Addis Ababa University

College of Health Sciences
School of Medicine
Department of Medical Biochemistry

Title: TOTAL OXIDANT-ANTIOXIDANT STATUS AMONG PSORIATIC PATIENTS ATTENDING AT ALERT HOSPITAL, ADDIS ABABA, ETHIOPIA

A thesis submitted to Addis Ababa University School of Graduate Studies, Department of Biochemistry in partial fulfillment of the requirements for the Degree of Master of Science in Medical Biochemistry.

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Declaration

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This is to certify that the thesis prepared by Asrat Endrias entitled: *Total oxidant-antioxidant status among psoriatic patients attending at Alert Hospital* is submitted in partial fulfillment of the requirements for degree of masters in medical biochemistry complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

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ABBREVIATION

CAT	Catalase
ERK	Extracellular- regulated kinase
GP	Glutathione peroxidase
IFN-α	Interferon-alpha
IL	Interlukin
iNOS	Inducible nitric oxide synthase
JAK-STAT	Janus kinase-Signal transducer and activator of transcription
MAPK	Mitogen-activated protein kinase
MDA	Manoldialdehyde
MEKK	Mitogen extracellular kinase kinase
MSK1	Mitogen- and- stress-activated protein kinase
NF-KB	Nuclear factor kB
NO	Nitric oxide
OSI	Oxidative stress index
PKC	Protein kinase C
PMN	Polymorphoneuclear
ROS	Reactive oxygen species
SOD	Superoxide dismutase
TAC	Total Anti-oxidant Capacity
TNF α	Tumor Necrosis Factor alpha
TOC	Total Oxidant Capacity

Abstract

Psoriasis is a chronic inflammatory skin disease characterized by the immune system activation in which the genetic and environmental factors are involved that trigger the development of skin lesions. Reactive oxygen species produced as a result of skin inflammation may cause disorders of the antioxidant defense systems and increased oxidative stress in psoriasis which was proposed to have to have a consequent function in psoriasis.

Aim of the study: *The presented research work was planned to evaluate oxidative stress by measuring Total Oxidant Capacity, Total Anti-oxidant capacity and Oxidative Stress Index in psoriatic patients.*

Methods: *A comparative cross-sectional study was undertaken with 45 clinically diagnosed psoriatic patients without any drug therapy and 45 age-sexes matched healthy controls. The severity of psoriasis was determined by Psoriasis area severity index. Out of total 45 Psoriasis patients, 22 were mild, 15 moderate and remaining 8 were severe psoriatic patients. Levels of Total Oxidant Capacity, Total Anti-oxidant Capacity and Oxidative Stress Index were measured in patients and controls.*

Results*: The present study showed significantly ($p<0.001$) increased levels of serum Total Oxidant Capacity as well as Oxidative Stress Index ($p<0.001$) in psoriasis patients as compared to controls and positively correlated with severity and duration of the disease. Total Anti-oxidant Capacity levels were significantly ($p<0.001$) lower in patients than in controls and negatively correlated with severity and duration of the disease.*

Conclusion*: These results provide some evidence regarding the role of increased reactive oxygen species with decreased antioxidant activity in psoriatic patients.*

Key Words*: Total Oxidant capacity, Total Antioxidant capacity, Oxidative stress index Psoriasis.*

1. INTRODUCTION

1.1. Psoriasis

The desire to have beautiful and healthy looking skin has been a centuries-old quest for humans. Skin with brighter complexion and smoother surface tends to be perceived as being healthier and more attractive. Beyond its obvious roles of keeping our insides in and the outside out, skin have many less conspicuous duties: its sweat glands help regulate temperature, its nerves provide the sense of touch, its deeper cells produce vitamin D, and its appearance advertises your age and health. For those who can read skin's language, a close examination reveals clues about the whole body's health. Changes in the skin color may indicate homeostatic imbalances in the body (Ng and Lau, 2015).

Skin makes up to 12–15% of an adult's body weight. Each square centimeter has 6 million cells, 5,000 sensory points, 100 sweat glands, and 15 sebaceous glands. It consists of three layers the epidermis, the dermis and the hypodermis. The epidermis is the protective skin layer in contact with the external environment. This skin layer consists mainly of a stratified squamous keratinized epithelium. The epidermis cells, the keratinocytes, divide in the basal layer and differentiate throughout their migration to the surface (Ojeh *et al*., 2015). The epidermis is divided into five different layers: stratum basale, stratum spinosum, stratum granulosum, stratum lucidum and stratum corneum. The dermis is the feeder layer of the epidermis and provides most of the skin's mechanical resistance and elasticity. It is mainly composed of fibroblasts, epidermal appendages, blood vessels, nerves and nerve endings. The hypodermis is the deepest layer of the skin. It varies in size and content, but is usually composed of adipocytes which form the adipose tissue (Jean and Pouliot, 2010).

Skin disease is one of the most common human illnesses which pervades all cultures, occurs at all ages, and affects between 30% and 70% of individuals. Its detrimental effects on health range from physical incapacity to death. The International Classification of human disease lists more than 1,000 skin or skin-related illnesses, a pattern dominated by a few conditions accounting for most of the skin disease burden. Skin remains the 18th leading cause of health burden worldwide (Hay *et al.,* 2014).

Patients of skin disease always experience physical, emotional and socio-economic embarrassment in the society. Psoriasis is one amongst these notorious auto-immune disorder having deep psychological and social impacts (Prashanth *et al.,* 2014). Psoriasis is non- contagious skin disease affecting both sexes equally, and can occur at any age, although it most commonly appears for the first time between the ages of 15 and 25 years (Dilnawaz *et al.,* 2013; Chong *et al*., 2010). The word 'psoriasis' is derived from the Greek word "psora" meaning "itch" + sis "action". It has been known since ancient times and was originally considered a type of leprosy. It is now considered as immune-mediated chronic inflammatory skin disease that is often associated with systemic manifestations (Al-Shobaili *et al*., 2013).

In the course of providing their protective function skin cells gets damaged so that they must be replaced. Therefore, our body produces new skin cells deep in the dermal matrix .These cells migrate upward towards the surface as they mature. Some mature skin cells undergo a process called keratinization-the conversion of squamous epithelial cells into keratin or simpler structural proteins. Keratinization eventually leads to cell death leaving a layer of drier, harder organic material (cell bodies) (Nagamani *et al*., 2015). In a normal healthy adult, the normal skin turnover occurs once in every 30-40 days. Psoriasis accelerates self-renewal due to excessive growth and reproduction of skin cells which leave them to live 4-10 days as a result, migration outside has no time to differentiate and they are not quite functional (Peslyak *et al*., 2011). Many psoriatic patients tolerate constant pain from cracking and bleeding lesions, and bear the humiliation and discomfort of continually flaking skin. Itching and pain can interfere with basic functions, such as self-care and sleep. Approximately 60% of psoriasis patients missed an average of 26 days of work a year due to their illness (Augustin and Radtke , 2014).

1.1.1. Epidemiology of psoriasis

Worldwide psoriasis is affecting, as presumed, approximately 120–180 million people. The population prevalence of psoriasis has been reported to range from 2% to 3%. Around 150,000 new cases of psoriasis are reported annually. Studies in developed countries have reported higher prevalence rates of on average about 4.6% (Chandran *et al*., 2010).

A total of 7.2 million US adults had psoriasis in 2010; an estimated 7.4 million US adults were affected in 2013. When stratifying the sample by race among those between ages 20 and 59 years, the psoriasis prevalence was highest in Caucasians at 3.6% followed by African Americans 1.9%, Hispanics 1.6% and others 1.4%. The prevalence of psoriasis among US adults has not changed significantly since 2003 to 2004 (Rachakonda *et al*., 2014). Population-based surveys from China and Japan have given a similar low prevalence ranging respectively from 0.05 to 1.23% and 0.29 to 1.18%. The same rates were found in India 0.5%-2.3% (Alexis *et al*., 2014).

Interestingly, clinic and population surveys have shown great variability between different Sub-Saharan African populations. These suggest that West African countries like Nigeria, Ghana, Mali, Senegal, Angola etc, have a lower prevalence of psoriasis 0.05 % to 1% than 3% in the eastern countries like Kenya, Uganda and Tanzania (Diallo *et al*., 2012).

Information on the prevalence of psoriasis in Ethiopia is extremely limited; however, data on the incidence of psoriasis at a specified clinic or hospital over a defined period of time are more frequently available. While the absolute incidence rate at a clinic may fail to reflect the local prevalence of psoriasis, the relative rates of psoriasis incidence, obtained from clinics were retrieved and reviewed. A total of 2342 dermatologic cases were diagnosed at ALERT pathology laboratory from January 2007 to December 2010 which represent a wide range of inflammatory, infectious, and neoplastic diseases. During this period out of 632 inflammatory dermatoses 43 were diagnosed with psoriasis (Gimbel *et al*., 2013).

1.1.2. Clinical and histological features of psoriasis

The most frequent clinical type of psoriasis which is affecting 85%–90% of the patients is psoriasis vulgaris (also called plaque psoriasis) (Jiang *et al*., 2015) characterized by erythematous plaques of the skin with well-defined borders and silvery scales that can be frequently observed on scalp, tips of fingers and toes, palms, soles, umbilicus, gluteus, under the breasts and genitals, elbows, knees, shins and sacrum (Kuchekar *et al*., 2011) (Figure1.1A). Less common phenotypes include guttate, inverse, pustular, erythrodermic and palmo-plantar psoriasis.

Histological analysis of psoriasis shows an increased thickening of the epidermis, in conjunction with incomplete keratinocyte differentiation of the upper layers, leading to the retention of nuclei in the stratum corneum (Figure1.1B). The basal layers of the epidermis are characterized by increased keratinocyte proliferation and elongated epidermal rete ridges. More dermal blood vessels are formed, responsible for the redness of the lesions. Moreover, a massive immune cell infiltrate can be observed in both dermis and epidermis, containing T cells, dendritic cells and others. Very characteristic is the presence of neutrophils in the epidermis and especially in the stratum corneum (Di Meglio *et al*., 2011; Lowes *et al*., 2014).

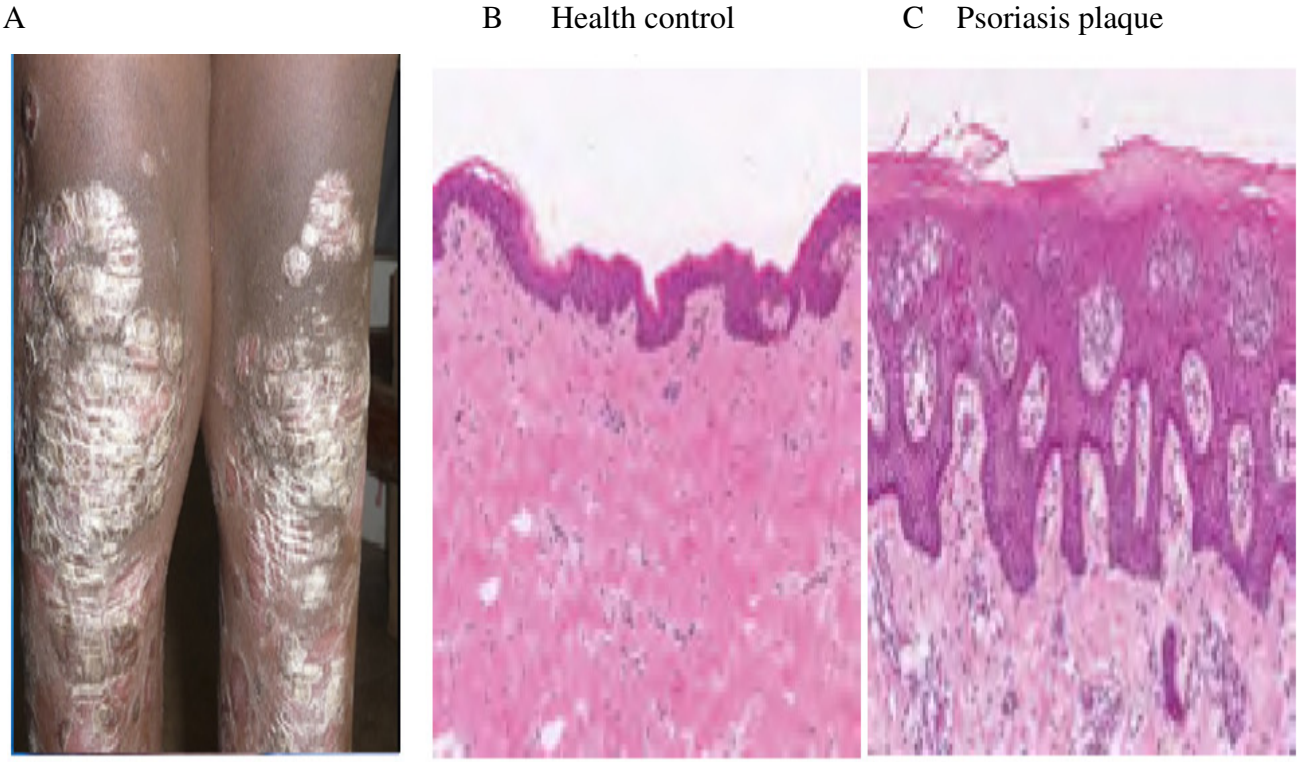

Figure1.1: Clinical manifestation of plaque type psoriasis. (A) Representative image of psoriatic plaques on the knee of a psoriasis patient. (B) Cross-sections through normal healthy and lesional (C) psoriatic skin (Wohn, 2015).

