.**

Dr. Sharada Metgud
Professor of Microbiology,
J. N. Medical College,
Belgaum- 590010

CONSENT FORM II

PARTICIPANT CONSENT FORM

Participant's name:

Address:

Phone No.:

Email ID:

Title of the project: **Study of Systemic inflammation, Oxidative stress and Cardiometabolic markers in Psoriasis.**

The details of the study have been provided to me in writing and explained to me in my own language. I confirm that I have understood the above study and had the opportunity to ask questions. I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without the medical care that will normally be provided by the hospital being affected. I agree not to restrict the use of any data or results that arise from this study provided. Such a use is only for scientific purpose(s). I have been given an information sheet giving details of the study. I fully consent to participate in the above study.

Signature of the Participant: _____ Date: _____

Signature of the Witness: _____ Date: _____

ಒಪ್ಪಿಗೆ ಪತ್ರ

ಅಧ್ಯಯನದಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳುವವರಿಗೆ ಮಾಹಿತಿ

ಯೋಜನೆಯ ಶೀರ್ಷಿಕೆ :

ಸೋರಿಯಾಸಿಸ್ ನಲ್ಲಿ ಕಂಡುಬರುವ ಉರಿಯೂತ , ಉತ್ಕರ್ಷಣಾ ಒತ್ತಡ ಮತ್ತು ಹೃದಯರಕ್ತನಾಳಗಳ ಚಯಾಪಚಯ ಗುರುತುಕಾರಕಗಳ ಅಧ್ಯಯನ.

೧ .**ಅಧ್ಯಯನಕಾರರ ಹೆಸರು, ಹುದ್ದೆ,ವಿಳಾಸ ಮತ್ತು ದೂರವಾಣಿ :**

ಡಾಮನ್ಮಂಗಿ ನೀಲಾ .

ಪಿ ಡಿ.ಎಚ್.ವಿಧ್ಯಾರ್ಥಿ (ಜೀವರಸಾಯನ ಶಾಸ್ತ್ರ)

ಬಿವಿಶ್ವವಿದ್ಯಾಲಯ .ಇ.ಡಿ.ಲ್.

ಸಹಾಯಕ ಪ್ರಾಧ್ಯಾಪಕರು (ಜೀವರಸಾಯನ ಶಾಸ್ತ್ರ)

ಎಸ್ಮಹಾವಿದ್ಯಾಲಯ ವೈದ್ಯಕೀಯ ನಿಜಲಿಂಗಪ್ಪ.

ಬಾಗಲಕೋಟ

ದೂರವಾಣಿ :9740174564

ಇ ಮೇಲ್ : nvs2579@gmail.com

೨ .**ಮಾರ್ಗದರ್ಶಕರ ಹೆಸರು, ಹುದ್ದೆ,ವಿಳಾಸ ಮತ್ತು ದೂರವಾಣಿ :**

ಡಾ ಬಸವರಾಜ .ದೇವರನಾವಡಗಿ

ಪ್ರಾಧ್ಯಾಪಕರು ಮತ್ತು ಮುಖ್ಯಸ್ಥರು ಜೀವರಸಾಯನ ಶಾಸ್ತ್ರ ವಿಭಾಗ

ಶ್ರೀ ಬಿಕಾಲೇಜ್ ಮೆಡಿಕಲ್ .ಪಾಟೀಲ್ .ಎಮ್.

ವಿಜಯಪುರ

ದೂರವಾಣಿ :9448745957

ಇ ಮೇಲ್ : rohit1234@gmail.com

೩ .**ಸಹಮಾರ್ಗದರ್ಶಕರ ಹೆಸರು, ಹುದ್ದೆ,ವಿಳಾಸ ಮತ್ತು ದೂರವಾಣಿ :**

ಡಾ.ಎಸ್ ಬಾಲಚಂದ್ರ ಅಂಕದ್ ..

ಪ್ರಾಧ್ಯಾಪಕರು ಮತ್ತು ಮುಖ್ಯಸ್ಥರು ಚರ್ಮಶಾಸ್ತ್ರ

ಎಸ್ ಮತ್ತು ಮಹಾವಿದ್ಯಾಲಯ ವೈದ್ಯಕೀಯ ನಿಜಲಿಂಗಪ್ಪ.

ಎಚ್.ಬಾಗಲಕೋಟೆ ಆಸ್ಪತ್ರೆ ಕೆ.ಎಸ್.

ದೂರವಾಣಿ :9980410056

ಇ ಮೇಲ್ : drbsankad@gmail.com

ಅಧ್ಯಯನದ ಉದ್ದೇಶಗಳು :

ನಲ್ಲಿ ಸೋರಿಯಾಸಿಸ್ * ಉರಿಯೂತದ ಗುರುತುಕಾರಕಗಳಾದ Th-1 Cytokine , IFN- Gamma , TNF-Alpha, IL-2 ಅಧ್ಯಯನ.

