Association of Skin Type with IL-6 Gene Polymorphism in Patients with Acne Vulgaris

By

QuraTulAin

Department of Biochemistry

Faculty of Biological Sciences

Quaid-i-AzamUniversity

Islamabad, Pakistan

(2013)

Association of Skin Type with IL-6 Gene Polymorphism in Patients with Acne Vulgaris

A thesisSubmitted in the partial fulfillment of theRequirements for the Degree of Master of Philosophy

In

Biochemistry/Molecular Biology

By

QuraTulAin

Department of Biochemistry

Faculty of Biological Sciences

Quaid-i-AzamUniversity

Islamabad, Pakistan

(2013)

CERTIFICATE

This thesis submitted by Miss QuraTulAin is accepted in its present form by the Department of Biochemistry, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad as satisfying the thesis requirements for the degree of Master of Philosophy (M. Phil) in Biochemistry/Molecular Biology.

Internal Examiner: _____
Dr. QamarJaved

External Examiner: _____
External

Chairperson: _____
Dr. BushraMirza

Dated:

DECLARATION

I hereby declare that the work presented here is my own effort and that the thesis is my own composition. No part of the thesis has been previously presented for the award of any other degree.

QuraTulAin

Dedicated to……

My beloved parents

Especially my Father

CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
AP-1	Activator protein-1
AR	Androgen receptor
Bcl2	B-cell lymphoma 2
BMB	Bromophenol blue
C/EPBβ	CAAT/enhancer binding protein β
CRE	Cyclic AMP response element
CRH	Corticotropin releasing hormone
CYP17	Cytochrome P450 17
CYP1A1	Cytochrome P-450 1A1
DHEAS	Dehydroepiandrosterone sulfate
ERK	Extracellular signal-regulated kinase
ESR	Erythrocyte sedimentation rate
GRE	Glucocorticoid response element
IBD	Inflammatory bowel disease
IFNβ	Interferon β
IGF-1	Insulin like growth factor-1
IL-6R	IL-6 receptor
JAK	Janus kinase
MAPK	Mitogen activated protein kinase

MCP-1	Monocyte chemoattractant protein-1
$MgCl_2$	Magnesium chloride
MRE	Multiple response element
MUC-1	Mucin-1
NF-κB	Nuclear factor of kappa light polypeptide gene enhancer in B-cells
$NH4_2SO4$	Ammonium sulfate
OR	Odds ratio
p	Short arm of chromosome
P. acnes	*Propionibacterium acnes*
PCO	Polycystic ovary
PgE2	Prostaglandin E2
PgF2	Prostaglandin F2
PI3K	Phosphoinositol-3 kinase
PIMS	Pakistan Institute of Medical Sciences
PMN	Poly morpho nuclear neutrophils
PPAR	Peroxisome proliferator activated receptor
q	Long arm of chromosome
RA	Rheumatoid arthritis
Rb	Retinoblastoma
RCE	Retinoblastoma control element
RFLP	Restriction fragment length polymorphism

rpm	Revolutions per minute
rs	Reference SNP ID number
RXR	Retinoid receptors
SDS	Sodium dodecyl sulfate
SHP2	Protein-tyrosine phosphatase
SLE	Systemic lupus erythematosus
SNP	Single nucleotide polymorphism
SRE	Serum response element
SREBP-1	Sterol response element binding protein-1
STAT	Signal transducers and activators of transcription
Taq	Thermusaquaticus
TBE	Tris borate EDTA
TE	Tris EDTA
TLR	Toll like receptor
TNF-α	Tumor necrosis factor-α
UD	Undigested
UTR	Untranslated Region
VNTR	Variable Number Tandem Repeat
vs.	Versus
χ^2	Chi square

ACKNOWLEDGEMENTS

All the praises and gratitude to **Almighty Allah**, who gave me the ability and strength to complete this project. All blessings and respects are for our beloved **ProphetHazrat Mohammed (S.A.W.W.)** whose teachings guide us towards light ofknowledge.

This thesis owes its existence to the guidance, patience, support and inspiration of many people. It is a pleasure to convey my gratitude to them all in my humble acknowledgment.

I am grateful to **Dr. BushraMirza**, Chairperson, Department of Biochemistry, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad for extending the research facilities of the department to accomplish this work.

I feel highly privileged in taking opportunity to extend my greatest gratitude and thanks to my thesis supervisor **Dr. QamarJaved**, Associate Professor, Department of Biochemistry, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad for his scholastic guidance, valuable suggestions, support and encouragement throughout the study and presentation of this manuscript. I am thankful to him for his supervision, inspiration, reassurance and counseling from time to time.

It is inevitable to thank **SabirHussain** (Ph.D. scholar) and**Dr. AfroseLiaquat** (Ph.D. scholar) for their keen interest, generous guidance, caring behavior and intellectual support during my research work. Their sustained grooming inspired me to strive for excellence in research.

Many thanks go in particular to **Sidra Younis** (Ph.D. scholar). I am much indebted for her valuable advice in discussion and technical assistance throughout my thesis writing and furthermore, using her precious times to read this thesis and gave her critical comments about it.

I express special thanks to my senior lab fellow **Tamar**for her kind guidance, continued encouragement and pieces of advice.

I would also acknowledge my lab fellows **Iqra, Saima, Saira and Afshan**for their cooperation, care and concern, moral support, necessaryguidance and pleasant company. I consider myself fortunate to have the presence of such a decent group of people around me during my research work in the lab.

I must acknowledge to the office staff especially **Muhammad Saeed, Tariq Mahmood and Fiaz-ur-Rehman** for all the instances in which their assistance helped me along the way. I also offer my thanks to the lab cleaner for providing a tidy and clean environment to work.

I do not have words to thanks my affectionate and adorable **family and friends** without whom I feel myself incomplete. With deepest vehement of gratitude, I regard vigorous tribute to my **parents** whose affection, endless love, guidance, strength and prayers have always been my greatest assets in life. No acknowledgement could ever adequately express my feelings to them for having complete faith in me. Thank you for your unconditional support with my studies. Thank you for giving me a chance to prove and improve myself through all walks of my life.

Finally, I offer my regards to all of those who supported, encouraged and assisted me in any respect to the successful completion of my study. I also need to apologize for not being able to mention them personally one by one. Thank you for your support.

QuraTulAin

ABSTRACT

Acne vulgaris is chronic, multifactorial and the most common inflammatory skindisorder of the pilosebaceous follicles, occurring mostly during adolescence. It is characterized by non-inflammatory and inflammatory lesions. The most significant pathophysiologic factors influencing the acne development are abnormal hyper ductal keratinization, high level of secreted sebum, abnormal microbial flora *i.e.Propionibacterium acnes (P. acnes)* propagationandintense inflammatory response. Among five skin types oily skin is more susceptible for acne development as compared tocombination, sensitive, normal and dry skin. IL-6 is a cytokine with multiple functions and it is involved in inflammation and other immune responses. IL-6 is thought to be involved in the acnepathogenesis. Numbers of single nucleotide polymorphisms have been discovered in human IL-6 gene, four of them are present in its promoter. The promoter polymorphism G/C at position -174, has been implicated in various inflammatory disorders like psoriasis vulgaris. Aim of this study was to determine IL-6 -174G/C polymorphism in patients with different skin types. Sixty five patients and seventy healthy controls were covered in this current study. In order to analyze the IL-6 gene (-174G/C) polymorphism present in promoter, polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) were employed. *Nla*III was used for restrictiondigestion.Genotypic and allelic frequency differences between acne patientsand healthy controls werestatistically not significant (P = 0.30, P = 1.00 respectively). Genotypic and allelic frequencies of acne patients with different skin types were compared and no association was found (P=0.38). Similarly, no link was observed between genotypes of IL-6 (-174) and the severity of acne (P = 0.38). In short, outcomes of this primary study conducted, suggest that IL-6 (-174G/C) promoter polymorphism is not associated with skin type of acne patients in Pakistani population.In future, further studies are required to delineate relationship between skin types and -174G/C IL-6 promoter polymorphism.

INTRODUCTION

Acne vulgaris is a common and complex disorder of the pilosebaceous folliclesoccurring mostly in facial skin, back and chest. It occurs mostly at the time of adolescence; when level of androgens reach at its peak in human body. In brief, acne vulgaris starts when pilosebaceous follicles become blocked with skin cells forming comedones, sebum is secreted in high amount which bloats the follicles and microbe, the *Propionibacterium acnes (P. acnes)*starts propagating in it. If comedons bursts into dermis, result is inflammation and a papule or pustule forms (Leyden and Shalita, 1986).

1.1 Types of acne

There are many types of acne including acne fulminans, acne neonatorum, acne infantum, acne conglobata, steroid acne, chloracne, acne venenata Infantum andacne vulgaris.

1.1.1 Acne fulminans

It is a type of acne which is very rare and severe because it involves immune reactions against *p. acnes* affecting adult boys mostly (Karvonen, 1993). Symptoms of this disease are high ESR (erythrocyte sedimentation rate), high fever, high white blood cells (WBC) count, arthralgia, obstructive bone lesions and ulcerative lesions (Knitzer and Needleman,1991)**.**

1.1.2 Acne Neonatorum

It is present at birth time or develops shortly after birth. Closed comedones are the most common lesions, but sometimes papules, pustules and even open comedons are also found (Giknis *et al.,* 1952).

1.1.3 Acne Infantum

It generally occurs between 3 and 6 months of age *i.e.*after acne neonatorum (Ayres, 1926).In addition to open and closed comedones; pustules, nodules, papules and scarring cystic lesions may be present (Chew *et al.,* 1990).

1.1.4 Acne Conglobata

Lesions primarily occur on the face of children, also include nodules, cysts, and strenuous sinuses, leading to scars, as in the adult acne. These children develop severe acne when they go to teen age and adult age (Arbegast*et al.,* 1991).

1.1.5 Acne Venenata Infantum

This problem is raised when parents apply some topical skin products like greasy creams, oils, salves and pomades to their infants. Acneiform reactions and comedones are developed. In U.S this problem is most commonly seen in black people, called pomade acne because this problem is associated mostly with hair products in adults (Menni and Brancaleone, 1992; Berlin, l954).

1.1.6 Steroid Acne

This problem is found in both children and adults and it is raised by systemic and topical corticosteroids. As children have not developed hair follicles, so they are less sensitive to side effect of corticosteroids (Latif and Laude, l982).

1.1.7 Chloracne

It is a type of acneiform reaction which is caused by the use of aromatic hydrocarbons with chlorine (Caputo*et al.,* 1988).

1.1.8 Acne Vulgaris

Acne vulgaris is a complex multi-factorial disease mainly affecting thepilosebaceous follicle and characterized by non inflamed lesion the comedones; inflamed lesions *i.e.* nodules, papules, pustules, and scars (Simpson and Cunliffe, 2008).

1.2 Prevalence of acne

Acne is a familiar disorder of skin affectingalmost all ages. It affects male patients more frequently than female patients. It affects > 80% of all persons during childhood and teenage life (O'Brien *et al.,* 1998; Kligman and Plewig, 1976).Nearly 85%of individuals of white Caucasian populations present a variant clinical picture of acne,

(Zouboulis *et al.,* 2005) while in America its prevalence is about 85 (White, 1998). AnAustralian study of undergraduates indicated that approx. 98% boys and about 90% girls had acne on facial areas including head and neck (Kilkenny *et al.,* 1998). Clinical acne is 30% prevalent in Asian women (Perkins *et al.,* 2011).

1.3 Acne vulgaris

Acne vulgaris is an inflammatorydiseasewhich is very common and involves pilosebaceous follicles. It ischaracterized by the comedones, pustules, papules, inflamed nodules and pus filled cysts (Healy and Simpson, 1994). The lesions are mostly present on face;howeverneck, shoulders, chest and upper back may also be affected. Acne maycause severe scarring and in turn considerable psychological distress (Mallon *et al.,* 1999).

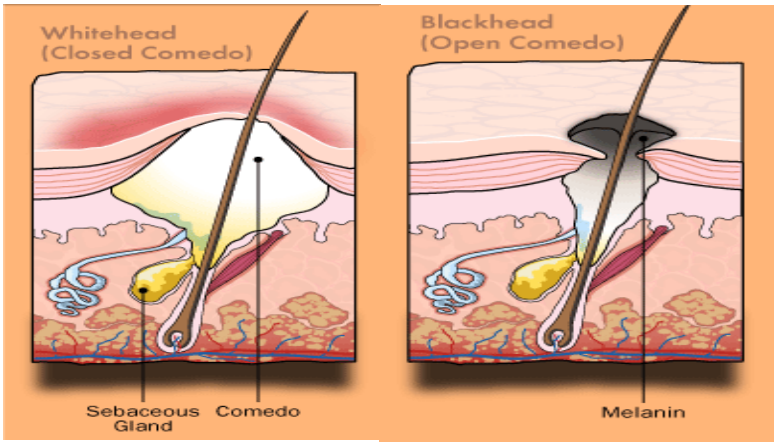
Acne is divided into two types of lesions noninflammatory and inflammatory (Berson and Shalita, 1995).Open and close comedones are present in noninflammatory acne (Figure 1.1). Comedo formation starts from microcomedones which are invisible. All other acne lesion starts from this micro comedo. Morphologically there are two types of comedo, whitehead(closed comedo) and blackhead (open comedo) (Toyoda and Morohashi, 1998).

Inflammatory acne lesions include papules and pustules. Papules are actually elevated lesions which measure$\leq$ 0.5 cm, while pustules have a huge collection of pus which is visible at the surface. Papule and pustule can be converted to nodule upon getting enlarged and firm.A related severetype of lesion with deep inflamed areas is called cyst (Figure 1.1). Any form of inflammatory acne can accompany scarring (Toyoda and Morohashi, 1998).

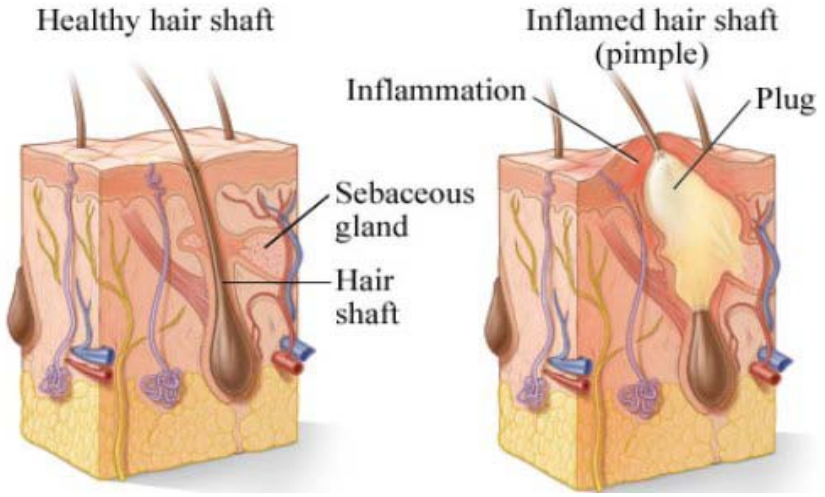
1.4 Classification

Acne vulgaris can bebroadly classified as mild, moderate and severe on the basis of severity of acne lesions.

Comedonal Acne



Inflammatory Acne



Nodulocystic Acne

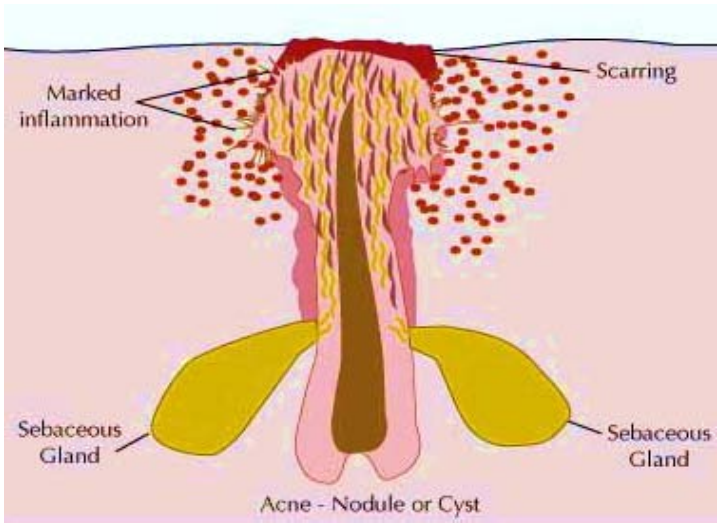


Figure 1.1 Types of acne lesions.

1.4.1 Mild

In case of mild acne noninflammatory lesion, known as comedones, are common. Mild acne is usually restricted to the face and it is described by the existence of non-inflammatory lesions accompanying few inflamed lesions (Dawson and Dellavalle, 2013) like papules and pustules but occur very few in number usually less than ten (Cunliffe *et al.,* 2003).

1.4.2 Moderate

In this case moderate numbers of lesions are present on face. Comedones, papules and pustules are present on face in ten to forty in number (Cunliffe *et al.,* 2003).

1.4.3 Severe

In this type of acne nodulocystic acne is present while acne conglobata with large, painful pustular or nodular lesions is also present. Many small comedones, papules and pustules are also present (Cunliffe *et al.,* 2003).In such severe cases, facial acne lesions are frequently accompanied by extensive lesions on trunk (Dawson and Dellavalle, 2013).

1.5 Factors affecting disease

Genetic factors have a large influence on excretion of sebum, but these factors are not enough to develop clinical picture of disease. Environmental factors are mainly involved in developing clinical picture of acne. In spite of the genetic regulation of the sebum excretion, environmental factors, such as food and diet, might act as modifiers of genetic expression (Walton *et al.,*1988).

1.5.1 Endocrinological Factors

There is a major association between hormones and acne vulgaris especially that of serum testosterone (Rahman *et al.,*2012). Androgens increase the production of sebum and also enhance the follicular keratosis which play main role in acne development (Cibula *et al.,* 2000). Association between the PCO (polycystic ovary) and acne has also been established (Betti *et al.,* 1990). High levels of androgens are

due to polycystic ovaries and excessive corticosteroids usage (Alsop, 2008), which then play role in acne development.

1.5.2 Food and Diet

Fatty foods, chocolates and oily products are mainly implicated in acne etiology (Rigopoulos *et al.,* 2007; El-Akawi *et al.,* 2006;Green and Sinclair, 2001). The studies of Adebamowo and colleagues showed that high milk intake can increase the acne susceptibility (Adebamowo *et al.,* 2008; 2006).Insulin-like growth factor-1 (IGF-1) is produced in high amount in the body due to increased milk consumption, soandrogen production is raised in ovaries, contributing to acne (Aizawa and Niimura, 1995; Cara, 1994). High glycemic food intake increases the insulin which further increases the sebocytes proliferation and sebum production hence contributing acne (Zouboulis *et al.,* 1998).IGF-1 stimulate the sebaceous gland for lipid synthesis and alsoenhanceexpression of a transcription factor; sterol response element binding protein-1 (SREBP-1), which regulates various gene which are involved in lipogenesis. IGF-1 activates the PI-3K and MAPK/ERK pathways hence contributes acne (Smith *et al.,* 2008).

1.5.3 Chemicals Including Cosmetics

Some substances are comedogenic which cause comedones, while some cause papules and pustules hence called acnegenic. Follicular plugging cause comedons, while follicular irritation is acnegenic (Mills and Berger, 1991). Vegetable oils, foundations, moisturizers and some petroleum oils (Alsop, 2008), hair gels and tar fumes (Mills and Kligman, 1982), polyhalogenated aromatic hydrocarbons, *e.g.*, dioxin (Ju *et al.,* 2012, Neuberger *et al.,* 1991;Moses and Prioleau, 1985; Tindall, 1985), halogen drugs (Belisario, 1951) cause chloracne.

1.5.4 Psychic Factors

Acne vulgaris is being related to psychiatricalmorbidity. Acne can be caused by emotional stress andpsychiatric problems may develop in patients with acne (Koo and Smith, 1991). Psychic problems originating due to acne include issues with self confidence, self esteem, social withdrawal, embarrassment, body image, depression,

anger, anxiety, confusion, frustration, preoccupation with acne, problems in family and relationships and limitations in life style (Koo, 1995).

1.5.5 Medication

It may be a causative factor or aggravating factor. Medication including systemic steroids, contraceptives, anabolic steroids, antiepileptics including carbamazepine, phenobarbital and phenytoin, antidepressants including lithium and medicines used in therapies to cure tuberculosis including isoniazid, may contribute to acne (Alsop, 2008).

1.5.6 Hereditary Factors

Most of the genes involved in lesion formation studied are key players of the innate immune system. While some genes involved in lesion developmenthave function in metabolism of hormones (Table1.1).In recent time some other polymorphisms,related to the metabolic processes of cell have also been published (Szabo and Kemeny, 2011).

The androgen receptors (AR) are structurally much conserved member of nuclear receptors' super family. CAG-repeat length in receptor may affect androgen gene expression, in hair follicles and in sebaceous glands in skin disorder like that of acne (Sawaya and Shalita, 1998).In acne pathogenesis, inflammation plays very important role and pro-inflammatory cytokines are the main factors. Tumor necrosis factor alpha (TNFα) is a major molecule which is coded by a gene with high level of genetic polymorphism especially in promoter region. Variants of the TNFα gene may affect the risk of the acne vulgaris (Szabo *et al.,* 2011).

Table 1.1 Genetic polymorphisms studied in the pathogenesis of acne vulgaris.

	Gene	Polymorphism	rs number	Type of polymorphism	Location
Innate immunity genes	TLR2	+2,258 (G>A)(Arg753 Gly)	rs5743708	SNP	Coding region
	TLR4	+896 (A>G) (Asp299Gly) +1,196(C>T) (Thr399Ile)	rs4986790 rs4986791	SNP SNP	Coding region Coding region
	MUC1	20-aa long tandem repeat		VNTR	Coding region
	TNFA	-238 (G>A) -308 (G>A) -857 (C>T) -863 (C>A) -1031 (T>C)	rs361525 rs1800629 rs1799724 rs1800630 rs1799964	SNP SNP SNP SNP SNP	Promoter Promoter Promoter Promoter Promoter
	IL-1A	+4,845 (G>T)	rs17561	SNP	Coding region
	IL1RN	86-bp-long tandem repeat		VNTR	Intron
Genes affecting steroid hormone metabolism	AR	CAG and GGN repeat polymorphism		VNTR	Promoter
	CYP1A1	+4,889 (A>G) (Ile462Val) +6,235 (T>C)	rs1048943 rs4646903	SNP SNP	Coding region 3' UTR
	CYP17	-34 (T>C)	rs743572	SNP	Promoter

(Adapted and modified from Szabo *et al.*, 2011)

1.6 Pathogenesis

Acne is a kind of disease which is related with pilosebaceous follicles which have many large sebaceous glands. The areas mostly affected with acne are nose, cheeks, forehead and chest which usually have large number of sebaceous follicles (Knutson, 1974; Wolff *et al.,* 1975).

Four steps and processes play important role in formation of acne lesions; increased sebum production under androgen control, modification of the keratinization process leading to comedones formation, follicular colonization by *P. acnes* and inflammation (Thiboutot *et al.,* 2009).

1.6.1 Increased Sebum Production

Increased sebum production is an important step leading towards acne development**.**Production of sebum is under complex hormonal control (Pochi and Strauss, 1974) especially by androgens released from the adrenals and testes (Pochi and Strauss, 1969). The greasiness of skin of acne patients indicates the disease severity (Knutson, 1974). All sebocytes possess enzymes which can convert weak androgens into powerful enzymes. The activity of enzymes including steroid sulfatase, 3β hydroxy steroid dehydrogenase, 17 β-hydroxysteroid dehydrogenase, 3 α-hydroxysteroid dehydrogenase, 5 α-reductase, and aromatase, is increased in the sebum producing glands of acne patients (Chen *et al.*,2002).

Two types of 5α reductase are present in nature. Type I dominate in the sebaceous glands of the scalp and face which explains the facial acne predominance (Thiboutot *et al.*,1995). In sebocytes three types of PPAR receptors have been identified which are α, β and γ, among all γ form is most important in acne.Androgens, free fatty acids and linoleic acid activate these PPAR receptors which bind to retinoid receptors RXR, leading to formation of the hetero dimers, inducing alteration in sebocyte differentiation and proliferation and also production of free fatty acids. Therefore they are involved in maturation of sebaceous gland and also in instigation of the inflammatory reaction present in acne (Deplewski and Rosenfield, 2000; Rosenfield *et al.,* 1998).

A neuro mediator called, substance P has receptors on sebaceous glands and stimulates the sebaceous secretion (Toyoda and Morohashi, 2001).High sebum production under stress might be a result of substance P production. The composition of the sebum is changed in case of high sebum production; particularly the linoleic acid level is decreased by the process of dilution (Cunliffe and Simpson, 1998). When a person eats meals highly rich in unsaturated fats level of squalene is increased in pilosebaceous follicle, which provides a possible link between diet and acne development (Thiboutot and Strauss, 2002).

1.6.2 Obstruction of Pilosebaceous Follicle

Obstruction of pilosebaceous ducts takes place in acne; these are the ducts which act as channel to move sebum toward skin surface.According to many histological studies, inflamed acne lesion is produced after non-inflamed lesion production. Non-inflamed acne lesions are presented clinically as whiteheads or blackheads (Strauss and Kligman, 1958).

Abnormalities of proliferation, differentiation and adhesion of keratinocytes lead to the obstruction of pilosebaceous follicle which results in the formation of a micro-comedo (Cunliffe *et al.,* 2000). Keratinocytes residing in the pilosebaceous canal possess various androgen receptors and also specific enzyme system which are required for metabolism of androgens (Chen *et al.,* 2002).Anomalies in the androgen metabolism in the infrainfundibulum of keratinocytes could lead to variances in proliferation, differentiation and adhesion of these cells (Thiboutot *et al.,* 2003). *In vitro*, if we add interleukin (IL)-1α to a culture medium containing a pilosebaceous canal (Guy and Kealey, 1998) then possibly formation of micro-comedo takes place.

Expression of the integrins could also play an important role in the formation of a micro comedo (Cunliffe, 1998). Integrins are usually adhesive molecules ensuring a strong cohesion between all keratinocytes. So these adhesive molecules notably act on the regulation of proliferation, differentiation and migration of keratinocytes (Pouliot *et al.,* 2002; Watt, 2002). Hyper-seborrhea results in decrease of linoleic acid concentration in sebum which could lead to an anomaly in keratinocytes differentiation in the infrainfundibulum, affecting the formation of micro-comedo (Cunliffe and Simpson, 1998).

1.6.3 Colonization by *P. Acne*

Staphylococcus epidermidis and *P. acnes* are mostly found on skin and in ducts which are prone to acne (Leyden *et al.*,1975; Gloor*et al.*,1978). *P. acnes'*infection of skin cell and follicles and biologically significant molecules production by bacteria, totally depends upon some physiological variants like pH and oxygen tension (Holland *et al.*,1993). Alternative complement pathway can be stimulated by substances, produced by*P. acnes*(Massey, 1978) and also exerts a chemotactic affect which converts non-inflammatory lesions into inflammatory lesions (Puhvel and Sakamoto, 1977).

P. acnes normally reside in follicular ducts and do not cause infection, but upon getting optimal conditions of pH and oxygen tension it produces small molecules of low molecular weight which travel towards dermis through diffusion process and initiates inflammation by activating alternative complement pathway and also by stimulating chemotaxis (Voss, 1976).

1.6.4 Immunologic Processes and Inflammation

Classically inflammation was thought of a process consisting of production of free fatty acids from triglycerides of sebaceous follicles. But now it is considered as a more complex process. Proliferation of *P. acnes* with in micro-environment of micro comedo is correlated with the production of pro inflammatory molecules and cytokines (Leyden, 2001). *P. acnes* produce chemo attractant small molecules for lymphocytes and neutrophils,which describe the reason for the accumulation of these cells in the peri follicular infiltrate and follicular epithelium (Webster and Leyden, 1980). If we observe the infiltrate of inflamed acne lesion in first four hours of inflammation, T CD4 lymphocytes are the main cells found there (Jeremy *et al.,* 2003; Layton *et al.,* 1998). *P. acnes* stimulate proliferation of T lymphocytes by two mechanisms production of non-specific mitogens and specific antigenic process of stimulation *in vitro*. Cells are activated directly by super antigens, independent of action of antigen-presenting cell, which actually produces rapid, fast and extensive activation of the effector cells (Jappe *et al.,* 2002).

Non-specific mechanisms of defense involve polynuclear neutrophils, Toll receptors, defensins, cytokines, free radicals and metalloproteases. Free fatty acids which are produced by the sebocytes play a very important chemotactic role (Zouboulis *et al.*,1998).Chemo taxis of polynuclear neutrophil do not only depend on the free fatty acids but also affected by substances produced by *P. acnes* which diffuse into comedo wall so play a chemotactic role to gather neutrophils around comedo (Vowels *et al.,* 1995). Many cytokines play roles in acne inflammatory process (Ingham *et al.*,1998,1992; Layton *et al.*,1998) but four play essential parts in control of acne lesion formation and also in its regression. These are interleukin-1α, interferon-γ, TGF-α and interleukin-4.Interleukin-1α plays a vital role (Ingham *et al.,* 1998; 1992; Layton *et al.*,1998). It is secreted by activated keratinocytes; it induces the formation of comedo and also stimulates non-specific immunity (Uehara *et al.*, 2001). IL-8 and IL-6 not only amplify polynuclear neutrophil chemotaxis but also inflammatory reaction taking place in pilosebaceous follicle (Ingham *et al.,* 1998, 1992; Layton *et al.,* 1998).

Toll receptors have also been linked with acne pathogenesis (Kurt-Jones *et al.*, 2002). Production of defensins is stimulated by interleukin-8α and play role in inflammation as they are found in perilesional keratinocytes (Liu *et al.*, 2002). PPAR receptors are involved in instigation of the inflammatory acne development as they induce sebaceous gland to produce of interleukin-1 and TNF-α (Deplewski and Rosenfield, 2000; Rosenfield*et al.,* 1998).

1.7 Skin types and Acne vulgaris

A lipid film has covered facial skin;this layer consists of epidermal lipids and sebum. Sebum secretions vary from individual to individual depending upon genetics,age, gender and topology (Youn *et al.*,2002). In general, there are four types ofskin: Normal, oily,combination and dry. This classification is based on hydration of skin, rate of sebum excretion and personal information, gathered by using questionnaire (Park *et al.*,1999).

Several researchers have observed that high amount of sebum is produced in acne patients as compared to controls (Pierard-Franchimont *et al.,* 1991;Pierard *et al.,* 1987; Harris *et al.,* 1983). But still the difference between skin types of acne patients

and controls is not established partly due to the absence of standard approaches to determine type of skin.

According toskin type determining software,there are 5 basic types of skin. They are normal, oily, combination, dry, and sensitive skin.http://www.prokerala.com/health/beauty/skin-type-test.php

1.7.1 Normal skin

Normal skin looks clear, smooth, glowing and clean and has a good circulation with healthy complexion.Normal skin exhibits perfect balance between moisture and the cell turnover in protective layer of skin. Skin has two-way barriers which protect internal body from external damaging elements and prevent excessive outward release of moisture. Hydro lipid film is very crucial to this barrier function, which is composed of moisture and natural oils. In normal skin, this hydro lipid film performs its job perfectly by keeping external damaging elements out and right amount of moisture inside body. http://www.eucerinus.com/skin-health/skin-type/normal-skin.html

1.7.2 Oily skin

Oily skin refers to a facial disorder, which is characterized by presence of large pores, feeling of greasiness and shiny appearance, due to increased production of sebum. Oily skin causes many cosmetic problems and it is also a cause of common skin diseases such as seborrheic dermatitis and acne vulgaris. Oily skin leads to low self-esteem, distress, depression and negative self-perception which is harmful to social interactions (Arbuckle *et al.,* 2009, 2008; Segot-Chicq *et al.,* 2007)

1.7.3 Dry skin

Appearance of dry skin is tight, scaly, flaky, cracked and dry. Wrinkles can easily develop. Dryness of skin can occur at any agehttp://www.prokerala.com/health/beauty/skin-type-test.php#characteristics. It isobvious that the horny layer converts intomore brittle and very less stretchable along with increased dryness. Therefore it is desirable to evaluate skin dryness to a sub-

clinical inflammatory process that can be enhanced by exposure to sun and more use of soaps, face cleanser and astringents (Leveque*et al.,* 1987).

1.7.4 Combination skin

Combination skin has two opposite ends of the skin care spectrum. People with combination skin have an oily T-zone and dry cheeks. People deal with infrequent breakouts and larger pores, particularly on the nose. Gentle oil control is required in certain areas while some areas need to be moisturized, to certify healthy skin http://www.prokerala.com/health/beauty/skin-type-test.php#characteristics.

1.7.5 Sensitive skin

Sensitive skin (hyper-reactive, irritable skin or intolerant) can be definedby the initiation of tingling sensations, erythema andburning, owing to various factors, which may be physical like cold, heat, wind and ultraviolet; chemical like cosmetics, water pollution and soap; psychological like mental stress or hormonal like menstrual cycle (Berardesca *et al.,* 2006; Muizzuddin, 1998).Initially this skin type was described on facebut some other body parts are possible mainly scalp and hands (Saint-Martory *et al.,* 2008). Reducedskin barrier function and increased trans epidermalwater loss have been reported(Seidenari *et al.,* 1998). Inflammation possibly results from the release substance P, from which vasodilatationand mast cell degranulation is triggered. Non-specific inflammatory reactionmay also be related with release of IL-1, IL-8, PgF2, PgE2 and TNF-α(Reilly *et al.,* 2000).

1.8 Interleukin-6 (IL-6)

In response to various stimuli like physical exercise, oxidative stress and endotoxins produced by bacteria, some cells like monocytes, macrophages and endothelial secrete a polypeptide named asIL-6. IL-6 plays its role via its level in blood. In healthy individuals detection of normal IL-6 can be done in the 1 pg/mL range (Stenvinkel*et al.,*2002). IL-6 function can be studied by its various names which were purposed before its final name proposal as IL-6 (Wolvekamp and Marquet, 1990). These names include hepatocyte stimulatory factor, interferon (IFN)-β2, macrophage granulocyte

inducing factor 2, myeloma/plasma cytoma growth factor and some others. IL-6 was primarily identified as a B-cell differentiation factor (Hirano *et al.,* 1986; Teranishi *et al.,* 1982) but now it is known to be multifunctional which regulating hematopoiesis, immunologic reactions and inflammation (Hirano, 1998).

IL-6 plays its function by using a receptor complex which is formedof IL-6 receptor (IL-6R) and gp130, a glycoprotein involved in transduction of signal. Soluble receptor of IL-6 is present in the serum and form a complex when become bound to IL-6. (Horiuchi *et al.,* 1994; Mullberg *et al.,*1993, 1992; Lust *et al.,* 1992). The receptor complexIL-6/sIL-6R bind to gp130 receptor that is expressed on endothelial cells and fibroblasts and signal transducers and activators of transcription protein 3 signaling pathway (STAT3) is activated (Jones and Rose-john, 2002).Furthermore, sIL-6R is also involved in transition of early inflammatory response to late response (Figure 1.2).

1.8.1 Effects of IL-6 on Inflammation and Acne Vulgaris

IL-6stimulatesinflammation by increasingproliferation of lymphocytes, leucocyte recruitment, activating differentiation of B cells and by inducing the acute phase proteins (Papanicolaou *et al.,* 1998). Acute to chronic inflammation transition is dictated by IL-6 and sIL-6R, by altering leucocyte infiltrate neutrophils to monocytes (Gabay, 2006).IL-6 has been also found to involve in initiation and propagation of chronic inflammatory reactions. In case of acute inflammation pro inflammatory cytokines promotes the accumulation of neutrophils and also play a role in IL-6 release.

Then neutrophils shed receptors of IL-6 in reaction to IL-8 like cytokines and differentially regulated cytokine production in endothelial cells is stimulated.Monocyte chemo attractant protein MCP-1 production is promoted while production of IL-8 is decreased. As a result of which accumulation of monocytes is favored. Moreover, IL-6 also plays its part in neutrophil apoptosis so it also resolves acute inflammation (McLoughlin *et al,* 2003; Afford *et al.,* 1992).IL-6 plays vital role in chronic inflammation. Level of IL-6 can be detected in high range in most inflammatory diseases as IL-6 is up regulated during inflammatory process (Gabay, 2006). IL-6, gp130 and sIL-6Rα are all elevated in most inflammatory conditions, for

example IBD (Mitsuyama *et al.*, 2006), cancer (Soresi, *et al.*, 2006) and rheumatoid arthritis (RA) (Cronstein, 2007).

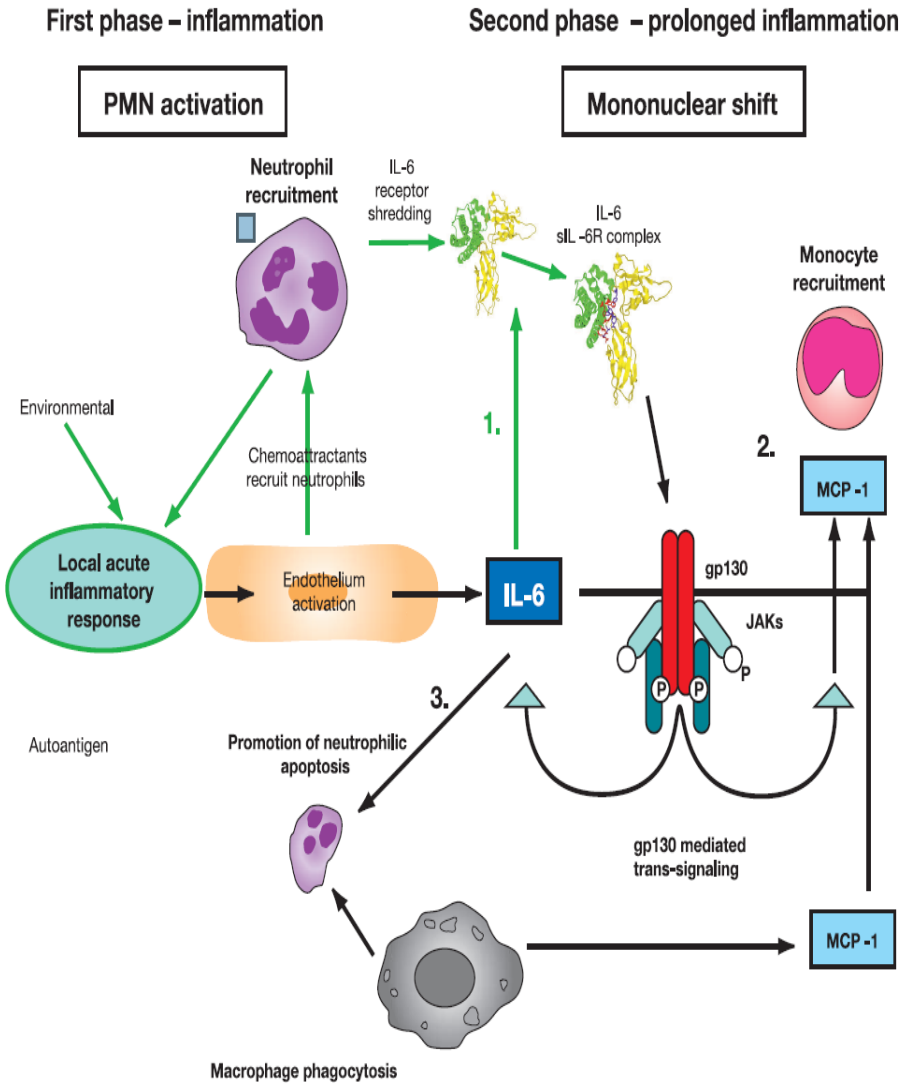


Figure 1.2 Possible role played by IL-6 in the shift from acute to chronic inflammation. Stage 1: following acute inflammatory response, IL-6 can bind with sIL-6R. Stage 2: trans-signalling through gp130 leads to monocyte recruitment. Stage 3: prolonged IL-6 leads to neutrophilic apoptosis, phagocytosis and mononuclear accumulation at the site of injury. IL, interleukin; JAK, Janus activated kinase; MCP,

monocyte chemoattractant protein; PMN, polymorphonuclear neutrophil; sIL-6R, soluble IL-6 receptor (Gabay, 2006).

Cytokines are predisposed by various factors in the normal sebaceous glands. When sebocyte culture is not under stress, cytokines like IL-6, IL-8 and IL-1α are discharged in supernatant. But under stress, level of released cytokines increases. Ifhuman skin cells are incubated with vitamin D, calcium and arachidonic acid, IL-6 expression is unregulated(Krämer *et al*., 2009; Alestas *et al*., 2005). Release of IL-6 from sebocytes can be triggered by corticotropin-releasing hormone (CRH) by stimulating immune processes leading to acne (Ganceviciene *et al*., 2009; Krause *et al*., 2007).

1.8.2 Signal Transduction

Two key signaling pathways are triggered by IL-6; JAK-STAT and ERK/MAPK pathway (Figure 1.3). Binding of IL-6 to IL-6R is followed by homo dimerization of gp130. Activation of a Janus kinase (JAK1) takes place. Upon activation it phosphorylates and activates another transcription protein STAT3 (signal transducer and activator of transcription) (Vanden Berghe *et al.,*2000). Phosphorylated STAT3 moves to nucleus after dimerization and transcription is initiated (Heinrich *et al.,*2003). This pathway promotes growth while it prevents apoptosis (Taub, 1996).Another pathway is triggered by IL-6*i.e.* mitogen-activated protein kinase (MAPK) pathway. After concerned analysis of MAPKs activated by gp130, tyrosine of SHP2-binding site was found crucial and it also activates Ras (Heinrich *et al.,* 2003).

IL-6 trans signaling is an other alternate pathway of IL-6 signaling. Free IL-6 can bound to its soluble receptor. This complex signals all cells having gp130 on their surfaces. Trans-signaling IL-6 is possibly a reason for the most of ill effects shown by IL-6 in chronic inflammatory reactions. Regulation of sIL-6Rα is unclear presently, butalternative mRNA splicingis the process through which it is produced. While proteolytic shedding of cells' surfaces can also produce it (Rose-John *et al.,* 2006).

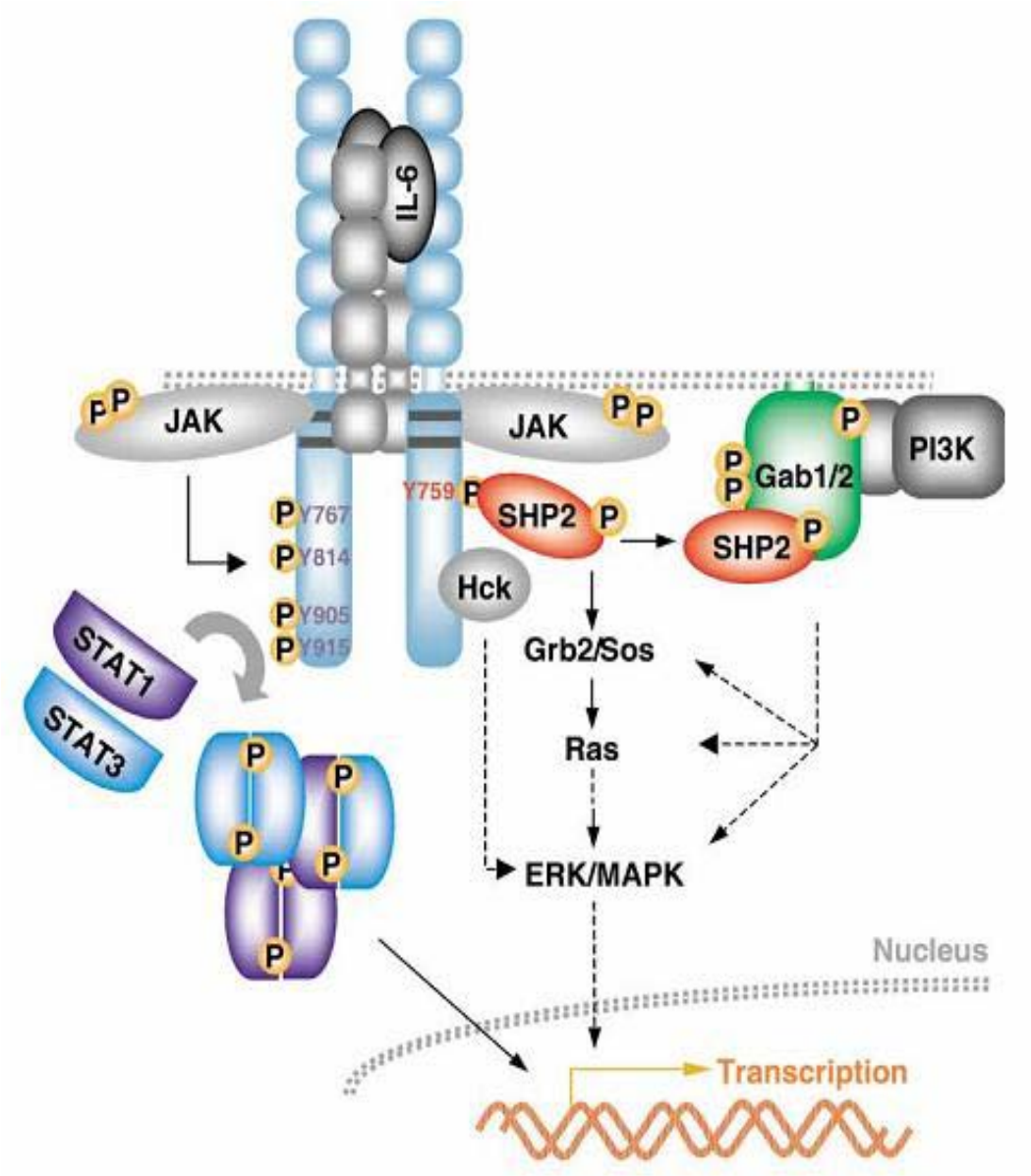


Figure 1.3 The intracellular signal transduction pathway generated by IL-6 (Kamimura *et al.*, 2003).

1.8.4 Molecular Properties of IL-6

IL-6 gene of Homo sapiens is located on the chromosome 7p21. Receptor gene (IL-6R) is present on the chromosome 1q21. While humans have two loci for gp130 at two different chromosomes; one is the genuine gene of gp130 located on the chromosome 5q11 while second gene is a pseudo gene present on chromosome 17(Ishihara and Hirano, 2002).

1.8.4.1 Polymorphisms in Promoter Region of the IL-6 Gene

For transcriptional control of the IL-6 gene many potential promoter elements are present in its 5´ flanking region like AP-1 binding site, c-fos serum-response element (SRE) homology, CCAAT/enhancer binding protein β (C/EBPβ), multiple response element (MRE), the glucocorticoid response element (GRE), cAMP response element (CRE) and the nuclear factor for IL-6 expression (NF-IL-6).IL-6 promoter activity can be suppressed by tumor suppressors, p53 and Rb proteins (Chauhan *et al.*, 1996; Santhanam *et al.*, 1991).

Four polymorphisms are present in the IL-6 gene promoter (-597G → A, -572G → C, -373A(n)T(n), -174G →C)(Figure 1.4), all of these are actually naturally occurring haplotypes. A polymorphism named -174 (G → C) is present in promoter of the IL-6 gene which is involved in transcription of IL-6 gene. It was observed after bypass surgery that patients with C/C allele at -174 position showed high level of IL-6as compared to G/G allele(Brull *et al.*, 2001). This G/C polymorphism is thought to be associated with IL-6 level;C at 174 positions mediates decreased production of IL-6 while G governs increased IL-6 production (Ishihara and Hirano, 2002; Sehgal, 1990).

The G/C variation is associated with the vulnerability to several inflammatory disorders, such as insulin (type 1) and non insulin dependent (type 2) diabetes mellitus. This G/C variation is also associated withKaposi's sarcoma,dementia, Alzheimer's disease, schizophrenia, artery occlusive disease, Eales' disease and post menopausal osteoporosis (Zakharyan *et al.,* 2012; Sen *et al.,* 2011; Flex *et al.,* 2002; Pola *et al.,* 2002a; Pola *et al.,* 2002b; Ferrari *et al.,* 2001; Fernández-Real *et al.,* 2000; Jahromi *et al.,* 2000; Foster *et al.,* 2000), No association was observed between systemic lupus erythematosus (SLE) or inflammatory bowel disease (IBD) with -174 G/C polymorphism (Linker-Israeli *et al.,* 1999) or in patients (Klein *et al.,* 2001).

High level of IL-6 expression has been found in the pathology of psoriasis, and relative deficiency of IL-10 in psoriatic patients appears to be significant in the development of psoriasis(Baran *et al.,* 2008).Association of IL-6 (-174G/C) with psoriasis vulgaris in cases from Egypt was studied and it was also found thatgenetic polymorphisms related to IL6 geneexhibited particular pattern of association with psoriasis which may have a possible impact on counseling of disease and itsmanagement(Settin *et al.,* 2009).

Role of IL-6 in thepathogenesis of acne vulgaris is established by recent studies, by demonstrating its rolein inflammation. However, so far no study was conducted to focusimplication of polymorphic regions of IL-6 gene in pathogenesis of acne. So, the present study was intended for findingassociation of IL-6 gene polymorphism (-174G/C) withacne vulgaris patients having different types of skin and acne severity in Pakistani population.

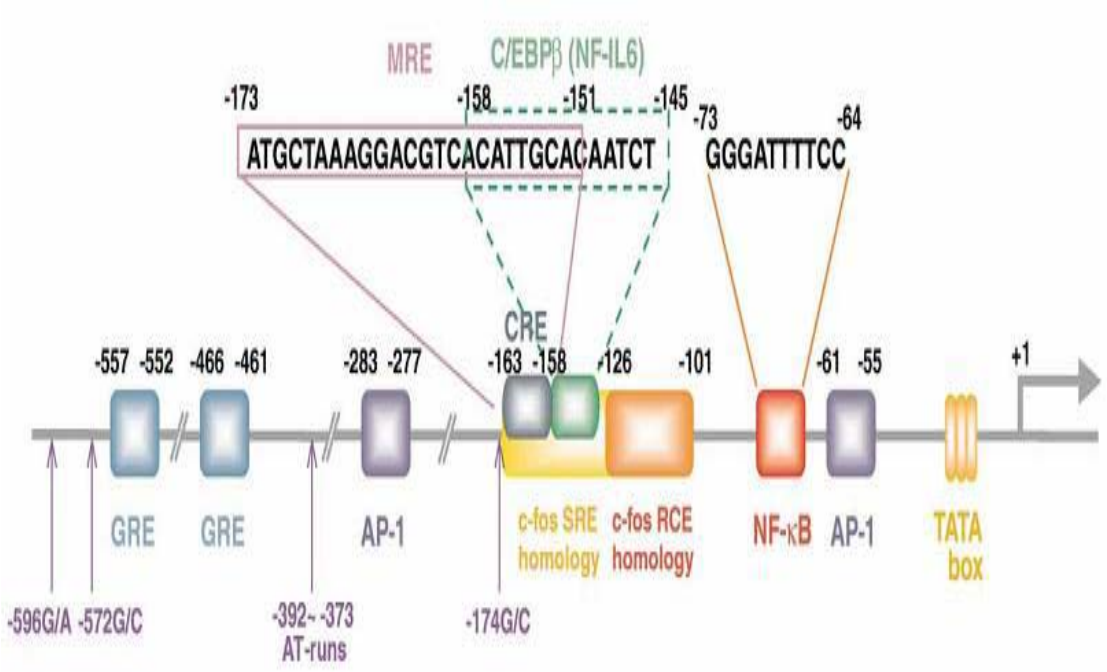


Figure 1.4A schematic illustration of the 5´ flanking promoter region of humanIL-6gene. The nucleotide position of the cis-regulatory elements and polymorphicregions is given relative to the major transcription start site (+1). Functional polymorphisms located in the 5´ flanking region are indicated with arrows (Kamimura*et al.*, 2003).

MATERIALS AND METHODS

2.1 Study Population

Currentstudy was conducted on135 case-control subjects, 65 were acne patients and 70 were healthy controls. All subjects included in this study, werePakistani and they were aged between 11 and 30years.

This study was approved by Institutional Review Board, Q.A.U., Islamabad.

2.2 Inclusion Criteria

Patients (n = 65) employed in this study, were havingseveral degreesofacne vulgaris severity. Acne vulgariswas described as a disorder depicted by non-inflammatory; open and closedcomedones and by inflammatory lesions; pustules, papules, nodules and cysts. Combined Acne Severity Classification byLehmann *et al*. (2002)was used for evaluating acne severity. Seventy subjects with no history of any diseasewere also enrolled for this study**.** All of controls were matched with patients according to age.

2.3 Questionnaire Filling

Questionnaire was filled by every subject of this study. Interviews were taken from every contributor personally. Samples were taken with patients' consent. Questionnaire given in Annex-I,collectedinformation of all subjects, including age gender, lesion type, drug response, occupation and family history.

2.4 Blood Sample Collection

Patients' blood samples were collected from Pakistan Institute of Medical Sciences (PIMS) Islamabad. While samples of control subjects were collected from PIMS and Quaid-i-Azam University Islamabad.Samples were collected in the 5 mL sterile syringes (Master®Plus, Pakistan) by usingvein puncture technique. For analysis of samples at molecular level, blood(2.5 mL) wasinstantlytransferred into the screw cappedEDTA vials(BD, USA).To transport samples in laboratory, they were kept in ice box. Samples were keptat 4°C in the laboratory.

2.5 Evaluation of Skin type

Skin types were determined by using online software for skin type testhttp://www.prokerala.com/health/beauty/skin-type-test.php. Skin type of each patient and control was evaluated by filling the online questionnaire and taking information from questionnaires of patients and controls. Normal skin was referred as type I, dry skin as type II, oily and combination skins as type III and sensitive skin as type IV.

2.6 Genetic Testing

Stock solutions were prepared as given in table 2.1.Stocksolutions were autoclaved and stored in refrigerator at 4ºC. These solutions were then used forpreparation of working solutions.Solution A (Erythrocyte lysis buffer), Solution B (Leukocyte lysis buffer), Solution C (Saturated phenol pH 8.0), Solution D (Chloroform: Isoamylalcohol), Na-Acetate (3 M), SDS (10%, pH 7.2) and 70% Ethanol were prepared for DNA extraction (table 2.2). Gel electrophoresis solutions;Gel loading dye (Bromophenol Blue BPB), Ethidium Bromide, 1X TBE buffer were prepared according to method given in table 2.3.

2.6.1 DNA Extraction

DNA extraction was carried out in laboratory by using liquid-liquid extraction *i.e.* standard phenol-chloroform method (Sambrook*et al*., 1989). From every sample, 500µL of whole blood was taken in an eppendorf tube. Solution A (750 µL)was added, mixed by inversion, kept them at room temperature for at least fifteen minutes and centrifuged at 13,000 rpm in a microcentrifuge (Eppendorf Centrifuge, Model 5417R, Germany) for two minutes. Pellet was settled in the bottom of eppendorf tube. After discarding supernatant, pellet was re-suspended in solution A (400 µL).Then centrifugation was done for 2 minutes at 13,000 rpm. After centrifugation supernatant was discarded and solution B (400 µL), SDS (25 µL) and proteinase K (6 µL) were added in pellet to re-suspend it. Tubes were incubated at 37°C overnight.

Table 2.1 Preparation of stock solutions

Stock solution	Method of preparation
Tris (1M,pH 7.6)	Tris-base (121.1 g) was dissolved in 800 mLof distilled water (dH_2O) and pH was adjusted to 7.6 with few drops of conc. HCl, then dH_2O was added to adjustthe volume up to 1000 mL.
$MgCl_2$ (1M)	For making 1M $MgCl_2$, 203.3 g $MgCl_2$ was dissolvedin 800 mL of dH_2O and final volume was adjusted to 1000 mL with dH_2O.
NaCl (5M)	NaCl (292.1 g) was dissolved in 800 mL dH_2O and final volume was adjusted to 1000 mL with dH_2O.
EDTA (0.5M, pH 8)	EDTA (186.1 g) was added into 800 mL dH_2O, stirred on magnetic hot plate stirrer, after dissolving, pH was adjusted to 8 by adding NaOH. Final volume was adjusted to 1000 mL with dH_2O.
TBE Buffer (10X)	In order to make 10X TBE buffer 108 g of Tris-base was dissolved in 700 mL of dH_2O. Then 55 g of boric acid was added and dissolved in the solution. In the next step 40 mL of EDTA (0.5 M, pH 8) was added to the solution and final volume was adjusted to 1000 mL with dH_2O.

Table 2.2 Preparation of working solutions used in DNA Extraction.

Working solution	Method of preparation
Solution A (Erythrocyte lysis buffer)	For solution A preparation, 54.72 g of 0.32M Sucrose, 5 mL of 10 mM Tris-HCl pH 7.5 (1 M stock) and 2.5 mL of 5 mM MgCl2 (1M stock) were dissolved in 400 mL of dH_2O and pH was adjusted to 7.5. Volumewas adjusted to 495 mL with dH_2O. The solution was autoclaved and 5 mL of 1% v/v Triton X-100 was added after cooling at room temperature.
SolutionB (Leukocyte lysis buffer)	Five mL of 10 mM Tris-HCl, pH 7.5 (1 M stock), 40 mL of 400 mM NaCl (5 M stock) and 2 mL of 2 mM EDTA,pH 8 (0.5 M stock) were dissolved in 300 mL of dH_2O. Final volume was adjusted to 500 mL with dH_2O. The solution was autoclaved.
Solution C (Saturated phenol pH 8.0)	Phenol was melted in its own container at 65°C in a hot water bath and 100 mL of melted phenol was transferred in a new bottle. Then 100 mL of deionized water was added in it. Bottle was vigorously shaken to mix the organic and aqueous phases until they formed a fine emulsion. The bottle was stored overnight at room temperature to separate phases. Following day, upper (aqueous) phase was pipetted off. Procedure was repeated twice by adding equal volume of deionized water. Finally, pH was adjustedto 8.0 and phenol was stored at 4ºC, protected from light in an amber bottle.
Solution D	For making solution D, 1 mL of isoamylalcohol was added in 24 mL chloroform. Final volume was adjusted to 25 mL.
Na-Acetate (3 M)	For 3 M Na-Acetate preparation, 24.61 g Na-Acetate was dissolved in 60 mL of dH_2O and final volume was adjusted to 100 mL with dH_2O.
SDS (10%, pH 7.2)	Firstly, 10 g of SDS was dissolved in 80 mL dH_2O and pH was adjusted 7.2 by adding few drops of HCl. Afterwards the final volume was adjusted to 100 mL with dH_2O.
70% Ethanol	In order to make 70% ethanol 30 mL of dH_2O was added in 70 mL

	of absolute ethanol and final volume was adjusted to 100 mL.

Table 2.3 Preparation of gel electrophoresis solutions.

Name of solution	Method of preparations
BPB (Gel loading dye)	For loading dye preparation, 40 g sucrose and then 0.25 g of BPB were dissolved in 80 mL of dH_2O; final volume was adjusted to 100 mL with dH_2O.
Ethidium Bromide	Ethidium Bromide (1 g) was dissolvedin 95 mL of dH_2O and final volume was adjusted to 100 mL with dH_2O.
1X TBE buffer	For 1X TBE, 100 mL of TBE buffer from 10X stock was added in 900 mL of dH_2O and final volume was adjusted to 1000mL.

On the next day, mixture of solution C and D (600 µL) was added in samples, mixed by inverting tubes several times and then centrifugation was performed for ten minutes at speed of 13,000rpm. Then upper phase, which was aqueous in nature, was collected to a micro-centrifuge tube. Solution D, equal involume with collected phase, was then added in it. Tubes were centrifugedfor ten minutes at 13,000 rpm. Upper phase was collected to a 1.5mL micro-centrifuge tube and 55 µL of sodium acetate and 500 µL isopropanol were added in it.Tubes were inverted several time in order to precipitate DNA and centrifuged at speed of 13,000 rpm for ten minutes. Now upper phase wasremoved by taking care thatthe DNA pellet is not disturbed, chilled ethanol (350 µL of 70% ethanol) was added in order to wash DNA pellet and again centrifuged at 13,000 rpm speed for ten minutes. Ethanol was removed after centrifugation, the DNA pallet was dried and then 100-250 µL of TE buffer (DNA dissolution buffer) depending upon size of pellet,was added to dissolve pellet. The tubes were left in incubator at 37ºC. On the very next day, the amount ofDNA in buffer was determined by running it on 1% agarose gelelectrophoresis (Table 2.4).

2.6.2 Polymerase chain reaction (PCR)

IL-6 gene part containing the -174G/C polymorphism, with reference SNP ID numberrs1800795, was amplified by PCR. Following pair of primer was used (Opdal and Rognum, 2007):

F: 5'-GCGATGGAGTCAGAGGAAAC-3'

R: 5'-ATCTTTGTTGGAGGGTGAGG-3'

PCR was performed in PCR tubes (Axygen, USA) with 0.2 mL capacity containing 25 µLmaster mix. Reaction mixture preparation is discussed in table 2.5.Reaction product was centrifuged for 20 seconds at speed 6,000 rpm to mix the DNA with master mix. Total size of the amplicon (the PCR product) was 408 bp (Annex-II). In order toaccomplish amplification, thermal cycler (Life Technologies, USA) Applied BiosystemsGeneAmp® PCR System 9700 was used.PCR conditions of temperature and time were specifically set, as shown in table(Table 2.6). After

successfulcompletion of the PCR program, the amplicons having 408 bp sizes were stored in the refrigerator. These PCR products were analyzed by 2% agarose gel(Table 2.7).

Table 2.4 One percent (1%) agarose gel electrophoresis.

Gel percentage	Procedure of agarose gel electrophoresis
One percent (1%) agarose gel electrophoresis	▪ One percent (w/v) agarose gel was prepared by dissolving 0.5 g of agarose in 50mL of 1X TBE buffer in a 250 mL flat bottom flask. ▪ The mixed solution was melted in a microwave oven. ▪ To stain the DNA, 7 µL of ethidium bromide solution (10mg/mL) was added to the melted agarose. ▪ From each sample, 5 µL DNA was mixed with loading dye and loaded into the wells of 1% agarose gel. ▪ Electrophoresis was performed at 120 volts for 30 minutes in 1X TBE running buffer. ▪ DNA bands were visualized by placing the gel on UV transilluminator and results were recorded by gel documentation system (Wealtec-Dolphin Doc).

Table 2.5 PCR reaction mixture.

Ingredients	Quantity
DNA template	3 µL
*Taq*buffer [750 mM Tris HCl (pH 8.8), 200 mM $(NH_4)_2SO_4$, 0.1% (v/v) Tween 20]	2.5 µL
$MgCl_2$ (25 mM)	2.0 µL
dNTPs (10 mMFermentas, England):	0.5 µL
FP (0.1 µM)	1.25 µL
RP (0.1 µM)	1.25 µL
*Taq*DNA polymerase (5 u/µL,Fermentas, England)	0.25 µL
PCR water	14.25 µL

Table 2.6 PCR programming cycle

	Steps		Temperature	Time
1	**Initial denaturation**		95ºC	5 minute
2	**40 cycles**	Denaturation	95ºC	1 minute
		Primer annealing	60ºC	1 minute
		Primer extension	72ºC	1 minute
3	**Final extension**		72ºC	10 minutes
	Holding temperature		4ºC	

Table 2.7 Procedure of 2% agarose gel electrophoresis.

Gel percentage	Procedure of agarose gel electrophoresis
Two percent (2%) agarose gel electrophoresis	▪ Agarose (1 g) of was dissolved in 50 mL of 1X TBE buffer in a 250 mL flat bottom flask and melted it. ▪ For staining PCR products, 7 µL of ethidium bromide solution (10 mg/mL) was added to the melted agarose. ▪ Amplified PCR product (4 µL) of was mixed with loading dye and loaded into the wells of 2% agarose gel. ▪ Electrophoresis was performed at 120 volts for 30 minutes in 1X TBE running buffer. ▪ The bands of amplified PCR products were visualized by placing the gel on UV transilluminator and results were recorded by gel documentation system.

2.6.3 Genotyping of IL-6 at position -174 (G/C)

The genotyping of IL-6 gene at -174 was carried out via restriction fragment length polymorphism (RFLP) technique. In order to detect G/C polymorphism at -174 promoter site, the PCR product with 408 bpsize was digested with *Nla*III (5'…CATG↓…3'). RFLP was accomplished by making 20 µL reaction mixture, (Table 2.8). Mixture was incubated in an incubator, at 37ºC for at least16 hours. The restricted PCR fragments (digestion products) were analyzed on 3% agarose gel electrophoresis (Table 2.9).

2.7 Statistical Analysis

The clinical and baseline characteristics like age of patients and the controls were characterizedby taking their mean value, calculation of standard deviation (SD). Both patients and controlswere comparedby using the Student's t-test. Numbers and percentages were used to express data. Allelic and genotypic frequencies were represented as percentages. For comparing genotypic and allelic frequencies between the two groups, Chi-square ($\chi 2$) testwere used. For comparing skin types with genotypes, acne severity and drug response one way ANOVA was applied. Statistical significance was set at < 0.05.Entire statistical tests were carried out by using GraphPad Prism version 6.00 (GraphPad Software, San Diego California USA).

Table 2.8RFLP Reaction mixture.

Ingredients	Quantity
PCR product	12 µL
1X G buffer [10 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 50 mM NaCl, 0.1 mg/mL BSA]	3 µL
(*Nla*III (5'…CATG↓…3') enzyme (5u/µL) (Fermantas, England)	0.5 µL
PCR water	4.5 µL

Table 2.9 Procedure of 3% agarose gel electrophoresis.

Gel percentage	Procedure of agarose gel electrophoresis
Three percent (3%) agarose gel electrophoresis	▪ Agarose (1.5 g) was mixed in 50 mL of 1X TBE buffer in a 250 mL flat bottom flask and melted it. ▪ To stain the digested PCR fragments, 7 µL of ethidium bromide solution (10 mg/mL) was added to agarose. ▪ The whole sample (20 µL) containing the digested PCR fragments was mixed with 6X DNA loading dye and loaded into the wells of 3% agarose gel. ▪ Electrophoresis was performed at 90 volts for 45 minutes in 1X TBE running buffer. ▪ The bands of digested PCR fragments were visualized by placing the gel on UV transilluminator and results were recorded by gel documentation system. ▪ The genotypes were determined according to the respective number of bands.

RESULTS

3.1 Characteristics of Acne Patients and Control Subjects

Characteristics of the patients with acne and healthy control subjects (both baseline and clinical)are given in Table 3.1 to Table 3.3 and Figure 3.1.

3.1.1 Baseline and clinical characteristics

Age of patients and controls (which is a baseline characteristic)was analyzed via Chi-square test. The study was carried out on 135 individuals including 65 acne patients and 70 control subjects. The study population of patient group consisted of36 Females (55%) and 29 male (45%) while control group consisted of 28 males (40) and 42 (60%) females. Age of the subjects under study ranged from 11 years to 30 years. Two age groups were made, 11-19 years and 20-30 years.Control group contained 33 (47%) aged 11-19 years and 37 (53%) aged 20-30 years while patients contained 24 (37%) aged 11-19 years and 41 (63%) aged 27-30 years (P = 0.22; Table 3.1).

For analysis of age (variable) of both patient and control group, mean values of age were calculated. Standard deviations (SDs) of age from both patient and control group was calculated. Unpaired Student's t-test was used for Comparing controls and patients. The results demonstrated that mean age of patients (20.82 ± 3.56 years) as compared to controls (21.39 ± 3.82 years) was not statistically significant (P = 0.38; t = 0.89; Table 3.2).

3.1.2 Clinical characteristics of patients

The clinical features of patients with acne vulgaris are shown in Figure 3.1. Among 65 patients, 10 (15%) patients reported mild acne; 34 (52%) patients reported moderate and 21 (33%) reported severe acne vulgaris. Out of 65 patients 26 (40%) patients had a positive family history of acne, while 39 (60%) had negative family history. Acne showed more prevalence in patients having a type III skin *i.e.* 54 (83%) as compared to type I skin 3 (5%), type II skin 4 (6%) and type IV skin 4(6%). Majority of the cases 44 (68%) were students, 12 (18%) were household and only 9 (14) were doing jobs. Scarring was the noticeable result of acne in 58 (89%) of the

patients, while only 7(11%) patients had skin deprived of scars. Many patients had facial acne *i.e.* 36 (55%), while 27 (42%) cases had acne breakout on other body parts and 2 (3%) reported acne on whole body. Family history, age groups,presence of scars and gender were compared with the acne severity*i.e.* with mild, moderate and severe acne. All of which showed no statistical significance when compared with acne severity (Table 3.3).There was no statistically significant difference found between skin type and acne severity (P = 0.64; Table 3.4). Moreover it was found that there is a significant difference between skin type of acne patient and drug response (P= 0.03; Table 3.5)

3.2 Genotype Distribution of Acne patients and controls

Genotype pattern of the acne patients was evaluated in order to associate -174 SNP of IL-6 with skin type of patients and vulnerability of the disease. Genotyping was done by using PCR and RFLPutilizing *Nla*III restriction enzyme. For conducting molecular study of the samples, *Nla*III enzyme recognized 5'…CATG↓…3' sequence and restricted amplified PCR product (408 bp). The restricted fragments were analyzed on 3% agarose gel. By observing band distribution pattern on 3% agarose gel, genotypes of patients were determined. Three band patterns were detected on the gels after digestion of PCR product.

Wild type genotype *i.e.* GG yielded three fragments(208 bp, 171 bp and 29 bp) upon digestion while variant heterozygous genotype GC yieldedfive different bands(208 bp, 171 bp, 122 bp, 86 bp and 29 bp). Whereas four bands (171 bp, 122 bp, 86 bp and 29 bp) were yielded by variant homozygous genotype CC. As small fragment (29 bp) could not be seen on the gels so GG, GC and CC were represented on gel by two, four and three bands respectively. Genotype patterns can be observed in Figures (3.2-3.18). Frequencies of all genotypes of IL-6 at -174 in the study population are demonstrated in Table 3.6. Data was found in consistence with Hardy-Weinberg equilibrium in the study population.When genotypes were compared between patient and control groups, non-significant P value (0.30) was obtained ($\chi 2 = 2.38$). In control subjects, distribution of genotype was GG = 46 (66%), GC = 21 (30%) and CC = 3 (4%) while in patients GG = 50 (77%), GC = 14 (21.5%) and CC = 1 (1.5%). When wild type GG and mutated GC+CC genotypic were compared, it was revealed that

GC+CC was more common in the control group (34%) as compared to patient group (23%) (P = 0.15; Table 3.7). Likewise, allelic frequencies were also compared between patients and controls,P=0.11 was obtained which is non-significant and interpret that mutant allele C was more frequent among controls (19% *vs*. 12%) while wild type allele G was more common in patients (88% *vs*. 81%) (Table 3.7).

3.2.1 Genotype distribution among patients

A non-significant P= 0.23 was obtained when genotype of wild type *i.e.* GG and mutant genotypes *i.e.* GC+CC were compared with skin type of acne patients (Table 3.8). In order to find IL-6 (-174G/C SNP)association with acne pathogenesis, genotypic frequencies were compared with severity of acne and family history of the patients. No statistically significance was found between severity of acne and IL-6 (-174G/C) genotypes polymorphism (P = 0.38; $\chi 2$ = 1.92; Table 3.9). Likewise Genotypic frequencies at -174 in IL-6 gene showed no association with family history (P= 0.55;$\chi 2$ = 0.36; Table 3.10).

Table 3.1 Baseline characteristics of controls and acne patients.

Characteristic	**Controls n (%age)**	**Patients n (%age)**	**$\chi 2$**	***P* value**
	70 (100)	**65 (100)**		
Age groups				
11-19 years	33 (47.14)	24 (36.92)	1.44	0.22
20-30 years	37 (52.86)	41 (63.07)		

Table represents column wise comparison of age groups between control subjects and acne patients.

Values are represented as numbers and percentages.

P value calculated by Chi-square test

n, number; %age, percentage

Table 3.2 Clinical characteristics of controls and acne patients.

Characteristic	**Controls (n= 70)**	**Patients (n=65)**	**t value**	***P* Value**
	Mean ± SD	**Mean ± SD**		
Age (years)	21.39 ± 3.82	20.82 ± 3.56	0.89	0.38

Table represents column wise comparison of age between control subjects and acne patients.

Values are represented as mean ± SD.

P value was calculated by Student's t-test (unpaired).

n, number; SD, Standard deviation

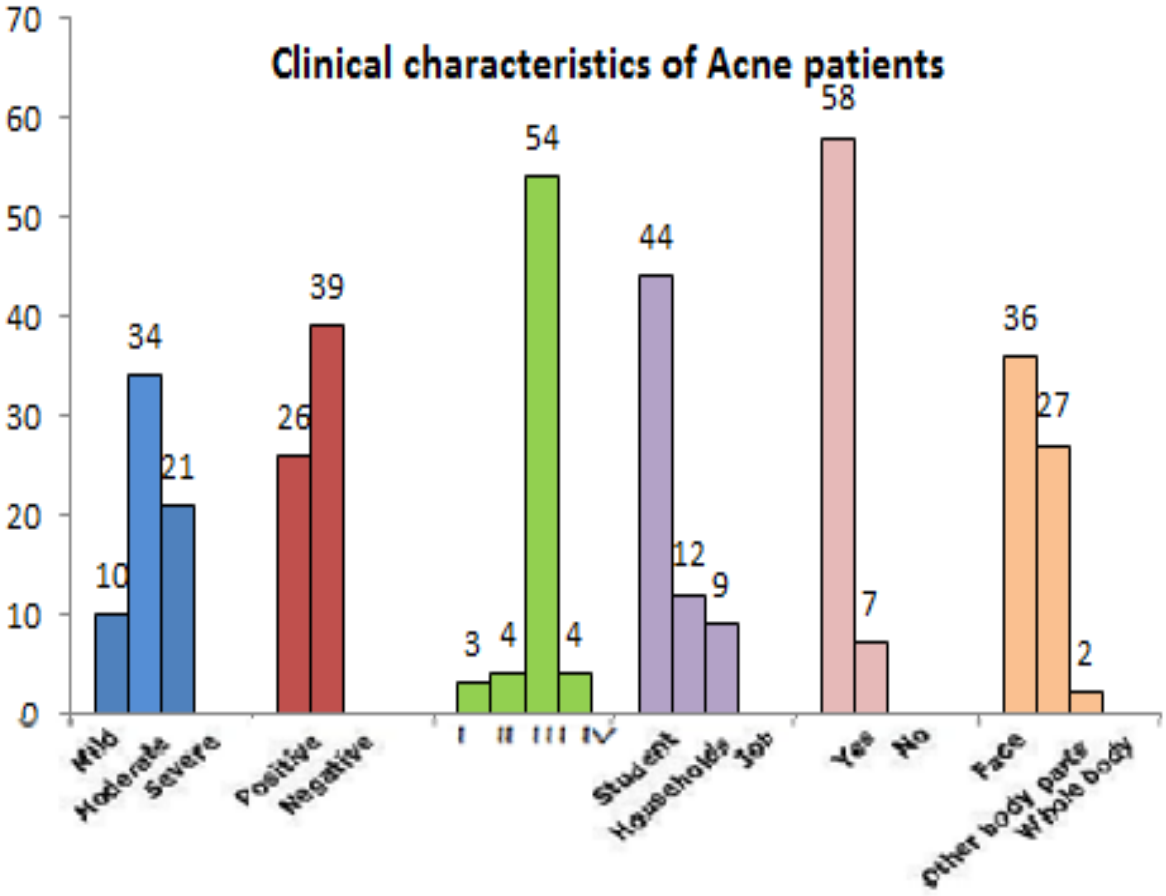


Figure 3.1 Graph showing clinical characteristics of acne patients.

Table 3.3 Family history, Scars, age groups and genders *vs.* severity of acne in the patients.

Characteristics	Severity of acne			χ^2 Value	*P* value
	Mild	Moderate	Severe		
Family history					
Positive	4	12	10	0.82	0.66
Negative	6	22	11		
Scars					
Present	8	30	20	1.71	0.42
Absent	2	4	1		
Age Groups					
11-19 years	1	12	11	5.30	0.07
20-30 years	9	22	10		
Gender					
Female	6	20	10	0.76	0.68
male	4	14	11		

Table represents column wise comparison of family history, scars on skin, age group and genders *vs.* severity of acne within the patients.

Values are represented as numbers.

P value was calculated by Chi-square test.

Table 3.4 Type of skin *vs*. severity of acne in the patients.

Skin Type	Severity of Acne			*P* value
	Mild	**Moderate**	**Severe**	
Type I	1	1	1	0.64
Type II	1	2	1	
Type III	7	30	13	
Type IV	1	1	2	

Table represents column wise comparison of type of skin *vs*. severity of acne within patients.

Values are represented as numbers.

P value was calculated by one way ANOVA test.

Table 3.5 Type of Skin *vs*. Drug response.

Drug Response	Type I/II	Type III	Type IV	*P* value
Positive	1	4	0	0.03
Negative	4	26	2	
Temporary	2	19	2	
Reaction	0	5	0	

Table represents column wise comparison of type of skin *vs*. severity of acne within patients.

Values are represented as numbers.

P value was calculated by One way ANOVA.

Table 3.6 Genotype frequencies of GG, GC and CC of IL-6 -174G/C polymorphism in the study population.

Genotype	Controls n (%age)	Patients n (%age)	χ^2 Value	*P* Value
	70 (100)	**65 (100)**		
GG	46 (65.71)	50 (76.92)	2.38	0.30
GC	21 (30.00)	14 (21.54)		
CC	3 (4.29)	1 (1.54)		

Table represents column wise comparison of genotypes (GG, GC, CC) between control subjects and acne patients.

Values are represented as numbers and percentages.

P value was calculated by Chi-square test.

n, number: %age, percentage

Table 3.7 Comparison of GG *vs.* GC + CC genotype frequencies and G/C alleles of the IL-6 -174 in the study population.

Characteristics	**Controlsn (%age)**	**Patientsn (%age)**	**$\chi 2$**	***P* value**
	70 (100)	**65 (100)**		
Genotype				
GG	46 (65.71)	50 (76.92)	2.06	0.15
GC+CC	24 (34.29)	15 (23.07)		
Alleles				
G	113 (80.71)	114 (87.69)	2.45	0.11
C	27 (19.29)	16 (12.30)		

Table represents column wise comparison of GG *vs.* GC + CC genotypes and G/C alleles between control subjects and acne patients.

Values are represented as number and percentages.

P value was calculated by Chi-square test.

n, number; %age percentage

Table 3.8 Genotype frequencies of GG and GC+CC of the IL-6 -174G/Cpolymorphism *vs*. skin type of the patients (n = 65)

Genotype	**Types of skin**				***P* value**
	Type I	**Type II**	**Type III**	**Type IV**	
GG	1	3	44	2	0.23
GC+CC	2	1	10	2	

Table represents column wise comparison of GG and GC+CC genotypes *vs*. skin type of acne patients.

Values are represented as numbers.

P value was calculated by One way ANOVA test.

Table 3.9 Genotype frequencies of GG and GC + CC of the IL-6 -174G/C polymorphism *vs.* severity of acne in the patients.

Genotype	Severity of acne			χ^2 value	*P*value
	Mild	Moderate	Severe		
GG	6	27	17	1.92	0.38
GC + CC	4	7	4		

Table represents column wise comparison of GG and GC + CC *vs.* severity of acne within patients.

Values are represented as numbers.

P value was calculated by Chi-square test.

Table 3.10 Genotype frequencies of GG and GC + CC of the IL-6 -174G/C polymorphism *vs.* family history of the patients.

Genotype	**Family history**		**$\chi 2$**	***P* value**
	Positive	**Negative**		
GG	19	31	0.36	0.54
GC + CC	7	8		

Table represents column wise comparison of GG and GC + CC genotypes *vs.* family history of acne patients.

Values represented as numbers.

P value was calculated by Chi-square test.

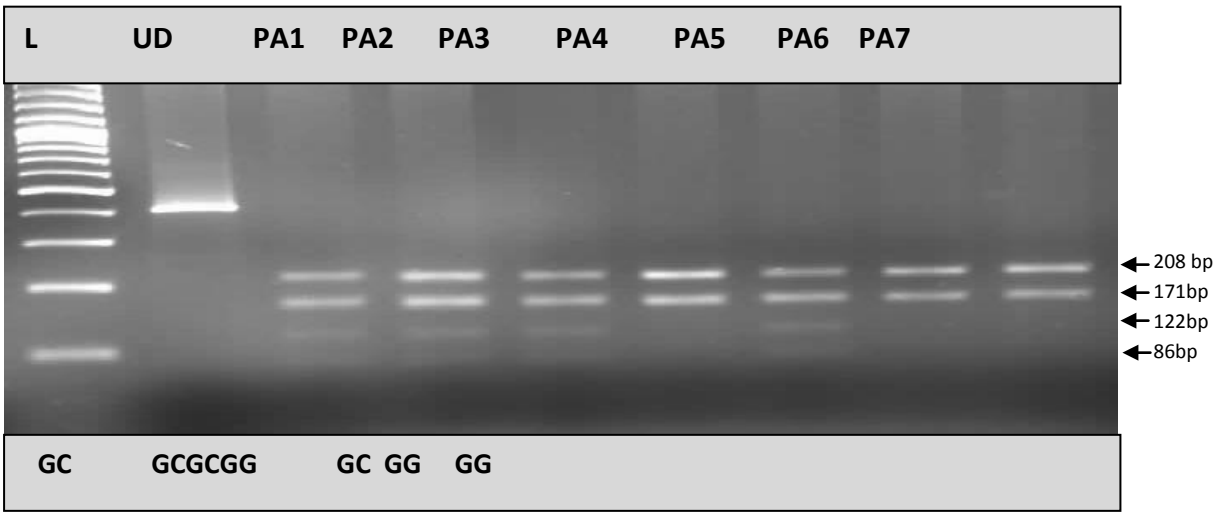


Figure 3.2 Electropherogram of ethidium bromide stained 3% agarose gel, showing genotype pattern obtained with *Nla*III restriction enzyme digest at -174G/C position of IL-6. Lane L represents 100 bp DNA ladder while lane UD refers to the undigested PCR amplified (408 bp) fragment. The other lanes (PA1-PA7) refer to genotype pattern of acne patients. Genotypes are characterized as GG (208 bp, 171 bp and 29 bp), GC (208 bp, 171 bp, 122 bp, 86 bp and 29). 29 bp fragment cannot be seen on the gel.

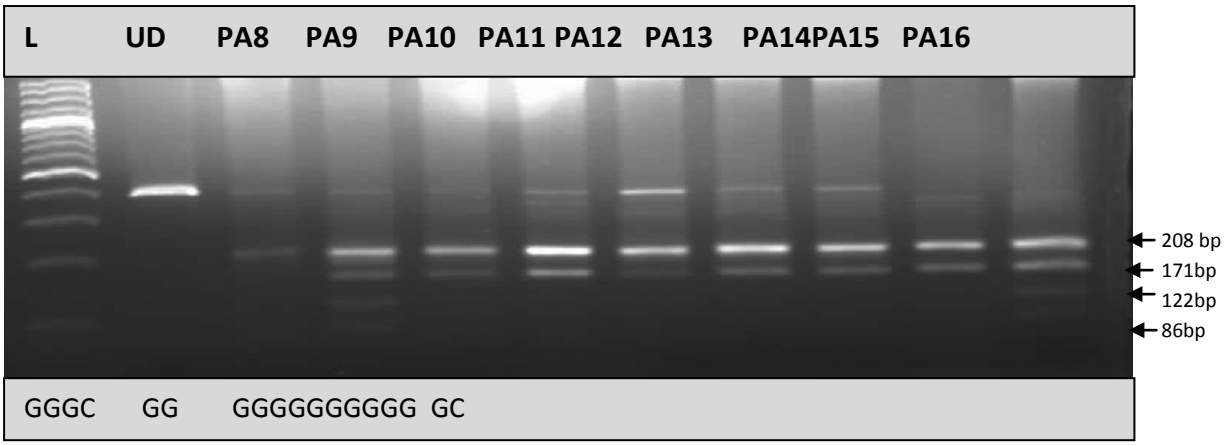


Figure 3.3 Electropherogram of ethidium bromide stained 3% agarose gel, showing genotype pattern obtained with *Nla*III restriction enzyme digest at -174G/C position of IL-6. Lane L represents 100 bp DNA ladder while lane UD refers to the undigested PCR amplified (408 bp) fragment. The other lanes (PA8-PA16) refer to genotype pattern of acne patients. Genotypes are characterized as GG (208 bp, 171 bp and 29

bp), GC (208 bp, 171 bp, 122 bp, 86 bp and 29). 29 bp fragment cannot be seen on the gel.

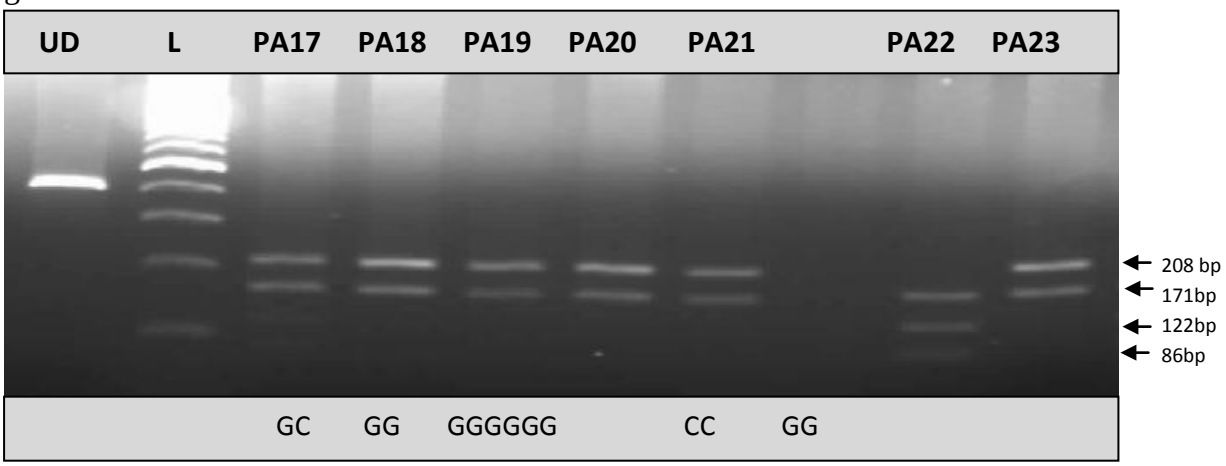


Figure 3.4 Electropherogram of ethidium bromide stained 3% agarose gel, showing genotype pattern obtained with *Nla*III restriction enzyme digest at -174G/C position of IL-6. Lane L represents 100 bp DNA ladder while lane UD refers to the undigested PCR amplified (408 bp) fragment. The other lanes (PA17-PA23) refer to genotype pattern of acne patients. Genotypes are characterized as GG (208 bp, 171 bp and 29 bp), GC (208 bp, 171 bp, 122 bp, 86 bp and 29) and CC (171 bp, 122 bp, 86 bp and 29 bp). 29 bp fragment cannot be seen on the gel.

Figure 3.5 Electropherogram of ethidium bromide stained 3% agarose gel, showing genotype pattern obtained with *Nla*III restriction enzyme digest at -174G/C position of IL-6. Lane L represents 100 bp DNA ladder while lane UD refers to the undigested PCR amplified (408 bp) fragment. The other lanes (PA24-PA36) refer to genotype pattern of acne patients. Genotypes are characterized as GG (208 bp, 171 bp and 29

bp), GC (208 bp, 171 bp, 122 bp, 86 bp and 29). 29 bp fragment cannot be seen on the gel.

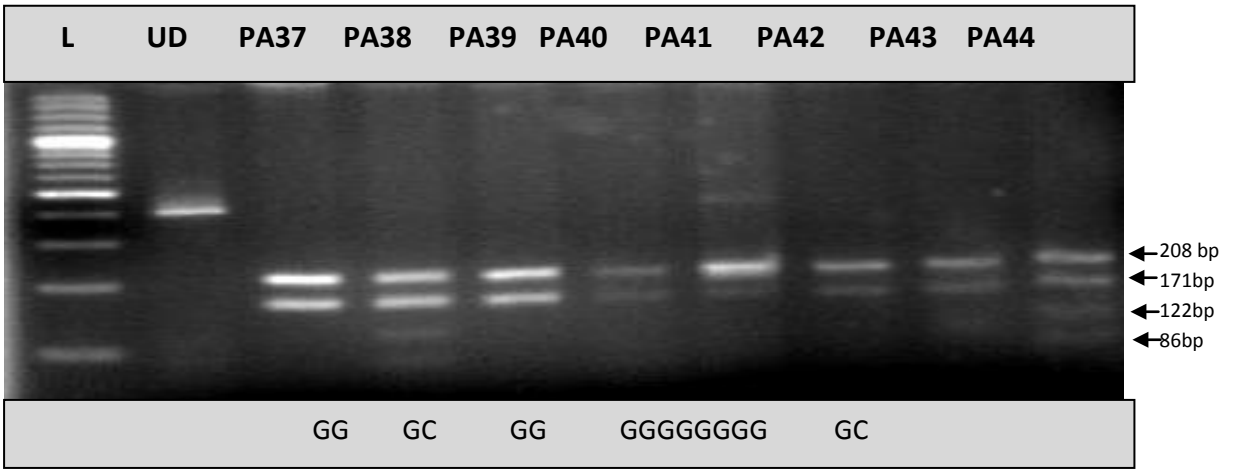


Figure 3.6 Electropherogram of ethidium bromide stained 3% agarose gel, showing genotype pattern obtained with *Nla*III restriction enzyme digest at -174G/C position of IL-6. Lane L represents 100 bp DNA ladder while lane UD refers to the undigested PCR amplified (408 bp) fragment. The other lanes (PA37-PA44) refer to genotype pattern of acne patients. Genotypes are characterized as GG (208 bp, 171 bp and 29 bp), GC (208 bp, 171 bp, 122 bp, 86 bp and 29). 29 bp fragment cannot be seen on the gel.

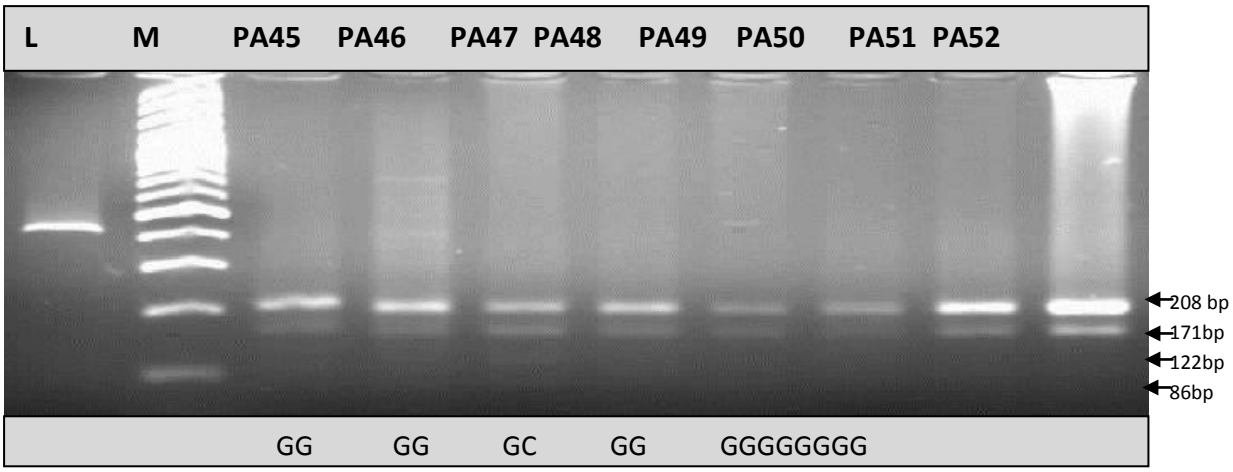


Figure 3.7 Electropherogram of ethidium bromide stained 3% agarose gel, showing genotype pattern obtained with *Nla*III restriction enzyme digest at -174G/C position of IL-6. Lane L represents 100 bp DNA ladder while lane UD refers to the undigested PCR amplified (408 bp) fragment. The other lanes (PA45-PA52) refer to genotype

pattern of acne patients. Genotypes are characterized as GG (208 bp, 171 bp and 29 bp), GC (208 bp, 171 bp, 122 bp, 86 bp and 29). 29 bp fragment cannot be seen on the gel.

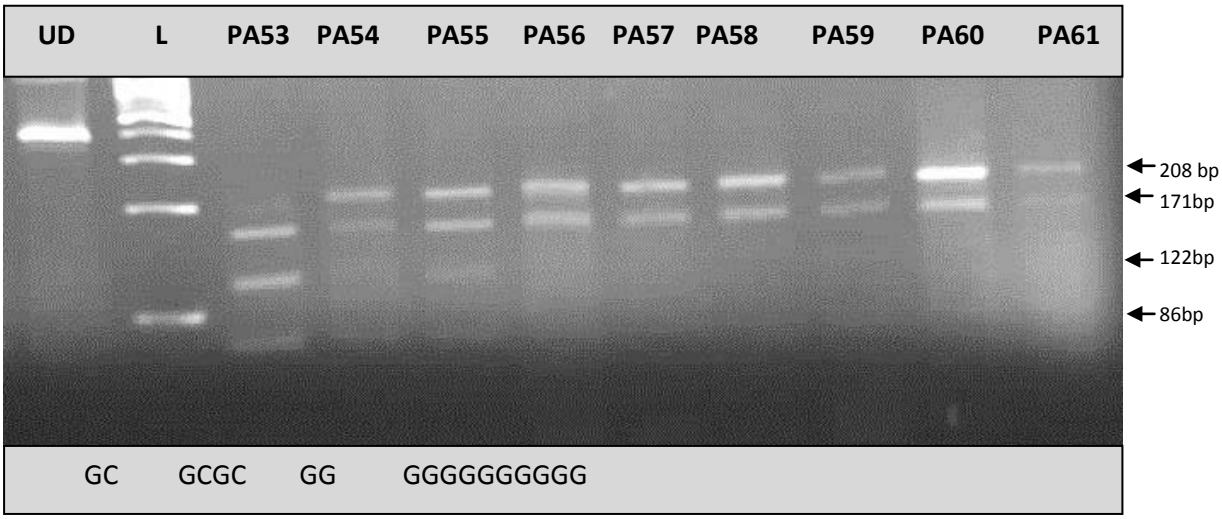


Figure 3.8 Electropherogram of ethidium bromide stained 3% agarose gel, showing genotype pattern obtained with *Nla*III restriction enzyme digest at -174G/C position of IL-6. Lane L represents 100 bp DNA ladder while lane UD refers to the undigested PCR amplified (408 bp) fragment. The other lanes (PA53-PA61) refer to genotype pattern of acne patients. Genotypes are characterized as GG (208 bp, 171 bp and 29 bp), GC (208 bp, 171 bp, 122 bp, 86 bp and 29). 29 bp fragment cannot be seen on the gel.

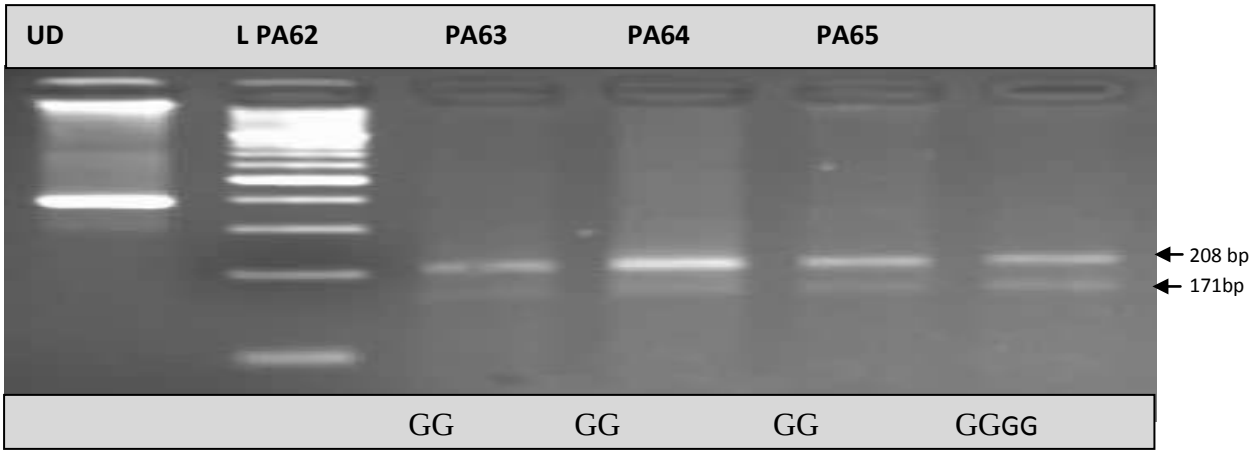


Figure 3.9 Electropherogram of ethidium bromide stained 3% agarose gel, showing genotype pattern obtained with *Nla*III restriction enzyme digest at -174G/C position of IL-6. Lane L represents 100 bp DNA ladder while lane UD refers to the undigested PCR amplified (408 bp) fragment. The other lanes (PA62-PA65) refer to genotype

pattern of acne patients. Genotypes are characterized as GG (208 bp, 171 bp and 29 bp). 29 bp fragment cannot be seen on the gel.

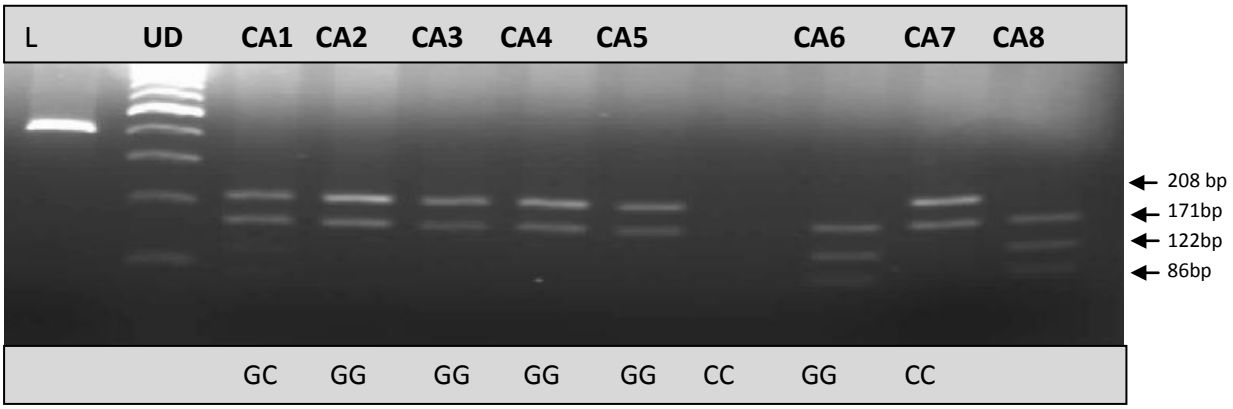


Figure 3.10 Electropherogram of ethidium bromide stained 3% agarose gel, showing genotype pattern obtained with NlaIII restriction enzyme digest at -174G/C position of IL-6. Lane L represents 100 bp DNA ladder while lane UD refers to the undigested PCR amplified (408 bp) fragment. The other lanes (CA1-CA8) refer to genotype pattern of control subjects. Genotypes are characterized as GG (208 bp, 171 bp and 29 bp), GC (208 bp, 171 bp, 122 bp, 86 bp and 29) and CC (171 bp, 122 bp, 86 bp and 29 bp). 29 bp fragment cannot be seen on the gel.

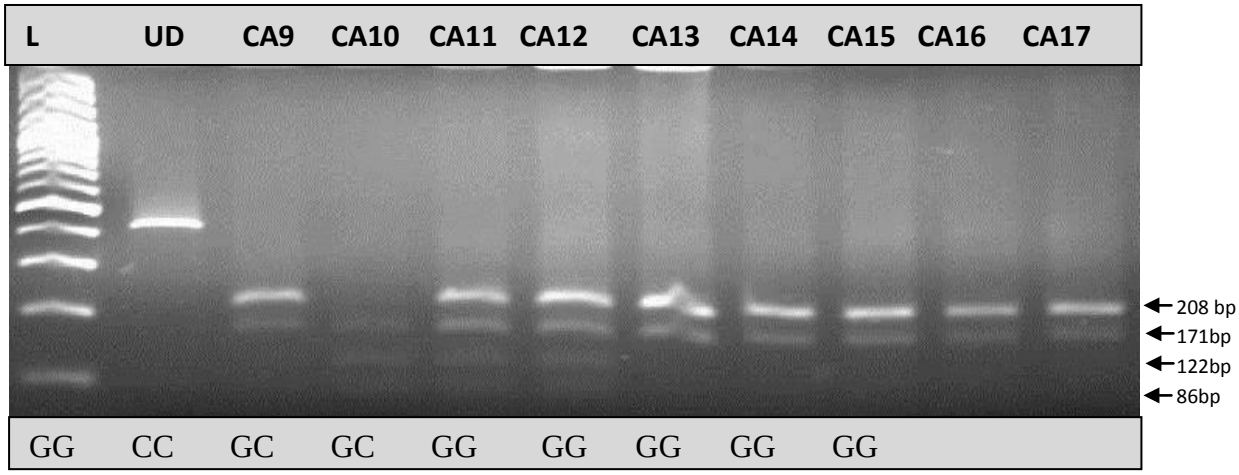


Figure 3.11 Electropherogram of ethidium bromide stained 3% agarose gel, showing genotype pattern obtained with NlaIII restriction enzyme digest at -174G/C position of IL-6. Lane L represents 100 bp DNA ladder while lane UD refers to the undigested PCR amplified (408 bp) fragment. The other lanes (CA9-CA17) refer to genotype

pattern of control subjects. Genotypes arecharacterized as GG (208 bp, 171 bp and 29 bp), GC (208 bp, 171 bp, 122 bp, 86 bp and 29) and CC (171 bp, 122 bp, 86 bp and 29 bp). 29 bp fragment cannot be seen on the gel.

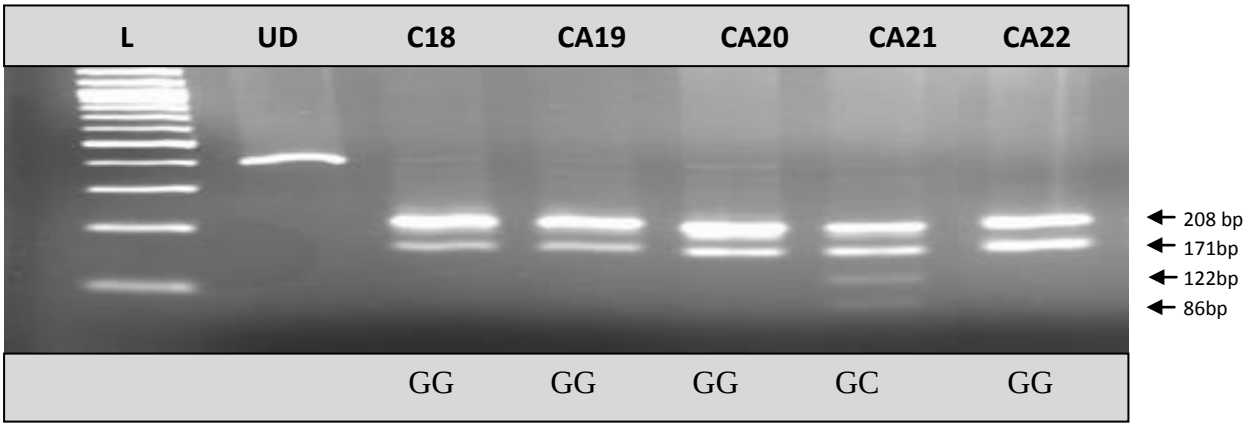


Figure 3.12 Electropherogram of ethidium bromide stained 3% agarose gel, showing genotype pattern obtained with NlaIII restriction enzyme digest at -174G/C position of IL-6. Lane L represents 100 bp DNA ladder while lane UD refers to the undigested PCR amplified (408 bp) fragment. The other lanes (CA18-CA22) refer to genotype pattern of control subjects. Genotypes arecharacterized as GG (208 bp, 171 bp and 29 bp), GC (208 bp, 171 bp, 122 bp, 86 bp and 29). 29 bp fragment cannot be seen on the gel.

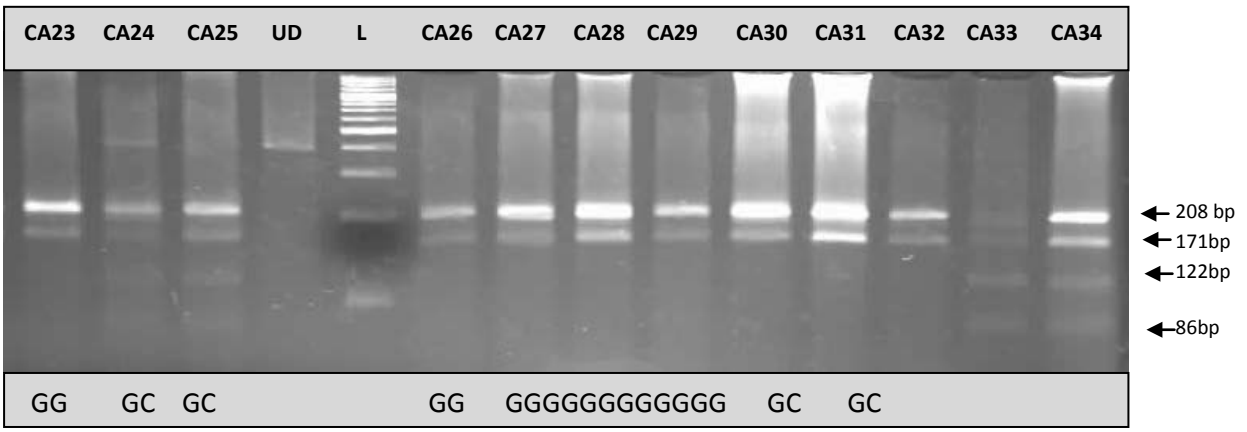


Figure 3.13 Electropherogram of ethidium bromide stained 3% agarose gel, showing genotype pattern obtained with NlaIII restriction enzyme digest at -174G/C position of IL-6. Lane L represents 100 bp DNA ladder while lane UD refers to the undigested

PCR amplified (408 bp) fragment. The other lanes (CA23-CA34) refer to genotype pattern of control subjects. Genotypes arecharacterized as GG (208 bp, 171 bp and 29 bp), GC (208 bp, 171 bp, 122 bp, 86 bp and 29). 29 bp fragment cannot be seen on the gel.

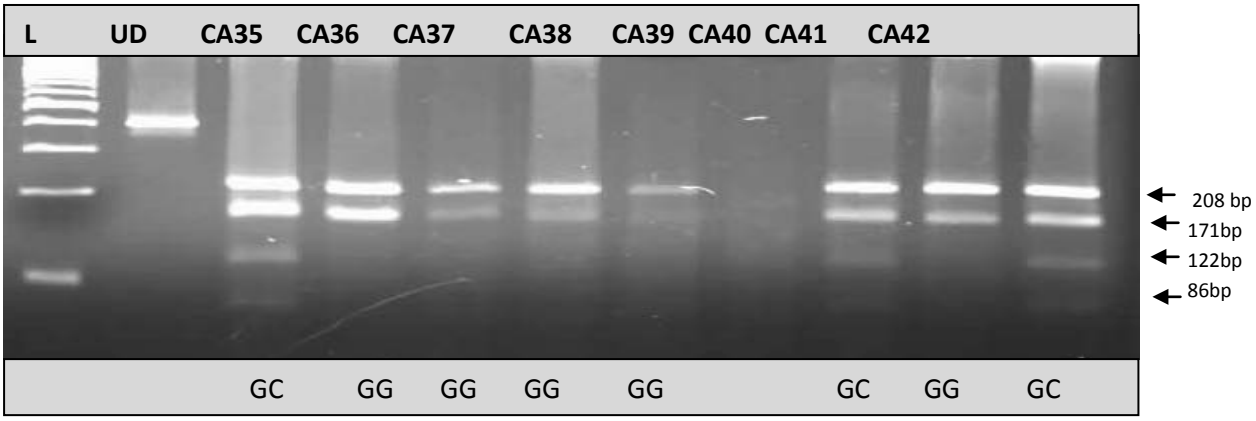


Figure 3.14 Electropherogram of ethidium bromide stained 3% agarose gel, showing genotype pattern obtained with NlaIII restriction enzyme digest at -174G/C position of IL-6. Lane L represents 100 bp DNA ladder while lane UD refers to the undigested PCR amplified (408 bp) fragment. The other lanes (CA35-CA42) refer to genotype pattern of control subjects. Genotypes arecharacterized as GG (208 bp, 171 bp and 29 bp), GC (208 bp, 171 bp, 122 bp, 86 bp and 29). 29 bp fragment cannot be seen on the gel.

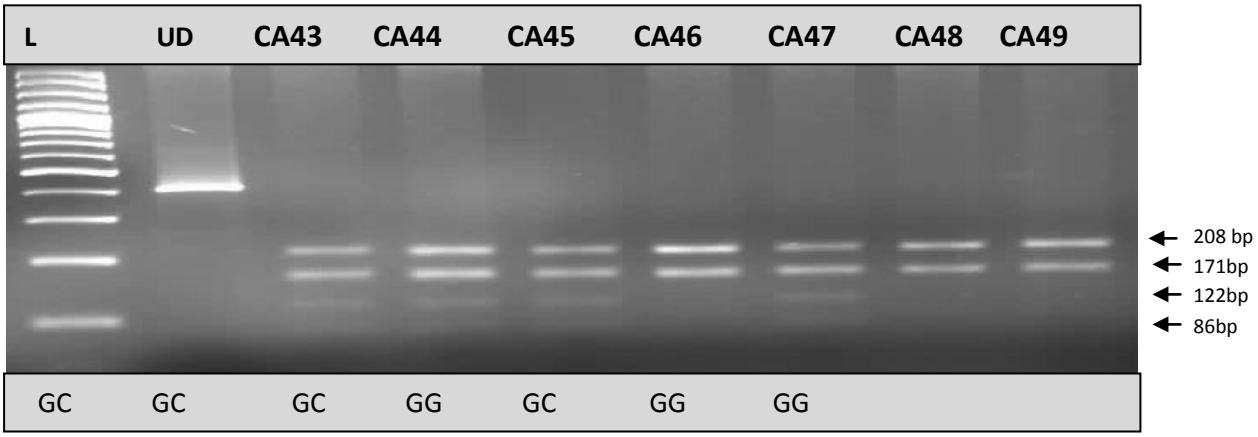


Figure 3.15 Electropherogram of ethidium bromide stained 3% agarose gel, showing genotype pattern obtained with NlaIII restriction enzyme digest at -174G/C position of IL-6. Lane L represents 100 bp DNA ladder while lane UD refers to the undigested PCR amplified (408 bp) fragment. The other lanes (CA43-CA49) refer to genotype

pattern of control subjects. Genotypes arecharacterized as GG (208 bp, 171 bp and 29 bp), GC (208 bp, 171 bp, 122 bp, 86 bp and 29). 29 bp fragment cannot be seen on the gel.

Figure 3.16 Electropherogram of ethidium bromide stained 3% agarose gel, showing genotype pattern obtained with NlaIII restriction enzyme digest at -174G/C position of IL-6. Lane L represents 100 bp DNA ladder while lane UD refers to the undigested PCR amplified (408 bp) fragment. The other lanes (CA50-CA57) refer to genotype pattern of control subjects. Genotypes arecharacterized as GG (208 bp, 171 bp and 29 bp), GC (208 bp, 171 bp, 122 bp, 86 bp and 29). 29 bp fragment cannot be seen on the gel.

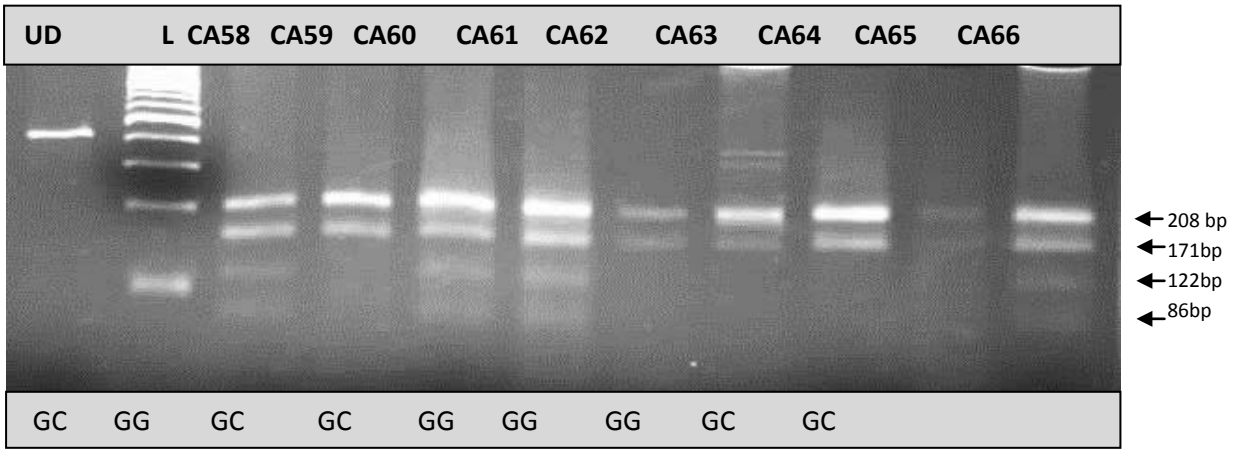


Figure 3.17 Electropherogram of ethidium bromide stained 3% agarose gel, showing genotype pattern obtained with NlaIII restriction enzyme digest at -174G/C position of IL-6. Lane L represents 100 bp DNA ladder while lane UD refers to the undigested PCR amplified (408 bp) fragment. The other lanes (CA58-CA66) refer to genotype

pattern of control subjects. Genotypes arecharacterized as GG (208 bp, 171 bp and 29 bp), GC (208 bp, 171 bp, 122 bp, 86 bp and 29). 29 bp fragment cannot be seen on the gel.

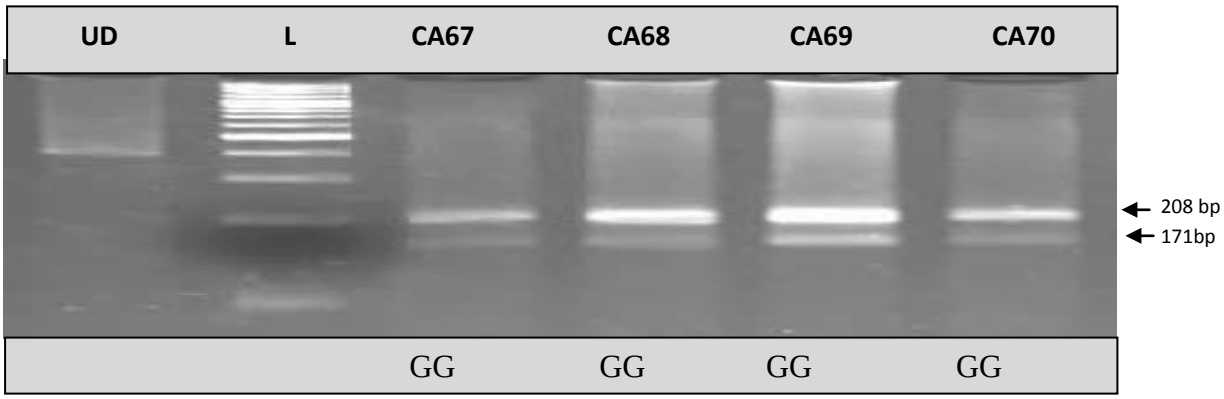


Figure 3.18 Electropherogram of ethidium bromide stained 3% agarose gel, showing genotype pattern obtained with NlaIII restriction enzyme digest at -174G/C position of IL-6. Lane L represents 100 bp DNA ladder while lane UD refers to the undigested PCR amplified (408 bp) fragment. The other lanes (CA67-CA70) refer to genotype pattern of control subjects. Genotypes arecharacterized as GG (208 bp, 171 bp and 29 bp). 29 bp fragment cannot be seen on the gel.

DISCUSSION

Acne vulgaris is a complex multi-factorial dermatosis mainly affecting the pilosebaceous follicle and clinically characterized by non inflamed acne lesion the comedones and inflamed acne lesions *i.e.* nodules, papules and pustules (Simpson and Cunliffe, 2008). The abnormal keratinization of infundibular duct of the hair follicles is starting point for micro comedo formation (Toyoda and Morohashi, 1998).Transition from non-inflammatory to inflammatory lesions, is favored by cytokines production, hormonal factors and high sebum production, characteristic of acne vulgaris (Kim, 2005; Gollnick, 2003).

Cytokines play major part in the inflammation and immune-regulatory reactions. Skin and chiefly keratinocytes in the skin are potent source for production of many cytokines including IL-6. So overproduction of cytokines, any change in cytokine receptors and dysregulation of cytokines have been associated with many inflammatory skin diseases (Sauder, 1990). High expression of IL-6 is shown by keratinocytes, in different skin disorders (Turksen*et al.*, 1992).

IL-6 is a pleiotropic cytokine, identified primarily as B cell differentiation factor. At beginning of the acute inflammation, acute phase response is mediated by IL-6. After its pro-inflammatory activity, acute inflammationis turned into chronic inflammation. In chronic inflammation, continuous signals are provided from IL-6 support,growth and survival of lymphocytes, due to which levels of IL-6 is increased. Thesecircumstances provide basis for amplification ofchronic inflammatory responses (Ishihara and Hirano, 2002).

Homo sapiens IL-6 gene is located on the chromosome 7. Guanine to cytosine (G→C) polymorphism in promoter IL-6 gene at position -174 is common and known bi-allelic SNP. The polymorphism (-174 G/C) seems to effect mainly the production of IL-6: C at 174 positions *i.e.* G→C at position -174 mediates decreased production of IL-6 while G at 174 position *i.e.* C→G at position -174 governs increased IL-6 production ((Ishihara and Hirano, 2002; Sehgal, 1990).However Endler*et al.*(2004) observed no association between -174G/C and level of IL-6. While in a different investigation it was observed that increased production of IL-6 cytokine is associated with C allele at -174 position (Brull*et al*., 2001).IL-6 gene promoter polymorphisms govern the

protein-DNA interaction at the transcription site and hence influence the IL-6 expression in different inflammatory diseases (Tanabe *et al*., 1988).

In order to understand the role of IL-6 gene in the pathogenesis of acne vulgaris, we investigated whether skin types are related with IL-6 gene polymorphism. We also investigated whetherIL-6 gene polymorphism (-174G/C) is involved in pathogenesis and severity of disease in Pakistani population. This is a primary study investigating role of -174 G/C SNP of IL-6 gene in acne vulgaris and its association with skin types.This study did not observe any relationship between type of skin and IL-6 -174G/C genotype frequencies.No significant difference wasfound, in the distribution of IL-6 genotypes between patients with acne vulgaris and healthy controls.No significant difference was found among acne susceptibility and IL-6 (-174G/C) single nucleotide polymorphism. These outcomes are similar to previous studies, which study that IL-6 -174G/C polymorphism have no effect in many inflammatory diseases (Godarzi*et al*., 2011; Lieb*et al*., 2004; Fedetz*et al*., 2001).

Relevant to this study, there were also no significant difference observed in the distribution of the polymorphism of IL-6 genotypes between patients with psoriasis and healthy controls in a Polish population(Baran*et al.*, 2008).While in contrast, Settin*et al.* (2009) observed a significant higher frequency of IL-6 (-174CC) among Egyptian psoriatic cases, compared to controls. Alternatively, IL-6 (-174GC) genotype showed a significant lower frequency among psoriasis cases when compared to controls.

IL-6 (-174) SNP have been studiedin several inflammatory diseases like psoriasis vulgaris (Villuendas*et al.*, 2002). However different studies can have varied resultsdue to ethnic differences of studied population, linkage disequilibrium with other genes and power of sample size.Irrespective of gender specificity in this study and small sample size, the similarity of genotypic and allelic frequencies of IL-6 gene (-174G/C) polymorphism between the patient and control groups, suggest that this polymorphism of IL-6 gene can not acts as a genetic markerin pathogenesis of acne vulgaris.

In conclusion, the result of current study proposedthat no association is present betweenIL-6 gene polymorphism (SNP) at-174 and patients of acne vulgaris with

different skin types. There was no association betweenIL-6 gene polymorphism at-174 and susceptibility to acne vulgaris. As acne is a disease affected by many factors, so possibly the result may be influenced bygenetic and some environmental factors. This is a preliminary study focused on a very small population. So further studies, concerning skin types and IL-6 gene polymorphismmust be performed in other population with large sample size. Analysis should also include expression analysis to highlight the importance of this polymorphism (SNP) in pathogenesis of acne vulgaris.

In addition, further studies conducted onother polymorphisms ofIL-6 gene locusand their association with skin type will explainsignificance of IL-6 gene in the pathogenesis of acne, which would provide helpful information infield of therapeutics and diagnosis.

REFERENCES

Adebamowo CA, Spiegelman D, Berkey CS, Danby FW, Rockett HH, Colditz GA, Willett WC and Holmes MD (2006). Milk consumption and acne in adolescent girls.*Dermatol Online J* 12(4): 1-12.

Adebamowo CA, Spiegelman D, Berkey CS, Danby FW, Rockett HH, Colditz GA, Willett WC and Holmes MD (2008). Milk consumption and acne in teenaged boys.*J Am AcadDermatol* 58(5): 787-793.

Afford SC, Pongracz J, Stockley RA, Crocker J and Burnett D (1992). The induction by human interleukin-6 of apoptosis in the promonocytic cell line U937 and human neutrophils.*J BiolChem* 267(30): 21612-21616.

Aizawa H and Niimura M (1995). Elevated serum insulin-like growth factor-1 (IGF-1) levels in women with postadolescent acne. *J Dermatol*22: 249–252.

Alestas T, Ganceviciene R, Fimmel S, Muller-Decker K and Zouboulis CC (2005). Enzymes involved in the biosynthesis of leukotriene B4 and prostaglandin E2 are active in sebaceous glands.*J Mol Med (Berl)* 84(1): 75-87.

Alsop R (2008). Acne vulgaris.*InnovAiT* 1(7): 470-473.

Arbegast KD,Braddock SW, Lamberty LF and Sawka AR (199I). Treatment of infantile cystic acne with oral isotretinoin: an ease report. *PediatrDermatol* 8: 166-168.

Arbuckle R, Atkinson MJ, Clark M, Abetz L, Lohs J, Kuhagen I, Harness J, Draelos Z, Thiboutot D, Blume-Peytavi U and Copley-Merriman K (2008). Patient experiences with oily skin: The qualitative development of content for two new patient reported outcome questionnaires. *Health and QOL Outcomes***6**:80.

Arbuckle R, Clark M, Harness J, Bonner N, Scott J, Draelos Z, Rizer R, Yeh Y and Copley-Merriman K (2009). Item reduction and psychometric validation of the Oily Skin Self Assessment Scale (OSSAS) and the Oily Skin Impact Scale(OSIS). *Value Health* 5: 828–837.

Ayres S (1926). Infantile acne vulgaris: a report of three cases. *Arch DermatolSyph*14(1): 12-13.

Baran W, Szepietowski JC, Mazur G and Baran E (2008).IL-6 and IL-10promotergenepolymorphisms in psoriasis vulgaris.*ActaDermVenereol*88(2): 113-116

Belisario JC (1951). Acne vulgaris: Its aetiology and treatment. *Aust. J. Dermatol*1: 85–111.

Berardesca E, Fluhr JW and Maibach HI (2006). What is sensitive skin? *Sensitive Skin Syndrome* 1–6.

Berlin C (1954). Acne comedo in children due lo paraffin oil applied on the head. *AMA Arch DermSyphilol* 69(6): 683-687.

Berson DS and Shalita AR (1995). The treatment of acne: the role of combination therapies. *J Am AcadDermatol* 32: 31–41.

Betti R, Bencini PL, Lodi A, Urbani CE, Chiarelli G and Crosti C (1990).Incidence of polycystic ovaries in patients with late-onset or persistent acne; hormonal reports.*Dermatologica*181(2): 109-111.

Brull DJ, Montgomery HE, Sanders J, Dhamrait S, Luong L, Rumley A, Lowe GD and Humphries SE (2001). Interleukin-6 gene −174g > c and −572g > c promoter polymorphisms are strong predictors of plasma interleukin-6 levels after coronary artery bypass surgery. *ArteriosclerThrombVascBiol* 21: 1458–1463.

Caputo R, Monti M, Ermaeora E, Canninaii G, Gelmetti C, Gianotti R, Gianni E and Puecinelli V (1988). Cutaneous manifestations of tetrachlorodibenzo-p-dioxin in children and adolescents. Follow-up 10 years after the Seveso, Italy, accident. *J Am AcadDermatol*19(5): 812-819.

Cara JF (1994). Insulin-like growth factors, insulin-like growth factor binding proteins and ovarian androgen production.*Horm Res*42: 49–54.

Chauhan D, Uchiyama H, Akbarali Y, Urashima M, Yamamoto K, Libermann TA and Anderson KC (1996). Multiple myeloma cell adhesion-induced interleukin-6 expression in bone marrow stromal cells involves activation of NF-κB. *Blood* 87(3): 1104-1112.

ChenW, Thiboutot D and Zouboulis C (2002). Cutaneous androgen metabolism: basic research and clinical perspectives. *J Invest Dermatol* 119: 992-1007.

Chew EW, Bingham A and Burrows D (1990). Incidence of acne vulgaris in patients with infantile acne.*ClinExpDermatol*15: 376-377.

Cibula D, Hill M, Vohradnikova O, kuzel D, Fanta M and Zivny J (2000). The role of androgens in determining acne severity in adult women.*Br J Dermatol*143(2): 399-404.

Cronstein BN (2007). Interleukin-6: a key mediator of systemic and local symptoms in rheumatoid arthritis. *Bull NYU HospJt Dis* 65(1): 11-15

Cunliffe WJ (1998). Poster in Mondial Congress of Dermatological Research Cologne.

Cunliffe WJ and Simpson NB (1998). Disorders of the sebaceous glands.In Textbook of Dermatology Champion RN, Burton JL, Burns DA.*Eds 6th Ed Oxford Blackwell Science* 1927-1984.

Cunliffe WJ, Holland DB, Clark SM and Stables GI (2000). Comedogenesis: some new aetiological, clinical and therapeutic strategies. *Br JDerm*142: 1084-1091.

Cunliffe WJ, Meynadier J, Alirezai M, George SA, Coutts I, Roseeuw DI, Hachem JP, Briantais P, Sidou F and Soto P (2003). Is combined oral and topical therapy better than oral therapy alone in patients with moderate to moderately severe acne vulgaris? A comparison of the efficacy and safety of lymecyclineplus adapalene gel 0.1%, versus lymecycline plus gel vehicle.*J Am AcadDermatol* 49(3): 218-226.

Dawson AL and Dellavalle RP (2013).Acne vulgaris*BMJ*. 346:f2634. doi:10.1136/bmj.f2634

Deplewski D and Rosenfield RL (2000).Role of hormones in pilosebaceous unit development.*Endocrine Rev* 21: 363-392.

El-Akawi Z, Nemr NA, Abdul-Razzak K and Al-Aboosi M (2006). Factors believed by Jordanian acne patients to affect their acne condition. *East Medit Health J*12: 840–846.

Endler G, Marsik C, Joukhadar C, Marculescu R, Mayr F, Mannhalter C, Wagner OF and Jilma B (2004). The interleukin-6 G(-174)C promoter polymorphism does not determine plasma interleukin-6 concentrations in experimental endotoxemia in humans. *ClinChem* 50(1): 195-200.

Fedetz M, Matesanz F, Pascual M, Martín J, Fernández O, Guerrero M and Alcina A (2001). The -174/-597 promoter polymorphisms in the interleukin-6 gene are not associated with susceptibility to multiple sclerosis. *J NeurolSci* 190(1-2): 69-72.

Fernández-Real JM, Broch M, Vendrell J, Gutiérrez C, Casamitjana R, Pugeat M, Richart C and Ricart W (2000). Interleukin-6 gene polymorphism and insulin sensitivity.*Diabetes* 49(3): 517-520.

Ferrari SL, Garnero P, Emond S, Montgomery H, Humphries SE and Greenspan SL (2001). A functional polymorphic variant in the interleukin-6 gene promoter associated with low bone resorption in postmenopausal women. *Arthritis Rheum* 44(1): 196-201.

Flex A, Gaetani E, Pola R, Santoliquido A, Aloi F, Papaleo P, Dal Lago A, Pola E, Serricchio M, Tondi P and Pola P (2002). The -174 G/C polymorphism of the interleukin-6 gene promoter is associated with peripheral artery occlusive disease. *Eur J VascEndovascSurg* 24(3): 264-268.

Foster CB, Lehrnbecher T, Samuels S, Stein S, Mol F, Metcalf JA, Wyvill K, Steinberg SM, Kovacs J, Blauvelt A, Yarchoan R and Chanock SJ (2000). An

IL6 promoter polymorphism is associated with a lifetime risk of development of Kaposi sarcoma in men infected with human immunodeficiency virus. *Blood* 96(7): 2562-2567.

Gabay C (2006). Interleukin-6 and chronic inflammation.*Arthritis Res Ther*8(2): 3

Ganceviciene R, Graziene V, Fimmel S and Zouboulis CC (2009). Involvement of the corticotropin-releasing hormone system in the pathogenesis of acne vulgaris.*Br J Dermatol*160(2): 345-352.

Giknis F, Hall WK and Tolman MM (1952).Acne neonatorum.*ArehDermatolSyph* 66: 7I7-721.

Gloor M, Kraft H and Franke M (1978).Effectiveness of topically applied antibiotics on anaerobic bacteria in the pilo-sebaceous duct.*Dermatologica* 157: 96-104.

Godarzi EM, Sarvestani EK, Aflaki E and Amirghofran Z (2011).Interleukin-6 gene polymorphism in Iranian patients with systemic lupus erythematosus.*ClinRheumatol* 30(2): 179-184.

Gollnick H (2003). Current concepts of the pathogenesis of acne: Iimplications for drug treatment. *Drugs* 63(15): 1579-1596.

Green J and Sinclair RD (2001).Perceptions of acne vulgaris in final year medical student written examination answers.*Australas J Dermatol*42: 98–101.

Guy R and Kealey T (1998).Modelling the infundibulum in acne.*Dermatology*196: 32-37.

Harris HH, Downing DT, Stewart ME and Strauss JS (1983). Sustainable rates of sebum secretion in acne patients and matched normal control subjects. *J Am AcadDermatol* 8: 200–203.

Healy E and Simpson N (1994).Acne vulgaris.*BMJ* 308: 831–833.

Heinrich PC, Behrmann I, Haan S, Hermanns HM, Müller-Newen G and Schaper F (2003). Principles of interleukin (IL)-6-type cytokine signalling and its regulation. *Biochem J*374: 1-20.

Hirano T (1998). Interleukin 6 and its receptor: ten years later. *Int Rev Immunol* 16: 249–284.

Hirano T, Yasukawa K, Harada H, Taga T, Watanabe Y, Matsuda T, Kashiwamura S, Nakajima K, Koyama K, Iwamatsu A, Tsunasawa S, Sakiyama F, Matsui H, Takahara Y, Taniguchi T and Kishimoto T (1986). Complementary DNA for a novel human interleukin (BSF-2) that induces B cell lymphocytes to produce immunoglobulin.*Nature* 324: 73-76.

Holland KT, Holland DB, Cunliffe WJ and Cutcliffe AG (1993). Detection of *Propionihacterium acnes* polypeptides which have stimulated an immune response in acne patients but not in normal individuals.*ExpDermatol* 2(1): 12-16.

Horiuchi S, Koyanagi Y, Zhou Y, Miyamoto H, Tanaka Y, Waki M, Matsumoto A, Yamamoto M and Yamamoto N (1994). Soluble interleukin-6 receptors released from T cell or granulocyte/ macrophage cell lines and human peripheral blood mononuclear cells are generated through an alternative splicing mechanism. *Eur J Immunol* 24(8): 1945–1948.

Ingham E, Eady AE, Goodwin CE, Cove JH and Cunliffe WJ (1992). Pro-inflammatory levels of interleukin-1α like bioactivity are present in the majority of open comedones in acne vulgaris. *J Invest Dermatol*98: 895-901.

Ingham E,Walters CE, Eady EA, Cove JH, Kearney JN and Cunliffe WJ (1998). Inflammation in acne vulgaris: failure of skin micro-organisms to modulate keratinocytes interleukin-1α production *in vitro*. *Dermatology* 196: 86-88.

Ishihara K and Hirano T (2002).IL-6 in autoimmune disease and chronic inflammatory proliferative disease.*Cytokine Growth Factor Rev* 13(4-5): 357-368.

Jahromi MM, Millward BA. andDemaine AG (2000). A. polymorphism in the promoter region of the gene for interleukin-6 is associated with susceptibility to type I diabetes mellitus. *J Interferon Cytokine Res* 20(10): 885-888.

Jappe U, Ingham E, Henwood J and Holland KT (2002).*Propionobacterium acnes* and inflammation in acne: *P. Acnes* has T-cell mitogenic activity. *Br J Dermatol* 146: 202-09.

Jeremy AH, Holland DB, Roberts SG, Thomson KF and Cunliffe WJ (2003). Inflammatory events are involved in acne lesion initiation. *J Invest Dermatol*121: 20–27.

Jones SA and Rose-John S (2002). The role of soluble receptors in cytokine biology: the agonistic properties of the sIL-6R/IL-6complex. *BiochimBiophysActa.*1592(3): 251–263.

Ju Q, Yang K, Zouboulis CC, Ring J and Chen W (2012). Chloracne: From clinic to research. *Dermatol Sin* 30(1): 2-6.

Kamimura D, Ishihara K and Hirano T (2003). IL-6 signal transduction and its physiological roles: The signal orchestration model. *Rev PhysiolBiochemPharmacol* 149(1): 1-38.

Karvonen SL (1993). Acne fulminans: report of clinical findings and treatment of twenty-four patients.*J Am AcadDermatol*28(4):572-579.

Kilkenny M, Merlin K, Plunkett A and Marks R (1998). The prevalence of common skin conditions in Australian school students: 3. Acne vulgaris. *Br J Dermatol*139(5): 840–845.

Kim J (2005). Review of the innate immune response in acne vulgaris: Activation of Toll-like receptor 2 in acne triggers inflammatory cytokine responses. *Dermatology* 211(3): 193-198.

Klein W, Tromm A, Griga T, Fricke H, Folwaczny C, Hocke M, Eitner K, Marx M, Epplen JT (2001). The polymorphism at position −174 of the IL-6 gene is not

associated with inflammatory bowel disease. Eur J GastroenterolHepatol 13: 45–47.

Kligman AM and Plewig G (1976).Classification of acne.*Cutis* 17: 520–522.

Knitzer RH and Needleman BW (1991). Musculoskeletal syndromes associated with acne. *SeminArrhritis Rheum* 20: 247-255.

Knutson DD (1974). Ultrastructural observations in acne vulgaris:the normal sebaceous follicle and acne lesions. *J Invest Dermatol*62: 288–307

Koo J (1995). The psychosocial impact of acne patients' perceptions.*J Am Acaddermatol*32: 26-30.

Koo JY and Smith LL (1991).Psychologic aspects of acne.*PediatrDermatol* 8: 185-188.

Kramer M, Dadon S, Hasanreisoglu M, Monselise Y, Avraham BR, Feldman A, Eldar I, Weinberger D and Goldenberg-Cohen N (2009). Proinflammatory cytokines in a mouse model of central retinal artery occlusion. *Mol Vis* 15: 885-894.

Krause K, Schnitger A, Fimmel S, Glass E and Zouboulis CC (2007). Corticotropin-releasing hormone skin signaling is receptor-mediated and is predominant in the sebaceous glands. *HormMetab Res* 39: 166-170.

Kurt-Jones EA, Mandell L, Whitney C, Padgett A, Gosselin K, Newburger PE and Finberg RW (2002). Roll of toll-like receptor 2 (TLR2) in neutrophil activation: GM-CSF enhances TLR2 expression and TLR2-mediated interleukin-8 responses in neutrophils. *Blood* 100: 1860-1868

Latif R and Laude TA (1982).Steroid acne in a 14-month-old boy.*Cutis* 29(4): 373-376.

Layton AM, Morris C, Cunliffe WJ and Ingham E (1998). Immunohistochemical investigation of evolving inflammation in lesions of acne vulgaris.*ExpDermatol* 7: 191-97.

Leveque JL, Grove G, Corcuff P, Kligman AM and Leger DS (1987).Biophysical characterization of dry facial skin.*j. Soc.CosmetChem* 82: 171-177

Leyden JJ (2001).The Evolving Role of *Propionibacterium Acnes*.SeminCutan Med Surg 20(3): 139-143.

Leyden JJ and Shalita AR (1986). Rational therapy for acne vulgaris: an update on topical treatment. *J Am AcadDermatol*15(4): 907-915.

Leyden JJ, McGinley KJ, Mills OH and Kligman AM (1975) Propionibacterium levels in patients with and without acne vulgaris. *J Invest Dermatol* 65: 382-384.

Lieb W, Pavlik R, Erdmann J, Mayer B, Holmer SR, Fischer M, Baessler A, Hengstenberg C, Loewel H, Doering A, Riegger GA and Schunkert H (2004). No association of interleukin-6 gene polymorphism (-174 G/C) with myocardial infarction or traditional cardiovascular risk factors. *Int J Cardiol* 97(2): 205-212.

Linker-Israeli M, Wallace DJ, Prehn J, Michael D, Honda M, Taylor KD, Paul-Labrador M, Fischel-Ghodsian N, Fraser PA, Klinenberg JR (1999).Association of IL-6 gene alleles with systemic lupus erythematosus (SLE) and with elevated IL-6 expression.*Genes Immun* 1: 45–52.

Liu AY, DestoumieuxD,Wong AV, Park CH, Valore V, Liu L and Ganz T (2002). Human _ defensin-2 production in keratinocytes is regulated by interleukin-1 bacteria, and the state of differentiation. *J Invest Dermatol*118: 275-281.

Lust JA, Donovan KA, Kline MP, Greipp PR, Kyle RA and Maihle NJ (1992). Isolation of an mRNA encoding a soluble form of the human interleukin-6 receptor.*Cytokine*4(2): 96–100.

Mallon E, Newton JN, Klassen A, Stewart-Brown SL, Ryan TJ and Finlay AY (1999). The quality of life in acne: a comparison with general medical conditions using generic questionnaires. *Br J Dermatol* 140: 672–676.

Massey A, Mowbray JF and Noble WC (1978). Complement activation by Corynebacterium acnes. *BrJDermatol* 98: 583-584.

McLoughlin RM, Witowski J, Robson RL, Wilkinson TS, Hurst SM, Williams AS, Williams JD, Rose-John S, Jones SA and Topley (2003). Interplay between IFN-γ and IL-6 signaling governs neutrophil trafficking and apoptosis during acute inflammation. *J Clin Invest* 112(4): 598-607.

Menni S and Brancaleone W (1992).Cosmetic acne in a child.*Eur J Dermatol* 2: 242-243.

Mills OH and Berger RS (1991).Defining the susceptibility of acne prone and sensitive skin populations to extrinsic factors.*Dermatologic Clinics* 9: 93-98.

Mills OH and Kligman AM (1982). Comodogenecity of sunscreens.Experimental observations in rabbits.*Arch Dermatol* 118(6): 417-419.

Mitsuyama, K. Sata M and Rose-John S (2006).Interleukin-6 trans-signaling in inflammatory bowel disease.*Cytokine Growth Factor Rev.* 17(6): 451-61.

Moses M and Prioleau PG (1985). Cutaneous histological findings in chemical workers with and without chloracne with past exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *J Am AcadDermatol* 12: 497-506.

Muizzuddin N, Marenus KD and Maes DH (1998).Factors defining sensitive skin and its treatment.*Am J Contact Dermat* 9: 170–175.

Müllberg J, Schooltink H, Stoyan T, Günther M, Graeve L, Buse G, Mackiewicz A, Heinrich PC and Rose-John S (1993). The soluble interleukin-6 receptor is generated by shedding. Eur J Immunol 23(2): 473-80.

Müllberg J, Schooltink H, Stoyan T, Heinrich PC and Rose-John S (1992). Protein kinase C activity is rate limiting for shedding of the interleukin 6 receptor. *BiochemBiophys Res Commun* 189(2): 794–800.

Neuberger M, Landvoigt W and Derntl F (1991). Blood levels of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in chemical workers after chloracne and in comparison groups. *Int Arch Occup Environ Health* 63(5): 325-327.

O'Brien SC, Lewis JB and Cunliffe WJ (1998). The Leeds Revised Acne Grading System. *J Dermatol Treat* 9: 215–220

Opdal SH and Rognum TO (2007).The IL6 -174G/C polymorphism and sudden infant death syndrome.*Hum Immunol*68: 541-543.

Papanicolaou DA, Wilder RL, Manolagas SC and Chrousos GP (1998). The pathophysiologic roles of interleukin-6 in human disease.*Ann Intern Med* 128: 127–137

Park SG, Kim YD, Kim JJ and Kang SH (1999). Two possible classifications of facial skin type by two parameters in Korean women: sebum excretion rate (SER) and skin surface relief (SSR).*Skin Res Technol*5: 189-194.

Perkins AC, Cheng CE, Hillebrand GG, Miyamoto K and Kimball AB (2011).Comparison of the epidemiology of acne vulgaris among Caucasian, Asian, Continental Indian and African American women.*J EurAcadDermatolVenereol* 25(9): 1054-1060

Pierard GE, Pierard-Franchimont C and Le T (1987).Seborrhoea in acne-prone and acne-free patients.*Dermatologica* 175: 5–9.

Pierard-Franchimont C, Pierard GE, Saint-Leger D, Leveque JL and Kligman AM (1991).Comparison of the kinetics of sebum secretion in young women with and without acne.*Dermatologica* 183: 120–122.

Pochi PE and Strauss JS (1969). Sebaceous gland response in man to the administration of testosterone A4-androstenedione and dehydroisoandrosterone. *J Invest Dermatol* 52: 32-36.

Pochi PE and Strauss JS (1974).Endocrinologic control of the development and activity of the human sebaceous gland.*J Invest Dermatol*62: 191-201.

Pola R, Flex A, Gaetani E, Lago AD, Gerardino L, Pola P and Bernabei R (2002b).The -174 G/C polymorphism of the interleukin-6 gene promoter is associated with Alzheimer's disease in an Italian population. *Neuroreport* 13(13): 1645-1647.

Pola R, Gaetani E, Flex A, Aloi F, Papaleo P, Gerardino L, De Martini D, Flore R, Pola P and Bernabei R (2002a). -174 G/C interleukin-6 gene polymorphism and increased risk of multi-infarct dementia: A case-control study. *ExpGerontol* 37(7): 949-955.

Pouliot N, Saunders NA and Kaur P (2002).Laminin 10/11: an alternative adhesive ligand for epidermal kératinocytes with a functional role in promoting proliferation and migration. *ExpDermatol*11: 387-397.

Puhvel SM and Sakamoto M (1977).An in vivo evaluation of the inflammatory effect of purified comedonal components in human skin.*J Invest Dermatol* 69: 401-406.

Rahman MM, Khondker L, Hazra SC and Khan MSI (2012). Association of serum testosterone with acne vulgaris in women-A case control study.*Jpad*22: 105-111.

Reilly DM, Parslew R, Sharpe GR, Powell S and Green MR (2000). Inflammatory mediators in normal, sensitive and diseased skin types.*ActaDermato-Venereologica*80: 171–174.

Rigopoulos D, Gregoriou S, Ifandi A, Efstathiou G, Georgala S, Chalkias J and Katsambas A (2007). Coping with acne: beliefs and perceptions in a sample of secondary school Greek pupils.*J EurAcadDermatolVenereol*21: 806–810.

Rose-John S, Scheller J, Elson G and Jones SA (2006). Interleukin-6 biology is coordinated by membrane-bound and soluble receptors: role in inflammation and cancer. *J. Leukoc. Biol.* 80: 227–236.

Rosenfield RL, Deplewski D, Kentsis A and Ciletti N (1998).Mechanisms of androgen induction of sebocyte differentiation.Dermatology 196: 43-46.

Saint-Martory C, Roguedas AM, Sibaud V, Degouy A, Schmitt AM and Misery L (2008). Sensitive skin is not limited to the face. *Br J Dermatol*158: 130–133.

Sambrook J, Fritsch EF and Maniatis T (1989). Molecular cloning: A laboratory manual. (2nd ed.): *Cold Spring Harbor Laboratory Press, New York, USA.*

Santhanam U, Ray A and Sehgal PB (1991).Repression of the interleukin 6 gene promoter by p53 and the retinoblastoma susceptibility gene product.*ProcNatlAcadSci USA* 88(17): 7605-7609.

Sauder DN (1990). The role of epidermalcytokines in inflammatoryskin diseases.*J Invest Dermatol* 95(5): 27-28.

Sawaya ME and Shalita AR (1998).Androgen receptor polymorphisms (CAG repeat length) in androgenetic alopecia, hirsutism, and acne.*J Cutan Med Surg* 3: 9-15.

Segot-Chicq E, Compan-Zaouati D, Wolkenstein P, Consoli S, Rodary C, Delvigne V, Guillou V and Poli F (2007). Development and validation of a questionnaire to evaluate how a cosmetic product for oily skin is able to improve well-being in women.*J EurAcadDermatolVenereol* 21:1181-1186.

Sehgal PB (1990). Interleukin-6: molecular pathophysiology. *J Invest Dermatol* 94: 2–6.

Seidenari S, Francomano M and Mantavoni L (1998).Baseline biophysical parameters in subjects with sensitive skin.*Contact Dermatitis* 38: 311–315.

Sen A, Paine SK, Chowdhury IH, Mukherjee A, Choudhuri S, Saha A, Mandal LK and Bhattacharya B (2011). Impact of interleukin-6 promoter polymorphism and serum interleukin-6 level on the acute inflammation and neovascularization stages of patients with Eales' disease.*Mol Vis* 17: 2552-2563.

Settin A, Hassan H, El-Baz R and Hassan T (2009).Association of cytokine gene polymorphisms with psoriasis in cases from the Nile Delta of Egypt.*ActaDermatovenerol Alp PanonicaAdriat*18(3): 105-112.

Simpson NB and Cunliffe WJ (2008).Disorders of sebaceous glands. In: Rook's textbook of dermatology. Burns T, Breathnach S, Cox N and Griffiths C, (eds.) (7th ed.): *Wiley, Blackwell Science Ltd, Malden, MA, USA.*

Smith TM, Gilliland K, Clawson GA and Thiboutot D (2008).IGF-1 induces SREBP-1 expression and lipogenesis in SEB-1 sebocytes via activation of the Phosphoinositide 3-kinase (PI3-K)/Akt pathway. *J Invest Dermatol* 128(5): 1286–1293.

Stenvinkel P, Barany P, Heimburger O, Pecoits-Filho R and Lindholm B (2002). Mortality, malnutrition, and atherosclerosis in ESRD: What is the role of interleukin-6? *Kidney Int* 61(80): 103–108.

Strauss JS and Kligman AM (1958). Pathologic patterns of the sebaceous gland. *J Invest Dermatol* 30: 51-61.

Szabo K and Kemeny L (2011).Studying the genetic predisposing factors in the pathogenesis of acne vulgaris.*Hum Immunol* 72(9): 766-773

Szabó K, Tax G, Teodorescu-Brinzeu D, Koreck A and Kemény L (2011).TNFα gene polymorphisms in the pathogenesis of acne vulgaris.*Arch Dermatol Res*303(1): 19-27

Tanabe O, Akira S, Kamiya T, Wong GG, Hirano T and Kishimoto T (1988). Genomic structure of the murine IL-6 gene.High degree conservation of potential regulatory sequences between mouse and human.*J Immunol*141(11):3875-3881.

Taub R (1996). Liver regeneration 4: transcriptional control of liver regeneration. *FASEB J.* 10: 413–427.

Teranishi T, Hirano T, Arima N and Onoue K (1982). Human helper T cell factor(s) (ThF). II. Induction of IgG production in B lymphoblastoid cell lines and

identification of T cell replacing factor (TRF)-like factor(s). *J Immunol* 128: 1903-1908.

Thiboutot D and Strauss JS (2002). Diet and acne revisited. *Arch Dermatol*138: 1591-1592.

Thiboutot D, Gollnick H, Bettoli V, Dréno B, Kang S, Leyden JJ, Shalita AR, Lozada VT, Berson D, Finlay A, Goh CL, Herane MI, Kaminsky A, Kubba R, Layton A, Miyachi Y, Perez M, Martin JP, Ramos-e-Silva M, See JA, Shear N and Wolf J JR (2009). New insights into the management of acne: An update from the global alliance to improve outcomes in acne group. *J Am AcadDermatol*60(5): 1-50.

Thiboutot D, Harris G, Iles V, Cimis G, Gilliland K and Hagari S (1995). Activity of the type 1,5 alpha-reductase exhibits regional differences in isolated sebaceous glands and whole skin. *J Invest Dermatol* 105(2): 209-214.

Thiboutot D, Jabara S, McAllister JM, Sivarajah A, Gilliland K, Cong Z and Clawson G (2003). Human skin is a steroidogenic tissue: steroidogenic enzymes and cofactors are expressed in epidermis, normal sebocytes and an immortalized seobcyte cell line (SEB-1). *JInvest Dermatol* 120: 905-14.

Tindall JP (1985). Chloracne and chloracnegens.*J Am AcadDermatol*13: 539-558.

Toyoda M and Morohashi M (1998).An overview of topical antibiotics for acne treatment.*Dermatology* 196(1): 130–134.

Toyoda M and Morohashi M (2001).Pathogenesis of acne.*Med ElextronMicrosc* 34: 29-40.

Turksen K, Kupper T, Degenstein L, Williams I and Fuchs E (1992). Interleukin6: insights to its function in skin by overexpression in transgenic mice. 89(11):5068-5072.

Uehara A, Sugawara S, Tamai R and Takada H (2001).Contrasting responses of human gingival and colonic epithelial cells to lipopolysaccharides,

lipoteichoic acids and peptidoglycans in the presence of soluble CD14.*Med MicrobiolImmunol*189: 185-192.

VandenBerghe W, Vermeulen L, De Wilde G, De Bosscher K, Boone E and Haegeman G (2000). Signal transduction by tumor necrosis factor and gene regulation of the inflammatory cytokine interleukin-6. *Biochem.Pharmacol*60(8): 1185-1195.

Villuendas G, San Millán JL, Sancho J and Escobar-Morreale HF (2002). The -597 G-->A and -174 G-->C polymorphisms in the promoter of the IL-6 gene are associated with hyperandrogenism. *J ClinEndocrinolMetab*87(3): 1134-1141.

Voss JG (1976). A microbial etiology of acne ?*Cutis* 17: 488-496.

Vowels BR, Yang S and Leyden JJ (1995). Induction of proinflammatory cytokines by a soluble factor of Propionibacterium acnes: implications for chronic inflammatory acne. *Infect Immun* 63: 3158- 3165.

Walton S, Wyatt EH and Cunliffe WJ (1988).Genetic control of sebum excretion and acne – a twin study.*Br J Dermatol*118: 393–396.

Watt FM (2002). Role of integrins in regulating epidermal adhesion, growth and differentiation.*EMBO J* 21: 3919-3926.

Webster GF and LeydenJJ ((1980).Characterization of serum-independent polymorpho nuclear leukocyte chemotactic factors produced by propionibacterium acnes.*Inflammation* 4: 261-269.

White GM (1998). Recent findings in the epidemiologic evidence, classification, and subtypes of acne vulgaris.*J Am AcadDermatol*39(2): 34–37.

Wolvekamp MC and Marquet RL (1990). Interleukin-6: historical background, genetics and biological significance. *ImmunolLett* 24: 1–9

Youn SW, Kim SJ, Hwang IA and Park KC (2002). Evaluation of facial skin type by sebum secretion: discrepancies between subjective description and sebum secretion. *Skin Res Technol* 8: 168–172.

Zakharyan R, Petrek M, Arakelyan A, Mrazek F, Atshemyan S and Boyajyan A (2012). Interleukin-6 promoter polymorphism and plasma levels in patients with schizophrenia.*Tissue Antigens* DOI: 10.1111/j.1399-0039.2012.01886.x.

Zouboulis CC, Xia L, Akamatsu H, Seltmann H, Fritsch M, Hornemann S, Rühl R, Chen W, Nau H and Orfanos CE (1998). The human sebocyte culture model provides new insights into development and management of seborrhea and acne. *Dermatology*196: 21–31.

Zouboulis CC, Eady A, Philpott M, Goldsmith LA, Orfanos C, Cunliffe WC and Rosenfield R (2005). What is the pathogenesis of acne?*ExpDermatol* 14(2): 143-152.

5.1 Electronic References

http://www.eucerinus.com/skin-health/skin-type/normal-skin.html

http://www.prokerala.com/health/beauty/skin-type-test.php

http://www.prokerala.com/health/beauty/skin-type-test.php#characteristics

ANNEXURE-I

Department of Biochemistry

Faculty of Biological Sciences

Quaid-i-Azam University, Islamabad, Pakistan

Acne Questionnaire

1	Serial No:	**2**	Name:
3	Gender:	**4**	Age:
5	Age of onset:	**6**	Duration of disease:
7	No. of pimples per week:	**8**	Treatment (Yes/No):
9	Drugs response (Yes/No):	**10**	Acne Breakout:
11	Type of skin:	**12**	Scar:
13	Redness/Swelling (Yes/No):	**14**	Duration of pimples (days):
15	Family History (Yes/No):	**16**	Occupation history:
17	Any other disease (Yes/No):	**18**	Cause of acne:
19	**Acne severity:** Mild: Moderate: Severe:	**20**	**Acne lesion types:** Blackheads: Whiteheads: Pustules: Nodules: Cysts: Ice pick scars:

ANNEXURE-II

Sequence of PCR product (408 bp)

Forward primer

5′-**GCGATGGAGTCAGAGGAAAC**TCAGTTCAGAACATCTTTGGTTTTTACA

AATACAAATTAACTGGAACGCTAAATTCTAGCCTGTTAATCTGGTCACTGA

AAAAAAATTTTTTTTTTTTCAAAAAACATAGCTTTAGCTTATTTTTTTCTC

TTTGTAAAACTTCGTGCATG↓ACTTCAGCTTTACTCTTTGTCAAGACATG↓C

CAAAGTGCTGAGTCACTAATAAAAGAAAAAAAGAAAGTAAAGGAAGAGT

GGTTCTGCTTCTTAGCGCTAGCCTCAATGACGACCTAAGCTGCACTTTTCC

CCCTAGTTGTGTCTTGC**G**ATG↓CTAAAGGACGTCACATTGCACAATCTTAA

TAAGGTTTCCAATCAGCCCCACCCGCTCTGGCCCCAC**CCTCACCCTCCAA**

CAAAGAT-3′

-174 G/C

Reverse primer

CATG↓: *Nla*III restriction enzyme cutting site

Association of skin type with IL-6 gene polymorphism in patients with Acne vulgaris.

ORIGINALITY REPORT

13 %	1 %	6 %	9 %
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to Higher Education Commission Pakistan *Student Paper*	8%
2	Naugler, W.E.. "The wolf in sheep's clothing: the role of interleukin-6 in imm... *Publication*	< 1%
3	Theresa C. Barnes. "The Many Faces of Interleukin-6: The Role of IL-6 in I... *Publication*	< 1%
4	Settin, Ahmad. "Molecular Study on Cytokine Gene Polymorphism among C... *Publication*	< 1%
5	Yalcin, S.. "Role of interleukin-6 -174 G/C promoter polymorphism in trace m... *Publication*	< 1%
6	Shi, Yuhui. "Mechanisms of N-3 polyunsaturated fatty acid inhibition of myco... *Publication*	< 1%
7	Hussain, Muhammad Sajid. "Molecular Genetic Analysis of Autosomal Rec... *Publication*	< 1%
8	M. Drozdzik. "Interleukin-6 gene polymorphism in renal transplant patients w... *Publication*	< 1%
9	www.nutriweb.org.my *Internet Source*	< 1%
10	Amorim, F. G., B. P. Campagnaro, C. L. Tonini, A. P. C. Norbim, I. D. Lour... *Publication*	< 1%
11	Laura Faggioli. "Nuclear Factor k B (NF-k B), Nuclear Factor Interleukin-6 ... *Publication*	< 1%
12	Vasconcelos, Romero H.T., Silvia M.L. Montenegro, Elisa A.N. Azevedo, Y... *Publication*	< 1%
13	carcin.oxfordjournals.org *Internet Source*	< 1%
14	Kato EH, Kishi R, Kondo T, Saijo Yasuaki, Sata F, Yamada H, サイジョウ *Publication*	< 1%
15	"Abstracts for the 22nd European Immunogenetics and Histocompatibility C... *Publication*	< 1%

16	dr.ntu.edu.sg *Internet Source*	< 1%
17	test2.jkarah.com *Internet Source*	< 1%
18	onlinelibrary.wiley.com *Internet Source*	< 1%
19	Rehman, Sadia, Naveed Akhtar, Nusrat Saba, Saeeda Munir, Waqar Ahme... *Publication*	< 1%
20	www.hausamberg-hasbergen.de *Internet Source*	< 1%
21	Holla, Lydie Izakovicova, Antonin Fassmann, Andrea Stejskalová, Vladimír ... *Publication*	< 1%
22	F. Ismail. "Specialist dermatology clinics for organ transplant recipients sign... *Publication*	< 1%
23	Igaz, P.. "Soluble interleukin-6 receptor (sIL-6R) makes IL-6R negative T c... *Publication*	< 1%
24	Pinchai, Nadthanun. "Search for genes involved in the synthesis of poly(L-m... *Publication*	< 1%
25	BIANCA MARCHETTI. "Transgenic Animals with Impaired Type II Glucoco... *Publication*	< 1%
26	Boschetti, Elisa <1981>(Bordoni, Alessandra and Tugnoli, Vitaliano). "Bioch... *Publication*	< 1%
27	Markus Schwaninger. "Adenosine-induced expression of interleukin-6 in as... *Publication*	< 1%
28	Kannan, Yashas. "Functional Characterization Of Human IkappaBzeta In M... *Publication*	< 1%

EXCLUDE QUOTES ON
EXCLUDE BIBLIOGRAPHY ON
EXCLUDE MATCHES OFF

INTRODUCTION

MATERIALS AND METHODS

RESULTS

DISCUSSION

REFERENCES