1.1.3. Co-morbidities of psoriasis

The relationship between psoriasis and other diseases has drawn increasing interest in recent years. Psoriasis is associated with several co-morbidities, including psoriatic arthritis, cardiovascular disease, obesity, diabetes, hypertension, dyslipidemia, metabolic syndrome, nonalcoholic fatty liver disease, cancer, anxiety and depression. Recently, also lymphoma, multiple sclerosis, chronic obstructive pulmonary disease, Chron's disease and inflammatory bowel disease are found at a higher prevalence in psoriatic patients compared to the general population. These disease associations may be due to the systemic inflammatory mediators generated in psoriasis, shared risk factors (i.e., smoking, alcohol consumption), or treatment (Ni and Chiu, 2014).

1.1.4. Treatment of psoriasis

Although treatment options have increased during the past years, no cure is known for psoriasis. Treatment in psoriasis can generally be categorized into topical and systemic therapies. The treatment choice is dictated by the severity, type, and location of psoriasis (Mrowietz *et al*., 2011). Patients with mild psoriasis can usually be managed with topical agents including: corticosteroids, tar, retinoids and vitamin D derivates. Moderate to severe psoriasis requires phototherapy or systemic therapies such as methotrexate, retinoids, cyclosporine or the biologic immune modifying agents including alefacept, the anti- Tumor Necrosis Factor (TNF) agents or anti-IL12/23 monoclonal antibody (ustekinumab) (Menter *et al*., 2010).

1.1.5. Pathogenesis of psoriasis

The exact cause and progress of psoriasis remains poorly understood but there is now increasing evidence that psoriasis is a result of a complex interaction between impaired barrier function and innate and adaptive immune systems, which is impacted by environmental and genetic contributions (Balato *et al*., 2012)

1.1.5.1. Genetic factors

Compared with the general population, a higher incidence of the disease has been identified among first-degree and second-degree relatives of psoriasis. The risk of psoriasis is greater in monozygotic twins than in dizygotic twins, confirming the genetic basis of the disease (Skroza *et al*., 2013). The mode of inheritance of psoriasis is complex. Several susceptibility loci for psoriasis PSORiasis Susceptibility locus (*PSORS*) have been identified, but the major genetic determinant of psoriasis is *PSORS1*, which is located within the major histocompatibility complex (MHC) on chromosome 6p. Current data suggest that human leukocyte antigen (HLA) Cw6 is the susceptibility allele within *PSORS1*. This association is particularly strong in patients with early onset psoriasis (Chandran *et al*., 2010). One of the most important features of HLA-C is its capacity to regulate both innate and adaptive responses at the levels of both antigen presentation and natural killer cell regulation. In addition to PSORS1, linkage analyses and association studies have highlighted psoriasis loci on several other chromosomes outside of the MHC region, designated *PSORS2-PSORS10* (Sun *et al*., 2014).

1.1.5.2. Environmental triggers

Psoriasis can be triggered by several environmental stimuli, all of them also known to worsen the ongoing disease. Direct skin trauma can trigger psoriasis (Koebner phenomenon), Streptococcal throat infection, allergies, diet, weather and use of specific medications such as Lithium, anti-malarial medications may also trigger the condition or exacerbate existing psoriasis. Human immunodeficiency virus infection has not been shown to trigger psoriasis, but can exacerbate existing disease. As the infection progresses, psoriasis often worsen (Roberson *et al.,* 2010). Smoking, Obesity and alcohol use and abuse are also associated with psoriasis. These associations may not be causative but patients with psoriasis may be more susceptible to unhealthy behaviors (Weigle *et al*., 2013).

1.1.5.3. Immunopathogenesis of Psoriasis

The innate immune system is activated in the skin in genetically predisposed individuals exposed to environmental triggers like infection or trauma. The initial spark initiating the inflammatory cascade in genetically predisposed individuals has not been known. However, data supports antimicrobial peptide (LL-37) and self-DNA complexes to be a potential explanation of the mechanism through which host DNA is turned into a pro-inflammatory stimulus that breaks immunologic tolerance in psoriasis. The hypothesis is that complexes of the antimicrobial peptide LL-37 cathelicidin and self-DNA released from necrotic cells in the stressed skin activate plasmacytoid dendritic cells and starts of the inflammatory cascade in psoriasis (Al-Shobaili *et al.,* 2013).

It was previously assumed that T-helper 1 (T_H1) cells played the dominant role in the initiation and maintenance of psoriasis but, in recent years, the view has changed in favor of a T_H17 mediated disease. Innate immune cells produce key cytokines; tumor necrosis factor alpha (TNF-α), interferon alpha (IFN-α), Interferon gamma (IFN-γ), interleukin 1 beta (IL-1β), and IL-6 that activate dendritic cells (Chu *et al.,* 2011). Activated dendritic cells present antigens and secrete mediators such as IL-12 and IL-23, leading to the differentiation of T_H1 and T_H17. IL-23 serves as a key master cytokine regulator. T cells secrete mediators (e.g., IL-17 and IL-22) that activate keratinocytes and induce the production of antimicrobial peptides, proinflammatory cytokines and chemokines. These mediators feed back into the proinflammatory disease cycle and shape the inflammatory infiltrate (DiMeglio *et al.,* 2011).

There is compelling evidence that oxidative stress drives the production of oxidation products, such as 4-hydroxy- 2-nonenal or malonaldehyde which can denature proteins, alter apoptosis, and influence the release of proinflammatory mediators, such as cytokines, which may be critical for the induction of some inflammatory skin diseases. This is also based on the recognition that ROS (reactive oxygen species) can act as second messengers in the induction of several biological responses, such as the activation of Nuclear factor kappa (NF-kB) or Activator protein 1 (AP-1), the generation of cytokines, the modulation of Pro-inflammatory signaling pathways (Yadav *et al.,* 2013).

Besides direct damaging effects of unregulated ROS production, dysregulation of several pro-inflammatory pathways, like Mitogen-activated protein kinase (MAPK), NF-κB, and Janus kinase-Signal transducer and activator of transcription (JAK-STAT) has been considered to contribute to psoriasis etiology. Several members of the MAPK signaling pathways, like Extracellular-signal-regulated kinase (ERK) 1/2, c-Jun N-terminal kinases (JNK) and MAPK were activated in psoriatic skin, further supporting this notion. The recent demonstration that the peroxisome proliferator-activated receptors, whose natural ligands are polyunsaturated fatty acids and their oxidation products and an increased ROS generation by infiltrated leukocytes into psoriatic has further strengthened the concept that ROS may play a significant role in the pathogenesis of psoriasis (Wagener *et al*., 2013).

Several studies have investigated the role of oxidants/antioxidants systems in psoriasis with discordant results. Hence, it was decided to investigate the total oxidant- anti oxidant status and oxidative stress index in serum of psoriatic patients.

1.2. Literature Review

1.2.1. Generation of Reactive Oxygen Species (ROS) in the skin

Free radicals can be defined as reactive chemical species having a single unpaired electron in an outer orbit and are continuously produced by the organism's normal use of oxygen. This unstable configuration creates energy that is released upon reaction with adjacent molecules, such as proteins, lipids, carbohydrates, and nucleic acids. The majority of free radicals that damage biological systems are derived from oxygen and more generally referred to as "reactive oxygen species (Bhattacharya *et al*., 2015).

ROS can be defined as oxygen-containing molecules, which are more reactive and potent than molecular oxygen itself. ROS can be grouped into oxygen-centered radicals: hydroxyl radical ($^{\cdot}OH$), peroxyl radical ($ROO^{\cdot}$), alkoxyl radical ($RO^{\cdot}$), Superoxide anion ($O_2^{\cdot -}$) and oxygen-centered non-radicals: singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2) (Oyinloye *et al.,* 2015). Reactive nitrogen species (nitrous oxide, peroxynitrite, nitroxyl ion, etc.) are also a class of free radicals derived from nitrogen and considered a subclass of ROS. Reactive nitrogen species (RNS) are generated as a result of sequential reactions that begin with nitric oxide synthase (NOS) mediated conversion of arginine to citrulline. In this reaction, nitric oxide (NO) is generated, which reacts with $O_2^{\cdot -}$ to produce peroxynitrite ($ONOO^-$) (Katiyar *et al*., 2015).

Reactive oxygen species performs beneficial functions in our body and should be maintained. It is the mediator of phagocytosis, apoptosis, detoxification reactions, executioner of precancerous cells and infections, etc. It is beneficially involved in signaling pathways to maintain cellular homeostasis in body. The ROS regulates many metabolic and cellular processes including proliferation, migration, gene expression, and immunity (Klaunig *et al*., 2010). In the healthy skin, practically all types of skin cells produce ROS and RNS. These free radicals are indispensable effectors in the homeostatic pathways leading to cell proliferation, differentiation, senescence, and death (Pai *et al*., 2014).

The sources of ROS which are enzymatic as well as non-enzymatic, in the cell are manifold. Enzymes that are ROS producing, on purpose or as a byproduct, include the mitochondrial electron transport chain, nicotinamide adenine dinucleotide phosphate-oxidase (NADPH oxidases) xanthine oxidoreductase (XOR), several peroxisomal oxidases, enzymes of the cytochrome P450 family, cyclooxygenases, and lipoxygenases (Rinnerthaler *et al*., 2015). Cellular ROS generation also include phagosomes located in specialized cells of the immune system, involved in killing of pathogen; and the endoplasmic reticulum, cell membranes and cytosol (Dickinson *et al*., 2011).

In human skin, superoxide anion radical $O_2^{\cdot -}$ is formed by one-electron reduction of molecular oxygen catalyzed by various mitochondrial and cytoplasmic enzymes (Kurahashi, *et al*., 2015). Mitochondria are the major site for ROS production and also a target of free radicals in the cells. The mitochondrial respiratory chain produces superoxide and H_2O_2 at complex I and III, which can be enhanced by inhibition of the respiratory chain owing to lack of oxygen (Pankotai *et al*., 2010).

More recent evidence reveals that complex II in the mitochondrial membrane also contributes to the overall $O_2^{\cdot -}$ Production in human skin cells (Rastogi and Pospısil , 2011). The contribution of mitochondria to the production of ROS in the skin is only substantial in the stem cells, but further on is small compared to other organs, because during the cornification process the keratinocytes degrade all their organelles including the nucleus, mitochondria, peroxisomes, and the endoplasmic reticulum (Anderson *et al*., 2014).

Apart from the mitochondria, $O_2^{\cdot -}$ is formed in the cytoplasm of skin keratinocyte cells by a reduction of molecular oxygen catalyzed by heme-flavoprotein NADPH oxidase (NOX) (Bazela *et al*., 2014). The cytosol has the capacity to produce ROS as a byproduct of the arachidonic acid metabolism.The enzymes cyclooxygenase (COX) and lipoxygenase(LOX) both use arachidonic acid as a substrate to synthesize prostaglandin H2 and the leukotrienes, respectively. Both enzymes have the capacity to produce superoxide in the presence of NADH or NADPH. The levels of arachidonic acid are relatively low in the skin, but increase in inflammatory skin diseases such as psoriasis, atopic dermatitis, and aging (Rahman *et al*., 2012).

The skin is at the interface between the body and the environment and is therefore in constant contact with pollutants, xenobiotics, and UV radiation. These exogenous factors represent the main contributor to the formation of ROS in human skin, therefore being very specific for this organ. Additionally, alcohol intake, false nutrition, and physiological and mechanical stress are believed to contribute to this kind of exogenous mediated ROS production. In addition the skin is also one of the very few organs that are in direct contact with atmospheric oxygen (Poljsak *et al*., 2012).

Inflammation begins when tissues react to a local irritation usually caused by a physical injury, infection or by exposure to toxicant. Fluid and accompanying white blood cells traverse the vascular barrier leading to swelling, erythema, further inflammation and attraction of further white blood cells. During the inflammatory phase, there is a burst of respiration leading to the creation and release of free radicals, including production of both ROS and RNS (Roberts *et al*., 2010).

Another important pathway that leads to ROS production in skin is formation of prostaglandin E_2 (PGH_2) from arachidonic acid. The reaction is catalyzed by cyclooxygenase (COX), a prostaglandin-endoperoxide synthetase. The enzyme catalyzes the synthesis of hydroxyl endoperoxides, followed by ROS production (Reuter *et al*., 2010).