ಮತ್ತು ಒತ್ತಡ ಆಕ್ಸಿಡೇಟಿವ್ ಸಿರಮ್ ನಲ್ಲಿ ಸೋರಿಯಾಸಿಸ್ *Anti Oxidant ಗಳ ಅಧ್ಯಯನ.

ನಿಯತಾಂಕಗಳ ಮೇಲಿನ * ಉತ್ಕರ್ಷಣ ನಿರೋಧಕಗಳ ಮತ್ತು ರೋಗದ ತೀವ್ರತೆಯ ಪರಸ್ಪರ ಸಂಬಂಧತೆ.

*ಹೃದಯರಕ್ತನಾಳಗಳ ಚಯಾಪಚಯದ ಗುರುತುಕಾರಕಗಳಾದ HTN, Lipid Profile , BMI , DM (Glucose) ಮತ್ತು ಮೇಲಿನ ಗುರುತುಕಾರಕಗಾಳ ಹೋಲಿಕೆ.

೪ .**ಅಧ್ಯಯನದ ಕಾರ್ಯವಿಧಾನ:**

ಅಧ್ಯಯನದಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳುವವರ ವಿವರವನ್ನು ತೆಗೆದುಕೊಳ್ಳಲಾಗುವುದು . ಅಧ್ಯಯನದ ಪ್ರಾಯೋಗಿಕ ಮಾದರಿ ವಿಧಾನದ ಮೂಲಕ ಅವರನ್ನು ಪರೀಕ್ಷಿಸಲಾಗುವುದು ರೋಗದ .ತೀವ್ರತೆಗಳ ಸೌಮ್ಯ , ಮಧ್ಯಮ ಮತ್ತು ತೀವ್ರತೆ ಯಾಗಿ ಸೋರಿಯಾಸಿಸ್ ಸ್ಕೋರ್ ಗಳ ಉಪಯೋಗದೊಂದಿಗೆ ವಿಂಗಡಿಸುವುದು. ಸೊಂಕುರಹಿತ ಮುನ್ನೆಚ್ಚರಿಕಾ ಜಾಗ್ರತೆಯಿಂದ ಒಳಗೊಂಡು ರಕ್ತದ ಮಾದರಿಯನ್ನು ಪಡೆದುಕೊಳ್ಳಲಾಗುವುದು. Serum Inflammatory Markers , Oxidants & Anti Oxidants ಗಳನ್ನು ELISA ಯಂತ್ರದ ಮೂಲಕ ತಿಳಿದುಕೊಳ್ಳಲಾಗುವುದು.

Lipid Profile ಗಳನ್ನು Enzymatic ವಿಧಾನದಿಂದ ಮತ್ತು Lp(a) ಯನ್ನು Immunoturbidimetry ವಿಧಾನದಿಂದ ಅಧ್ಯಯನ ಮಾಡಲಾಗುವುದು.

೫: ಅವಧಿ ನಿರೀಕ್ಷಿತ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆಯ ಅಧ್ಯಯನದಲ್ಲಿ . ೨೫ ನಿಮಿಷಗಳು.

೬: ಪ್ರಯೋಜನಗಳು ಸಂಶೋಧನೆಯಿಂದಾಗುವ .

ಅಧ್ಯಯನದಿಂದ ಬಂದ ಫಲಿತಾಂಶವು ಸೋರಿಯಾಸಿಸ್ ರೋಗದ ಉಂಟಾಗುವಿಕೆ ಮತ್ತು ರೋಗದ ನಿರ್ವಹಣೆಗೆ ಸಹಕಾರಿಯಾಗಿದೆ.

೭: ಅಪಾಯಗಳು ಉಂಟಾಗಬಹುದಾದ ಪಾಲ್ಗೊಳ್ಳುವವರಿಗೆ ಅಧ್ಯಯನದಲ್ಲಿ . ಏನೂ ಇಲ್ಲ.

೮ : ನಿರ್ವಹಣೆ ಗೌಪ್ಯತೆ ದಾಖಲೆಗಳ .

ಅಧ್ಯಯನದ ದಾಖಲೆಗಳನ್ನು ಗೌಪ್ಯವಾಗಿಡಲಾಗುವುದು ವೈಯಕ್ತಿಕ ನಿಮ್ಮ .
ಮಾಹಿತಿಗಳನ್ನು ಯಾವುದೇ ಪ್ರಕಟಣೆಗಳಲ್ಲಿ ಬಹಿರಂಗಪಡಿಸುವುದಿಲ್ಲ .
.ಇರಿಸಲಾಗುವುದು ವಿಶ್ಲೇಷಣೆಗಾಗಿ ಅನಿರ್ದಿಷ್ಟವಾಗಿ ದಾಖಲೆಗಳನ್ನು ಅಧ್ಯಯನದ

೯:ನೀಡುವಿಕೆ ಚಿಕಿತ್ಸೆ ಉಚಿತ ಗಾಯಗಳಿಗೆ ಸಂಬಂಧಿತ ಸಂಶೋಧನಾ .
ಅಧ್ಯಯನದ ಕಾರ್ಯವಿಧಾನ ಕನಿಷ್ಠ ಅಪಾಯವನ್ನು ಹೊಂದಿದ್ದರೂ , ಯಾವುದೇ ಅಪಾಯ ಉಂಟಾದಲ್ಲಿ ಉಚಿತ ಚಿಕಿತ್ಸೆಯನ್ನು ನೀಡಲಾಗುವುದು.

೧೦ ಉಂಟಾಗಬಹುದಾದ ಗಾಯಗಳಿಂದ .ಅಂಗವೈಕಲ್ಯತೆ ಮತ್ತು ಸಾವಿನ ಪರಿಹಾರ : ಅನ್ವಯಿಸುವುದಿಲ್ಲ.
೧೧ಸಮಯದ ಯಾವುದೇ ಅಧ್ಯಯನದ .ಲ್ಲಾದರೂ ಭಾಗವಹಿಸುವವರ ಹಿಂಪಡೆಯುವ ಸ್ವಾತಂತ್ರ್ಯತೆ :
ಇದು ಅಧ್ಯಯನದಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳುವವರ ನಿರ್ಧಾರವಾಗಿದೆ ನೀವು .
ಬಯಸದಿದ್ದರೆ ಮುಂದುವರೆಸಲು ಅಧ್ಯಯನದಲ್ಲಿ , ಯಾವುದೇ ಕಾರಣವನ್ನು ತಿಳಿಸದೆ ಹೊರನಡೆಯಲು ನೀವು ಮುಕ್ತರಾಗಿದ್ದೀರಿ ಹಿಂಪಡಿಕೆಯಿಂದ ನಿಮ್ಮ .
ಆರೈಕೆಯಲ್ಲಿ ವೈದ್ಯಕೀಯ ಸಾಮಾನ್ಯ ಸಂಸ್ಥೆಗೆ ನಮ್ಮ ಯಾವದೇ ಪರಿಣಾಮ ಬೀರುವುದಿಲ್ಲ.
೧೨. ಸಂಶೋಧನೆಯಿಂದ ಉತ್ಪತ್ತಿಯಾದ ಜೈವಿಕ ವಸ್ತುಗಳು ಮತ್ತು ಉಪಯುಕ್ತ ಮಾಹಿತಿಗಳನ್ನು ಪ್ರಸ್ತುತ ಮತ್ತು ಭವಿಷ್ಯದಲ್ಲಿ ಬಳಸಿಕೊಳ್ಳಲಾಗುವದು ಮತ್ತು ಇತರರೊಂದಿಗೆ ಹಂಚಿಕೊಳ್ಳಲಾಗುವ ಒಡಂಬಡಿಕೆಯಿಂದ ಕೂಡಿರುತ್ತದೆ.
೧೩: ದೂರವಾಣಿ ಮತ್ತು ವಿಳಾಸ ಅಧ್ಯಯನಾಕಾರರ ಮತ್ತು ಮಾರ್ಗದರ್ಶಕರು .

ಡಾಮನ್ನಂಗಿ ನೀಲಾ .
ಪಿ ಡಿ.ಎಚ್.ವಿದ್ಯಾರ್ಥಿ)ಜೀವರಸಾಯನ ಶಾಸ್ತ್ರ(
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ಸಹಾಯಕ ಪ್ರಾಧ್ಯಾಪಕರು) ಜೀವರಸಾಯನ ಶಾಸ್ತ್ರ(
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ಡಾ ಬಸವರಾಜ .ದೇವರನಾವಡಗಿ
ಪ್ರಾಧ್ಯಾಪಕರು ಮತ್ತು ಮುಖ್ಯಸ್ಥರು ಜೀವರಸಾಯನ ಶಾಸ್ತ್ರ ವಿಭಾಗ
ಶ್ರೀ ಬಿಕಾಲೀಜ್ ಮೆಡಿಕಲ್ .ಪಾಟೀಲ್ .ಎಮ್.
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ಮಾರ್ಗದರ್ಶಕರ ಸಹಿ.