1.2.2. Role of Oxidative Stress in the skin

The skin is one of the major targets of ROS attack since it is exposed to Ultraviolet (UV) radiation and a variety of environmental pollutants, high pressure of molecular oxygen and, in addition, is rich in polyunsaturated fatty acids. After unbalanced ROS exposure, resulting in lipid and protein modifications, the redox status of the intercellular milieu is shifted toward oxidizing conditions (Amaro-Ortiz *et al*., 2014).

Excess levels of ROS due to overproduction or because of insufficient scavenging generate oxidative stress, leading to injurious effects via: (1) oxidative modification and damage of biomolecules, altering lipid/protein/DNA structure and function; (2) further irreversible oxidation of reactive protein thiol groups which is hallmark of oxidative stress (Mailloux *et al*.,2012) and (3) dysregulation of cell signaling pathways (Lenaz *et al*.,2012) , triggering

downstream signaling cascades leading to altered cytokine release and exacerbation of inflammation. Combined, excess ROS lead to pathological changes in cells and tissues, as exemplified by inflammatory skin conditions like psoriasis (Garcia-Bailo *et al*., 2011).

Reactive oxygen species participate in the pathogenesis of many dermatologic diseases both as initiators being primarily involved in their pathology or being the secondary initiating agents generated during the respiratory burst of activated polymorphonuclear leukocytes (Kruk *et al*., 2014).

Ultraviolet radiation of sunlight has been demonstrated to generate ROS leading to oxidative stress in skin due to depletion of endogenous antioxidant enzymes. ROS generated by UV radiation primarily cause damage to DNA through oxidative modifications and mutations, but also by inducing expression of different genes, such as matrix metalloproteinases and collagenases, thereby affecting collagen integrity and skin aging (Amaro-Ortiz *et al*.,2014). Additionally, UV-mediated ROS generation also indirectly affects cellular function and survival via its effect on cell signaling pathways. For instance, activation of MAPK proteins occurs after UV exposure, suggesting that it may be responsible for executing the effects of UV-induced oxidative stress (Akasaka *et al*., 2010).

Reactive oxygen species may participate in the allergic reactions in the skin. It has been reported that nickel triggered allergic reactions in patients are characterized by an elevated level of free iron ions and decreased Glutathione/ glutathione disulfide (GSH/GSSG) ratio in the skin tissue, indicating the presence of oxidative stress (Sicherer *et al*., 2013).

Reactive oxygen has a paradoxical action on melanocytes because it not only enhances depigmentation, but also increases pigmentation in the skin. An example of melanocyte degeneration induced by oxidative stress is vitiligo, characterized by circumscribed depigmented macules in the skin. The skin of patients with vitiligo vulgaris contains high levels of Superoxide dismutase (SOD) and low levels of catalase (Venza *et al*., 2015). An imbalance of the ROS scavenging system results in the accumulation of H_2O_2 in the skin. H_2O_2 readily crosses the cell membrane and is therefore easily transferred to melanocytes from the keratinocytes. The transfer of H_2O_2 is thought to be one of the pathogenetic mechanisms of vitiligo.

ROS can also accelerate skin pigmentation.Keratinocytes adjacent to melanocytes intensively contribute to UV-induced skin pigmentation. Among ROS, NO^- derived from keratinocytes acts to induce melanogenesis by increasing the amount of the melanogenic factors tyrosinase and tyrosinase-related protein (Masaki *et al*., 2010).

Another important role of ROS is their participation in the skin aging. The free radical theory of aging is supported by finding that oxidative damage to biomolecules accumulates and increases with age. In the skin aging the process oxidative damage involves not only proteins, lipids and DNA but also is linked with alteration of the collagenous extracellular matrix in the dermis (Rinnerthaler *et al*., 2015).

The involvement of persistent oxidative stress in melanoma and non-melanoma skin cancers (known as the most prevalent cancers in humans) is due to the fact that ROS can activate oncogene by induction of proto-oncogenes, such as NF-κB, c-jun, c-fos and inactivate certain protease inhibitors. Activation of some transcription factors, like NF-γB, AP-1, p53, Hypoxia-inducible factor 1-alpha (HIF-1α) can lead to the expression of over 500 genes. The latter activity leads to increased proteases activity and rates of cells migration, thus to tumor progression and metastasis (Reuter *et al*. 2010).

1.2.3. Antioxidants in the skin

It has been known for some time now that the skin possesses an anti-oxidative system for oxidative stress reduction and maintenance of cellular redox balance. In order to cope with constant and excess efflux of damaging reactive metabolites, the skin has developed several lines of defense. In defense against oxidative stress, the skin engages all of these four mechanisms: reparation, prevention, stabilization and, as an important defense mechanism, antioxidant defense where enzymes and scavengers react directly with ROS, preventing them from reaching their biological target (Stojiljković *et al*., 2014). Their neutralizing capabilities reside in their ability to donate an electron toward off the deleterious effects of the highly reactive radicals or by converting ROS into different, less harmful, molecules (H Kashou *et al*., 2011).

Antioxidants can be categorized into primary antioxidants and secondary antioxidants. Primary antioxidants are water soluble and lipid soluble. SOD, Catalase (CAT) and Glutathione peroxidase are the primary antioxidant enzymes which inactivate the ROS into intermediates. Ascorbate, glutathione, uric acid etc. are water soluble, and lipids soluble are tocopherols, ubiquinols and carotenoids, etc (Poljsak *et al*., 2012).

Superoxide dismutase and Catalase are the most important antioxidant systems that protect the epidermis. SOD converts superoxide anions into hydrogen peroxide, whereas CAT catalyzes the decomposition of hydrogen peroxide to water and oxygen (Stojiljković *et al*., 2014). In addition to catalase, glutathione peroxidase (GPx) also breaks down H_2O_2 in the presence of the reduced form of glutathione (GSH). GPx also decomposes lipid hydroperoxides into their corresponding alcohols. Thioredoxin, a ubiquitous oxidoreductase enzyme, breaks down H_2O_2 in a NADPH-dependent reaction within cells. Metallothionine, a heavy metal ion-induced cysteine-rich peptide, also functions as a ROS scavenger (Masaki *et al*., 2010).

Secondary antioxidant enzymes are Glutathione reductase, Glucose-6-Phosphate dehydrogenase, glutathione-S-transferase and ubiquinone work directly to detoxify ROS by decreasing the peroxides level and continuously supplying the NADPH and glutathione for primary antioxidant enzymes to maintain their proper functioning. Copper, iron, manganese, zinc, selenium enhances the antioxidant enzyme activities (Klaunig *et al*., 2010).

These Secondary antioxidant enzymes are mainly derived from food and other dietary sources. Several herbs, spices, vitamins, foods, vegetables etc exhibits antioxidant activities. Flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins, isocatechins, epicatechin, etc found in natural foods are called phytochemicals. These groups of naturally occurring compounds can scavenge free radicals and thus they are effective as inhibitors of lipid peroxidation (Pandel *et al*., 2013).

1.2.4. Oxidative stress in the pathogenesis of psoriasis

Oxidative stress is believed to be a key factor in the pathogenesis of psoriasis. Studies have suggested the involvement of increased ROS levels in psoriasis pathogenesis. Increased ROS generation by infiltrated leukocytes into psoriatic lesions is accompanied by substantial biomolecular damage, like psoriatic skin lesions containing oxidized LDL. Indeed, a relationship between psoriasis severity, lipoprotein levels and oxidative damage has been proposed (Wagener *et al*., 2013).

Oxidative stress and inflammation are inextricably tied processes. Chronic inflammation is associated with elevated reactive oxygen species levels; anti-inflammatory cascades are linked to diminished reactive oxygen species concentrations. And the converse is true elevated oxidative stress triggers inflammation, whereas redox balance inhibits the cellular response. Thus, oxidative stress and inflammation may be seen as both causes and consequences of cellular pathology (Terlecky *et al*., 2012).

Reactive oxygen species are generated through different cellular pathways including calcium dependant pathway, protein tyrosine kinase, protein tyrosine phosphatase, serine threonine kinase, phospholipase, mitogen activated protein kinase, NF-κB, cytokines receptors, growth receptor, G–Protein coupled receptor, ion channel receptor and epidermal growth factor (Shafaq *et al*., 2012).

It is known that ROS, acting as second messengers, influence the cellular signal transduction pathways such as proinflammatory signaling pathways and modulate the expression of numerous genes. Dysregulation of several pro-inflammatory pathways, like MAPK/AP-1, NF-κB, and JAK–STAT signaling pathways have been regarded as early events in inflammatory disorders such as psoriasis (Armstrong *et al*., 2011). The activation of these signal transduction cascades triggers cell responses to growth factors, cytokines, neurotransmitters, and other intercellular signal molecules, leading to cell proliferation, differentiation, and apoptosis (Zhou *et al*., 2009).

1.2.4.1. MAPK\AP-1 pathway

Studies have demonstrated that ROS can induce or mediate the activation of the MAPK pathways. A number of cellular stimuli that induce ROS production also in parallel can activate MAPK pathways in multiple cell types. The prevention of ROS accumulation by antioxidants blocks MAPK activation after cell stimulation with cellular stimuli indicating the involvement of ROS in activation of MAPK pathways (Son *et al*., 2011).

ERK, p38, and c-Jun N-terminal kinase (JNK) pathways belong to a group collectively termed ‘‘MAPK signaling pathways’’. ROS are capable of inducing the ERK pathway in a variety of cell types, including epithelial, endothelial, cardio-myocytes, hepatocytes, and T lymphocytes. Compared to non lesional psoriatic skin, lesional psoriatic skin has increased ERK1/2 expression and treatment of psoriasis results in reduced ERK1/2 expression (Jiang *et al*., 2011).

TNF can induce cellular proliferation through TNF-receptor-associated factor 2 (TRAF2), Mitogen extracellular kinase kinase (MEKK1), and JNK, which results in c-Jun activation. Therefore, it has been hypothesized that, by activating c-Jun, JNK may mediate TNF overproduction and subsequent keratinocyte hyperproliferation in psoriasis. p38MAP Kinase (MAPK) participates in a signaling cascade that controls cellular responses to cytokines and stress. Compared to controls, lesional psoriatic skin had elevated levels of phosphorylated p38 MAPK (Armstrong *et al*., 2011).

1.2.4.2. NF-κB pathway modulation by ROS

IL-1, TNF-α and NK-κB are among the first receptors to generate ROS in non-phagocytic cells. TNF-α and oxidants may synergistically activate NK-κB by ROS independent mechanism (Shafaq *et al*., 2012). NF-κB, another redox-sensitive transcription involved in cellular processes like inflammation, cell proliferation and survival, has recently been demonstrated to be upregulated and active in psoriatic skin. Dysregulation of NF-κB-mediated signaling may further exaggerate disease severity, as NF-κB-mediated upregulation of pro-inflammatory cytokines activates NF-κB via a positive feedback loop.

Importantly, inhibition of NF-κB nuclear translocation and DNA binding activity dampens the inflammatory component of psoriasis (Wagener *et al*., 2013). Many of the cytokines involved in psoriasis pathology induce keratinocyte proliferation and these signals are via ROS-mediated action transmitted to transcription factors and associated proliferation pathways (Bito *et al*., 2012).

1.2.4.3. Transactivation between MAPK/AP-1 and NF-κB

Although ROS involved in the pathogenesis of psoriasis activates MAPK/AP-1 and NF-κB signaling pathways, there is compelling evidence showing the cross talk between these signal transduction pathways. As a downstream effector kinase of ERK1/2 and p38 MAPKs, Mitogen- and- stress-activated protein kinase 1 (MSK1) phosphorylates NF-κB, leading to the expression of multiple NF-κB-dependent genes that are involved in psoriasis. It may be assumed that activation of the transcription factors MAPK/AP- 1 and NF-κB by ROS as well as subsequent cross talk between these signaling transduction cascades triggers and replenishes the pathogenesis of psoriasis (Zhou *et al*., 2009).

1.2.4.4. JAK–STAT pathway modulation by ROS

The JAK-STAT pathways are central to a number of inflammatory and immune processes. For example, IL-22 in keratinocytes activates the STAT3 pathway, an important factor in psoriasis inflammation, resulting in increased ß-defensin 2/3 expression. Furthermore IFN-γ and IL-20 upregulate STAT1 which leads to a cascade of inflammatory mediators that contributes to the formation of psoriasis plaques (Armstrong *et al*., 2011).

Among the downstream genes of the JAK–STAT cascades, Inducible nitric oxide synthase (iNOS) is the most important one involved in the generation of oxidative stress contributing to the pathogenesis of psoriasis. It has been reported that iNOS is over-expressed in psoriatic lesions compared to uninvolved skin iNOS expression is mediated by the activation of transcriptional factors such as p38 MAPK , NF-κB , and JAK–STAT (Hsu and Armstrong, 2014).

1.2.4.5. Protein kinase pathways

Protein kinase C (PKC), whose activity is sensitive to the intracellular redox status, is a family of serine–threonine kinases. PKCζ is an atypical isoform and regulates multiple cellular functions. PKCζ mRNA and protein are increased in psoriatic lesions with membrane translocation of the phosphorylated form of PKCζ compared with uninvolved skin. Phospho-PKCζ can activate MAPK pathways and NF-κB by phosphorylating Reactive arthritis (RelA) p65 protein, crucial for its DNA binding after translocation into the nucleus to switch on the transcription of a variety of important proinflammatory molecules (Rundhaug *et al*., 2010). There from, redox-sensitive PKC can trigger the cytokines, chemokines, and adhesion molecules produced in psoriasis. Indeed, ROS stimulate cell proliferation and trigger undifferentiated keratinocytes to form a defective stratum corneum, an event central to the development of psoriasis. Abnormal keratinocyte growth in psoriasis, therefore, may be partially due to the elevated $O_2^{\bullet -}$ generated by Polymorphoneuclear (PMN) and psoriatic dermal fibroblasts (Zhou *et al*., 2009).

1.2.5. Evidence of oxidative stress in psoriasis patients

Malondialdehyde (MDA) is a stable end product of the peroxidation of membrane lipids by ROS, and, thus, it is used as an indicator of increased lipid peroxidation. Interactions between MDA and membrane components result in disturbed structure and function of cell membranes (Avci *et al*., 2014).

As a potent regulator of keratinocyte growth and differentiation, the multifunctional signaling molecule NO has been considered to be a strong candidate in the pathogenesis of psoriasis. This heat-labile and unstable compound is synthesized in endothelial cells as well as neurons by constitutive NO synthase (cNOS), while iNOS is found in leucocytes, macrophages, and mesengial cells (Singh *et al*., 2011).

A small amount of NO produced by cNOS in endothelium is responsible for the relaxation of adjacent smooth muscles and prevents adhesion of platelets and leucocytes to the endothelium. This is the anti-inflammatory effect of NO. However, when produced in large amounts NO can destroy tissues and impair immune response (El-Rahman, *et al*., 2014).

Notably, decreased antioxidant levels have been found together with increased levels of lipid peroxidation markers in blood of psoriasis patients. Also, serum levels of catalase were elevated in psoriatic patients and increased activity of superoxide dismutase (SOD) and expression levels of peroxiredoxin (Prdx) and glutathione peroxidase (GPx) have been found in psoriatic skin lesions. It is tempting to speculate that increased compensatory antioxidant levels counteract the skewed redox balance (Wagener *et al*., 2013).

In a study conducted with Egyptian population, erythrocyte concentrations of MDA and activity of CAT and SOD in blood samples of 34 patients with psoriasis and 30 healthy subjects. Statistically significant higher levels of plasma MDA and CAT were detected in patients when compared with control subjects. No significant correlations with severity of psoriasis were found. Levels of MDA were positively correlated with levels of CAT in psoriatic patients. Erythrocyte SOD levels were significantly lower in patients than in controls and negatively correlated with severity of the disease but insignificantly correlated with levels of MDA in psoriatic patients (Abdel-Mawla *et al*., 2013).

An Indian case control study with a total of 50 patients with confirmed diagnosis of psoriasis before starting treatment and 25 healthy controls showed that higher levels of MDA have been observed in Psoriasis patients when compared to controls (Nagamani *et al*., 2015). In another Indian study, Priya and colleagues also showed a significant role of ROS in pathogenesis of psoriasis where in 40 psoriasis patients between age group of 25 – 60 the levels of serum MDA and NO were significantly increased and SOD and Total Antioxidant Capacity (TAC), were decreased as compared to 40 normal controls subjects (Priya *et al*., 2013).

In a case-control study from Romania with 34 psoriasis patients in which all had active disease and none of them received any treatment the susceptibility of erythrocyte to lipid peroxidation (expressed as manoldialdehyde concentration) was found higher compared to 32 healthy volunteers (Boda *et al*., 2013).

In a separate Turkish study, the total oxidant and oxidative stress index levels of 40 psoriasis patients was found higher than the corresponding parameters in 47 healthy control groups. The total antioxidant level was significantly lower (Sürücü, *et al*., 2015). In another Turkish case-control study, evaluating 23 patients with active psoriasis found a significantly higher Plasma levels of NO and MDA levels than those in controls (Şikar *et al*., 2011).

Multiple measures of ROS were affected in a cross-sectional case-control study, showing increased levels of serum MDA and significantly decreased serum vitamin E as well as erythrocyte catalase activity in a total 90 psoriasis patients without any drug therapy for preceding two months as compared to 90 matched healthy controls (Pujari *et al*., 2014).

1.3. Statement of the problem

Skin problems are generally the most common diseases seen in primary care setting all over the globe and its prevalence ranges from 20-50% in developing countries. Compared with other diseases, skin diseases have a lower mortality rate but can affect the wellbeing, quality of life and health conditions (Baur *et al*., 2013). In Ethiopia, according to some studies, up to 80% of people have one or more skin diseases. It was found that skin diseases represented the sixth most frequent cause of outpatient visits to health care facilities nationwide. Many elements influence the pattern and burden of skin diseases in resource-poor settings, including poverty, environmental, and climatic factors (Gimbel *et al*., 2013). Psoriasis is one such type of a disease among various skin diseases affecting more than 125 million people, or nearly 3% of the world's population (IFPA 2015). Unfortunately, Ethiopian specific information regarding the true epidemiology of psoriasis is sadly lacking. This suggests that we are inadequately informed to manage a silent epidemic with considerable societal impact, both socially and economically.

Certainly, evidence has shown that psoriasis can affect a patient's quality of life to a level comparable with other chronic conditions, including myocardial infarction and some cancers. It has even been independently associated with suicidal ideation. Though it has long been considered that, with psoriasis, "torture is skin deep", evidence has been mounting that it is a systemic inflammatory disease with an increased risk of mortality when severe (Oliveira *et al*., 2015). The pathogenesis of psoriasis is not fully understood but evidence suggests that there is a strong genetic component which is mediated by abnormal T lymphocyte function. Recently studies have suggested the involvement of increased reactive oxygen species levels in pathogenesis of psoriasis (Mantovani *et al*., 2016).

As far as the investigator is aware, no previous study exists in Ethiopia about the status of oxidative stress among psoriatic patients. Therefore, this study was designed to evaluate the total oxidant–antioxidant capacity and oxidative stress index among newly diagnosed psoriatic patients in Alert Hospital Addis Ababa.

1.4. Significance of the study

Although psoriasis is rarely life threatening, its morbidity and associated co-morbidities have a severe negative impact on the quality of life of the patients and also confer a certain socioeconomic burden. Especially in developing countries, people with psoriasis have to face severe problems with stigmatization, embarrassment, discrimination, low self-esteem and negative attitudes in general among the public, and often bear the brunt of public rejection.

Until recently, it was difficult to treat psoriasis, due to incomplete understanding of the factors behind pathogenesis of psoriasis which points out that there may be some gap in our understanding of etiopathology of psoriasis. Oxidative stress is believed to be a key factor in the pathogenesis of psoriasis as studies have suggested the involvement of increased ROS levels in psoriasis pathogenesis however it is questionable whether the observed abnormalities are responsible for the onset of psoriasis, or resultant from ongoing pathologic process. Therefore, the hypothesis of an imbalance between oxidants and antioxidants in psoriasis and its role in the pathogenesis of the disease still remain to be elucidated.

Based on increasing awareness of the importance of oxidative stress in skin diseases and the limited knowledge of the Pathophysiology , the research was conducted to understand the status of oxidative stress in a cross sectional participants diagnosed with psoriasis. This is based on an assumption that oxidative stress as one of the important factor in pathogenesis of psoriasis. Evaluation of oxidative stress therefore will provide early indication of the disease progression and institution of possible treatment for psoriatic patients to prevent the development of the associated morbidity and mortality. Besides, it can also serve as baseline information to undertake further studies on similar settings in the future.

1.5. Hypothesis

The level of oxidative stress is not associated with severity of psoriasis.

2. OBJECTIVE

2.1. General objective

To evaluate the status of Total Oxidant - Antioxidant Capacity and Oxidative stress index in Psoriatic patients.

2.1.1. Specific objectives

1. To assess the status of Total Oxidant Capacity in Psoriatic patients.
2. To assess the status of Total Antioxidant Capacity in Psoriatic patients.
3. To estimate Oxidative stress index in Psoriatic patients.
4. To assess the correlation of Total Oxidant - Antioxidant Capacity and Oxidative stress index with severity of Psoriatic patients.
5. To investigate the correlation between Total Oxidant and Antioxidant Capacity and Oxidative stress index in Psoriatic patients.
6. To investigate the correlation between Total Oxidant and Antioxidant Capacity and Oxidative stress index with severity of Psoriatic patients.
7. To investigate the correlation between psoriasis disease duration and the status of Total Oxidant - Antioxidant Capacity and Oxidative stress index in Psoriatic patients.

3. MATERIAL AND METHODS

3.1. Study design and period

This study was a comparative Cross sectional study conducted during the period of September 2015 to January 2016.

3.2. Study area and study subjects

The study was conducted in ALERT Hospital which is located in kolfe keranio kiefle ketema and the analysis of data collected was done at the Department of Biochemistry, College of Health Sciences Addis Ababa, Ethiopia. A total of 90 participants comprising 45 newly diagnosed psoriatic patients in ALERT Hospital and who had given informed written consent and 45 healthy controls were enrolled in the study.

3.3. Inclusion criteria

Subjects who fulfilled the following criteria were involved in the study

1. Age between 18-65 years.
2. All patients who signed written informed consent
3. Newly diagnosed Patients confirmed with psoriasis

3.4. Exclusion criteria

Patients with the following conditions were excluded from the study.

1. Patients who had received any systemic or any local steroid medication or any phototherapy treat
2. Patients who don't signed written informed consent
3. Patients with diabetes, hypertension, family history of hyperlipidemia, renal and liver failure, endocrine disorders
4. Smokers and alcoholism (characterized by: a prolonged period of frequent, heavy alcohol use).

3.5. Sample size determination

A convenience sampling technique was used to determine the sample size. When calculating the sample size requirements for study, a number of factors are taken into consideration including effect size, cooperation and attrition, practical constrains such as time, subject availability and finance, subgroup analysis and sensitivity of the measurement used (Suen *et al*., 2014)

Many studies are based on relatively small sample size due to practical constraints such as time and subject availability, which often limits sample size. Examining the sample size of other comparable studies carried out internationally assessing oxidant-antioxidant levels in psoriasis sample size varies from 23 to 50 in these studies. However, power analysis and sample size calculation was not reported in any of these studies.

Based on the above reasons the present study recruited 45 psoriatic patients and 45 age-sexes matched healthy controls from Alert Hospital, Addis Ababa, Ethiopia. Psoriasis patients were graded and further divided into three groups on the basis of Psoriasis area and Severity index (PASI). Therefore, they were grouped as mild, moderate and severe.

3.6. Study variables

3.6.1. Dependent variables

- Total antioxidant capacity (TAC)
- Total oxidant capacity (TOC)
- Oxidative stress index (OSI)

3.6.2. Independent variable

- Age
- Sex
- BMI
- PASI
- Psoriasis disease duration

3.7. Ethical Consideration

All participants in study had information on study. A consent form was prepared with detailed explanation of objectives, risks, and benefits to the study subjects and the assurance of confidentiality of responses were given to participants. Data were collected after obtaining informed consent and agreement from the patients under study. Sample collection was performed by trained health professionals following ethical steps and procedures.

Only patients who had signed the informed consent were enrolled in the study. Strict confidentiality of all information collected was maintained and all protocols of safety were followed throughout the study. Assurances were given to the participants on confidentiality of collected data. Ethical approval was given for research from ethical committee of the Department of Biochemistry (DRERC) with a protocol number of 04/15 DRERC, and ALERT/AHRI ethics review committee /AAERC/ with a protocol number P011/15.

3.8. Data and Specimen collection handling and storage

Data was collected by professional laboratory technologists, nurses and or physicians with involvement of principal investigator. Under preceding instructions psoriatic patients and healthy controls were checked regarding fasting by interview on the morning of the examination by the physician or Nurse. Pertinent information was obtained from standardized clinical files designed for the program and extracted from it using a questionnaire and other demographic details were subsequently collected (See appendix 1).

5 ml of fasting venous blood samples were obtained by vein puncture from vein of the arm of each participant using sterile syringes and transported into anti-coagulant free blood collection vacutainer tubes and allowed to stand for 30 min to coagulate. After centrifugation at 3000 rpm for 10 minutes the serum was separated by sterile by sterile pipette and transferred to 3ml epyndrophs tests tube and was incubated at -80 ^{0}C until analyzed. The serum was used for the analysis of Total Antioxidant capacity (TAC), Total Oxidant capacity (TOC) and Oxidative stress index (OSI).

3.9. Biochemical assays and Laboratory analysis

3.9.1. Determination of Total Oxidant Capacity (TOC)

Principle

Absolute oxidant status of plasma was measured by robotized colorimetric estimation way for Total oxidant capacity (TOC). In this process, oxidants present in the plasma were determined by oxidation of ferrous particle by o-dianisidine complex to ferric complexes. The oxidation reaction is upgraded by glycerol molecules, which are richly present in the reaction medium. A colored compound is formed when the ferric ion reacts with xylenol orange in an acidic medium. The color strength, which was measured by ELISA microplate reader LT 4000, was correlated to the total quantity of oxidant molecules present in the plasma. The assay was calibrated with hydrogen peroxide, and the results were expressed as mmol H_2O_2 Equiv./l. (Erel, 2005).

Procedure

Reagent 1 and 2 were prepared. Reagent 1: [114mg of xylenol orange and 8.18gm of NaCl] were dissolved in 900mL of H_2SO_4 solution 25mM. The final reagent was composed of 150mM xylenol orange, 140mM NaCl, and 1.35M glycerol, pH 1.75; Reagent 2: [1.96 gm of ferrous ammonium sulfate and 3.17 gm of o-dianisidine dihydrochloride] were dissolved in 1000mL of H_2SO_4solution 25mM. TOC of serum was measured by adding 225µL reagent 1 into 35µL serum sample and then 11µL of reagent 2 was added. Finally absorbance was measured by ELISA microplate reader LT 4000 at wavelength 560 nm.

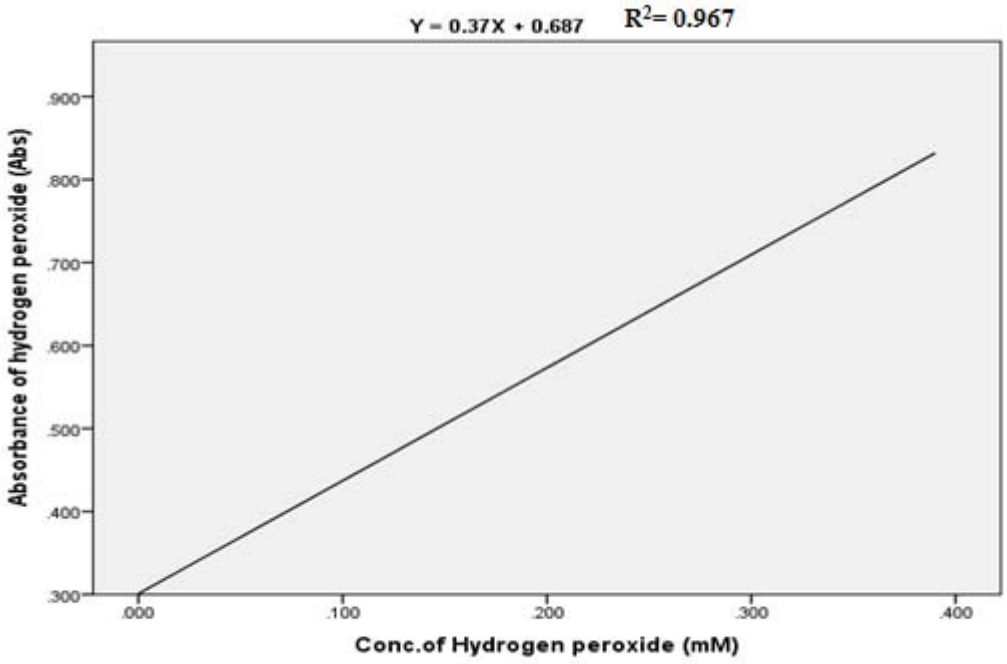

Figure 3.1: Standard curve for estimation of Total oxidant Capacity (TOC)

3.9.2. Determination of Total Antioxidant Capacity (TAC)

Principle

Total antioxidant capacity of plasma was measured by robotized colorimetric estimation system for Total antioxidant capacity (TAC). In this technique, the hydroxyl radical, the most powerful natural radical, was generated by the Fenton reaction and it responds with the colorless substrate O-dianisidine to create the dianisyl radical, which is splendid yellowish-brown in color. Upon the addition of a plasma sample, the oxidative responses started by the hydroxyl radicals present in the reaction are scavenge by the antioxidant agents of the plasma, keeping the color change and consequently giving a viable estimation of TAC. The assay was calibrated with Trolox, and test results were expressed as mmol TroloxEq/l (Koracevic *et al.,* 2001).

Procedure

For measurement of total antioxidant capacity reagent 1 and 2 were prepared. Reagent 1: 3.17gm of orthodianisidine dichloride and 0.01764gm of $Fe(NH_4)_2(SO_4)_2.6H_2O$ were dissolved in 1000mL of clark and Lubs solution. Reagent 2: 0.641mL of H_2O_2 solution (35%) was diluted in 1000ml with clark and lubs solution. TAC of serum was measured by adding 200 µL reagent 1 to 5 µL serum sample and then 20 µL of reagent 2 was added. Finally absorbance was measured by ELISA microplate reader LT 4000 at wavelength 660 nm.

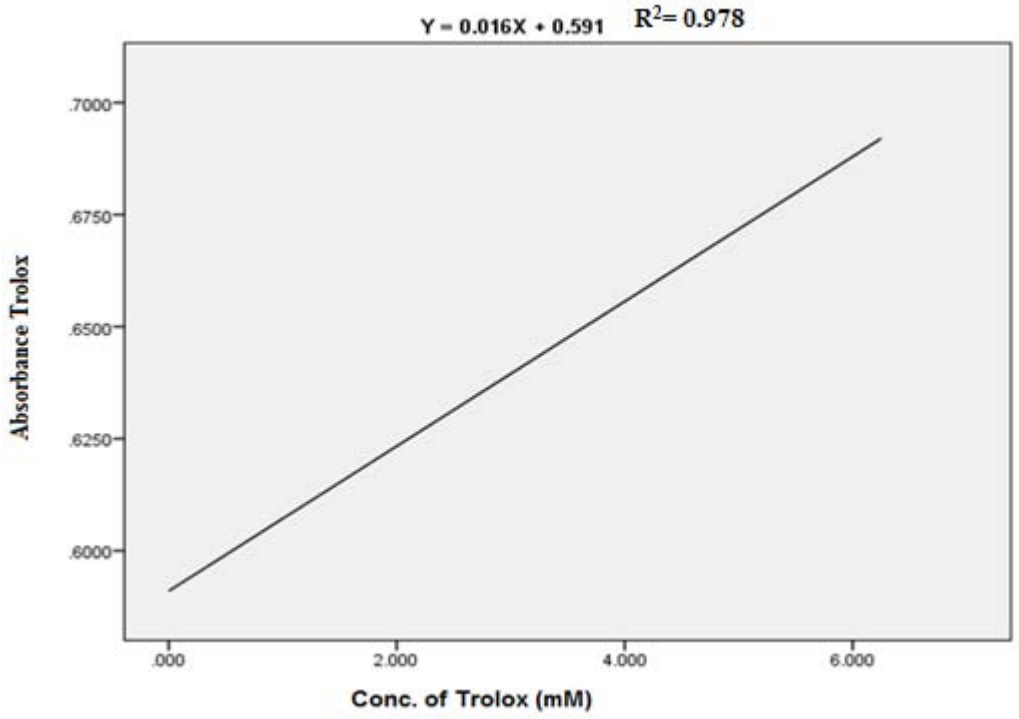

Figure 3.2: Standard curve for estimation of Total antioxidant capacity (TAC)

3.9.3. Measurement of oxidative stress index (OSI)

The TOC/TAC ratio provides the OSI, which is an indicator of the degree of oxidative stress. OSI value was calculated using the following formula: OSI (arbitrary unit) = TOC (mmol H_2O_2 equivalent/l)/TAC (mmol Trolox equivalent/l) (Erel et al., 2005).

3.10. Statistical Analysis

All data was expressed as mean ±SD. The statistical significance was evaluated by one way ANOVAs. All the data were entered to Epi Info (version 3.5.1, 2008) and statistical analysis was performed by using SPSS (version 20.0, 2007, America). While post hoc was used to assess statistical comparisons between groups. Correlations between variables were calculated by Pearson's correlation test. A P-value of less than <0.05 was considered statistically significant.

3.11. Dissemination of results

Clinical findings of the study will be submitted and presented to Department of Biochemistry, Addis Ababa University. Based on the findings conclusions and recommendations will also forwarded to Executive bodies and organizations for further action.

3.12. Quality control

The English version of the questionnaire was translated into the local language, Amharic. The data collectors were professional nurses and they were trained about data collection procedure and during the data collection the patient history and other information from the patients' medical record cards were cross checked. The questionnaire was pre-tested to check consistency a week before the actual data collection was done.

4. RESULTS

4.1. Demographic characterization

Among 90 participants 45 of them were psoriatic patients (24 males and 21 females) with mean age 39.69 ± 12.67 and BMI of 21.84 ± 2.63. The rest of 45 were age-and sex- matched healthy subjects as a control group (26 males and 19 females) with mean age 38.53 ± 12.05 and BMI of 23.38 ± 2.79.The clinical severity was determined according to the Psoriasis Area and Severity Index (PASI) with mean score 20.08 ± 16.94. Psoriasis patients were further graded according to the PASI as, mild (22 patients), moderate (15 patients) and severe (8 patients). Disease duration of the patients ranged from 3 weeks to 5 years with mean 2.39 ± 1.61 (Table 4.1).

Table 4.1: Demographic characteristics and clinical features of psoriatic patients and healthy Controls

	Healthy Controls(n= 45)	Psoriatic Patients (n=45)
Sex [M/F]	26/19	24/21
Age [years]	38.53 ±12.05	39.69 ±12.67
Height[m]	1.60 ± 0.09	1.62 ± 0.08
Weight[kg]	60.13 ± 8.86	57.53 ± 9.36
Body mass index [kg/m^2]	23.38 ± 2.79	21.84 ± 2.63
PASI		20.08 ± 16.94
Mild		22(48%)
Moderate		15(33%)
Sever		8(17%)
Type of lesion		
Plaque		38(84%)
Erythema		1(2%)
Papules		3(6%)
Pustule		3(6%)
Psoriasis duration [years]		2.39 ± 1.61

Results presented as mean ± SD and frequency (%) (Except male: female ratio)

4.2. Estimated biochemical parameters

4.2.1. Total oxidant- antioxidant between psoriasis and health control

Total oxidant capacity (TOC) and oxidative stress index (OSI) (13.94±2.28, 1.32±0.59) were significantly ($p<0.01$) higher in psoriatic patients compared to control subjects (11.60 ±1.43).On the other hand, the levels of Total Antioxidant capacity (TAC) were significantly decreased ($p<0.01$) in psoriatic patients (1.15±0.21) compared to normal healthy controls.(1.36±0.16) (Table 4.2).

Table 4.2: TOC, TAC and OSI levels in patients versus controls

Parameter	Healthy Contorts (n=45)	Psoriatic Patients (n=45)	*p*-value
TOC [mmol H_2O_2 eqv./l]	11.60 ±1.43	13.94±2.28	<0.01
TAC [mmol Trolox eqv./l]	1.36±0.16	1.15±0.21	<0.01
OSI [AU]	0.86±0.15	1.32±0.59	<0.01

Results presented: as mean ± SD, TAC – Total Antioxidant capacity, TOC – Total Oxidant capacity, OSI – Oxidative Stress Index.

4.2.2. Total oxidant- antioxidant status and severity of disease

Present study shows that, TOC and OSI levels were significantly (P<0.01) increased in moderate psoriatic patients than the patients presenting with mild psoriasis, whereas, severe psoriatic patients exhibited significant increase in TOC and OSI (P<0.001) as compared to moderate psoriatic patients. Furthermore, TAC activity in moderate psoriatic patients were significantly (P<0.01) lower than the patients presenting with mild psoriasis, whereas, severe psoriatic patients exhibited significant decrease in TAC (P<0.01) as compared to moderate psoriatic patients (Table 4.3).

Table 4.3: Changes in levels of TOC, TAC and OSI within different groups of psoriasis patients

Parameters	Control(n=45)	Psoriatic patients (n=45)		
		Mild(n=22)	Moderate(n=15)	Sever(n=8)
TOC [mmol H_2O_2 eqv./l]	11.60 ±1.43	12.47 ± 1.11^{b1c2}	14.03 ± 1.52^{a2c2}	17.81 ± 0.62^{a2}
TAC[mmol Trolox eqv./l]	1.36±0.16	1.29 ± 0.52^{b1c2}	1.14 ± 0.04^{a2c2}	0.76 ± 0.16^{a2}
OSI [AU]	0.86±0.15	0.96 ± 0.10^{b1c2}	1.26 ± 0.16^{a2c2}	2.43 ± 0.57^{a2}

Results presented as mean ± SD, TAC – Total Antioxidant capacity, TOC – Total Oxidant capacity , OSI – Oxidative stress index ; [a]compared to control, [b]compared to moderate, [c]compared to severe;, [1]p<0.01, [2]p<0.001

4.2.3. Psoriasis disease duration and changes in Total oxidant- antioxidant status

Disease duration of psoriatic patients were ranged from 3 weeks to 5 years with mean 2.39 ± 1.61(Table 4.1) and further categorized into <1, 1-3 and > 3 years. Psoriatic patients with a year duration greater than 3 years have showed a significant increase in TOC and OSI (*P* <0.001) with a decreased TAC levels when compared to patients with a year duration less than a year and patients between 1 to 3 years (Table 4.4)

Table 4.4: Psoriasis disease duration and changes in levels of TOC, TAC and OSI in psoriatic patients.

Parameters	Psoriasis disease duration [years]		
	<1	1-3	>3
TOC [mmol H_2O_2 eqv./l]	12.41±1.23[c1]	12.39±0.87[c1]	15.86±1.89 [ba]1
TAC [mmol Trolox eqv./l]	1.29±0.05[c1]	1.25±0.08[c1]	0.98±0.21 [ba]1
OSI [AU]	0.96±0.11[c1]	0.1±0.08[c1]	1.75±0.68 [ba]1

Results presented as mean ± SD, TAC – Total Antioxidant capacity, TOC – Total Oxidant capacity , OSI – Oxidative stress index ; [a]compared to <1, [b]compared to 1-3, [c]compared to >3; [1]p<0.001

4.2.4. Correlations between Total oxidant –antioxidant in psoriatic patients

The levels of TOC with TAC showed a negative correlation with significant ($p<0.001$) association. On the other hand TOC showed a positive correlation with levels of OSI with significant ($p<0.001$) association in psoriatic patients (Table 4.5).

Table 4.5: Correlations between TOC levels and TAC, OSI levels in psoriatic patients

Parameter	r	*P-value*
TOC and TAC	-0.81	<0.001
TOC and OSI	0.86	<0.001

*Total Antioxidant capacity, TOC – Total Oxidant, OSI – Oxidative Stress Index, **p<0.001, Results were expressed in pearson correlation coefficient (-) negative and (+) Positives correlation*

(a) r =-0.81P= <0.001 (b) r= 086 p= <0.001

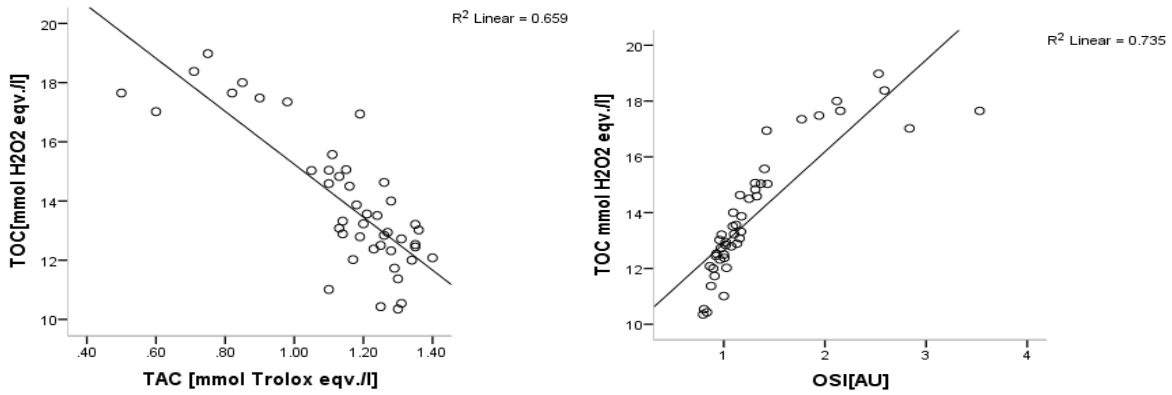

Figure 4.1: Regression fit of (a) TOC Vs TAC and (b) TOC Vs OSI

4.2.5. Correlations between Total oxidant –Antioxidant and severity of disease

It was found a negative correlation between TOC and TAC but it was not significant (p> 0.05) among mild, moderate and severs psoriatic patients. Conversely a positive correlation was found between TOC and OSI among the three groups with significant correlation in mild and moderate (p<0.001) whereas, severe psoriatic patients exhibited insignificant (P>0.05) correlation (Table 4.6).

Table 4.6: Correlations between TOC levels and TAC, OSI levels among the different groups of psoriasis patients

	Mild	Moderate	Severe
TOC and TAC	-0.22[a]	-0.047[a]	-0.018[a]
TOC and OSI	+0.927[b]	+0.949[b]	+0.065[a]

Results were expressed in pearson correlation coefficient (-) negative and (+) Positives correlation among variables a -p>0.05, [b]p<0.001

5. DISCUSSION

Skin cells are a major target of oxidative stress mainly due to ROS originating from the environment and skin metabolism itself. Although endogenous antioxidants attenuate the harmful effects of ROS, increased or prolonged presence of free radicals can override ROS defense mechanisms and mediate numerous cellular responses that contribute to the development of a variety of skin disorders, including psoriasis (Zhou *et al*., 2009).

The histological studies of epidermis of psoriasis patients revealed that polymorphonuclear leukocytes get infiltrated in it. These polymorphonuclear leukocytes might be releasing cytokines like TNF-α and interleukins in the surrounding tissues, for which ROS are known to be the mediators of their action. This in turn might trigger the inflammatory reactions that are the basis of psoriasis (Balato *et al*., 2012).

Generation of ROS from neutrophils, keratinocytes and fibroblasts can contribute to neutrophil activation which may play an important role in psoriatic process. ROS can act as second messengers in the induction of several biological responses such as the activation of NF-kB or AP-1, the generation of cytokines, the modulation of signaling pathways and the activation of peroxisome proliferator-activated receptors (shafaq *et al.,* 2012). It is now known that psoriasis can occur due to abnormalities in essential fatty acid metabolism, lymphokine secretion, free radical generation, lipid peroxidation and eicosanoid metabolism. Toxic effects of free oxygen radicals induce through production of lipid peroxidation and the reduction of natural antioxidants. An impaired antioxidant skin barrier may result in an increase of free oxygen radicals in psoriatic plaques (Ala *et al*., 2013).

Although there have been extensive studies on the roles of serum oxidants and antioxidants levels in psoriasis, their importance in the etiology or in the enhancement of the disease remains controversial (Gurupadappa *et al.,* 2011).

The present work investigated the possible role of oxidative stress in the pathogenesis of psoriasis through determination of serum TOC, TAC and OSI and their correlations with disease severity as expressed by PASI score in 45 psoriatic patients and age and sex matched 45 healthy individuals.

Oxidant generation is part of normal human metabolism and host defense mechanism, however when produced in excess oxidants and their products are known to play an important role in the pathogenesis of chronic inflammatory disorders (Bouayed and Bohn, 2010). Many free radicals are highly reactive and can either donate an electron to or extract an electron from other molecules therefore behaving as oxidants or reductants. The most important free radicals in many diseases are oxygen derivatives, particularly superoxide anion and the hydroxyl radical. The hydroxyl radical or a closely related species is probably the final mediator of most free radical induced cellular damage (Rahman *et al.,* 2012).

It is believed that in addition to genetic predisposition, ROS and mediated oxidative stress may play a role in the pathogenesis of inflammatory skin diseases, such as psoriasis. It is also believed that the ROS produced by keratinocytes, fibroblasts and endothelial cells causes chemotaxis and accumulation of neutrophils, as a result there is a production of superoxide in phagocytic reactions in psoriatic lesions (Güven *et al.,* 2013). Overproduction of ROS during the inflammatory process in psoriasis may result in oxidative stress leading to damage of cellular structures such as lipids, proteins and DNA and interference with production of cytokines such as IL-1 and TNF-α. It is also responsible for a decrease in the cAMP/cGMP ratio leading to epidermal hyperproliferation (Nassiri *et al.,* 2009).

The present work shows a significantly ($p<0.001$) increased concentration of TOC was observed in psoriatic patients (13.94±2.28) compared to healthy controls (11.60 ±1.43) (Table 4.2). This significantly elevated TOC could be due to high free radical load and insufficient antioxidant which consequently indicates a high oxidative stress condition in psoriatic patients. In concordance with this study, Sürücü *et al,.*(2015), Karababa *et al.,* (2013)*,* Gabr *et al*.,(2012) and Hashemi *et al*.,(2010) have reported increased TOC levels in psoriatic patients as compared to healthy controls.

There are compelling evidences which indicate that oxidative stress drives the production of oxidation products, such as MDA, which can denature proteins, alter apoptosis, and influence the release of proinflammatory mediators, such as cytokines, which may be critical for the induction of some inflammatory skin diseases (Aly and shahin, 2010).

This argument is substantiated by reports sikar *et al*., (2012), Mohamed (2014), Pujari *et al.,* (2010), Attwa and Swelam, (2011) which showed that psoriatic patients are normally in oxidative imbalance as a consequence of elevated peroxidation products like MDA.

The infiltrated and activated leukocytes might lead to release of ROS via processes like respiratory bust. Polymorphoneuclear (PMN) leukocytes have the potential to damage surrounding tissue by releasing superoxide anion radical produced via NADPH oxidase/myeloperoxidase which further gives rise to other activated oxygen species which all together were known to induce lipid peroxidation (Andrade *et al.,* 2012). ROS in turn also stimulates PMN recruitment by increasing PMN adhesion to endothelium. The increased generation of ROS, by increased infiltration and activation of PMNs might target cellular polyunsaturated fatty acids for lipid peroxidation, which might be the indicative factor for increased concentration of MDA in serum of psoriatic patients (Kiran *et al*., 2015).

Nitric oxide is a gaseous free radical that is released by the family of NO synthetase enzymes. It is a potent vasodilator, thus contributing considerably to the cardinal signs of inflammation. It is also known to exhibit cytotoxic effects in human skin (Aly and shahin, 2010). As a potential regulator of keratinocyte growth and differentiation, the multifunctional signaling molecule $NO^{\bullet}$ has been considered to be a strong candidate in the pathogenesis of psoriasis. $NO^{\bullet}$ is an important marker of inflammation. Expression of iNOS is involved in the pathogenesis of cutaneous inflammation in psoriasis which is confirmed by increase in mRNA expression of iNOS in skin lesions as compared to uninvolved skin as a result nitrite levels and nitrite–nitrate ratios appear to be good indicators for the increased $NO^{\bullet}$ production in psoriatic patients(Kadam *et al.,* 2010). This concept is supported by Sikar *et al.,* (2012) and Kadam et al., (2010) who demonstrated significantly elevated serum NO levels in different types of psoriatic patients when compared to normal individuals indicating deranged level of oxidative stress in psoriatic patients.

Results of the present study showed that the values of serum TOC were significantly higher ($p<0.001$) in all psoriasis patients than normal healthy controls. It was further found that the concentrations of serum TOC were increased with severity of the psoriasis (Table 4.3). The values of TOC were significantly higher in, moderate psoriatic patients than mild psoriatic patients ($p<0.001$) as well as severe psoriatic patients than moderate psoriatic patients ($p<0.001$) respectively and also the research considered increased TOC levels as psoriasis year duration increases ($P <0.001$) thereby confirming that the load was decidedly higher with the progression of psoriasis (Table 4.4). In very early phase of developing psoriasis lesions, macrophages were seen within the epidermis followed by lymphocytes. During subsequent development neutrophils began to appear between the upper layers forming pockets (Micro-abscesses). Neutrophil migration into the epidermis was most pronounced in active disease and occurred in a rhythmic pattern (Pujari *et al*., 2014). These presumptions were well supported by findings of Attwa and Swelam, (2011) and Gurupadappa *et al.,* (2011).

Skin cells are equipped with both enzymatic [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GP)] and non-enzymatic mechanisms (vitamin C, vitamin E, carotenoids, thiol antioxidants, flavinoids, selenium and others) to deal with the harmful effects of ROS (Şikar *et al.,* 2012). TAC is a biochemical parameter suitable for evaluating known and unknown antioxidants and their synergistic interaction and consumption by normal or increased levels of ROS production therefore assessed giving an insight into the delicate balance between oxidants and antioxidants in living cells (Bansal and Bilaspuri, 2010). Its value expresses the number of antioxidant molecules present in serum. An individual antioxidant level or activity indicates the antioxidant characteristics of only one antioxidant, whereas TAC may represent the total antioxidant characteristics of all antioxidants found in the serum (Kayar *et al.,* 2015).

Antioxidants can protect the epidermis from the events that contribute to epidermal toxicity and diseases. Deficiencies in any of the antioxidant defense system can cause a reduction in the total antioxidant status of an individual (Wagener *et al.,* 2013).

The results of this study showed statistically significant ($p<0.001$) differences in TAC values (1.15±0.21) between psoriatic patients and healthy controls (1.36±0.16) (Table 4.2). This is in concordance with the studies of Sürücü *et al,.*(2015), Karababa *et al.,* (2013), Gabr *et al.,*(2012) and Hashemi *et al.,*(2010) which suggest that antioxidant defense mechanisms are exhausted in psoriatic patients. The decreased TAC may be possibly due to depressed state of antioxidant system or due to the exaggerated inflammatory processes and oxidative stress in these patients. Antioxidants prevent oxidative injury of structural lipids and proteins contributing to barrier integrity, which is essential for healthy skin condition. This suggests that cellular redox environment plays a pivotal role in skin homeostasis and that skin disease could result from an imbalance between pro-oxidant and antioxidant stimuli (Galassetti *et al.,* 2012). However, in a study performed by Nassiri *et al.,* (2009) and his colleagues found no significant difference in serum TAC levels between cases and controls. They explained this by the younger age of Iranian patients and also because Iranian regular diet which contains more fruit and vegetable and less saturated fatty acid in comparison with western diets.

One important line of defense is a system of enzymes, including glutathione peroxidase, superoxide dismutase (SOD) and catalase (CAT) which decreases the concentration of the most harmful oxidants. During oxidative stress there can be the overload of superoxide radicals in the psoriasis (Benedetti *et al.,* 2012). Since, the natural antioxidant defense system SOD limits the development of inflammatory and immune processes, the tendency to decreased SOD activity in psoriasis patients is one of the reasons for the aggravated dermatological status. This over formation of the superoxide radical also was proposed to inhibit the activity of catalase (Shamsi *et al.,* 2010). This might be one of the reasons for significantly low serum vitamin E, CAT and SOD activities in psoriatic patients compared to healthy controls which are explained by the work of Reshma *et al.,* (2011), Mohammed *et al.,* (2014) and Pujari *et al.,* (2014).

The present study serum TAC levels were decreased significantly in; moderate psoriasis patients than mild psoriasis patients ($p<0.001$) whereas, severe psoriatic patients exhibited significant decrease in TAC activity ($P<0.001$) as compared to moderate psoriatic patients (Table 4.3).

Moreover a decreased TAC levels was observed as psoriasis year duration increases (P<0.001) (Table 4.4). The low TAC level can be explained with excessive depletion of the antioxidant capacity caused by free radical elimination to reduce the detrimental effects of ROS and their products in order to maintain pro-oxidant-antioxidant homeostasis. Pujari *et al* (2011) also found positive correlation between increased MDA, NO in serum of psoriatic patients with severity of psoriasis whereas negative correlation between decreased TAC with severity of psoriasis. The reduced level of TAC may reflect a decrease in the antioxidant capacity.

When the oxidant/antioxidant balance is tilted towards oxidants and oxidative stress arises, there is a significant negative correlation between the TAC and TOC values (Kayar *et al.,* 2015). In the present investigation a negative correlation was demonstrated between the TAC and TOC, which is one of the scales indicating the severity of psoriasis. This corresponds to previously reported findings (Sürücü *et al,.*(2015) and Karababa *et al* (2013). These findings may indicate the importance of the anti-oxidant system in the pathogenesis and severity of psoriasis.

Oxidative stress index (OSI), a proportional value between TAC and TOC, is a recently investigated parameter that is affected directly by oxidant and antioxidant status and that can present the degree of oxidative stress more clearly. Because OSI serves as an element of balance, its use is likely to be more important than TAC and TOC values in interpreting the study results. Therefore, OSI may be a useful and practical parameter for evaluating oxidative injury in psoriatic patients (Kose *et al.,* 2014).

The OSI estimated in this study was significantly (p<0.001) higher in psoriatic patients (1.32±0.59) than in healthy controls (0.86±0.15) (Table 4.2). This indicates the exact degrees of imbalance of Oxidative stress towards the oxidant status. This finding is in keeping with most previously published findings as Gabr and Al-Ghadir (2012) and Hashemi *et al.,* (2010) have reported high OSI values in psoriasis patients compared with control groups. On the basis of these results, we can deduce that a compromised antioxidant defense mechanism, accompanied by increased oxidant levels and OSI values in psoriasis patients, might play an important role in the pathogenesis of this disease.

Present study showed that there was statistically significant increase in OSI value in mild ($P<0.01$), moderate ($P<0.01$) and severe ($P<0.01$) psoriasis patients when compared to healthy controls. In addition, OSI in moderate psoriatic patients were significantly ($P<0.01$) lower than the patients presenting with mild psoriasis, whereas, severe psoriatic patients exhibited significant decrease in OSI ($P<0.01$) as compared to moderate psoriatic patients (Table 4.3) and also the research considered increased OSI as psoriasis year duration increases ($P <0.001$) (Table 4.4). Similar results were reported by Sürücü *et al.,* (2015) Gabr and Al-Ghadir (2012). This might be due to the prolonged release of excess ROS in the skin cells which can aggravate inflammatory injury and promote chronic inflammation. The decrease in TAC and increase in TOC was more pronounced in severe patients comparing to mild and moderate indicating that the anti-oxidants were nearly completely utilized to scavenge the superoxide free radicals.

6. CONCLUSION

The presented study concluded oxidative stress in psoriatic patients, indicated by increased TOC as well as OSI and decreased TAC levels when compared to healthy controls. Furthermore the study found worsened oxidant and antioxidant status according to the severity and duration of disease which may be the crucial point in the pathogenesis of psoriasis and need further elucidations. These findings suggest that a weakened antioxidant defense system may play a significant role in the increased oxidative stress found in psoriatic patients. Therefore, it is possible to conclude that increase of oxidative stress in psoriatic patients as severity and duration of the disease increases. Moreover the study also supports the possibility of involvement of oxidative stress in pathogenesis of psoriasis.

7. RECOMMENDATION

- Ethiopian specific information regarding the true epidemiology of psoriasis has to be studied adequately to manage the health, societal and economic impact of psoriasis nationwide.

- Psoriatic patients have to elucidate their feeding habit with high intake of fruit and vegetables, legumes, grains and cereals, fish and antioxidant levels with their therapy.

- Further research should continue to enhance the understanding of the genetics and causes of psoriasis in Ethiopia and result in improvements in diagnosing and treating this disease.

- Estimating DNA metabolites and important enzymatic antioxidants (superoxide dismutase and catalase activities) needed to be considered in subsequent studies in the future.

- ROS lie downstream as inflammatory driver therefore an effective biomarker of ROS may have even greater potential.

- Finally, of considerable interest is the possibility of using this information to develop novel strategies for diagnosis, prognosis and treatment of psoriasis patients are warranted.

8. LIMITATION

Small patient numbers: Invariably, this is only a cross sectional study with small number of patients. However, a cohort study with larger number of patients needs to be attempted in Ethiopia. Another limitation of this study is that we did not use an objective method to assess stress reactions with reference to nutrition.

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Appendix I: Questionnaire

Thank you for your willingness to participate; your cooperation is very important to the success of the study. This is a questionnaire you are asked to fill out. Please answer the questions as frankly and accurately as possible. All information obtained in the study will be kept confidential.

1. Personal identification: a. Full name of the subject : _____
   b. Subject code number: _____
2. Demographic detail
   a. Age : _____
   b. Gender : Male ☐ Female ☐
   c. Region :_____ Residence area _____
   d. Height :_____
   e. Weight : _____
   f. Marital status: Single ☐ Married ☐ Widowed ☐
   g. Education level: Illiterate ☐ High School or less ☐ College or above ☐
   h. Occupation : _____
3. Group: Study ☐ Control ☐
4. Date of diagnosis :_____
5. Age of onset : _____
6. Disease duration :_____

7. **Dermatology History**	Yes	No
a. Prior skin cancers	☐	☐
b. Has an immediate family member had skin cancer or skin problems?		
c. Trauma	☐	☐
d. Operation wound	☐	☐
e. Vaccination	☐	☐
f. Insect or animal bite	☐	☐
g. Sun burn	☐	☐
h. Obesity	☐	☐
i. Stress	☐	☐
j. Seasonal variation	☐	☐
k. Are you currently pregnant or breast feeding?	☐	☐
l. Any other health problems _____		

8. Treatment history -Medications and Allergies **yes** **No**

a. Tropical ☐ ☐
b. Phototherapy ☐ ☐
c. systemic ☐ ☐
d. None ☐
e. Other : _____

9. Social history

a. Do you smoke? ☐ ☐
b. How often do you drink alcohol?
None ☐ 1-3 ☐ 4-6 ☐ 11-15 ☐ 21+ ☐
c. What is your occupation or former occupation? _____
d. Number of people living in household? _____

10. **Medical History**

Do you have the following medical conditions?	Yes	No
1. Artificial joints	☐	☐
2. Cancer	☐	☐
3. Asthma	☐	☐
4. Depression psychiatric diseases/ disorder	☐	☐
5. Stomach ulcers	☐	☐
6. Tuberculosis	☐	☐
7. hay fever	☐	☐
8. Bleeding disorders	☐	☐

Other _____

For physician or clinician use only

11. General physical examinations

    a. Severity of psoriasis and percent of body surface affected

        - Mild up to 3%:_____
        - Moderate 3%-10%:_____
        - Severe > 10%:_____

    b. Types of lesion

        - Papules : _____
        - Pustule : _____
        - Plaque : _____
        - Erythema :_____

Appendix II: Consent form

PART I: patient information

You are invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take a time to read the following information carefully and discuss it with others if you wish.

General description of the disease: psoriasis

Psoriasis is a long lasting disease characterized by patches of abnormal skin. These skin patches are typically red, itchy, and scaly. They may vary in severity from small and localized to complete body coverage. Psoriasis is generally considered a genetic disease which is triggered by environmental factors. Psoriasis is associated with an increased risk of psoriatic arthritis, lymphomas, cardiovascular disease, Crohn's disease and depression. Psoriasis is not contagious. There is no cure for psoriasis. However, various treatments can help control the symptoms.

Purpose of the study

The aim of the study is to evaluate levels of some chemical components among patients of psoriasis at Alert Hospital, Addis Ababa, Ethiopia. This will be important in the monitoring of the risk of developing of cardiovascular diseases.

Procedure

If you are volunteers to participate in this study, we will ask you the following

- you will be asked to come the hospital after fasting overnight and compliance regarding fasting will be checked by on the Moring of the examination
- you are required to reply to the questions raised by physician or nurse
- you need to allow withdrawal of a 5ml of a serum blood from your hand

Potential risk

The only risk is that may result is mild pain associated with the withdrawal procedure. This is just the result of the routine work which is experienced by all patients. There is nothing else which may make the patient feel discomfort.

Benefits

This study is designed to determine the correlation between psoriasis and oxidative stress, total antioxidant capacity and levels of lipid profile which have a strong effect on developing cardiovascular diseases. Thus the patients involved in the study will have got information about their status and they may take measures to control the disease, before it is going worse. This will be done without any payment.

Confidentially

Any information that is obtained from the result and that can be identified with you will be remained confidential and will be disclosed only with you permission. We will not use your name in any of the information we get from the study or in any of the research report.

Sharing the result

At the end of the study, the study finding will be published and it will be disseminated to all responsible bodies for taking actions and other purpose.

The right to refuse

Please know that your participation in this study is entirely voluntary and you are free withdrawn at any time from the study. Taking of your blood sample to be used for this research is completely voluntary. Your decision will not affect your right to take medication or any other health service facility now or in future

Appendix III -Informed consent

I volunteer to participate in a research project conducted Asrat Endrias from Addis Ababa University.

I have been informed about a study that plans to measure the status of oxidative stress in psoriasis. For this purpose, blood needs to be taken. The aims of the study and the possible risks, include mild pain during blood collection were explained to me.

I am also informed that all the information contained within the questionnaire is to be kept confidential. Moreover, I have also been well informed of my right to kept hold of information, decline to cooperate and make myself withdraw from the study.

It's therefore, with full understanding of the situation that I gave the informed consent voluntarily to the researcher to use the blood taken for the investigation. Moreover, I have had the opportunity to ask questions about it and received clarification to my satisfaction. I have also been informed that the nature of the questionnaire is private.

I have been given a copy of this consent form and I have read and understood the explanation provided to me. I have had all my questions answered to my satisfaction, and I voluntarily agree to participate in this study.

.

Name of participant _____Signature _____Date_____

Name of investigator _____ Signature _____ Date _____

For participants who cannot read and write

- Witness name_____
- Signature _____
- Date _____

Appendix IV-Amharic Version

አዲስ አበባ ዩንቨርሲቲ፡ የሕክምና ፋካልቲ፡ ባዮኬሚስትሪ ት/ክፍል

የጥናት ተሳታፊዎች የመረጃና የስምምነት ቅፅ

መግቢያ፡

በዚህ ጥናታዊ ዕሁፍ እንዲሳተፉ እየተጋበዙ ነው፡፡በጥናቱ ላይ ለመሳተፍ ከመወሰነን በፊት ጥናቱ ለምን እንደሚካሄድና ምንም ዓይነት ነገሮች እንደሚያጋጥሞት ማወቅ ነው፡፡ ስለዚህ ጥቂት ጊዜ ይወስዱና የሚከተለውን ስለጥናቱ በተመለከተ መረጃ ይመልከቱ አስፈላጊ ከሆነም ከሌሎች ሰዎች ጋር ይወያዩ፡፡ ማንኛውም ግልጽ ያልሆነ ነገር ካለ ወይም ተጨማሪ መረጃ ከፈለጉ የጥናቱ ባለቤት መጠየቅ ይችላሉ፡፡

የጥናቱ ዋና ተመራማሪ ፡ አስራት አንድርያስ ኢ-ሜይል - endasrias@gmail.com

አድራሻ፡- ሞባይል +251 924437711

የበአለርት/ አህሪሆስፒታልየምርምርድጋፍሰጭኮሚቴ ስልክ ቁጥር፡0113-48-12-89

ሶርያሊስ

ሶርያሊስ የማይድን በዙር የሚተላለፍ ለረጅም ግዜ አብሮ የሚኖር መድሃኒት ያልተገኘለት የቆዳ በሽታ ነው፡፡ በሽታው ከትንሻ የሰውነት ክፍል እስከ ሙሉ የሰውነት አካል የሚሸፍን እና ቆዳን የማሳከክ እንዲሁም የመቆጣት ባህሪ ያለው ሲሆን በተለያዩ መድሃኒቶች በሽታው ወደ ሌላ የሰውነት ክፍል እንዳይሰፋፋ መከላከል ይቻላል፡፡

በሽታውምክ ልብ ህመም ለየመገጣጠሚያ ችግሮች ለስካር በሽታ ለአንጀት መቆጣት ለአእምሮ ጭንቀት ያጋልጣል፡፡ ስለዚህም አሁን የምናደርገው ጥናት ከላይ የተጠቀሱትን በሶርያሲስ በሽታ ምክንያት ሊከሰቱ የሚችሉትን ችግሮች ለመቆጣጠር ይረዳል ብለን እናስባለን፡፡

የጥናቱ አላማ፡

የዚህ ጥናት ዋና አላማ በአለርት ሆስፒታል ውስጥ በተለያዩ መረጃ ለሚገኙ ሶርያሲስ የቆዳ ሁሙማን በህመሙ ምክንያት ሊመጣሉ ተብለው የሚታሰቡ ተያያዥ ችግሮች ምን እንደሚመስል ጥናት ማድረግ ነው፡፡

የተሳታፊዎች ሁኔታ፡-

በጥናቱ ሊዳተፉ ከተስማሙ

1. ሀኪሙ ለሚጠይቁት ጥያቄዎች መልስ መስጠት
   ለምሳሌ፡- ዕድሜ፤ በሽታው መች አንደተያዙ/ ከዚህ በፊት ምን ኣይነት መድሃኒቶች እንደደተተቀሙ.....
2. ለምርመራው በመርፌ ከእጅ ላይ ትንሽ ናሙና 5ml የደምመጠን፣ለወስድ መስማማት፡፡

ሊከሰቱ ስለሚችሉ ስጋቶች እና የምቾት መጋደሎች

ለጥናቱ በሚወሰደው ደም ምክንያት የተለየ ችግር ኣይከሰትም፡፡ ምክንያቱም የጥናቱናሙና ኣወሳሰድ ከወትሮው በሽተኛው ለራሱ ብሎ ከሚሰጠው የተለየ ኣይደለም፡፡ እንዲያውም ለበሽተኛው ያለበትን ሁኔታ እንዲያውቅ እና እርምጃ እንዲወስድ ያደርገዋል፡፡ ከዚህ ጥናት ጋር በተያያዘ በጤናም ሆነ በሚያገኙት ተገቢ ህክምና ምንም አይነት ጉዳት ስለማያስከትል አይስጉ፡፡ ሊከሰት የሚችለው ችግር በደም ኣወሳሰድ ግዜ ከመርፌ መወጋት የተያያዘ ህመም ብቻ ነው፡ ይህ ደግሞ ከተለመደው የተለየ ኣይደለም፡፡

ጥቅሞች

የምርመራው ውጤት ለህሞማኖቹ እንዲሁም ኣጠቃላይ ለሶርያሲስ ታማሚዎች በተደጋጋሚ ላሚያጋጥማቸው ከበሽታው እና ጋር ለተያያዙ እና ሌሎችም ችግሮች ከመከሰታቸው በፊት በቅድሚያ እርምጃ እንዲወስዱ እንዲሁም ጥንቃቄ እንዲያደርጉ መረጃ ይሰጣል፡፡ ሆስፒታሉም በዚህ ምክንያት የሚመጣውን ወጪ እንዲሁም የሰው ሃይል እና ግዜን ይቆጥባል፡፡

ምስጥር ስለመጠበቅ

ለሁሉም ጥያቄዎች የሰጡንን መልሶች የሚስጥረር እንደምንይዝ ሊያውቁ ይገባል፡፡ የተወሰደው ናሙና ለጥናት ብቻ የሚውል ነው፡፡ ስለሚወሰደውማንኛውም መረጃዎችሆነየጥናትውጤት ለማሰራጨትበስምሳይሆንበሚስጥር (ኮድ) የሚመዘገብይሆናል፡፡

የጥናቱ ያለመሳተፍ ወይም ራስም የማግለል መብት

በዚህጥናትመሳተፍሙሉበሙሉበርሶፍቃድየተመሰረተመሆኑንልናሳስብ እንወዳለን፡፡ በጥናቱ ውስጥ ማሳተፍ ኣለመፈለግዎ በጤና እንካብካቤዎ ላይ ምንም ተፅዕኖ ኣይኖረውም :: በመሆኑም በማንኛውም ግዜ ምንም ዓይነት ምክንያት ሳይሰጡ ከጥናቱ ራስን የማግለል መበት የተጠበቀ ነው፡፡ የህንን ከወሰኑም ማንም ምክንያቱን እንዲገልፁ ሊያስገድደዎት ኣይችልም፡፡ የሰጡት ደም ለዚህ ጥናት እንደሚውል ማድረግ በእርሶሙ ሉፈቃድ ብቻ ሲሆን በጥናቱ ላለመሳተፍ መወሰን ወይም ኣለመወሰን መድሐኒት ወይም ሌላ የጤና ኣገልግሎት የማግኘት መብት ኣሁንም ሆነ ለወፊቱ ምንም አይነት ተፅኖ ኣያሳድርብትም፡፡

መረጃ ስለማግኘት

ይህጥናት በኣዲስ ኣባበ የኒቨርስቲ ሜዲካል ፋኩልቲ የባዮኬሚስትሪ ትምህርት ክፍል እና በኣለርት ሆስፒታል የምርምር ድጋፍ ሰጭ ኮሚቴ ድጋፍ ኣግኝቷል፡፡ ማናኛው ኣይነት ጥያቄዎች ካለዎት እርግጠኛ ሆነው ለጥናት ለማሳተፍ እስኪወስኑ ድረስ ተገቢ የሆነውን ማንኛውንም መረጃ የመጠየቅ መብት ኣለዎት፡፡

ክፍልሁለት፡-የስምምነትቅፅ

የዚህጥናት መሰረታዊ ዓላማ እና ሌሎች መረጃን በሚገባ ተገንዚቤለሁ፡፡ተሳትፎዬ በፍቃደኝነት ላይ ብቻ የተመለኮዘ እደሆነም ተረድቻለሁ፡፡ ማንኛዉም ሰብዓዊም ሆነ ህጋዊ ሙብት ሳይነካ ከጥናቱ ራሴን ማግለል እደምችልም እንደሆነ፡፡ ስለጥናቱ ዝርዝር ጉድይ በግልፅ ከተረዳሁት ባሻገር በተጨማሪ ማብራሪያ ብፈልግ መጠየቅ እደምችል ተረድቻለሁ፡፡ በመሆኑም በፈቃዴ የዚህ ጥናት አካል እንድሆን ስፈልግ የምጠብቅብኝ ሁሉ ለምድረግ በመወሰን መሆኑን በፊርማዬ አረጋግጣለሁ፡፡

የተሳታፊ ስም ፡_____

- ቀን: _____
- ፊርማ፡ _____

የጥናቱ ዋና ተመራመሪ ስም _____

- ቀን _____
- ፊርማ _____

ማንበብ እና መፃፍ ለማይችሉ የጥናቱ ተሳታፊዎች

- የምስክር ስም _____
- ፊርማ _____
- ቀን _____

መጠይቅ

አዲስ አበባ ዩንቨርሲቲ- የሕክምና ፋካልቲ -ባዮኬሚስትሪ ት/ክፍል

ይህን መጠይቅ ለመሙላት ፍቃደኛ በመሆንዎ ከልብ እናመሰግናለን ። የእርስዎ ትብብር ለምርምሩ ስኬት አስፈላጊ እንደመሆኑ መጠን ይህን መጠይቅ በተቻለዎ መጠን በግልፅ እና በትክክል እንዲሞሉት በአክብሮት እጠይቃለሁ፡፡ ማንኛውም እርስዎን የተመለከተ መረጃ ሚስጥራዊነቱ የተጠበቀ ነው፡፡

የማንነት መለያ

1. የሚስጢር ቁጥር:_____የካርድ ቁጥር:_____

ማህበራዊ ና ሰነ ህዝብ መራጃ

1. ዕድሜ: _____
2. ፆታ: ወንድ ☐ ሴት ☐
3. ክልል ኣድራሻ ከተማ፡ _____
4. ቁመት:_____ ክብደት:_____
5. የጋብቻ ሁኔታ: ያገባ ☐ ላጤ ☐ የፈታ ☐
6. የትምህርት ደረጃ: ኣንደኛ ደረጃ ☐ ሁለተኛ ደረጃ ☐ ኮሌጅ ☐
7. የስራ ዓይነት: _____
8. ምድብ: 1. የምርምር ☐ 2. ቆጥጥር ☐
9. የታከሙበት ቀን፡_____
10. በሽው የታየበት ዕድሜ:_____
11. በሽታው የቆየበት ግዜ:_____

የቆዳ ህመም መንሲዎኤች	አዎ	አይ
1. የቆዳ ካንስር	☐	☐
2. ኣደጋ	☐	☐
3. በተመሳሳይ ህማም የተጠቁ የቤተስብ ኣባል	☐	☐
4. የቆዶ ጥገና ቁስል	☐	☐
5. ክትባት	☐	☐
6. የተባይ እና የእንስሳት ንክሻ	☐	☐
7. የወቅትመቀያየር	☐	☐
8. እርግዝና ወይሞ ጡት ማጥትባት	☐	☐
9. መድሃኒት	☐	☐
10. ከፍተኛ የፀሃይ ብርሃን	☐	☐
11. ከልክ ያለፈ ውፍረት	☐	☐
12. ጭንቀት	☐	☐

ሌላ_____

ከዚህ በፊት የወሰዱት ወይም የተጠቀሙት የህክምና ኣይነት

1. በቆዳ የሚቀቡ የቅባት /ክሬም መድሃኒቶች ☐ ☐
2. የብርሃን ህክምና(ፎቶ ቴራቢ) ☐ ☐
3. መዲሃኒቶች(የእንክብል፣ ሽሮፕ/ፈሳሽ) ☐ ☐

(ለየተኛው ኣይነት በሽታ ጥቀስ)_____

4. የለም _____
5. ሌላ ካለ ጥቀስ

ማህበራዊሁኔታዎ ኣዎ ኣይ

1. የማጨስ ልምድ ኣልዎት ☐ ☐
2. የመጠጥ ልምድ ኣልዎት ☐ ☐
3. የስራዎ ኣይነት ምንድ ነው ወይም ምን ነበር _____
4. የቤተሰብ ብዛት ቁጥር_____

ከዚ በታች ከተዘረዘሩት የጤና ችግሮች ያለቦትን ምልክት ያርጉ፡፡ ኣዎ ኣይ

1. ስው ሰራሽ ኣካል ☐ ☐
2. ካንሰር ☐ ☐
3. ኣዝማ ☐ ☐
4. የ ኣእምሮ ጭንቀት/ችግር ☐ ☐
5. የጨጓራ ቁስትል ☐ ☐
6. ቲቢ ☐ ☐
7. የብናኝ አለርጂ/hay fever ☐ ☐
8. የደም መፍሰስ ችግር ☐ ☐

ሌላ _____

Appendix VII –PASI

PSORIASIS AREA AND SEVERITY INDEX (PASI) WORKSHEET
HOSPITAL NO.: …….
PATIENT NAME: …….
DATE OF VISIT: …….

The Psoriasis Area and Severity Index (PASI) is a quantitative rating score for measuring the severity of psoriatic lesions based on area coverage and plaque appearance.

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.					

PASI Score = ☐