ಸಹಮಾರ್ಗದರ್ಶಕರ ಹೆಸರು, ಹುದ್ದೆ,ವಿಳಾಸ ಮತ್ತು ದೂರವಾಣಿ :

ಡಾಅಂಕದ್ .ಎಸ್ ಬಾಲಚಂದ್ರ .
ಪ್ರಾಧ್ಯಾಪಕರು ಮತ್ತು ಮುಖ್ಯಸ್ಥರು ಚರ್ಮಶಾಸ್ತ್ರ
ಎಸ್ ಮತ್ತು ಮಹಾವಿದ್ಯಾಲಯ ವೈದ್ಯಕೀಯ ನಿಜಲಿಂಗಪ್ಪ.
 ಎಚ್.ಬಾಗಲಕೋಟೆ ಆಸ್ಪತ್ರೆ ಕೆ.ಎಸ್.
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೧೪:ವಿಳಾಸ ಸಂಪರ್ಕಿಸಬೇಕಾದ ಉಲ್ಲಂಘನೆಯಾದಲ್ಲಿ ಹಕ್ಕುಗಳ .

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ದೂರವಾಣಿ: 0831-2477477
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ಒಪ್ಪಿಗೆ ಪತ್ರ

ಅಭ್ಯರ್ಥಿಗಳ ಒಪ್ಪಿಗೆ ಪತ್ರ

ಹೆಸರು :
ವಿಳಾಸ:
ದೂರವಾಣಿ :
ಇ ಮೇಲ್ :

ಯೋಜನೆಯ ಶೀರ್ಷಿಕೆ :

ಸೋರಿಯಾಸಿಸ್ ನಲ್ಲಿ ಕಂಡುಬರುವ ಉರಿಯೂತ , ಉತ್ಕರ್ಷಣಾ ಒತ್ತಡ ಮತ್ತು ಹೃದಯರಕ್ತನಾಳಗಳ ಚಯಾಪಚಯ ಗುರುತುಕಾರಕಗಳ ಅಧ್ಯಯನ.

ಅಧ್ಯಯನದ ವಿವರಗಳನ್ನು ಬರವಣಿಗೆಯ ರೂಪದಲ್ಲಿ ಮತ್ತು ನನ್ನದೇ ಭಾಷೆಯಲ್ಲಿ ತಿಳಿಸಿರುತ್ತಾರೆ ಅಧ್ಯಯನವನ್ನು ನಾನು .ಅರ್ಥಮಾಡಿಕೊಂಡಿರುತ್ತೇನೆ ಮತ್ತು ಪ್ರಶ್ನೆಕೇಳುವ ಅವಕಾಶವನ್ನು ಹೊಂದಿದ್ದೇನೆಂದು ದೃಡೀಕರಿಸುತ್ತೇನೆ . ಯಾವುದೇ ಮತ್ತು ಸ್ವಯಂಪ್ರೇರಿತವಾಗಿದೆ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆ ನನ್ನ ಅಧ್ಯಯನದಲ್ಲಿ ವೈದ್ಯಕೀಯ ಸಾಮಾನ್ಯ ಮತ್ತು ಸರಿಯಲು ಹಿಂದೆ ಅಧ್ಯಯನದಿಂದ ಸಮಯದಲ್ಲಿ ಮುಕ್ತವಾಗಿದ್ದೇನ ಎಂದು ನಷ್ಟವಿಲ್ಲ ಆಸ್ಪತ್ರೆಗೆ ಆರೈಕೆಯಲ್ಲಿ ೆ . ಡೇಟಾಗಳನ್ನು ಮತ್ತು ಅಂಶಗಳನ್ನು ಉತ್ಪತ್ತಿಯಾದ ಅಧ್ಯಯನದಲ್ಲಿ . ಒಪ್ಪಿರುತ್ತೇನೆ ಎಂಬುದನ್ನು ನಿರ್ಬಂಧಿಸಲಾಗುವುದಿಲ್ಲ

ಇದನ್ನು ವೈಜ್ಞಾನಿಕ ಕಾರಣಗಳಿಗಾಗಿ ಬಳಸತಕ್ಕದ್ದು ವಿವರಣಾ ಅಧ್ಯಯನದ . .ನೀಡಲಾಗಿದೆ ನನಗೆ ಪತ್ರವನ್ನು
ಈ ಅಧ್ಯಯನಕ್ಕೆ ನನ್ನ ಸಂಪೂರ್ಣ ಸಮ್ಮತಿಯಿರುತ್ತದೆ.

ಅಭ್ಯರ್ಥಿಯ ಸಹಿ ದಿನಾಂಕ:

ಸಾಕ್ಷಿದಾರರ ಸಹಿ ದಿನಾಂಕ: