A HOSPITAL BASED CROSS-SECTIONAL STUDY TO ESTIMATE SERUM LEVELS OF INTERLEUKIN-6 AND HIGH SENSITIVITY C - REACTIVE PROTEIN AND THEIR CORRELATION WITH THE VITILIGO DISEASE ACTIVITY AND EXTENT

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A HOSPITAL BASED CROSS-SECTIONAL STUDY TO ESTIMATE SERUM LEVELS OF INTERLEUKIN-6 AND HIGH SENSITIVITY C-REACTIVE PROTEIN AND THEIR CORRELATION WITH THE VITILIGO DISEASE ACTIVITY AND EXTENT

DERMATOLOGY, VENEROLOGY AND LEPROSY

LIST OF ABBREVIATIONS

IL-6 – Interleukin-6

HsCRP – High sensitivity C-reactive protein

Th – T-helper

CRP – C-reactive protein

TNF- α – Tumour necrosis factor-α

IL – Interleukin

PASI – Psoriasis area severity index

TYRP1 – Tyrosinase-related protein 1

TYRP2 – Tyrosinase-related protein 2

MITF – Microphthalmia transcription factor

bFGF – Basic fibroblast growth factors

NRG1 – Neuregulin 1

SCF – Stem cell factor

TGF-β – Transforming growth factor

α-MSH – Melanocyte stimulating factor

NO – Nitric oxide

PAR2 – Proteinase-activated receptor-2

KGF – Keratinocyte growth factor

IGF-1 – Insulin growth factor

UV – Ultraviolet

TYR – Tyrosinase

DOPA – Dihydroxyphenylalanine

DHI – Dihydroxyindole

DCT – DOPAchrome tautomerase

DHICA – Dihydroxyindole-2-carboxylic acid

ROS – Reactive oxygen species

Bcl-2 – B-cell lymphoma-2

RER – Rough endoplasmic reticulum

B.C. – Before Christ

H_2O_2 – Hydrogen peroxide

FOXP1 - Forkhead box P1

MHC – Major histocompatibility complex

XBP1 – X-box binding protein 1

HLA – Human leukocyte antigen

ACE – Angiotensin converting enzyme

CAT – Catalase

PTPN22 – Protein tyrosine phosphatase non-receptor type 22

COMT – Catechol-Omethyltransferase

CTLA-4 – Cytotoxic T lymphocyte antigen-4

ESR – Estrogen receptor

MBL2 – Mannan-binding lectin

NALP1 – NACHT leucine-rich repeat protein 1

IL2RA – Interleukin-2 receptor A

AIS – Autoimmune susceptibility

SLEV1 – Systemic lupus erythematosus vitiligo related gene

miRNAs – Micro ribonucleic acids

TLRs – Toll-like receptors

SNPs – Single nucleotide polymorphisms

CD – Cluster of differentiation

IFN γ – Interferon γ

CXCL10 – Chemokine ligand 10

Tregs – Regulatory T-cells

ET-1 – Endothelin-1

mRNA – Messenger ribonucleic acid

SOX – SRY- Box transcription factor

APS1 – Autoimmune polyendocrine syndrome type 1

ATP – Adenosine triphosphate

LXR – liver X receptor

VDR – Vitamin D receptor

VGICC – Vitiligo Global Issues Conference

SV – Segmental Vitiligo

NSV – Non-segmental Vitiligo

AIDS - Acquired immunodeficiency syndrome

NB-UVB – Narrowband Ultraviolet B

PUVA – Psoralen with ultraviolet A

OMP – Oral Minipulse

HPA – Hypothalamic pituitary-adrenal

TCS – Topical corticosteroids

ABSTRACT

Introduction:

Vitiligo is an autoimmune disorder which is characterized by progressive destruction of melanocytes and clinically presents as hypopigmented or depigmented lesions. The exact mechanism remains unclear but there is a definitive part that cell mediated immunity plays in the pathogenesis of vitiligo. Interleukin-6 and HsCRP are sensitive markers which tend to rise in association with systemic inflammation.

Aim:

To measure serum interleukin 6 and high sensitivity C-reactive protein levels in cases of vitiligo and to correlate those levels with the activity and extent of the disease.

Materials and methods:

It is a hospital-based prospective cross-sectional study. Patients with typical clinical features of vitiligo irrespective of age, gender and on-going or past treatment were included, whereas patients with any other co-existing chronic inflammatory disorders, any active cutaneous or systemic infections, co-morbidities, history of smoking, congenital and acquired causes of depigmentation disorders were excluded. After taking a complete history and performing physical examination, the severity of vitiligo was assessed with Vitiligo Disease Activity Score and the extent was calculated using Vitiligo Area Severity Index. Serum IL-6 and HsCRP levels were measured.

Results:

A mild negative, statistically insignificant correlation was found between VIDA and IL-6 (p=0.092) There was an absolute absence of correlation between VIDA and HsCRP levels (p=0.998). There was a mild positive correlation between VASI and both the markers, that is IL-6 and HsCRP, though it was statistically not significant (p=0.175 and p=0.238 respectively) Within the subgroups of patients who were on immunosuppressive therapy while being investigated and the ones who were not, the group that was not on immunosuppressants showed a higher mean value of IL-6 and HsCRP than the group that was on them, however the difference was not significant (p=0.105 for IL-6 values and p=0.943 for HsCRP values)

Conclusion:

The mild negative correlation between VIDA and IL-6 levels can be explained by the fact that patients on immunosuppressives, which are preferred in higher disease activity, at the time of investigation showed a lesser mean value as compared to those who were not, implying that there is a role of immunosuppressive agents in reducing these inflammatory markers. Therefore, it is crucial to also consider the treatment status, and not just the disease activity while evaluating levels of inflammatory markers in such conditions.

Keywords: Vitiligo, VIDA, VASI, IL-6, HsCRP, Activity, Extent

LIST OF TABLES

LIST OF FIGURES

LIST OF CONTENTS

INTRODUCTION

Vitiligo is a frequently encountered depigmenting skin disease which has a surveyed prevalence of 0.5–2%. The disease is typified by presence of selective melanocyte loss that leads to development of typical nonscaly, chalky-white macules. More often than not, this disorder is adjourned as a non-essential concern, though its consequences can be emotionally disturbing with a significant morbidity.[1]

Across studies from India, vitiligo has been observed to affect between 0.25% and 4% of dermatological outpatients.[2] Most affected populations are groups with a varied age when vitiligo sets in and two summits are noted in some studies. The initiation of vitiligo generally occurs by the age 30 and many reports show that around half of all patients would have developed it by 20 years of age.[1,3] Presence of family history should be sought for in cases of pediatric cases.[3]

Vitiligo is a multifaceted disorder that typically shows destruction of functional melanocytes. Different theories have been put forward to explain melanocyte destruction in vitiligo. They are as follows: genetic, responses of autoimmunity, oxidative stress, inflammatory mediator production and melanocyte detachment mechanisms. There seems to be an involvement of both innate and adaptive forms of immunity.[1]

IL-6 is a T- helper 2 (Th2) cytokine which has various functions in inflammation and regulation of immunity. It is noted in association with numerous autoimmune and inflammatory diseases.[4] Changes in keratinocyte-derived mediator levels have now been revealed in vitiligo epidermis, implying a role for epidermal cytokine imbalance in vitiligo pathogenesis.[4-6]

C-reactive protein (CRP) is a susceptible indicator for inherent inflammation. Tumour necrosis factor-α (TNF- α), interleukin (IL)-1, IL-6 are mediators which are involved in regulation of the synthesis of acute phase reactants by the liver. Test for HsCRP detects extremely low amounts of CRP in the blood. It is utilized not only as a diagnostic marker, but also a prognostic marker for diseases of the cardiac system and is a comprehensive index for estimating the risk for coronary sequalae.[7,8]

The status of activity of the disease is an essential guide to choose appropriate therapeutic options, knowing prognosis and monitor patient's response to treatment. However, there is an absence of a clear protocol on assessment of activity in vitiligo during a solo consultation. Skin findings like trichrome/hypopigmented lesions, confetti-like lesions and Koebner's phenomenon are observed to be possible indicators of activity of disease.[10]

A commonly used method which utilizes 'the last time point of disease progression' (VIDA), is an outcome measure that is reported by the patient and takes into account retrospective information (recall). The VIDA Scale is a commonly used tool that pin points the time when activity (worsening) of the lesions was last observed.[10] It is a six-point scale for assessment of stability of vitiligo over time. The patients themselves report of disease activity. It aids in monitoring the efficacy of intervention at halting and reversing the extent of the disease. Development of new lesions or extension of pre-existing lesions is said to be an active disease.[11]

Being a quantitative tool, VASI is a theorectical derivative of the psoriasis area severity index (PASI) score that is used in evaluation of psoriasis. VASI is calculated by inclusion of all body areas (range, 0–100). One hand unit means the palm along with the ventral aspect of the fingers, and approximates to 1% of the total surface area of the body and it is used to measure the

reference base percentage of total body area involved in vitiligo. The body is divided into five distinct areas: Trunk, upper limbs, hands, lower limbs and feet.[11,12]

AIM OF THE STUDY:

To estimate serum interleukin-6 and high sensitivity c-reactive protein levels and their correlation with the vitiligo disease activity and extent.

OBJECTIVE OF THE STUDY:

To measure the serum interleukin 6 and high sensitivity C-reactive protein levels in patients with vitiligo.

To correlate those levels with the activity of the disease using VIDA.

To correlate those levels with the extent of the disease using VASI.

REVIEW OF LITERATURE

The skin comprises of three layers: the epidermis, dermis and subcutaneous tissue. The epidermis consists of four important types of cells, namely the keratinocyte, the melanocyte, the Langerhans cell and the Merkel cell.[13]

Melanocytes:

Melanocytes are cells that are derived from the neural crest cells (retinal pigment cells being an exception, which develop from the optic cup of the forebrain). This cell group emerges from the embryonic germinal layer known as the ectoderm. The mid portion of the embryonic disc, develops in the neuroectoderm which is perceivable as a neural plate in a 4-week-old human embryo. This plate then folds and gives rise to the neural tube along with future brain, spinal cord. This process is known as neuralation, during which a set of cells separates from edges of the neural plate and there is a change of phenotype from epithelial to mesenchyme, after which they move out from neuroepithelium. This group of neuroectodermal cells moving to different areas of the evolving body are the neural crest cells – NCC. Formation of melanoblasts takes place when neural crest cells get committed to the melanogenic lineage, which have the ability to move to different destinations and evolve into melanogonia, finally to mature melanocytes. Signals responsible for migration of the melanoblasts to their final destinations have not been described yet.[14,15]

Melanocytes are present in minority in the skin, ear, hair matrix, eye, central nervous system and mucous membranes. They may also be present in dermis along with the epidermal

basal layer.[14] Tyrosinase-related protein 1 and 2 (TYRP1, TYRP2/DCT), tyrosinase (TYR), melanosomal matrix proteins, microphthalmia transcription factor (MITF) are a few of the various melanocyte-specific proteins which are useful in detecting these cells on a molecular level.[15]

Melanocyte as epidermal melanin unit in the skin:

Fitzpatrick and Breathnach in 1963 had initially explained the concept of a melanocyte interacting with a specific group of keratinocytes and termed it as "the epidermal melanin unit." As proposed by them, it was a functional unit consisting of one melanocyte and 36 keratinocytes. This ratio, although, is not constant and depends on the stage of development from fetal life to birth to adulthood.[13] There are around 1200 melanocytes/mm2 of skin. In the cytoplasm of these melanocytes are special membrane-bound oragnelles known as melanosomes which have a function of production of melanin.[15] The primary function of the melanocyte, which is providing pigmentation to the skin, takes place due to the dendrites that assist the transfer of melanosomes to the keratinocyte[13,14] and also plays a role in photoprotection. Melanin granules cover the nucleus of keratinocytes like a cap.[15] The characteristics of melanosomes like number, size, type ascertains the skin colour. It is the melanocyte genotype, but not the keratinocyte's, which determines quantity of melanin and size of melanosomes.[13] The melanocyte plasma membrane encloses the packed melanosomes, which are then released from numerous melanocyte dendrites into the extracellular space, phagocytosed by keratinocytes, and disseminated throughout the perinuclear region.[16]

Melanocytes, keratinocytes, and fibroblasts present in the dermis interact through cell-to-cell contact and certain secreted factors. There is an interaction between melanocytes, keratinocytes and fibroblasts present in the dermis by the cell to cell contact and certain secreted factors. A system of paracrine growth factors namely, basic fibroblast growth factors (bFGF)[13], cell adhesion molecules like E- and P-cadherins are responsible for control of melanocyte growth and activity by keratinocytes. These cells, namely, melanocytes and keratinocytes also are a medium of various hormones that balance proliferation of melanocyte, melanogenesis and dendrites' formation. Factors secreted by the dermal fibroblasts, e.g. neuregulin 1 (NRG1), stem cell factor (SCF) also hold a place in maintenance of melanocyte biology. These cytokines regulate melanocyte growth and proliferation as well as their shape, mobility, dendricity and adhesive properties. Melanocytes secrete various signal molecules that target not only the keratinocytes, but also cells of the cutaneous immune system. They lead to the release of transforming growth factor (TGF-β), melanocyte stimulating factor (α-MSH), proinflammatory cytokines (IL-1α, IL-2, IL-3, IL-6, IL-10 and TNF-α), chemokines (IL-8, chemokine ligand 2), eicosanoids, serotonin, nitric oxide (NO) and catecholamines, when stimulated. Melanogenesis is inhibited when these substances start acting like autocrine factors, e.g. IL-1, IL-6 and TNF-α, and melanin production is upregulated by eicosanoids and α-MSH.[15]

Hair follicle melanocytes:

Proximal bulb of the hair follicle is where the melanocytes are situated and also in the sebaceous gland. Bulbar melanocyte bodies are found at the apex of the dermal papillae. Dendrites of melanocytes enter between the keratinocytes of cortex and medulla. Ratio of melanocytes to keratinocytes is comparatively denser than in the epidermis, that is 1:5. Follicular

pigmentation results from the structural and functional interplay of melanocytes in the follicle, keratinocytes of the matrix, and dermal papilla fibroblasts. The trilateral complex is known as the follicular melanin unit or the hair melanin unit. The activity of melanogenesis by the follicular melanocytes, migration of melanin granules in the keratinocytes, development of pigmented shafts of hair all seem to be elements of the pigmentation process for hair. It is contemplated this migration of granules of melanin to the keratinocytes that is growing is indistinguishable from the Proteinase-activated receptor-2 (PAR2) receptor mediated epidermal melanosome phagocytosis. But degradation of melanosomes and their quality is where the dissimilarity lies. The melanocytes here are bigger in size and more dendritic than those found in the epidermis. Furthermore, whereas evolving keratinocytes in the epidermis degrade delivered melanin almost in its entirety, hair cortical keratinocytes only partially digest pigment granules. Products that get secreted by the nearby cells like the keratinocytes, endothelial cells and fibroblasts influence the synthesis of melanin occurring in the hair. The biochemical pathway of pigment generation and regulation in addition to melanosomes biogenesis run parallel to that seen in the epidermis, though it is important to note that melanocytes of the hair follicle are more vulnerable to the consequences of ageing than epidermal melanocytes, which causes greying of the hair. It emerges that keratinocyte growth factor (KGF), insulin growth factor (IGF-1), SCF and noggin, which are generated from fibroblasts of the dermal papilla, have a specific role in regulating the activity and proliferation of keratinocytes and melanocytes in the hair matrix during hair growth. Epidermal melanocytes live longer, while hair melanocytes get depleted in around 3-8 years which is when the hair cycle ends. The task of melanogenesis occurs only in the course of the growing phase (anagen stage); development of pigment is ceased in the regressing phase (catagen stage) and completely lacking in the resting phase (telogen stage). Moreover, it is

notable that while catagen is in progress, fully differentiated melanocytes in the hair bulb perish via apoptosis, however melanoblasts that reside in the hair bulge give rise to new melanocytes.[15]

Melanogenesis:

The biochemical pathway in charge of melanin production is called melanogenesis. It materializes in melanocytes, in specialized organelles in the cytoplasm called melanosomes. There is genesis of two main types of melanin, namely pheomelanin and eumelanin. They vary from each other in the way of synthesis as well as color. The characteristics of melanin, that include free radical scavenging, absorption and scattering of ultraviolet (UV) light, ion storage, oxidation-reduction processes, and are all advantageous to the body. The kind of melanin formed depends on whether the substrates are available and the role of enzymes involved in melanogenesis.[15]

Tyrosinase (TYR) executes the process of hydroxylation of tyrosine to L-3,4-dihydroxyphenylalanine (DOPA) that is then rapidly oxidized to DOPAquinone. In case where cysteine is available, DOPAquinone reacts with it, forming 3- or 5-cysteinylDOPAs, that then oxidizes and polymerizes, leading to formation of yellow-red soluble melanin, that is pheomelanin. In case where thiols (cysteine, glutathione or thioredoxin) are absent, there is production of brown-black melanin, that is eumelanin. DOPAquinone cyclizes spontaneously to DOPAchrome. There is spontaneous loss of carboxylic acid from DOPAchrome and generation of 5,6-dihydroxyindole (DHI), that oxidizes rapidly and undergoes polymerization to produce dark brown-black insoluble melanin, that is DHI-melanin. Nevertheless, if there is presence of (TYRP2/DCT), DOPAchrome produces DHI-2-carboxylic acid (DHICA). Tyrosinase and

TYRP1 act as catalysts for further reactions after which a lighter brown color melanin, that is DHICA-melanin is obtained finally.[15]

All types of melanin are present in the human skin, and their ratio is what determines visible pigmentation. Varied pigmentation of the skin among different races and ethnicity depends on the content of eumelanin. The color of the skin is based on the ratio of eumelanin to total melanin. Almost equivalent quantity of pheomelanin is seen in the dark and light skin, which brings us to a conclusion that pheomelanin is not contributory to pigmentation of the skin. Whereas in case of hair, the color is determined by the ratio of eumelanin to pheomelanin. When compared to pheomelanin, eumelanin has greater photoprotective qualities, including greater resistance to deterioration and the capacity to neutralize reactive oxygen species (ROS).[15]

Cytotoxic substances are formed (quinones, hydrogen peroxide) as intermediate metabolites during the process of melanogenesis. In order to safeguard itself, the melanocyte elevates the concentration of the antiapoptotic protein B-cell lymphoma-2 (Bcl-2) and partitions the melanogenesis-related regions of its melanosomes. Whether endoplasmic reticulum is the originator of melanosomes or not, is still a controversy. TYR along with TYRP1 and TYRP2 is required for their genesis. Tyrosinase, the most important of these three enzymes needed for melanogenesis, is produced on the rough endoplasmic reticulum (RER) ribosomes, transferred to the Golgi complex, and then glycosylated, which is an activity necessary for maintaining its structural and functional integrity.[15]

Melanosome development occurs in four stages:

- Premelanosomes (Stage I): small, rounded vesicles which consist of an amorphous matrix.

- In stage II, melanosomes have a well-organized fibrillar matrix (from glycoprotein100 family mainly) with presence of tyrosinase, although no trace of pigment production.
- Stage III marks the initiation of melanin development where deposition of this pigment on protein fibrils takes place.
- Pigment gets filled up in the entire melanosome in stage IV.

Tyrosinase activity is lost in completely melanized melanosomes when they are transferred by the cytoskeletal system elements to surrounding keratinocytes.[15]

VITILIGO:

History of vitiligo:

In the period of Aushooryan was a description of Kilăsa in 2200 B.C., that was the earliest known. In 1550 B.C., there was some knowledge of this disorder in the Ebers Papyrus as well. It has been spoken about in the tomes of various religions. Even though there is an historic awareness, it has been confused with leprosy since ages and given rise to extreme stigma of vitiligo patients. This is a social issue persists even today in some parts of the globe, especially in rural setups where there is lack of awareness and medical facilities.[2]

Epidemiology:

The prevalence is evaluated to be around 0.5–2% worldwide. It does not vary depending on different races and ethnicity; nonetheless there seems to be diversified prevalence to in different areas of the world.[1-3,17]

In India, vitiligo prevalence has been outlined to be around 0.25% and 4% of the outpatients and as much as 8.8% in Rajasthan and Gujarat. It affects males and females both with

a similar frequency but most of the reports show a female preference.[2,18,19] Mixed age groups and two peaks have been noted in some studies with onset generally under 30 years and most studies pointing towards onset by 20 years. Presence of family history is of significance in cases of early onset vitiligo seen in children.[3]

Etiology:

It is difficult to pinpoint the exact etiology and often misunderstood. It is a multifactorial disorder with a pathogenesis involving multiple hypotheses like oxidant– antioxidant, autoimmune, viral, biochemical, cytotoxic and neural mechanisms for melanocytic loss in intrinsically susceptible individuals. Certain triggers such as mental stress, autoimmunity, poor nutrition, trauma, medication, infections, sepsis, sun exposure, chemicals and toxins are also contributory to it.[2]

Pathophysiology:

Few major postulations for vitiligo pathogenesis are:

(i) Autoimmune pathogenesis: a long-prevailing hypothesis.

(ii) Neural hypothesis says that the release of certain neurochemical molecules by nerve endings can destroy melanocytes or decrease production of melanin.

(iii) Biochemical theory hypothesizes aggregation of intermediate products of melanin synthesis pathway that are toxic and defective defense against free radicals, and aggregation of high amount of hydrogen peroxide (H_2O_2) as a reason for melanocyte damage. Another causes such as genetic factors, melanocyte structural and functional defects, melanocyte growth factors deficiency also play a part in the depigmentation process.[1,2]

Genetics: Given the fact that vitiligo is a multifactorial disorder, many candidate genes which include forkhead box P1 (FOXP1), major histocompatibility complex (MHC), X-box binding protein 1 (XBP1), , human leukocyte antigen (HLA), angiotensin converting enzyme (ACE), catalase (CAT), protein tyrosine phosphatase non-receptor type 22 (PTPN22), catechol-Omethyltransferase (COMT), cytotoxic T lymphocyte antigen-4 (CTLA-4), estrogen receptor (ESR), (MBL2), NACHT leucine-rich repeat protein 1 (NALP1) and interleukin-2 receptor A (IL2RA), have been tested to have a genetic association with generalized forms of vitiligo.[2] Tyrosinase acts as a catalyst in the rate-limiting steps in the pathway of synthesis of melanin and is encoded by the gene TYR. It is the main autoantigen involved in generalized vitiligo.[1] HLA haplotypes, particularly HLA-A2, −DR4, −DR7 and −DQB1*0303, are said to have a part in cases of various vitiligo-associated autoimmune/auto-inflammatory syndromes. PTPN22, NALP1 and XBP1 are found to be causative in cases of vitiligo alone. Autoimmune susceptibility (AIS) loci associated with vitiligo has been disclosed by genome-wide linkage analysis. AIS1 was found to be present on chromosome 1p31.3– p32.2, whereas AIS2 is on chromosome 7 and AIS3 is located on chromosome 8. Where AIS1 and AIS2 linkages were associated with families having vitiligo as well as other autoimmune disorders, AIS3 was predominant in the subgroup with a non-autoimmune type. Other gene found in patients of generalized vitiligo in concordance with other autoimmune diseases was systemic lupus erythematosus vitiligo related gene (SLEV1) that is present on chromosome 17. Of late, microRNAs (miRNAs) and toll-like receptors (TLRs) involvement in the pathology of this disease is also being researched.[2] A report found increased levels of miR-125b, miR-155, miR-99b and miR199a-3p levels and decreased levels of miR-145 in the skin of patients with vitiligo. An over-expression of MiRNAs (miR-155) inhibits genes associated with melanogenesis and

interferon-regulated gene alteration in the melanocytes as well as keratinocytes. Single nucleotide polymorpisms (SNPs) in TLR7 were found to be in association with this depigmenting disorder in a study which confirmed the part of TLRs in disease pathogenesis, also opening new gates towards future targeted treatment.[2]

Cellular immunity: The main culprits, when it comes to explaining the concept of cellular immunity, are the cluster of differentiation 8 (CD8+) cytotoxic T-cells.[2] Melanocytes are targeted specifically by them and are destructed. They show anti-melanocyte cytotoxic reactivity as well as expression of the skin-homing marker cutaneous lymphocyte antigen. Histologically, their infiltration has been exhibited in the skin. Majority of cutaneous lymphocyte antigen-positive T cells around the lesional area expressed perforin and granzyme-B. It is casual for destruction of melanocytes and correlates well with the disease activity. Normal or stressed melanocytes give rise to certain antigenic proteins like Melan-A/MART-1, tyrosinase, gp100, tyrosinase related proteins 1 and 2.[1] Interferon γ (IFN γ) has come to light being a part of the "signature cytokine profile" and is in connection with the vitiligo pathogenesis. The expression of CXCL10 increases, which ultimately synchronizes CD8+ T-cell invasion into epidermal and follicular tissues. IL-17 together with Th17 cells are increasingly being known to contribute in elaborating this cytokine and contributing to autoimmunity. Increased levels of IL-17 in blood and tissue samples of patients with vitiligo was found in a study done by Singh *et al*. Recent reports have also shed light on the role of regulatory T-cells (Tregs) in contributing to the disease pathology. Their function is to combat autoimmunity it is known that the levels of these are decreased in vitiligo, particularly in lesional and perilesional skin. Alongside being lesser in

number, their functioning is also impaired. Moreover, TGF-β and IL-10, that induce Tregs physiologically, have been conclusively proven to be decreased in active vitiligo lesions.[2]

Role of IL-6: It is a Th2 cytokine with a varied functions in regulation of immunity and inflammation. The gene for human IL-6 gene is located on chromosome 7p21. IL-6 is in association with numerous autoimmune and inflammatory disorders namely as rheumatoid arthritis, systemic lupus erythematosus, gout, type-1 diabetes, atherosclerosis, etc. High levels of this cytokine have also been noted in patients of vitiligo. In vitro studies indicate that it acts as an 'intracrine' molecule and holds a post in the biology of melanocyte by regulating melanogenesis and proliferation of these cells.[4] Keratinocytes from affected areas exhibit unique alterations, which are supported by their challenging in vitro culture, due to the varied expression of keratinocyte-derived paracrine factors like endothelin-1 (ET-1), SCF, relevant cytokines like IL-1, IL-6, basic fibroblast growth factor (bFGF) and TNF- α. In more precision, higher expression of IL-6 along with TNF-α along with decreased levels of SCF have a negative impact on melanogenesis, and have been described by studying mRNA levels.[5,6]

Role of HsCRP: Another sensitive indicator of inherent inflammation is the CRP. It is an acute phase protein produced in the liver, this synthesis being modulated by mediators like IL-6, IL-1 and TNF-α. Very low levels of CRP in the serum can be picked by HsCRP test, which is a quantitative test. Till date, this test has been used more commonly for cardiac conditions and is an index for monitoring its related sequelae. For years, standard CRP has been utilized in patients with acute inflammation or to evaluate obvious persistent inflammation. Additionally, HsCRP has been optimized as a reliable indicator of milder systemic inflammation in a variety of

disorders, including diabetes, heart disease, and chronic obstructive pulmonary disease. In a recent study, cases of vitiligo and controls showed no significant difference in serum levels of HsCRP. In yet another study, association between CRP levels and local inflammation in vitiligo could not be found. Nevertheless, a bigger sample of cases with more body surface involvement and a longer duration of disease ought to be taken into consideration to demonstrate a clear picture on significance of this association.[7,8]

Humoral immunity: In cases with vitiligo, several antibody groups have been observed and are categorised as those directed against non-pigment cell antigens, intracellular pigment cell antigens and cell surface pigment cell antigens. A group of antigens, that is VIT 40/VIT 75/VIT 90, which are termed after their weights respectively are demonstrated 83% of vitiligo patients. VIT 75 and VIT 40 are shared amongst both the pigment as well as the non-pigment cells, though VIT 90 is exclusive to pigment cells. There is presence of antibodies in opposition to these antigens which are non-specific. Melanocytes are far more vulnerable to injury that is immune-mediated than other cells, it is likely that even slightest damage by the non-specific antibodies could lead to major destruction to melanocytes while sparing the nearby cells. Antibodies against tyrosinase and TYRP-1 and TRP-2, SOX10 and SOX9, are also discovered in cases with autoimmune polyendocrine syndrome type 1 (APS1) and those with vitiligo in absence of a concurrent illness. Anti-melanocyte antibodies are noted to be concentrated in the cytoplasm of melanocytes. In a different analysis, cases of vitiligo were shown to contain antibodies against membrane along with cytoplasmic antigens. These antigens on the membrane were confirmed as Vimentin X along with Lamin A/C via protein mass spectrometry. Therefore, it cannot be disproved that there is involvement of antibodies in opposition to melanocytes in the

pathogenesis of this disorder.[2] Exosomes are secreted by the melanocytes in times of stress in a process of conveying it to the innate immune system. Specific antigens of melanocytes, heat shock proteins, miRNAs along with few others that serve as damage-associated molecular patterns are expressed in these exosomes. These exosomes are responsible for transferring these target antigens to accessible dendritic cells and promote their evolution to antigen-presenting cells. Amongst all these, inducible heat shock protein 70 is distinct because it functions as a chaperone to peptides specific to the host which prevent cells from going through apoptosis. Inducible heat shock protein 70 is known to hold an essential position in the development of vitiligo lesions in a mouse model by induction of dendritic cells to deliver melanocyte-specific antigens to T lymphocytes in lymphoid organs. Recently, it was discovered that a altered form of inducible heat shock protein 70, Hsp70iQ435A, can cause repigmentation in lesions of vitiligo in Sinclair swine, providing hope for a new treatment for vitiligo patients.[1]

Oxidative stress: It plays a great part in starting the vitiligo onset by melanocyte damage. Redox homeostatic imbalance is caused by oxidative stress, which reflected as unrestricted genesis and insufficient breakdown of ROS. Melanocyte apoptosis takes place as an outcome of ROS stimulation by endogenous and exogenous stimuli. There is overproduction of ROS in the melanogenesis process, creating a prooxidative environment that makes melanocytes vulnerable to oxidative stress. Contribution by endogenous along with exogenous trigger factors leads to the uncontrolled genesis of ROS. As far as exogenous stressors are concerned, numerous factors like exposure to the environment (e.g. cytotoxic chemicals, trauma, ultraviolet irradiation), other disorders (calcium imbalance, malignancy, infections and neural diseases), and drugs (e.g.hormones and vaccination) trigger the overproduction of ROS to a specific degree,

after which generation of ROS is nearly an instantaneous reaction. Whereas a sequence of inner stimuli can activate ROS production: (a) processes of cellular metabolism like melanogenesis, that are genetically determined, require more energy; (b) altered metabolism of energy in mitochondria ultimately results in cell division, proliferation, and apoptosis. ROS are generated during the melanogenesis process as a result of the transformation of DOPA to DOPAquinone and later to dopachrome, making melanocytes prone to oxidative damage. Furthermore, melanogenesis is a process that needs high amount of energy and hence, increased quantity of adenosine triphosphate (ATP). The ATP production, in conjunction with ROS formation in mitochondria, creates a pro-oxidative environment in the epidermis. Membrane lipid redox mismatch may result in impaired functioning as well as altered structure, impacting intracellular transduction facilitated by membrane receptors, mitochondrial energy and electron transport. All these events collectively end up making the melanocyte an epicentre for buildup of ROS. Melanocytes release ROS as a response to stress. As a result, the antioxidant system is substantially distorted, with a disparity of raised indicators of oxidative stress (ROS, superoxide dismutase, malondialdehyde) and a considerable exhaustion of mechanism of anti-oxidation (catalase, thioredoxin reductase and thioredoxin, glutathione peroxidase, superoxide dismutases, glutathione reductase, and the repair enzymes methionine sulfoxide reductases) in the skin and the blood. It is proposed that the variance of pro-oxidants and antioxidants is to blame for the heightened susceptibility of melanocytes to external stressors that are pro-oxidant in nature and, over time, the induction of a presenescent state. ROS production and accumulation can result in harmful effects to DNA, oxidation and fragmentation of protein and lipid peroxidation, all of which hinder functions of the cell.[1,2] There is emergence of a new theory, haptenation theory, that suggests a significant role in vitiligo. This theory states that high levels of H2O2 cause an

increase in tyrosinase enzyme levels and activity. Tyrosinase is an enzyme that can bind to a variety of substrates, including noradrenaline (during periods of extreme emotional stress), oestrogen, tri-iodothyronine produce orthoquinone metabolites. By acting as probable substrates for tyrosinase that are haptogenic, these metabolites transform this enzyme into a newly formed antigen, which the immune system recognizes as an autoantigen. As a result, an autoimmune response is induced, resulting in loss of pigmentation via specific damage to melanocytes consisting of the autoantigen in the face of a modified tyrosinase enzyme.[2] Oxidative byproducts can be generated by exogenous stimuli also. Monobenzone is the commonest used agent causing depigmentation; it stimulates the secretion of antigen-containing exosomes related to the melanosome in response to melanocyte ROS overproduction. Due to oxidative stress, melanocyte adhesiveness is reduced at the perilesional area, which could explain the Koebner phenomenon. Simple molecules for adhesion such as cadherins and integrins are required for interaction of melanocyte-keratinocyte, unlike desmosomes which require specific structures. E-cadherin expression is downregulated in non lesional skin in vitiligo patients while tenascin, an antiadhesion molecule, is upregulated. Persistent friction in the affected skin can stimulate the cells of the epithelium, that turn mechanical forces to biochemical signaling, leading to stress in the cell and modified expression of cadherin.[1,20]

Melanocytorrhagy: This theory puts forward that there is apoptosis, separation, and consequent transepidermal loss due to altered melanocyte responses to friction in non-segmental vitiligo (NSV). The occurrence of Koebner's phenomenon appropriately explained since it suggests that weakly anchored melanocytes separate from the basement membrane when subjected to minimal friction and/or other stress, ascend upward through the epidermis and are

lost causing vitiligo lesions at the sites of trauma. In a study done by Kumar *et al*., it was established that the melanocytes showed weak adherence to collagen type IV in cases of active vitiligo, when in turn, in patients with a stable illness, this adhesion was reasonably robust. An even more important finding in this study was that the perilesional melanocytic dendrites were tiny, retracted and clubbed in patients with an unstable disease, rendering them unable to cohere melanocytes to the surrounding keratinocytes as well as the basement membrane, promoting their transepidermal loss. It has come to notice recently that tenascin, which is a matrix molecule outside the cell and prevents melanocyte adhesion to fibronectin, is found to be higher in lesional skin which is a factor leading to the loss of melanocytes. This contributes to form focal gaps and defective basement membrane development contributing to weakness of melanocytes' attachment to the basal layer and ultimately melanocyte loss of a chronic nature called as melanocytorrhagy. Melanocytes that are damaged are capable initiating an immune response during their transepidermal migration that can perpetuate vitiligo. Kumar *et al.* had conducted a study in which it was discovered that melanocytorrhagia in NSV patients is mostly caused by changes in nuclear receptor protein "liver X receptor alpha (LXR)". Furthermore, they delineated an elevated LXR-α expression, which is an apoptosis promoter, in the skin around the lesions in NSV. It was shown that the LXR-α agonist, 22(R)-hydroxycholesterol dramatically reduced the adhesion and proliferation of melanocytes in the same analysis. Thus, they came to a conclusion that an increased LXR-α expression in the melanocytes around the lesions dramatically reduced the attachment and proliferating capacity along with increased death of melanocytes.[2]

Vitamin D deficiency: Vitamin D or 1,25-dihydroxyvitamin D3, a fat-soluble vitamin, is acquired via food and is formed from 7-dehydrocholesterol under the impact of UV light in the

skin. Regulation of calcium as in addition to bone metabolism, controlling proliferating and differentiating ability of the cell together with immunoregulatory activities are functions of vitamin D, to name a few. It consists of a nuclear receptor known as vitamin D receptor (VDR). The cells responsible for the metabolism of calcium and bone, as well as the immune system cells of the skin, keratinocytes, melanocytes and fibroblasts all contain VDRs. Multiple ways exist by which vitamin D significantly affects keratinocytes and melanocytes. Invitro studies have indicated vitamin D3 promotes repigmentation of vitiligo in the context that it increases the genesis of melanin and content of tyrosinase in cultured melanocytes and provides them protection from UVB-associated death. There is evidence that analogues of vitamin D, namely calcipotriol and tacalcitol, have been known to augment normal pigmentating properties in vitiligo. Furthermore, vitamin D has shown immunomodulatory action causing suppression of cytokines that are proinflammatory in nature, like IL-6, IL8, TNF-α and TNF-γ.[2]

Risk factors:

- Physical stress to the body: major and chronic diseases, surgical procedures, accidents
- Chemical triggers: thiols, phenols, mercaptoamine, quinones together with their derivatives
- Endocrine triggers
- Infections as well as repeated intake of antibiotics
- Malnutrition: poor dietary habits, intake of stale, preserved and junk diet, UV radiation and sunburns.[21]

Classification and clinical features:

Vitiligo can be categorized as generalized or localized subtypes, based on the lesional extent in addition to its distribution. The localized category is then subclassified as focal subtype, segmental subtype, and mucosal subtype, while the generalized category, into acrofacial subtype, vulgaris subtype and universal subtypes. In case of overlapping features of different types are presents, it can be termed as 'mixed type'. On the basis of whether the lesions of vitiligo cross the midline, it is subdivided into non-segmental type, segmental type together with mixed type. At the Vitiligo Global Issues Conference (VGICC), a universally accepted classification was created and updated in 2012. The most significant consensus in this classification is the distinction between segmental subtype of vitiligo and other subtypes.[22]

A distinct distribution of the lesions that is segmental is referred to as segmental vitiligo (SV). 'Vitiligo' is the proposed nomenclature for all subtypes of vitiligo that are non-segmental, though vitiligo or non-segmental vitiligo may be mentioned as a transitory term. The term 'vulgaris' meaning 'common' was decided to not be used due to a negative undertone. The group of vitiligo/non-segmental vitiligo includes mixed subtype, which represents the simultaneous existence of vitiligo along with segmental vitiligo. Vitiligo or non-segmental vitiligo is usually preceded by the occurrence of segmental vitiligo. And ultimately, long-standing variety of focal and mucosal vitiligo both are encompassed in the group of unclassified or undetermined kind. Focal type of vitiligo is considered to be unclassified till one to two years of follow-up which may permit a more precise classification. Clinically, the focal type of vitiligo can transition into the segmental, generalized, or, in rare cases, the universal types of vitiligo.[22]

Vitiligo/ Non-segmental Vitiligo clinical characteristics:

It is marked by well-defined circular to oval hypopigmented to depigmented macules or patches not associated with symptoms involving the body bilaterally. The commonest type of vitiligo is the non-segmental type, attributing to around 85-90% of all cases. NSV has shown to have an incidence between 50-90%. The commonest site for initiation is over face (39%), then the anterior part of the trunk (23.6%), neck region (10.4%), and posterior part of the neck (9.1%).[22]

Acrofacial Vitiligo: It constitutes of depigmenting lesions over distal areas of the limbs (hands more than feet) and orifical area of the face in a circumference (Figure 1). The quickest rates of advancement are typically seen in the body regions closest to the original sites. However, progression to face is most commonly seen when the hands are the site of onset. Clinically, this could be attributed to the observation that one-third of all cases with lesions that originated in the hands had acrofacial lesions. Furthermore, since there is an association of mucosal lesions with acrofacial lesions, it is described that mucosal type of vitiligo may rather be a subtype of this type of vitiligo.[22]

Mucosal Vitiligo: Lesions are present on mouth and mucosal membranes, which includes the genital region. Where cases of mucosal vitiligo are associated with non- segmental vitiligo, it is simple to categorize it as non-segmental vitiligo. Whereas, when mucosal lesions occur as an isolated finding, it ought to be classified under unclassified vitiligo. Rather frequently involved mucosal lesions are seen around orifices of the body like the lips and genitalia. A poor prognostic indicator for vitiligo is the fact that patients in whom mucosae has been noted, exhibited more frequent progress in the condition.[22]

Generalized Vitiligo: Initially known to be vitiligo vulgaris, this kind is the commonest type of the disorder (Figure 2). In a prior study that examined clinical features, 1002 (69.8%) of the 1436 vitiligo patients had vitiligo vulgaris, now known as generalized type of vitiligo.[22]

Universal Vitiligo: Here, there is loss of pigmentation over the whole body, and complete or near-complete lesions are seen. Yet, there is absence of clear guidelines about the minimum percent of involvement of the body that is required to make a diagnosis of universal vitiligo.[22]

Mixed Type: There is co-occurrence of SV and NSV in this type. Non-segmental vitiligo is usually preceded by presence of segmental vitiligo, which can show a delay of about half a year to more than 2 years and is comparatively unresponsive to therapy than NSV. On unusual instances where NSV and SV show simultaneous existence, segmental lesions are more resistant to treatment compared to the non-segmental ones.[22]

Figure 1: Acrofacial vitiligo

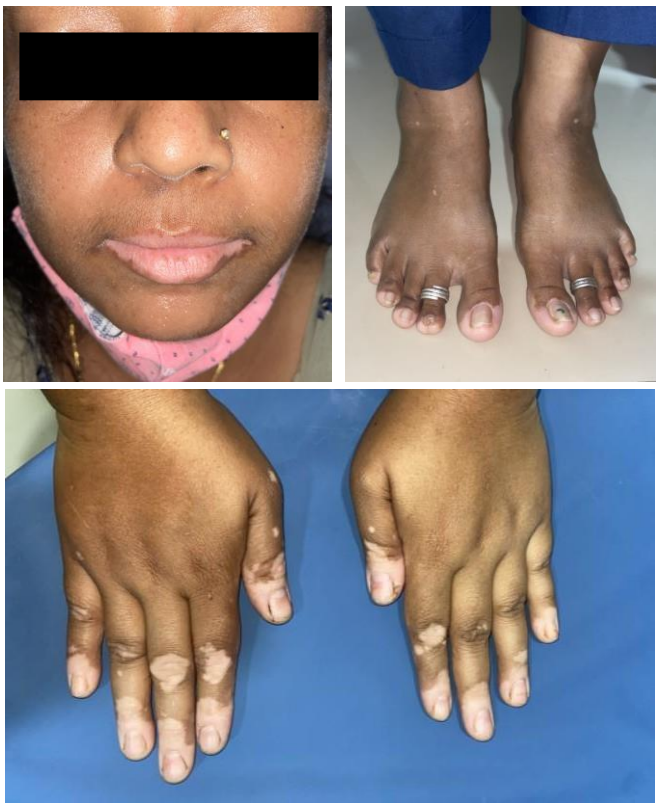
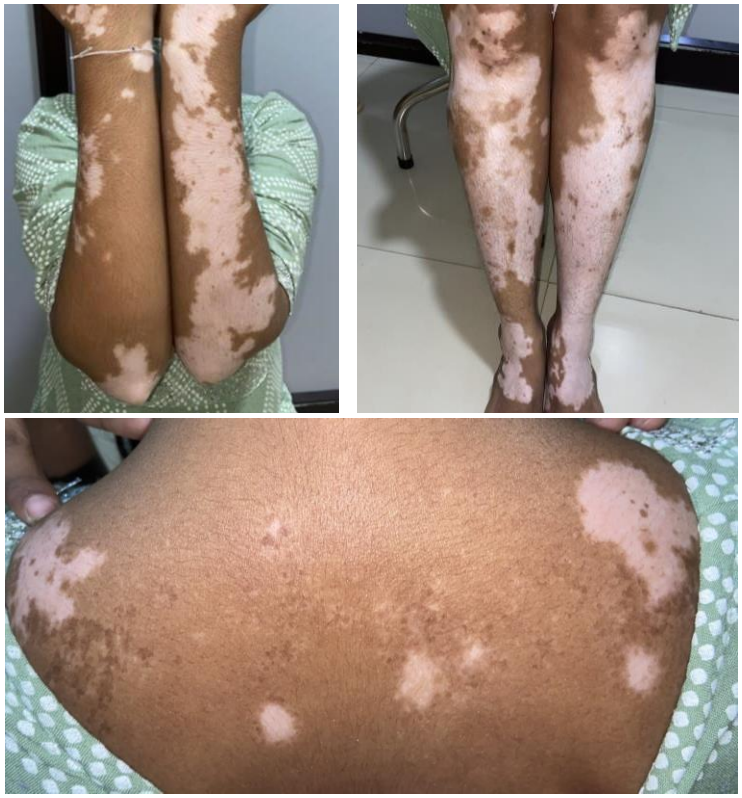


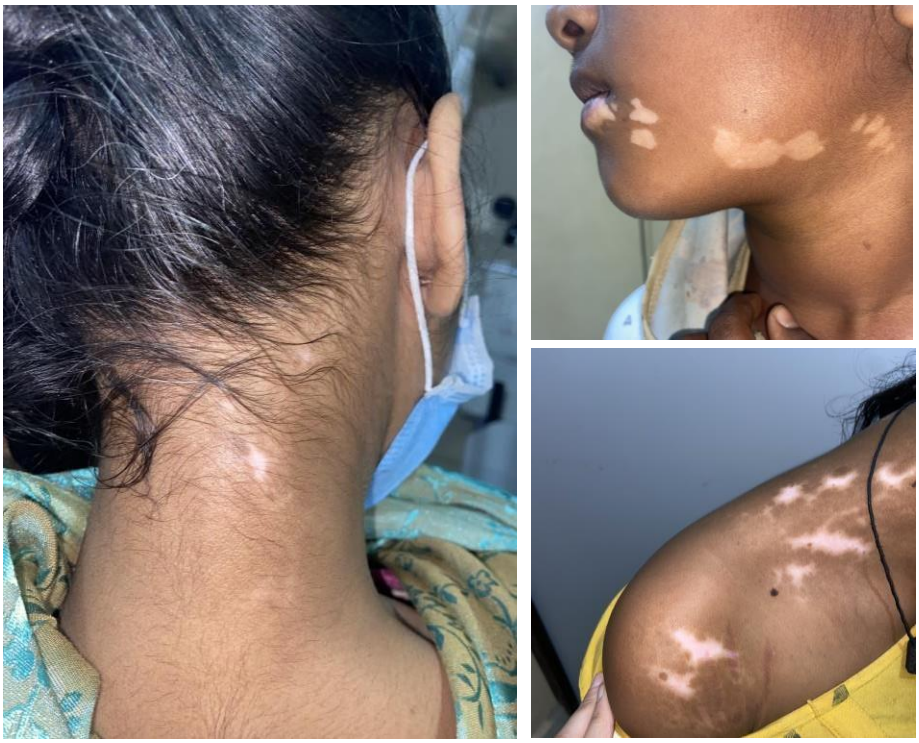
Figure 2: Generalized vitiligo/vitiligo vulgaris



Segmental Vitiligo characteristics:

Segmental vitiligo typically develops early in life and affects only one segment of the integument, spreading rather quickly within the affected area (Figure 3). Segmental vitiligo can often be very tough to distinguish from focal type of vitiligo till a later stage where lesions are differentiable, since the segment may be made up of many neighboring dermatomes or parts of them, or it may not be related to any dermatome at all. Moreover, segmental type of vitiligo may follow a linear distribution following Blaschko's line or not follow any other lines. It is frequently assumed that the distribution of SV may follow an unidentified route, consisting of a clonal cell group. Only in very infrequent cases, lesions can be termed as 'mixed' type when they become bilateral and generalized.[22]

Figure 3: Segmental vitiligo



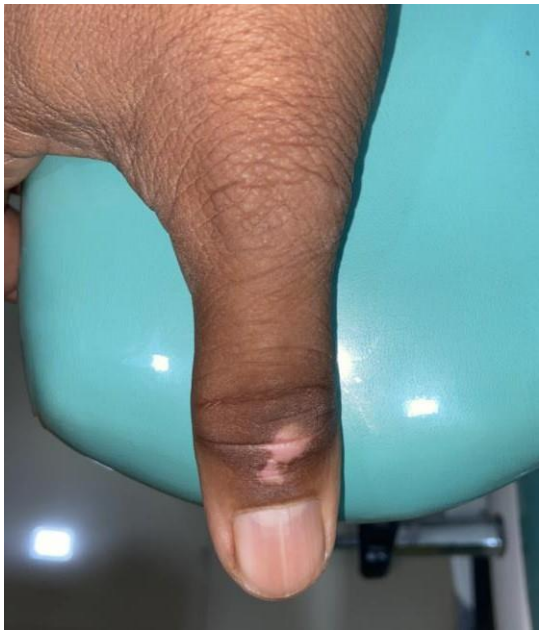
Undetermined/Unclassified Vitiligo

This comprises of two types of vitiligo that which fit neither in NSV nor SV. They are:

Focal vitiligo: Small, distinct, isolated patches of vitiligo that, after at least two years of initiation, do not fit into either the SV or NSV classification but may subsequently evolve into either of the 2 types[23] (Figure 4).

Mucosal vitiligo: Isolated oral and genital mucosae involvement without skin involvement till 2 years of follow-up.[23]

Figure 4: Focal vitiligo



Rare variants:

Trichrome vitiligo: It is characterized by presence of 3 zones: central depigmentation, narrow/broad band of intermediate pigmentation in between and normal skin.[23]

Quadrichrome vitiligo: Presence of four colours of pigmentation, usually seen in dark individuals; 4th colour being dark brown in areas of repigmentation occurring in the perifollicular region.[23]

Pentachrome vitiligo: Five sequential shades of pigmentation are present in the order white color, tan color, brown color, blue gray color and normal colour.[23]

Inflammatory vitiligo: Erythematous lesions with raised borders associated with an itching and burning sensation.[23]

Blue vitiligo: Lesions of vitiligo occurring over areas of post-inflammatory hyperpigmentation. This type of lesions has been observed in cases with acquired immunodeficiency syndrome (AIDS).[23]

Occupational/contact vitiligo: A unique kind of vitiligo precipitated by exposure to chemicals (aliphatic or aromatic derivatives of phenols and catechols). Depigmented lesions, in such cases, may be limited to the site of contact or may progress gradually to involve other body parts as well, simulating NSV.[23]

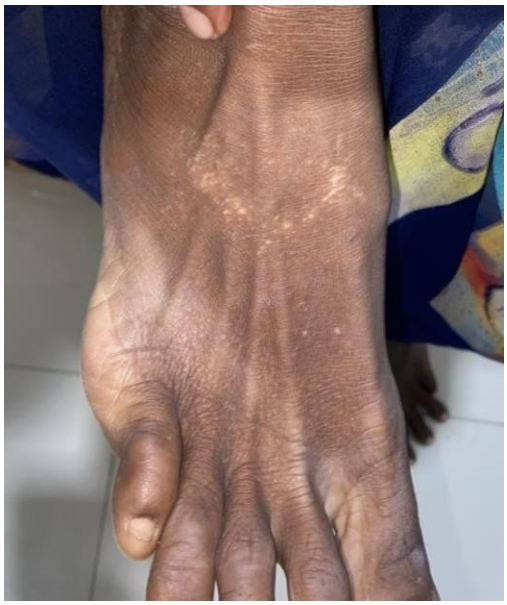
Vitiligo punctata: Well defined, sharply demarcated, punctate sized (1-1.5 mm) macules over the body at any site.[23]

Vitiligo minor: Presence of hypopigmented lesions rather than depigmented ones representing partial defect of pigmentation.[23]

Follicular vitiligo: It is one of the uncommon types of vitiligo, where hair follicle melanocytes are targeted primarily. Leukotrichia, that is, whitening of hair can be noticed in vitiliginous and non-vitiliginous areas. Despite the lesser likelihood of leukotrichia in NSV lesions, NSV is frequently found before or after the development of follicular vitiligo.[23]

Koebner's phenomenon: The emergence of lesions at the area of traumatised, unaffected skin is known as Koebner phenomenon. It is also known as an "isomorphic response" (Figure 5). By history, it is found in about one-third of the cases. A greater risk of Koebner phenomenon is to the patients with greater involvement of the body surface and a relatively early age of onset. Knowledge regarding this phenomenon is crucial so as to carefully examinate for linear depigmented macules at the areas of scratching, abrasions and other traumatic incidences in all cases of vitiligo, since it is an important sign of disease activity.[24]

Figure 5: Koebner's phenomenon



Diagnosis:

The diagnosis of this condition is usually uncomplicated, which is made on a clinical basis when there are features of acquired, amelanotic, chalky-white macules with well-defined borders with absence of scaling and presence in a typical distribution.[1,2]

Wood's lamp may aid in diagnosing, which is a UV irradiation tool from which there is emission of UVA. It aids in recognizing focal melanocyte loss and detection of areas where lesions may not be seen with a naked eye, especially in skin that is pale. The vitiligo lesions appear bright blue-white under the Wood's light and have distinct borders.[1]

Dermoscopy is a tool that can be used to distinguish between vitiligo and other disorders of depigmentation. Other hypopigmentation disorders lack telangiectasia and residual perifollicular pigmentation, which are common features of vitiligo. More significantly, it can be helpful in determining the stage of evolution and the disease activity in vitiligo: progressive

lesions show perifollicular pigmentation, whilst stable or remitting lesions show perifollicular depigmentation.[1]

Confirmatory tests are not necessary for the diagnosis. In-vivo confocal microscopy can non-invasively assess the melanocytes being absent in a lesion, or it can also be assessed by a skin biopsy. Histologically, there is a total melanin pigment loss in the epidermis in the center of a vitiligo lesion along with absence of melanocytes. Advancing border of lesions show occasional lymphocytes.[1]

Indices used in vitiligo:

Vitiligo Disease Activity Score: It is a six-point index for assessing the activity of the disease or stability over a period of time. The patient himself reports the activity. It aids in judging the efficacy of therapy in halting and reversing the disease progression and extent. The description of disease activity includes either expansion of the currently present areas of depigmentation or neogenesis of lesions. The grades are as follows: VIDA Score +4 – activity lasting 6 weeks or less; +3 – activity lasting 6 weeks to 3 months; +2 – activity lasting 3–6 months; +1 – activity lasting 6–12 months; 0 – stable for 1 year or more; and – 1 – stable with spontaneous repigmentation for 1 year or more. A higher VIDA score corresponds to a more active disease while a low score points towards the disease being less active or comparatively more stable. As of now, the activity is measured by the history that the patient gives along with cutaneous examination. Generally, during follow-up visits, photography is used to monitor the size, quantity, and grade of lesions as well as the repigmentation.[11]

Vitiligo Area Severity Index: Hamzavi *et al*. had put forward VASI, which was theoretically taken from the commonly used PASI (Psoriasis Area and Severity Index) score.

VASI is a sensitive, standardised approach for determining the extent and percentage of re/depigmentation. The hands, upper limbs, trunk, lower limbs and feet are the five distinct and mutually exclusive zones that comprise the patient's body in the VASI. Upper limbs include the axillary region, whereas lower limbs include the inguinal area and the buttocks. Although they can be evaluated separately, the face and neck are not considered in the all-inclusive assessment. For every body area, the VASI is calculated as the product of the area of involvement in hands units (1% per unit; rule of palm) and the depigmentation extent that fits in every hand unit-measured lesion (possibilities of 0%, 10%, 25%, 50%, 75%, 90% or 100%). Since it necessitates the physician to estimate the quantity of pigment present and the area of involved, it has a subjective component to it.[11]

Differential diagnoses:

Localized Depigmentation:

- Treponemal diseases like secondary syphilis and late stage of pinta.
- Postinflammatory depigmentation like atopic dermatitis, lichen planus, psoriasis, pityriasis alba, lichen sclerosus.
- Tuberous sclerosus
- Chronic or severe inflammation seen in cases of atopic dermatitis and contact dermatitis
- Lichen sclerosus
- Scleroderma
- Systemic sclerosis can show a presence of depigmented lesions dotted with pigmentation in the perifollicular area ("salt and pepper" pattern).

- Melanoma or as an adverse event due to immunotherapy with inhibitors of interleukin-2, interferon-alpha and immune checkpoint.
- Chemical leucoderma
- Piebaldism[24]

Localized Hypopigmentation:

- Pityriasis alba
- Tinea versicolor
- Tuberculoid leprosy
- Sarcoidosis
- Naevus depigmentosus
- Naevus anemicus
- Idiopathic guttate hypomelanosis[24]

Widespread Hypopigmentation and Depigmentation:

- Progressive macular hypomelanosis
- Hypopigmented mycosis fungoides (MF)
- Achromic pityriasis lichenoides chronica
- Oculocutaneous albinism[24]
- Hypomelanosis of Ito[25]

Treatment:

Medical therapy:

Narrowband Ultraviolet B (NB-UVB) treatment, Targeted Ultraviolet B, excimer laser, topical immunosuppresant treatment by topical usage of calcineurin antagonists, pseudocatalase, and Vitamin D analogues combined with use of UV light are the latest advancements in medicinal front.[2]

NB-UVB therapy: NB-UVB utilizes UV-lamps with a peak emission of about 311nm and has surfaced as the first choice of treatment in cases of generalized type of vitiligo and vitiligo vulgaris (patchy kind of vitiligo).[2] According to recent guidelines, NB-UVB (entire body or local) is to be offered as first choice phototherapy for patients with vitiligo having an insufficient response to topical treatment and/or who have extended or active disorder.[25] It acts via inducing of localized immunosuppression and stimulating the growth of melanocytes, not only in skin, but also in the outer root sheath of the hair. Melanocyte Stimulating Hormone biosynthesis and melanogenesis are both stimulated. Studies comparing NB-UVB to topical psoralen in addition to ultraviolet A (PUVA) treatment have found a greater rate of response with NB-UVB. Moreover, the occurrence of adverse effects like photosensitivity or phototoxicity with the usage of NB-UVB is much lesser as compared to those seen with topical PUVA. Different topical agents like topical tacrolimus, pimecrolimus, analogues of vitamin D and/or topical pseudocatalase are used as a combined therapy with to increase the efficacy of treatment and hence reduce the total tenure of therapy.[2]

Laser Therapy: Excimer laser, that makes use and generates a monochromatic laser light of 308nm wavelength and makes use of Xenon-Chlorine (gas, is another therapeutic modality. The laser can be used as a monotherapy or combined with topical immunosuppressant or PUVA-sol therapy.[2] Guidelines suggest considering excimer laser in patients with a localized form of vitiligo, combined with topical usage of calcineurin inhibitors.[25] According to studies, both localized and segmental vitiligo potentially benefit from treatment with this laser and rather quick visible results have been observed. What makes this laser a better choice than conventional UVB therapy is that the uninvolved skin is not exposed due to targeted mode of treatment. And compared to UVB therapy, the initiation of response occurs earlier when excimer laser therapy is used.[2]

Targeted UVB therapy: One more revolution in the field of therapeutics when it comes to vitiligo is targeted UVB therapy which delivers high intensity UVB light only over lesions with avoidance of any exposure on the normal skin. This is believed to dramatically increase treatment efficacy while simultaneously lowering the cumulative UVB radiation that each patient receives. Targeted UVB therapy, as the name suggests, is used widely for the therapy of focal type and segmental type. Few advantages that targeted UVB therapy gives as compared to excimer laser therapy include increased treatment safety and efficacy with a comparatively lesser cost.[2]

Systemic immunomodulator therapy: Systemic steroid therapy has been, by far, the most commonly used treatment amongst immunosuppressants. Nevertheless, due to its high incidence of adverse events, it is to be used with caution, particularly in pediatric population,

which is the most affected age. Hence, steroids are prescribed in pulse or even in mini-pulse forms to overcome this limitation.[2] Oral Minipulse (OMP) therapy, which involves giving systemic steroids in mini-pulse form twice a week on consecutive days, has also been administered. The primary study which showed the effectiveness of OMP in which oral betamethasone was given (0.1mg/kg with 5mg being the maximum dose) was reported in the year 1991.[26] In a study carried out at a later date on vitiligo in childhood group, oral methylprednisolone replaced betamethasone and it was used in combination with topical usage of fluticasone on the affected areas. In >90% of patients, the disease was arrested and significant (>50%) repigmentation of areas was achieved by >65% of children.[27] According to guidelines, oral betamethasone 0·1 mg/kg given two times per week on two continuous days for a period of 3 months should be considered, following which tapering of the dose by 1 mg every month should be done for a further duration of 3 months in addition with NB-UVB in cases with a rapidly progressing disease to contain the activity.[25]

Topical Vitamin D analogues: Topical calcipotriol has been used as monotherapy or combined with topical usage of steroids for vitiligo treatment. Vitamin D3 influences the growth and differentiation of not only melanocytes, but also of keratinocytes. When used as a combined therapy with UV-light and topical steroids, they have shown to give variable results.[2]

Topical immunomodulators: Topical agents like tacrolimus along with pimecrolimus have shown some encouraging effects off late in the management of vitiligo. They are being used consistently over the past few years in the light of their exceptional safety profile. Some advantages of these over topical steroids are that they do not cause telangiectasia or atrophy

which makes them a safe treatment option in young children. The risk of is suppression of hypothalamic pituitary-adrenal (HPA) axis is also absent as compared to that encountered with the usage of potent topical steroids. Parts like the face and neck, that are exposed to the sun, show the maximum improvement as opposed to the acral areas, which show minimum response.[2] Topical corticosteroids (TCS) have been in practice for their immunomodulatory and anti-inflammatory effects since the 1950s, though there is an absence of reports estimating the optimal period of treatment with them. Few specialists recommend using it daily for two to three months, whereas some others advise using a discontinuous regimen (once per day application for 15 days every month for a period of six months). They are usually applied twice a day.[1] Guidelines advocate topical usage of a potent or very potent corticosteroid once every day, to reduce adverse effects, as the first-line therapy avoiding the periocular area. Topical tacrolimus 0·1% two times a day is considered in cases with facial lesions as a substitute to potent or very potent topical corticosteroids, and under occlusion on sun exposed parts only in cases of nonfacial lesions. There is scope for an intermittent regime which would consist of applying potent or very potent topical corticosteroids once per day with or without inclusion of topical usage of calcineurin antagonists. Some examples include:

7 days of potent or very potent corticosteroids and minimum 7 days off, or

7 days of potent or very potent topical corticosteroids alternating with ≥7 days of topical usage of calcineurin antagonist.

Topical corticosteroids can be applied for more than 7 days after considering the risks and benefits.

Reassessment of the patients who are on topical treatment is to be done every 3–6 months to monitor the improvement. Periodic medical photography may be used to evaluate these changes.[25]

Pseudocatalase: Pseudocatalase is being used in conjunction with UVB radiation or Dead Sea climatotherapy to manage vitiligo. There is evidence suggesting high levels of H_2O_2 and oxidative stress in the affected site which supports the utilization of this in cases of vitiligo. It is applied over the lesion following which there is UVB exposure either to the entire body or to the affected skin. The combination is said to rectify oxidative stress that the melanocytes face in case of vitiligo leading to repigmentation.[2]

Topical 5-Fluorouracil: Topical 5-fluorouracil overstimulates melanocytes in the follicle which move to the epidermal layer during the process of epithelialization thereby inducing repigmentation. To enhance the repigmentation response, this type of topical treatment may be used in conjunction with spot dermabrasion of the affected areas.[2]

Surgical therapies:

The first and most important step in surgical management is patient selection. Since not all patients are benefitted from a surgical modality, selection of suitable patients for surgical is crucial. Patients' immunologic response seems to be the reason for variation in improvement to therapy. A stable disease or disease not associated with an autoimmune etiology generally show a better response to surgical intervention. Focal or segmental type of vitiligo shows a promising response to surgical intervention and it is usually the first-line therapeutic option for such

patients; whereas, surgeries are not as successful in case of other subtypes of vitiligo and should be considered only when medical therapy has led to failure and the cases have a disease that has been stable.[28]

Stability of the disease is one more criterion which should be taken into consideration before opting for a surgical line of treatment and is termed as a state where there is absence of any new or expanding lesions in a certain time period; most guidelines have fixed it to a year (generally between 6 months to 24 months). Reporting by the patient, serial photographs and usage of scoring systems are a few means by which disease stability can be noted. Another reliable indicator of an unstable disease is Koebnerization, which implies development of depigmentation at sites of trauma. Few methods with a fair potential to assess disease stability in the future include reflectance confocal microscopy, status of total antioxidants, levels of antibodies to the melanocytes, serum catecholamine levels together with their metabolites. Estimation of other cellular indicators like IL-17, IL-6, CXCL 9 and 10, and mircoRNA may come handy in evaluating stability.[28]

Location of recipient area also is a factor of accountability since certain areas like head and neck, which have a rich blood supply and density of follicles, show a more significant response to surgery than the limbs. Presentation like the acrofacial variant has a relatively poor outcome. Skin over the joints also show an unfavorable response which is attributed to its proneness to repeated friction and injury. Few contraindications to surgical procedures include patients having keloidal tendencies, significant bleeding pathologies and blood-borne infections.[28]

Surgical treatment for vitiligo has enhanced the cure rates of the disease by a significant amount, leading to an increase in their utilization in the treatment of vitiligo that is unresponsive, both in India and abroad. The main plus point with this modality of treatment is that the odds of repigmentation of affected site are around 90-100%. Various procedures are tried in the management such as autologous suction blister grafting, split-thickness grafting, punch grafting, smash grafting, single follicular unit grafting, cultured epidermal suspensions and autologous melanocyte culture grafting. There is no requirement of specialized instruments for these, and they are easy to perform, except the melanocyte culture grafting. These grafting techniques are now classified as tissue grafts or cellular grafts, dependent on whether the epidermis/dermis in its entirety or individual components of the cell are transplanted.[2]

Tissue grafting technique:

Suction blister grafting: By creating adequate negative pressure on the skin, suction blisters are created at the donor area, commonly over the thighs or buttocks. For this, suction apparatus or syringes with three-way cannulae can be used. Thin epidermal grafts are harvested from these blisters and then relocated on dermabraded recipient sites. Repigmentation occurs as a result of this, and the cosmetic matching is excellent. Suction blister grafting has become the first-line technique in grafting in vitiligo due to its ease, greater rate of improvement, and brilliant cosmetic outcome.[2]

Split thickness grafting: With a Silver's knife, a dermatome, Humby's knife or simply a shaving blade, a thin split thickness graft is harvested from a donor area and transplanted on the dermabraded recipient areas. Partial thickness grafting has one advantage over the suction blister

technique: it can treat a comparatively greater area of vitiligo in just one session. NB-UVB therapy can be started/continued following both partial thickness skin grafting along with suction blister grafting to accomplishing quicker and better results.[2]

Miniature punch grafting: After taking 1.0 to 2.0 mm diameter full-thickness punch grafts from a suitable donor area, they are relocated on to the recipient sites, on which there are similar punch shaped beds. Treatment with PUVA/PUVA-sol or topical corticosteroids is given to the receiving site which promotes spreading of the pigment from these transplanted punches onto the neighboring areas. Over time, the recipient region, in its entirety, acquires repigmentation. It is an easy procedure with an ability to treat greater affected region compared to the above mentioned two methods. This technique can also be used to alleviate vitiligo lesions with unusual or geographically shaped patterning. But there are certain risks and disadvantages with this procedure that one must be aware about and look out for, actively. Hypertrophic changes at the recipient area, 'cobblestone appearance' and 'polka-dot appearance' are few of the many examples of limitations. Meticulous selection of the patient and usage of a small punches (1.0 to 1.5 mm) are some measures by which these adverse events can be reduced. In India, miniature punch grafting is the most common surgical method carried out for vitiligo.[2]

Follicular unit grafting: Similar to the method used in hair transplantation, single-hair follicular units are taken from a donor site. These follicular units are then transferred into vitiligo lesions after being cut above the follicular bulb. The very concept of this technique is to transfer the follicular unit melanocytes to the lesional skin which then acts as a pigment source at the recipient area. The repigmentation process used here closely resembles how vitiliginous skin

normally repigments, producing a brilliant cosmetic effect. However, this procedure is tedious and requires experience.[2]

Smash grafting: On an appropriate surface, like a glass slide, a partial thickness graft is taken and "smashed," or sliced into minute pieces using a surgical blade. The recipient area, after being dermabraded, is transplanted with this "smashed" tissue which is subsequently coated with a specialized powder to prevent the graft from being disturbed. Here, thicker grafts are used which give a good cosmetic outcome which is of benefit over a partial thickness graft.[2]

Cellular grafting techniques:

Recent guidelines suggest considering cellular grafting in cases with stable, segmental or nonsegmental vitiligo that is not improving with other treatment.[25]

Non-cultured epidermal suspensions: After taking a split-thickness graft from a donor site, it is incubated overnight. The cells are manually segregated the next day using trypsin-EDTA solution and centrifuged to make a suspension. The recipient area is dermabraded and this suspension is applied on the affected sites, over which a collagen dressing is done to prevent any dislodgement. This technique can take care of a comparatively greater area affected, approximately ten times the size of the graft. However, the recipient site has to be treated for two to three months with NB-UVB or PUVA therapy for acquiring the desired result.[2]

Melanocyte culture transplantation: One of the latest techniques than non-cultured epidermal suspensions, it also requires a split-thickness graft to be harvested from a donor site

and incubated in a culture medium for melanocytes' or keratinocytes-melanocyte growth in vitro. Application of these cultured cells is carried out on the laser dermabraded or manually abraded lesional site. A single donor graft can effectively tackle a greater area of involved skin and the outcome with this method has been satisfying.[2]

METHODOLOGY

SOURCE OF DATA

Patients of vitiligo presenting in Outpatient Department of Dermatology, Venerology and Leprosy, in B.L.D.E (Deemed to be University), Shri B.M. Patil Medical College, Hospital and Research Centre, Vijayapura, were enrolled for the study.

Period of study:

The study was conducted from January 2021 to May 2022.

Study design:

A hospital-based cross-sectional study.

Sample size

With anticipated Mean±SD of HsCRP levels 3.22±6.98 mcg/ml, the study required a sample size of 51 with a 95% level of confidence and a precision of 2.

Formula used

- $n=\frac{z^2 S^2}{d^2}$

Where Z= Z statistic at α level of significance

d^2= Absolute error

S—Common standard deviation

q= 100-p

Note: Total number of cases collected was 58.

METHOD OF COLLECTION OF DATA:

Inclusion criteria:

- Patients with typical clinical features of vitiligo irrespective of age, gender and on-going or past treatment.

Exclusion criteria:

- Patients with any other co-existing chronic inflammatory disorder like rheumatoid arthritis, psoriasis, etc.
- Patients with any active cutaneous or systemic infection.
- Patients with co-morbidities like hypertension, diabetes mellitus, ischaemic heart disease, pulmonary tuberculosis, etc.
- Patients with any history of smoking.
- Patients with congenital (e.g., piebaldism, Waardenberg syndrome, albinism, etc.) causes and acquired (e.g., post-inflammatory depigmentation, contact leucoderma, etc.) causes of depigmentation disorders.

Methods:

Informed consent for the study was undertaken from all the patients. Details of the present illness, including the time of onset, duration of the lesion in each case, past history of any disease, and family history was recorded as per the proforma.

A thorough clinical examination to determine the exact distribution, and morphology of the cutaneous lesions was done.

Methodology:

- Initial clinical examination was done and signs and symptoms of the lesions were recorded in the proforma.
- Disease activity and disease extent was calculated using Vitiligo Disease Activity Score (+4 - Activity of disease since 6 weeks or less; +3 - Activity of 6 weeks to 3 months; +2 - Activity of 3 to 6 months; +1 - Activity of 6 to 12 months; 0 - Stable for 1 year or more; and -1 - Stable since 1 year or more with spontaneous repigmentation) and Vitiligo Area Severity Score (100% - complete depigmentation; 90% - only specks of pigment; 75% - depigmented areas exceed the pigmented area; 50% - pigmented and depigmented areas are equal; 25% - pigmented areas exceed depigmented areas; and 10% - specks of depigmentation) respectively.
- After obtaining consent, a blood sample of 5 ml was collected in a plain tube and the sample will be loaded in an autoanalyzer and serum IL-6 levels and serum HsCRP levels was estimated. A blood sample of 3 ml will be collected in a plain tube, and sample will be loaded in an autoanalyze, and complete blood count, and liver function tests was estimated as and when required.
- These values were represented in a table, and their values will be correlated with the activity and extent of vitiligo.

INVESTIGATIONS:

Following investigations will be done:

1. Serum IL-6 levels.
2. Serum HsCRP levels.

Other investigations (as and when required):

1. Complete haemogram
2. Liver function tests

STATISTICAL ANALYSIS:

- The data obtained was entered in a Microsoft Excel sheet, and statistical analysis was performed using a statistical package for the social sciences (Version 20).
- Results were presented as Mean (Median) ±SD, counts, and percentages and diagrams.
- Categorical variables were compared using Chi-square test.
- Correlation between variables was calculated by Person's/ Spearman's Correlation.
- Receiver Operating Curve (ROC) analysis was applied to find the sensitivity.
- $p<0.05$ will be considered statistically significant. All statistical tests performed were two-tailed.

ETHICAL CLEARANCE:

Institutional ethical committee clearance was undertaken for the study.

RESULTS

A hospital based cross sectional study was conducted from a period of January 2021 to May 2022. A total of 58 patients with a clinical diagnosis of vitiligo were included in the study. After taking a complete history and performing a full clinical examination, disease activity and extent were evaluated using VIDA and VASI, respectively. Serum levels of IL-6 and HsCRP were estimated via an autoanalyzer.

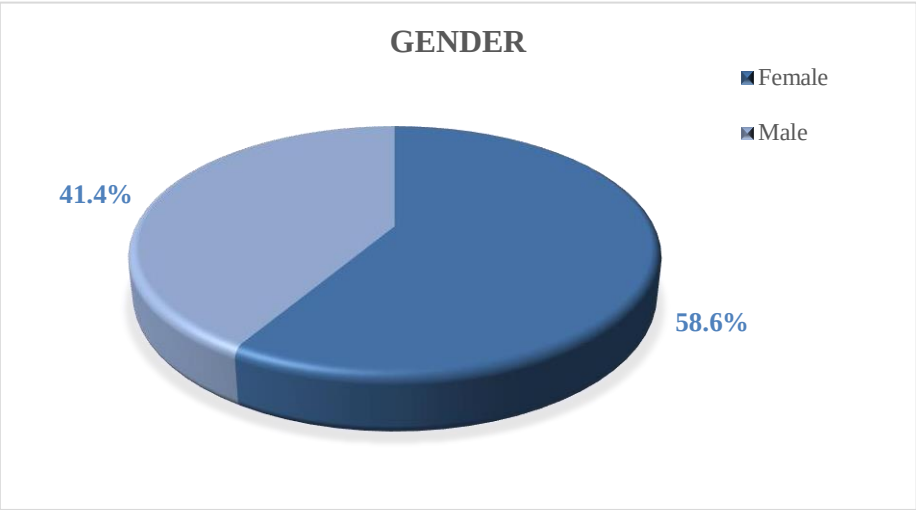
Gender distribution

Amongst the 58 cases enrolled, 34(58.6%) were females and 24(41.4%) were males (Table 1, Figure 6). Females outnumbered males in the study with a ratio of 1.41:1.

Table 1: Distribution of cases according to sex

Sex	Number	Percent
Male	34	58.6
Female	24	41.4
Total	58	100.0

Figure 6: Distribution of cases according to sex



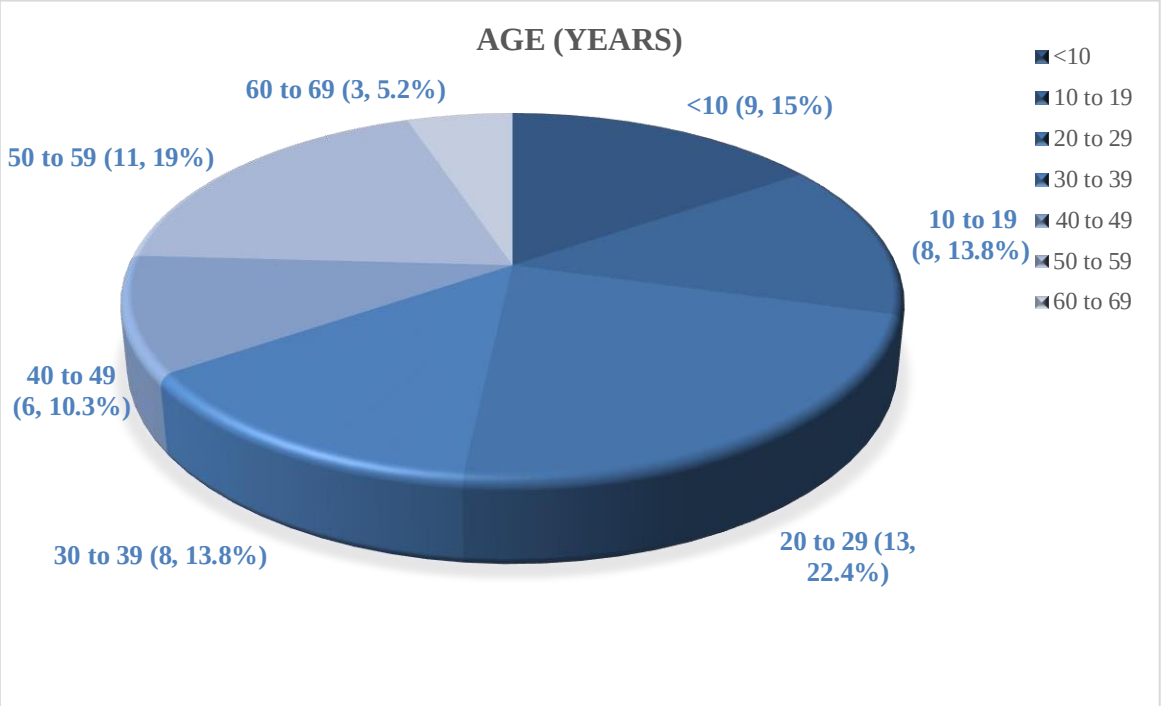
Age group

The age group of patients included in the study ranged from 4 years to 65 years. Most patients in the study belonged to the 2nd decade (13 patients – 22.4%) (Table 2, Figure 7).

Table 2: Distribution of cases according to age

Age groups	Number	Percent
<10	9	15.5
10-19	8	13.8
20-29	13	22.4
30-39	8	13.8
40-49	6	10.3
50-59	11	19.0
60-69	3	5.2
Total	58	100.0

Figure 7: Distribution of cases according to age



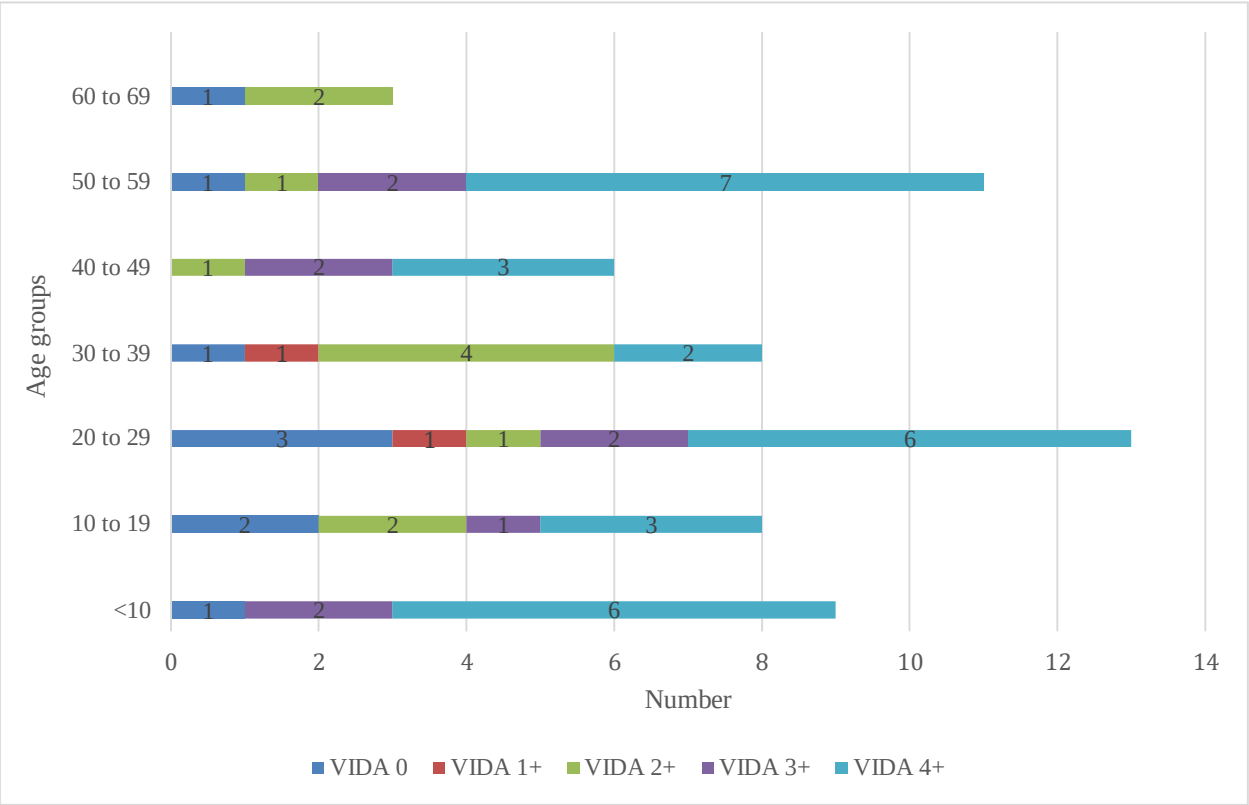
Relation between age and VIDA:

Age group below 10 years showed the highest number of patients with VIDA score of 4+ (6 out of 9, 66.66%) while the age group that showed maximum number of patients having a stable disease compared to other age groups was 60 to 69 years (1 out of 3, 33.33%) (Table 3, Figure 8).

Table 3: Distribution of VIDA within age groups

Age groups (in years)	VIDA					Chi-square value	p-value
	0	1+	2+	3+	4+		
<10	1	0	0	2	6	24.887	0.412
10-19	2	0	2	1	3		
20-29	3	1	1	2	6		
30-39	1	1	4	0	2		
40-49	0	0	1	2	3		
50-59	1	0	1	2	7		
60-69	1	0	2	0	0		
Total	9	2	11	9	27		

Figure 8: Distribution of VIDA within age groups



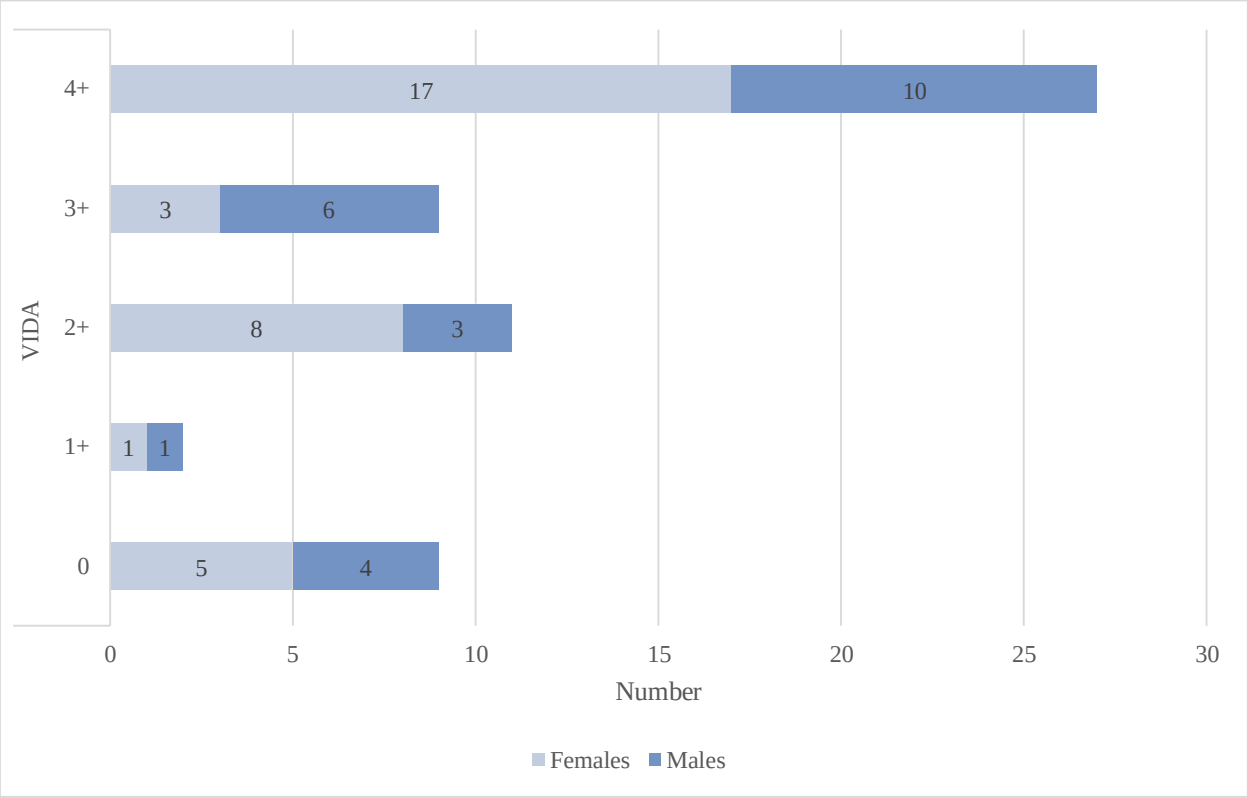
Relation between gender and VIDA:

Out of a total of 58 patients, almost half (27 out of 58, 46%) patients showed a highly active disease (VIDA 4+). 17 out a total of 34 female patients (50%) showed a VIDA score of 4+, while VIDA score of 4+ was noted in 10 out of 24 male patients (41.6%) (Table 4, Figure 9). Male to female ratio of an active disease was 1:1.7.

Table 4: Distribution of VIDA between gender groups

Gender	VIDA				
	0	1+	2+	3+	4+
Females	5	1	8	3	17
Males	4	1	3	6	10
Total	9	2	11	9	27

Figure 9: Distribution of VIDA between gender groups



Relation between gender and VASI:

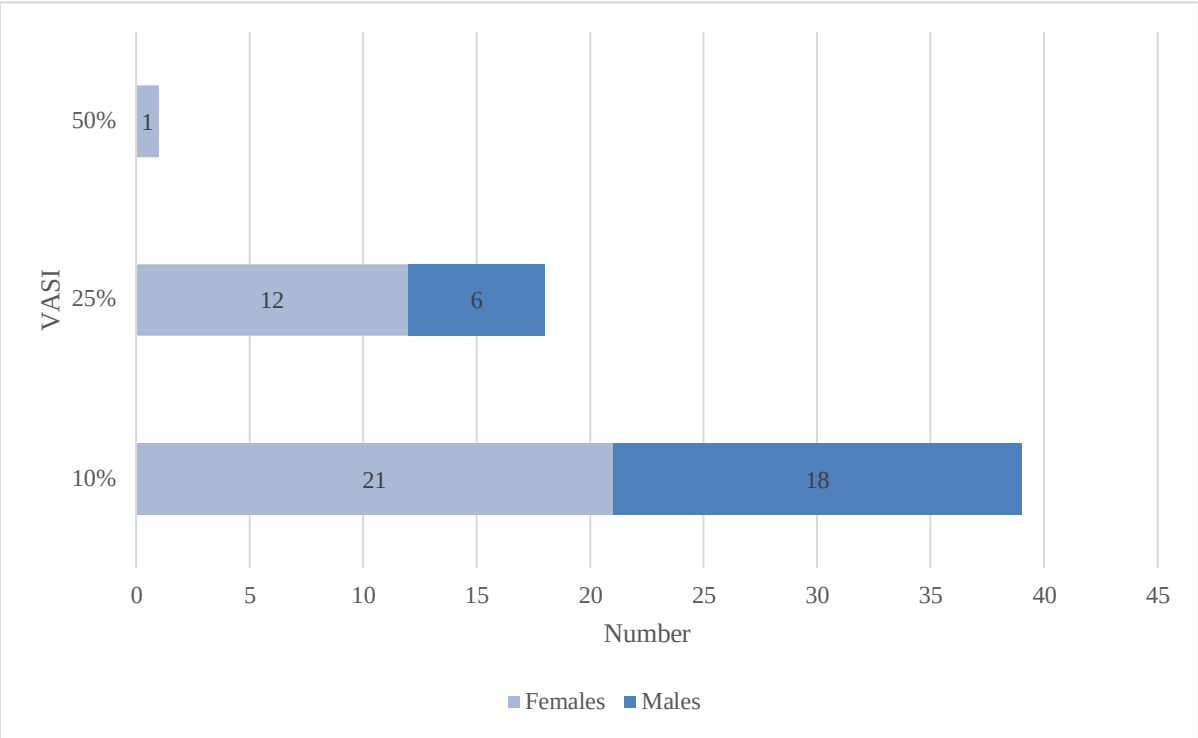
Amongst all 58 patients, 39 (67.2%) patients showed a VASI of 10%. This group had 21 out of all 34 females (61.7%) and 18 out of 24 males (75%).

18 of 58 total patients (31%) showed a VASI of 25%. 12 out of 34 females (35%) and 6 out of 24 males (25%) were found to be in this group. And 1 female patient (1.7%) showed a VASI of 50% (Table 5, Figure 10).

Table 5: Distribution of VASI between gender groups

Gender	VASI		
	10%	25%	50%
Females	21	12	1
Males	18	6	0
Total	39	18	1

Figure 10: Distribution of VASI between gender groups



Types of vitiligo:

Types of vitiligo encountered in our patients were vitiligo vulgaris, segmental, acral, acrofacial and focal. The commonest type was vitiligo vulgaris (42 out of 58, 72.4%) followed by segmental (7 out of 58, 12%), acral (5 out of 58, 8.6%), focal (3 out of 58, 5.2%) and acrofacial (1 out of 58, 1.7%).

Relation between age and types of vitiligo:

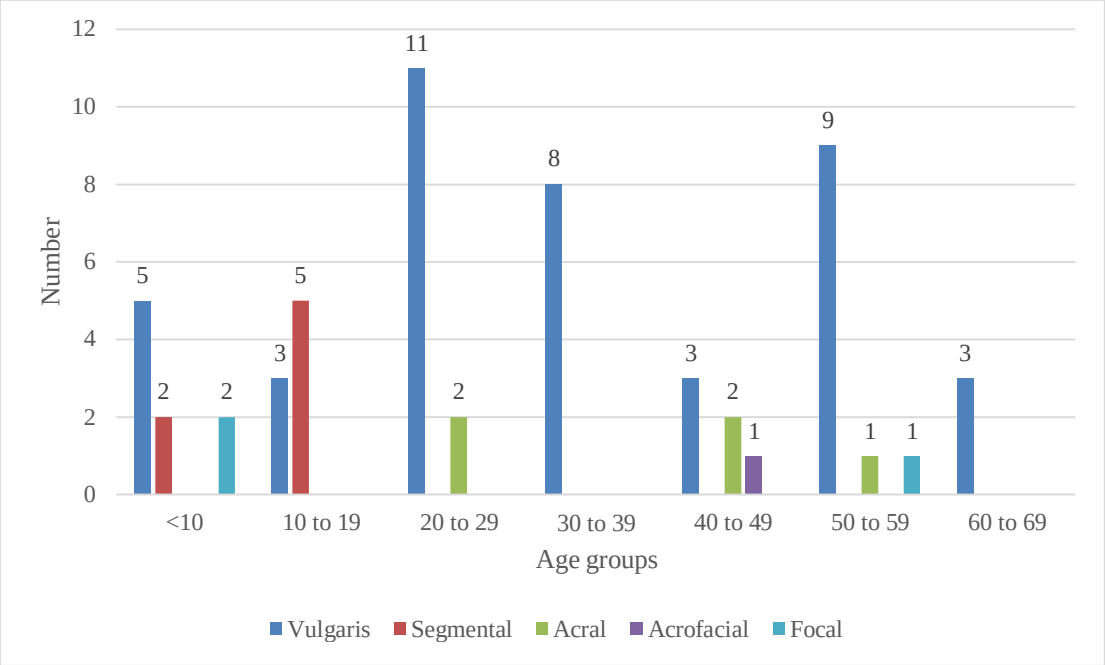
Out of a total of 42 vitiligo vulgaris cases, maximum cases were seen in the 2nd decade (11 out of 42, 26.2%) followed by the 5th decade (9 out of 42, 12.51%). Segmental vitiligo was exclusively

found in the pediatric age group ranging from 5 to 16 years of age. Focal vitiligo was also found mainly in age less than 10 years, but a single case aged 52 years also had a focal variety. Acral vitiligo was noted in the middle aged, that is in the 2nd and 4th decade. A solitary case of acrofacial variety was found in a 45-year-old patient (Table 6, Figure 11).

Table 6: Distribution of types of vitiligo within age groups

Age groups (in years)	Vulgaris	Segmental	Acral	Focal	Acrofacial
<10	5	2	0	2	0
10-19	3	5	0	0	0
20-29	11	0	2	0	0
30-39	8	0	0	0	0
40-49	3	0	2	0	1
50-59	9	0	1	1	0
60-69	3	0	0	0	0
Total	42	7	5	3	1

Figure 11: Distribution of types of vitiligo within age groups



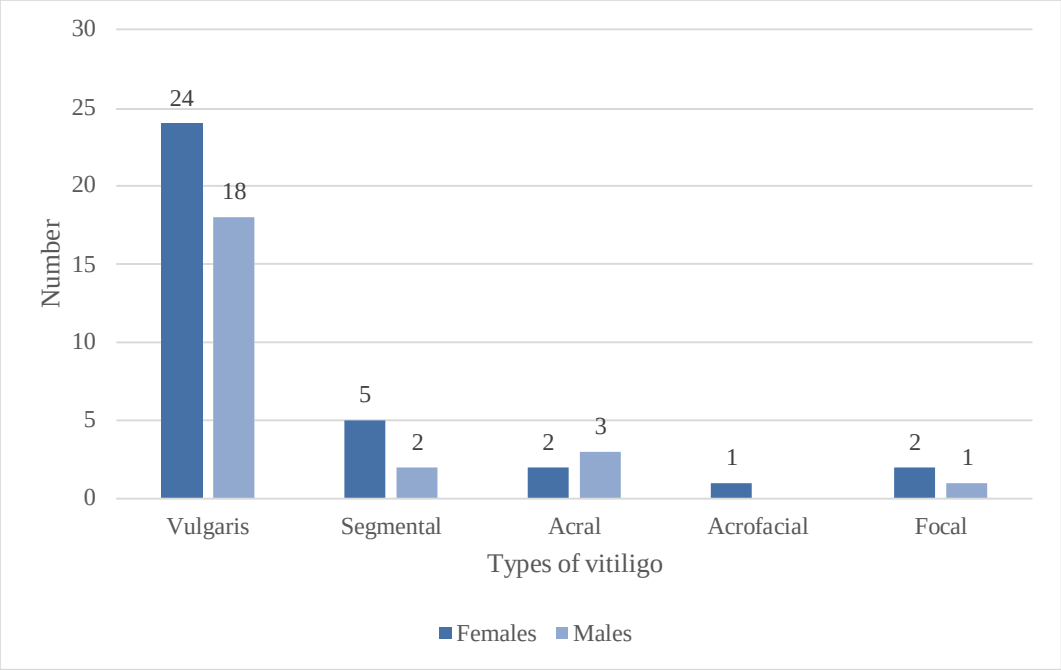
Relation between gender and types of vitiligo:

About similar percent of males (18 out of 24, 75%) and females (24 out of 34, 70.6%) had vitiligo vulgaris. The other types also showed no significant difference based on gender of the patient (Table 7, Figure 12).

Table 7: Distribution of types of vitiligo within gender groups

Gender	Vulgaris	Segmental	Acral	Focal	Acrofacial
Females	24	5	2	1	2
Males	18	2	3	0	1
Total	42	7	5	1	3

Figure 12: Distribution of types of vitiligo within gender groups



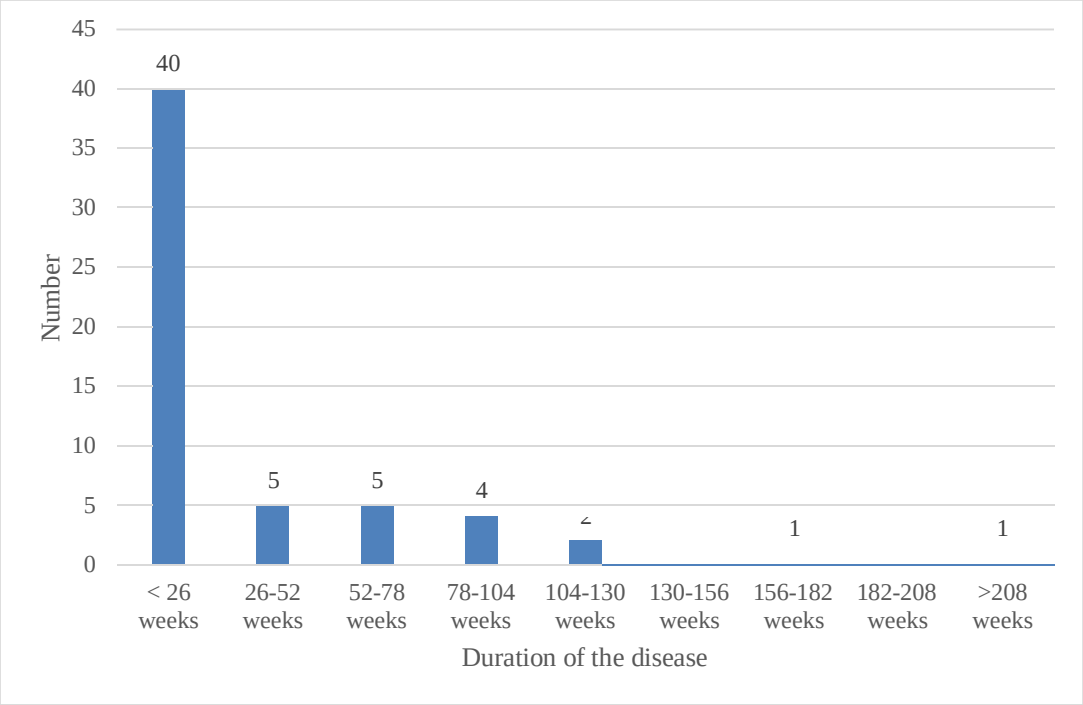
Duration of the disease:

Duration of the disease in this study showed a range from 0.5 months (2 weeks) to 240 months (20 years) with a mean of 34.258 months (approximately 137.032 weeks). Maximum number of patients had a rather acute onset of vitiligo, that is less than 6 months (around 26 weeks) duration (Table 8, Figure 13).

Table 8: Distribution of cases based on duration of disease

Duration (in months)	Number	Percent
< 26 weeks	40	68.96%
26-52 weeks	5	8.62%
52-78 weeks	5	8.62%
78-104 weeks	4	6.89%
104-130 weeks	2	3.44%
130-156 weeks	0	0.00%
156-182 weeks	1	1.72%
182-208 weeks	0	0.00%
<208 weeks	1	1.72%
Total	58	100.00%

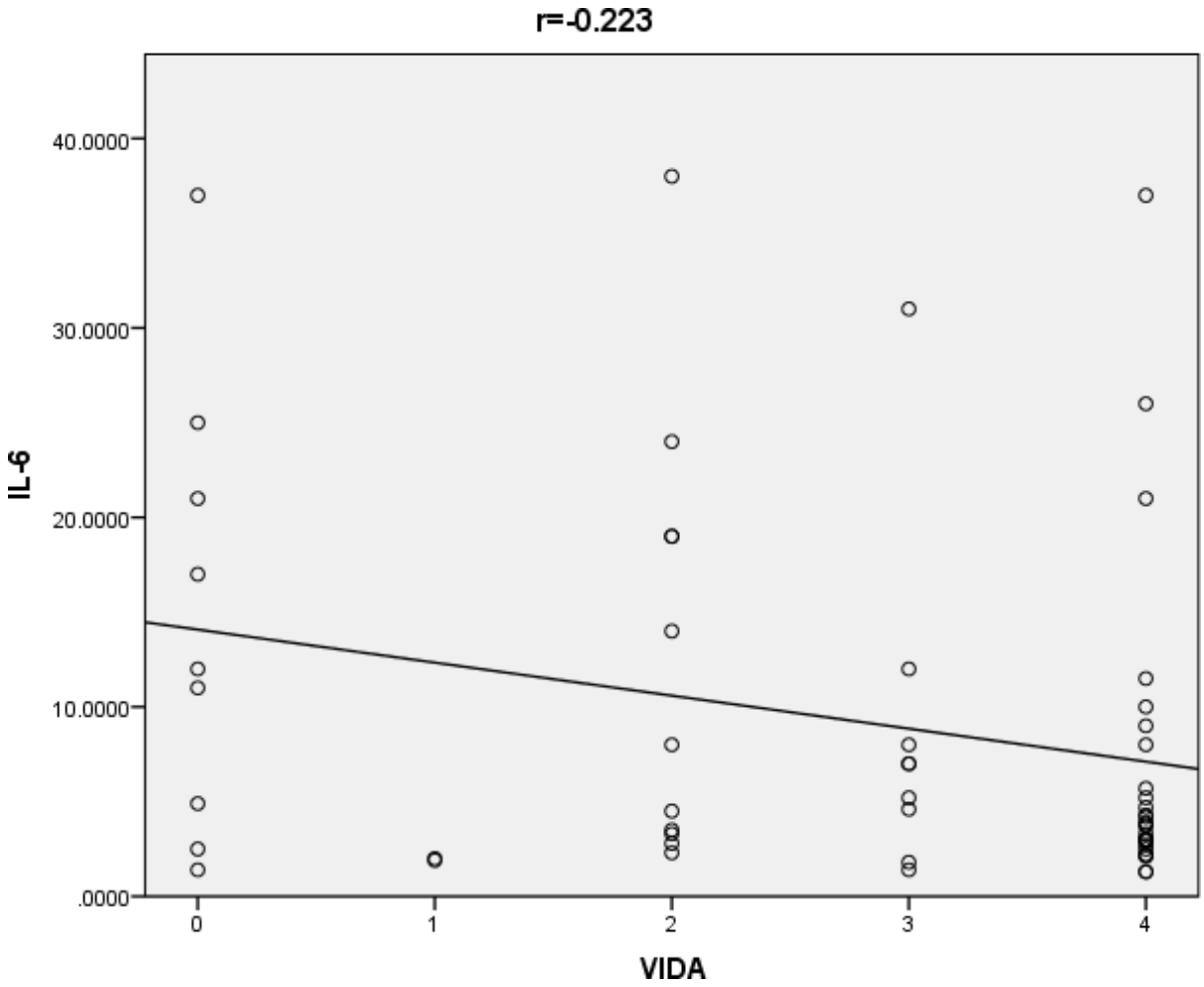
Figure 13: Distribution of cases based on duration of disease



Relation between VIDA and IL-6:

There was a mild negative correlation found between VIDA and IL-6 levels which was statistically insignificant (Figure 14).

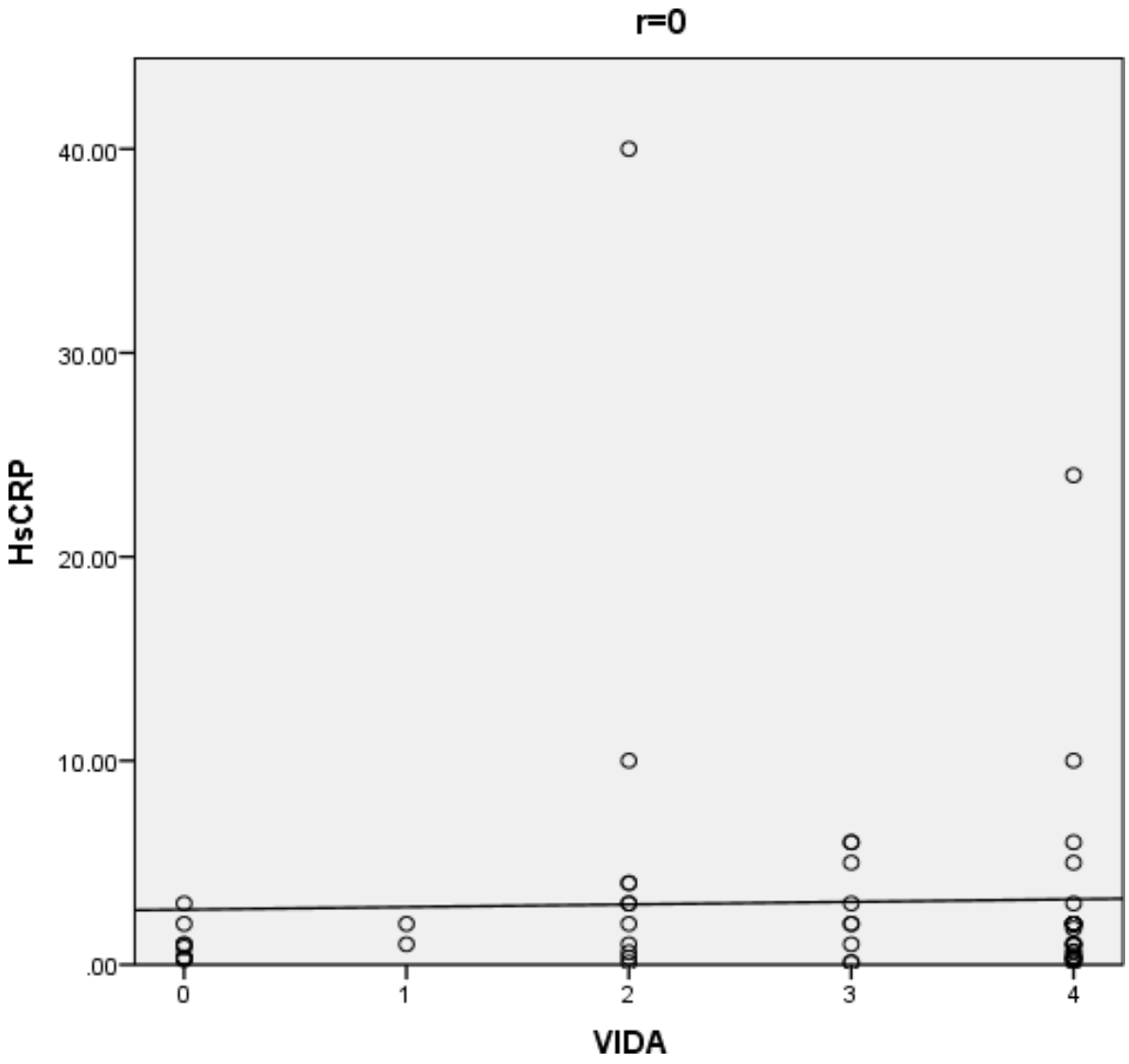
Figure 14: Scatter diagram showing a correlation of VIDA and IL-6



Relation between VIDA and HsCRP:

There was absolute absence of any correlation between VIDA and HsCRP levels (Table 9, Figure 15).

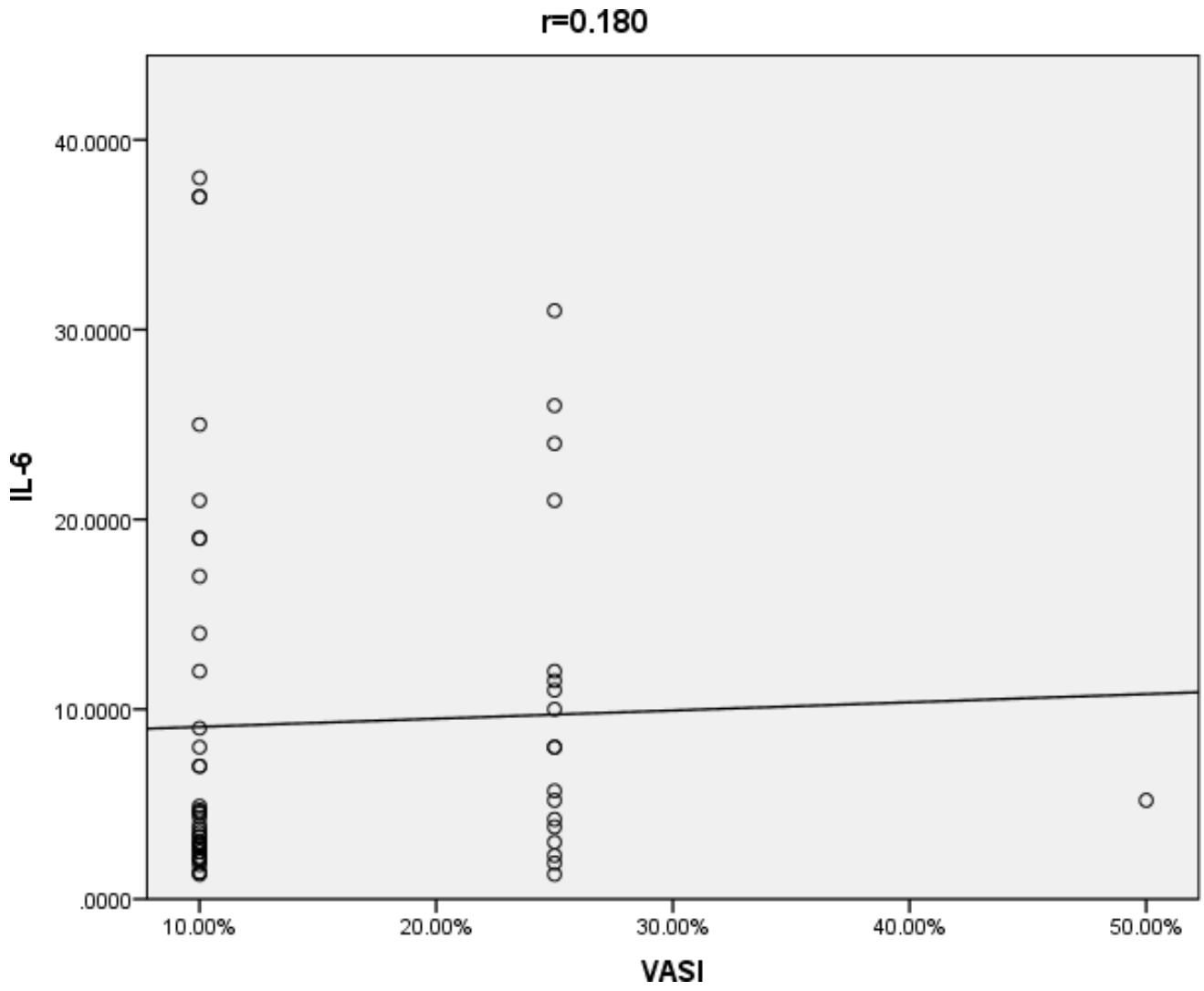
Figure 15: Scatter diagram showing a correlation of VIDA and HsCRP



Relation between VASI and IL-6:

There was a mild, statistically insignificant correlation between VASI and IL-6 levels (Table 9, Figure 16).

Figure 16: Scatter diagram showing a correlation of VASI and IL-6



Relation between VASI and HsCRP:

There was a mild, statistically insignificant correlation between VASI and HsCRP levels (Table 9, Figure 17).

Figure 17: Scatter diagram showing a correlation of VASI and HsCRP

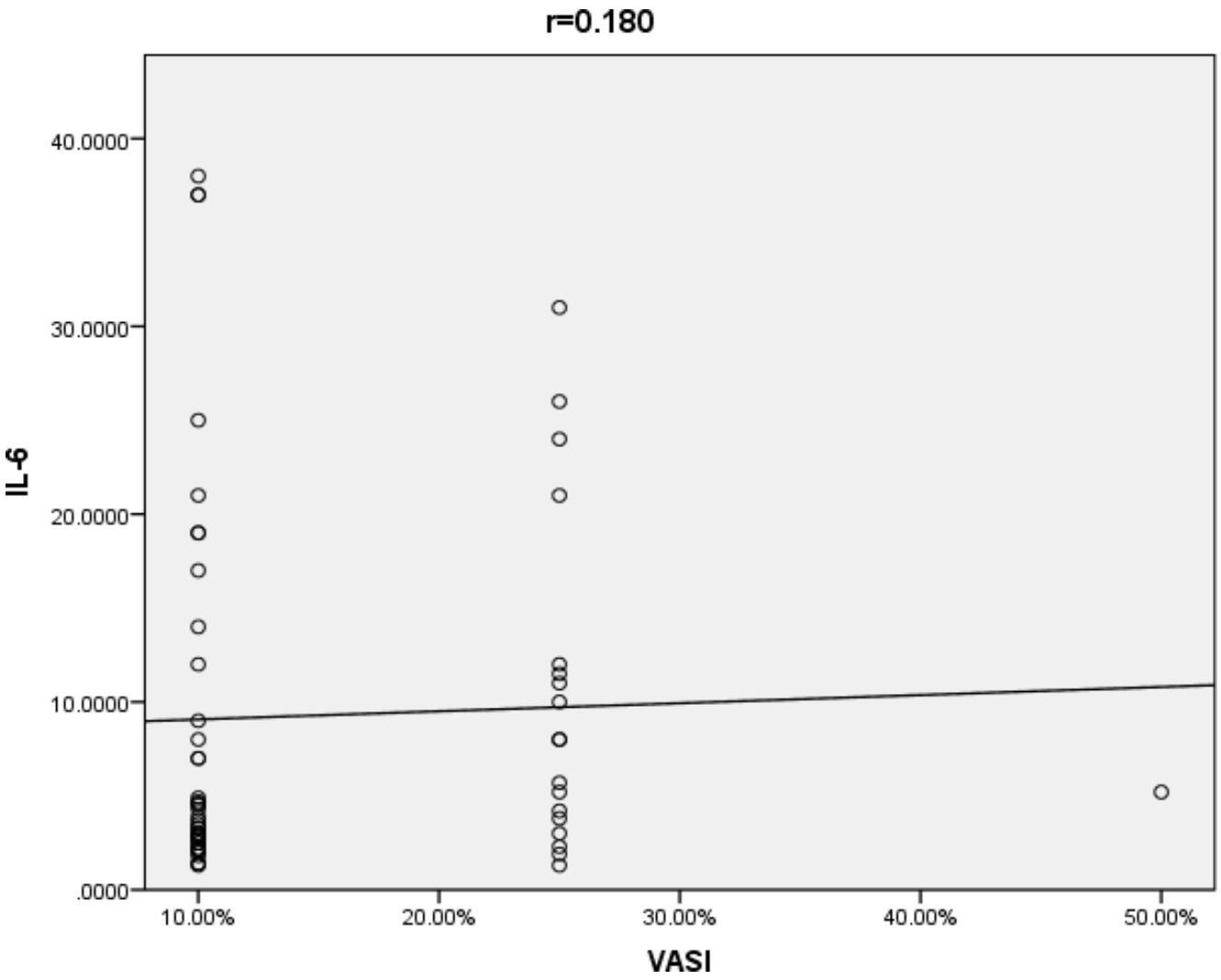


Table 9: Non-parametric correlations

Variables	Spearman's rho correlation coefficient	p-value
VIDA and IL-6	-0.223	0.092
VIDA and HsCRP	0	0.998
VASI and IL-6	0.180	0.175
VASI and HsCRP	0.157	0.238

**. Correlation is significant at the 0.01 level (2-tailed).

VIDA in association with other parameters:

When compared with other parameters, duration of the disease was found to vary significantly as VIDA score changed. Rest of the parameters, namely VASI, IL-6 and HsCRP levels did not show much variation with a changing VIDA score (Table 10).

Table 10: Non-parametric correlation of VIDA with other parameters

Variables	VIDA	N	Mean	Std. Deviation	Kruskal-Wallis H Test	p-value
VASI	0	9	13.33%	6.61%	2.549	0.636
	1	2	17.50%	10.61%		
	2	11	12.73%	6.07%		
	3	9	16.67%	7.91%		
	4	27	16.48%	9.79%		
	Total	58	15.34%	8.37%		
IL-6	0	9	14.64444	11.67841	8.67	0.07
	1	2	1.95	0.070711		
	2	11	12.58182	11.47692		
	3	9	8.666667	8.974965		
	4	27	6.944444	8.319501		
	Total	58	9.303448	9.813399		
HsCRP	0	9	1.0111	0.93013	4.351	0.361
	1	2	1.5	0.70711		
	2	11	6.1855	11.55261		
	3	9	2.8011	2.35601		
	4	27	2.6411	4.78092		
	Total	58	3.0459	6.12182		
Duration	0	9	46.000	30.8869	12.103	0.017
	1	2	66.000	25.4558		
	2	11	33.091	33.5036		
	3	9	51.111	76.3486		
	4	27	22.852	40.2042		
	Total	58	34.259	45.4034		

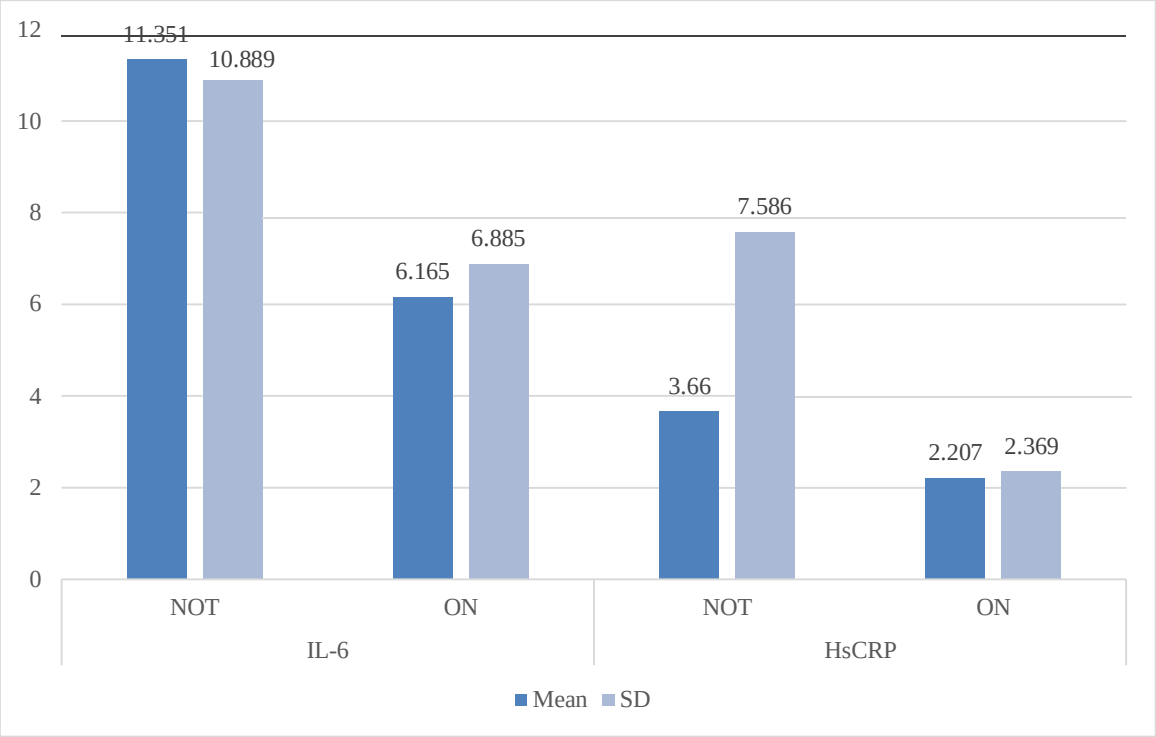
Values of IL-6 and HsCRP within subgroups of patients:

The patients were further categorized into two subgroups based on whether they were on any type of immunosuppressive therapy while being investigated or not. The mean values of IL-6 and HsCRP levels were noted. (Table 11, Figure 18).

Table 11: Distribution of mean values and SD of IL-6 and HsCRP levels in subgroups (NOT signifies the group not taking any immunosuprressive therapy, while ON denotes the group taking some form of immunosuppressant)

Variables	Group	N	Mean	SD	Mann-Whitney U test value	p-value
IL-6	NOT	35	11.351	10.889	505	0.105
	ON	23	6.165	6.885		
HsCRP	NOT	35	3.66	7.586	397.5	0.943
	ON	23	2.207	2.369		
Non Significant						

Figure 18: Distribution of mean values and SD of IL-6 and HsCRP levels in subgroups on and not on immunosuppressive therapy



The mean values of both IL-6 and HsCRP levels were found to be higher in the subgroup not taking immunosuppressive treatment as compared to the subgroup on immunosuppressants, although this difference was not statistically significant.

DISCUSSION

Vitiligo, a common acquired depigmenting disorder, is typified by selective melanocytic loss which leads to appearance of well defined, non-scaly, hypopigmented to depigmented macules and/or patches practically over any part of the body along with leukotrichia. It has a negative psychological impact on the patients and is found to be associated with various other autoimmune conditions.[1-5]

There are different theories proposed to explain the etiopathogenesis of this disorder. One of those is cellular immunity which suggests that there is the presence of antibodies to the surface and cytoplasmic melanocyte antigens that leads to the death of melanocytes via complement-mediated lysis and antibody-mediated cytotoxicity. CD8+ T cells have been found in the epidermis together with the dermis in the skin of affected patients. These T cells are capable of producing various cytokines like IFN-γ and TNF- α. CXCL9, 10, 11, along with STAT1, IL-6 and HsCRP, are also contributory to the inflammation produced.[1,2,4-7,20]

Various indices have been used over the years so as to assess the dynamic changes in the disease like activity, severity, extent, etc. One such index is VIDA, which is based on patient's recall of the latest noted disease worsening, that is development of new lesions or increase in size of the pre-existing lesions; and a lower score denotes less active/more stable disease. There is a question about its reliability due to a possible error in noting slow changes over time, variation in skin colour, etc. Although, at present, it is one of the most frequently used tool.[10,11]

VASI is an indicator for disease severity that is based on the extent of involvement. It divides the body into five exclusive regions and the percentage of affected area is calculated. It, again, has a subjective component since the physician is required to assess the involvement.[11,12]

This study was undertaken to assess the serum levels of IL-6 and HsCRP in vitiligo patients and correlate these values with VIDA and VASI.

Amongst the 58 cases enrolled, 34(58.6%) were females and 24(41.4%) were males. Females outnumbered males in the study with a ratio of 1.41:1. Out of 55 patients in a study by Abdallah *et al*. (29), female preponderance was seen with female to male ratio of 1.62:1. Another study done by Ranjkesh *et al*.[30] also showed similar results with a female to male ratio of 1.86:1. The age group of the patients in this study ranged from 4 years to 65 years with a mean of 30.43 years and a predominance of patients in the age group of 20-29 years. Other studies done by Namazi *et al*.[8], Shah *et al*.[19], Abdallah *et al*.[29], Ranjkesh *et al*.[30] showed majority of patients belonging to the 2nd decade.

Duration of the disease in this study showed a range from 0.5 months (2 weeks) to 240 months (20 years) with a mean of 34.258 months (approximately 137.032 weeks, 2.85 years). A maximum number of patients had a rather acute onset of vitiligo, that is less than 6 months (around 26 weeks) duration. Another study by Dave *et al*.[31] revealed mean duration of disease to be 3.5 years.

Age group below 10 years showed the highest number of patients with VIDA score of 4+ (6 out of 9, 66.66%) while the age group that showed maximum number of patients having a stable disease compared to other age groups was 60 to 69 years (1 out of 3, 33.33%). Ali *et al*.[32], Sheth *et al*.[33] have found similar results of high activity in pediatric age. According to studies done by Palit *et al*.[34] and Silverberg *et al*.[35], higher disease activity in children with vitiligo can be attributed to autoimmunity being the culprit in the pathogenesis since there is a more common association of paediatric vitiligo with other autoimmune conditions like hypothyroidism,

alopecia areata, diabetes mellitus, Addison's disease and a positive family history. Two patients in this study had a positive family history (5 years old and 21 years old).

Out of the total number of patients, almost half (46%) patients showed a highly active disease (VIDA 4+). Out of this, the proportion of females having an active disease (50%) was slightly higher as compared to males (41.6%). Similar findings were noted by Patil *et al*.[36] In context with VASI, the extent of the disease was found to be more in cases of females as opposed to males. A possible explanation for both these findings, which is supported by studies done by Schallreuter *et al*.[37] and Kotb El-Sayed *et al*.[38], is the role of estrogen which is contributory to production of hydrogen peroxide and subsequent DNA damage in peripheral lymphocytes leading to the depigmentation that is apparent clinically.

The commonest type of vitiligo encountered was vitiligo vulgaris (72.4%) followed by segmental (12%), acral (8.6%), focal (5.2%) and acrofacial (1.7%). This finding was similar to that found by Silverberg *et al*.[3], Shah *et al*.[19] and Dave *et al*.[31] Most patients in the study fell into the age category of 2nd decade and this finding is in concordance with that by Ezzedine *et al*.[3,39] Segmental vitiligo was exclusively found in the paediatric age group ranging from 5 to 16 years. Focal vitiligo was also found mainly in age less than 10 years. Overall, amongst 17 patients aged less than 19 years, the incidence of vitiligo vulgaris was the highest (8, 47.05%) followed by segmental (7, 41.17%) and focal (1, 11.76%) subtypes with no cases of acral/acrofacial and mucosal variants. Acral vitiligo was noted in the middle aged, that is in the 2nd and 4th decade. A solitary case of acrofacial variety was found in a 45-year-old patient.
Similar findings were noted in studies by Palit *et al*.[34] and Silverberg *et al*.[35], where they stated that incidence of segmental and focal types is more common in childhood vitiligo while that of acral/acrofacial is rarer than adults.

There was a negative, although not statistically significant, correlation that was found between VIDA score and serum IL-6 levels. This implies that with increase in the vitiligo disease activity, there occurs a fall in the levels of IL-6. A significant elevation in serum levels of IL-6 was found when vitiligo cases were compared with healthy controls by Singh *et al.*[40] and Habib *et al.*[41] but they could not find a significant correlation between these levels and VIDA.

There was absolutely no correlation found between VIDA and HsCRP. A study by Namazi *et al.*[8] found no significant difference between the HsCRP levels in vitiligo case group and control group suggesting a lack of significant inflammation to raise HsCRP levels.

A mild positive, statistically insignificant correlation was found between VASI and IL-6, VASI and HsCRP. Not only did Sarkar *et al.*[42] and Rahman *et al.*[43] find a statistically significant difference in HsCRP levels between the vitiligo case and control groups, but also a positive correlation between HsCRP and VASI.

A further subcategorization of patients was done into two groups, that is patients who were on at least one type of immunosuppressive therapy while being investigated and those who were not on any immunosuppressants. The mean levels of both IL-6 as well as HsCRP were noted to be higher in the group which was not on immunosuppressants as compared to the one that was, though this difference was not statistically significant. This implies that there is a role of immunosuppressive therapy in reducing the levels of these inflammatory markers in cases of vitiligo. Immunosuppressive treatment (corticosteroids or steroid sparing immunosuppressive agents) is preferred in patients whose disease is highly active so as to halt and curb the progression of vitiligo, whereas patients with a stable disease can be managed with therapeutic modalities other than immunosuppression. This explains why the levels of IL-6 showed a falling

trend with an increase in disease activity since most patients with an active disease were on immunosuppressive therapy.

Limitations of this study include lack of a control group hence, it is not a case-control study. Patients with on-going treatment were not excluded since the study intended to evaluate activity in all patients that presented to us. Treatment could have influenced the disease activity in such patients.

CONCLUSION

This study was conducted to estimate the serum levels of IL-6 and HsCRP in patients of vitiligo and correlate their values with disease activity and extent using VIDA and VASI, respectively. A total of 58 patients were included with a female to male ratio of 1.41:1. The age of the patients included ranged from 4 years to 65 years with a majority being in the 2nd decade. Duration of the disease ranged from 2 weeks to 20 years, with a mean of 2.85 years.

Females showed a higher disease activity and extent as compared to males in the study which could be probably attributed to certain hormonal contribution to the disease pathogenesis. While comparing disease activity across all age groups, paediatric age group of <10 years showed maximum number of cases (66.66%) having a VIDA score of 4+. Autoimmune causality of vitiligo in such patients and higher association of other autoimmune conditions in them can be a speculation for this finding.

Vitiligo vulgaris was the commonest encountered type (72.4%) followed by segmental (12%), acral (8.6%), focal (5.2%) and acrofacial (1.7%). All cases of segmental and most cases of focal type (66.66%) were seen paediatric age group while acral and acrofacial type was seen in the middle aged.

There was a statistically insignificant negative correlation of VIDA with serum IL-6 levels. The negative correlation could be explained by the fact that patients on immunosuppressives, which are preferred in higher disease activity, at the time of investigation showed a lesser mean value as compared to those who were not, implying that there is a role of immunosuppressive agents in reducing these inflammatory markers. There was absolutely no correlation found between VIDA and HsCRP. The correlation of VASI with both the markers was mildly positive and insignificant.

Therefore, it is crucial to also consider the treatment status, and not just the disease activity while evaluating levels of inflammatory markers in such conditions. Apart from the treatment status of patients, other coexistent conditions of immunosuppression which can influence the levels of these sensitive markers also ought to be kept in mind.

SUMMARY

A hospital-based cross-sectional study was conducted from a period of January 2021 to May 2022 to estimate serum levels of IL-6 and HsCRP in patients of vitiligo and correlate their values with vitiligo disease activity and extent using VIDA and VASI. A total of 58 patients were enrolled in the study. After an initial clinical evaluation, VIDA and VASI scores were calculated, and blood samples were sent for analysis of levels of IL-6 and HsCRP. The following findings were noted-

- Females were predominant in the study.
- Age of the patients varied from 4 years to 65 years, with a majority patients belonging to the 2nd decade.
- Female patients showed a higher disease activity and extent as compared to males.
- While comparing disease activity across all age groups, paediatric age group of <10 years showed maximum number of cases having a VIDA score of 4+.
- Vitiligo vulgaris was the commonest encountered type followed by segmental, acral, focal and acrofacial.
- There was a statistically insignificant negative correlation between VIDA and serum IL-6 levels. When the two subgroups of cases, that is the one which was on immunosuppressive therapy while being investigated and the one which was not, were compared, the mean values of IL-6 and HsCRP were higher in the group not on immunosuppressants as opposed to the other group.
- There was absolutely no correlation found between VIDA and HsCRP levels.
- There was a mild positive correlation found between VASI and both the markers, that is serum IL-6 and HsCRP, though it was statistically insignificant.

Higher mean values of IL-6 and HsCRP in patients not on immunosuppressants as compared to those on them, points towards the role of immunosuppressive therapy in decreasing these markers. Immunosuppressive therapy is a preferred form of treatment in a highly active disease (higher VIDA score) which can explain the finding of lower IL-6 values in patients with a higher disease activity.

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ETHICAL CLEARANCE CERTIFICATE

IEC/NO-09/2021
Date-22/01/2021

B.L.D.E. (DEEMED TO BE UNIVERSITY)
(Declared vide notification No. F.9-37/2007-U.3 (A) Dated. 29-2-2008 of the MHRD, Government of India under Section 3 of the UGC Act, 1956)
The Constituent College
SHRI. B. M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Institutional ethical committee of this college met on 11-01-2021 at 11-00 am to scrutinize the synopsis of Postgraduate students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected and revised version synopsis of the Thesis has been accorded Ethical Clearance

Title: A hospital based cross-sectional study to estimate serum levels of interleukin-6 and high sensitivity c-reactive protein and their correlation with the vitiligo disease activity and exten.

Name of PG student: Dr Bhargavi Uttmani, Department of Dermatology

Name of Guide/Co-investigator: Dr Keshavmurthy Adya, Associate Professor of Dermatology

DR .S.V.PATIL
CHAIRMAN, IEC
Institutional Ethical Committee
B L D E (Deemed to be University)
Shri B.M. Patil Medical College,
VIJAYAPUR-586103 (Karnataka)

Following documents were placed before Ethical Committee for Scrutinization:

1. Copy of Synopsis / Research project
2. Copy of informed consent form
3. Any other relevant documents.

5

CONSENT FORM

B.L.D.E. (Deemed to be University) SHRI B.M PATIL

MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTRE,

VIJAYAPURA-586 103

INFORMED CONSENT FOR PARTICIPATION IN DISSERTATION/RESEARCH

TITLE OF THE PROJECT: A HOSPITAL BASED CROSS-SECTIONAL STUDY TO ESTIMATE SERUM LEVELS OF INTERLEUKIN-6 AND HIGH SENSITIVITY C-REACTIVE PROTEIN AND THEIR CORRELATION WITH THE VITILIGO DISEASE ACTIVITY AND EXTENT

PG GUIDE: DR. KESHAVMURTHY ADYA

PG STUDENT: DR. BHARGAVI UTTMANI

PURPOSE OF RESEARCH:

I have been informed that this project will determine the serum levels of IL-6 and HsCRP in patients with vitiligo and correlate its values with the disease activity and extent.

BENEFITS:

I understand that my participation in this study will help the investigator to know the serum levels of IL-6 and HsCRP in patients with vitiligo and its correlation with the disease activity and extent.

PROCEDURE:

I understand that relevant history will be taken and I will undergo a detailed clinical examination after which treatment will be given.

RISK AND DISCOMFORTS:

I understand there is no risk involved and I will experience no discomfort during the clinical examination.

CONFIDENTIALITY:

I understand that medical information produced by this study will become a part of my hospital records and will be subjected to the confidentiality and privacy regulation of the said hospital. Information of a sensitive personal nature will not be a part of the medical records but will be stored in the investigator's research file.

If the data are used for publication in the medical literature or teaching purposes, no names will be used and other identifiers such as photographs and audio or videotapes will be used only with my special written permission. I understand I may see the photographs, videotapes and hear the audiotapes before giving this permission.

REQUEST FOR MORE INFORMATION:

I understand that I may ask more questions about the study at any time concerned. Dr. Bhargavi Uttmani is available to answer my questions or concerns. I understand that I will be informed of any significant new findings discovered during the course of this study, which may influence my continued participation.

REFUSAL OR WITHDRAWAL OF PARTICIPATION:

I understand that my participation is voluntary and I may refuse to participate or may withdraw consent and discontinue participation in this study at any time without prejudice. I also understand that Dr. Bhargavi Uttmani may terminate my participation in this study at any time after she has explained the reasons for doing so and has helped arrange for my continued care by my own physician if this is appropriate.

INJURY STATEMENT:

I understand that in the unlikely event of injury to me resulting directly from my participation in this study and if such injury were reported promptly, then medical treatment will be available to me, but no further compensation will be provided. I understand that by my agreement for my participation in this study, I am not waiving any of my legal rights.

I have explained to (patient's/relevant guardian's name) the purpose of the research, the procedures required, and the possible risks and benefits to the best of my ability in the patient's own language.

_____ _____

Investigator/P. G. Guide Date

I confirm that …….(Name of the PG guide/chief researcher) has explained to me the research, the study procedures that I undergo and the possible risks and discomforts as well as benefits that I may experience. I have read and understood this consent form. Therefore, I agree to give my consent for my participation as a subject in this research project.

_____	_____
Participant/Guardian	Date
_____	_____
Witness to signature	Date

PROFORMA

B.L.D.E. (Deemed to be University)

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

DEPARTMENT OF DERMATOLOGY,VENEREOLOGY AND LEPROSY

SCHEME OF CASE TAKING

A HOSPITAL BASED CROSS-SECTIONAL STUDY TO ESTIMATE SERUM LEVELS OF INTERLEUKIN-6 AND HIGH SENSITIVITY C-REACTIVE PROTEIN AND THEIR CORRELATION WITH THE VITILIGO DISEASE ACTIVITY AND EXTENT

GENERAL INFORMATION

Sl. No.:

Date:

I.P/O.P No:

Hospital:

Name:

Age:

Sex:

Address:

Occupation:

PRESENTING COMPLAINTS AND DURATION:

HISTORY OF PRESENTING ILLNESS

- Skin lesions: 1. Onset – Sudden/ Gradual

  2. Duration

  3. Associated complaints:
  Redness/Burning/Scaling/Erosions/Exudation/Lichenification
- Site: Unilateral/Bilateral
- Distribution of lesions: Unilateral/Bilateral
- Associated features: Present/Absent
- Constitutional symptoms: Present/Absent
- Other symptoms
- History of similar complaints in the family: Present/Absent
- History of associated diseases:
- Treatment history: Topical/Systemic

PAST HISTORY

History of similar complaints in the past: Present/Absent

PERSONAL HISTORY

- Diet: Veg/Non-veg
- Appetite: Normal/Poor
- Bowel/Bladder: Regular/Disturbed
- Sleep: Normal/Disturbed
- Habits:
  Smoker/Alcoholic/Drug addiction/No habits

FAMILY HISTORY

- Married/Unmarried
- If married, no. of children

GENERAL PHYSICAL EXAMINATION

- Built: Well/Moderate/Poor
- Nourishment: Well/Moderate/Poor
- Others: Pallor/Icterus/Cyanosis/Clubbing/Oedema/Lymphadenopathy – Present/Absent
- Vital signs: Pulse rate R.R

  B.P Temperature

CUTANEOUS EXAMINATION

- Hypopigmented/Depigmented lesions/Both
- Site:
- Borders: Regular/Irregular
  - Side: Unilateral/Bilateral
- Body surface area (According to the rule of palm):
- Examination of mucous membranes:

  Oral

  Genital
- Others:

  Hair

  Nails

Genitals

- Other cutaneous lesions elsewhere:

VITILIGO DISEASE ACTIVITY SCORE

Disease Activity	Score	
The activity of 6 weeks or less duration	4+	
The activity of 6 weeks to 3 months	3+	
The activity of 3-6 months	2+	
The activity of 6-12 months	1+	
Stable for 1 year or more	0	
Stable with spontaneous repigmentation for 1 year or more	-1	

VITILIGO AREA SEVERITY SCORE

Degree of pigmentation	Percentage	
Complete depigmentation, no pigment +	100	
Specks of pigmentation +	90	
Depigmented area exceeds the pigmented area	75	
Pigmented and depigmented areas are equal	50	
Pigmented area exceeds depigmented areas	25	
Only specks of depigmentation +	10	

SYSTEMIC EXAMINATION

CVS:

CNS:

RS:

Per abdomen:

PROVISIONAL DIAGNOSIS:

INVESTIGATIONS:

ROUTINE	SPECIFIC
Complete haemogram	Serum IL-6 levels
	Serum hs-CRP levels

FINAL DIAGNOSIS:

KEY TO MASTERCHART

y – years

M – Male

F – Female

m – months

pg – picogram

ml – millilitre

mg – milligram

l – litre

ON – On immunosuppressive therapy

NOT – Not on immunosuppressive therapy

MASTERCHART

Serial number	Age (y)	Sex	Duration of disease (m)	VIDA	VASI (%)	IL-6 (pg/ml)	HsCRP (mg/l)	Type	Immunosuppressants
1	17	F	60	2	25	2.3	0.1	Vulgaris	ON
2	50	M	42	4	25	5.7	5.39	Vulgaris	ON
3	4	F	24	0	25	10.55	0.23	Vulgaris	NOT
4	42	F	24	4	25	4.2	2.3	Acral	ON
5	7	M	12	4	25	3.8	0.33	Vulgaris	ON
6	9	F	1	4	10	3.3	0.6	Segmental	NOT
7	25	M	96	4	25	1.3	1.28	Vulgaris	ON
8	15	F	1	4	10	1.3	0.34	Vulgaris	NOT
9	30	M	84	1	25	1.9	1.53	Vulgaris	ON
10	20	M	18	0	10	1.4	0.34	Acral	ON
11	25	F	180	4	10	2.2	1.03	Vulgaris	NOT
12	55	M	18	3	25	30.9	5.68	Vulgaris	ON
13	53	M	1	4	10	20.7	5.9	Acral	NOT
14	16	F	24	2	10	13.8	0.34	Segmental	NOT
15	45	F	36	2	10	19.3	3.86	Acrofacial	NOT
16	13	F	60	0	10	17.2	0.34	Segmental	NOT
17	30	F	108	2	25	23.6	1.39	Vulgaris	NOT
18	51	M	240	3	25	12.5	3.48	Vulgaris	ON
19	55	F	72	0	25	20.9	2.13	Vulgaris	ON
20	30	M	108	0	10	37.2	0.85	Vulgaris	NOT
21	11	F	24	0	10	24.8	0.34	Segmental	NOT
22	45	F	96	3	25	8.5	5.3	Vulgaris	NOT
23	60	F	72	2	10	19.1	2.96	Vulgaris	NOT
24	52	F	24	2	10	37.9	39.9	Vulgaris	NOT
25	50	F	24	4	25	7.7	1.84	Vulgaris	NOT

26	54	F	96	4	25	25.64	2	Vulgaris	NOT
27	25	F	60	0	10	11.8	1.09	Vulgaris	NOT
28	11	M	6	3	10	7.3	1.69	Vulgaris	ON
29	25	M	8	4	10	3.9	0.95	Vulgaris	NOT
30	33	F	5	2	10	8.5	10.47	Vulgaris	ON
31	10	M	1	4	10	2.9	0.33	Segmental	NOT
32	39	M	7	4	10	2.7	0.66	Vulgaris	ON
33	52	F	24	4	25	3	0.42	Vulgaris	NOT
34	26	M	6	3	10	7.1	6.35	Acral	NOT
35	52	F	0.5	4	10	4.7	1.39	Focal	NOT
36	25	F	36	3	10	4.6	1.16	Vulgaris	NOT
37	21	F	48	1	10	2	1.1	Vulgaris	NOT
38	25	M	5	2	10	3.5	2.34	Vulgaris	NOT
39	21	F	5	4	10	2.2	0.19	Vulgaris	NOT
40	60	M	12	2	10	3.3	3	Vulgaris	NOT
41	9	M	18	3	10	1.8	0.1	Focal	NOT
42	40	F	4	3	25	5.2	2.1	Vulgaris	ON
43	5	F	1	4	25	10	1.78	Segmental	NOT
44	32	F	12	2	10	4.5	0.6	Vulgaris	ON
45	45	F	24	4	10	8.7	2.43	Acral	NOT
46	30	F	6	4	50	5.2	2.1	Vulgaris	ON
47	65	M	24	0	10	2.5	1.09	Vulgaris	NOT
48	28	M	24	0	10	4.9	2.83	Vulgaris	ON
49	9	F	6	4	10	3	0.26	Vulgaris	ON
50	5	F	2	4	10	3	0.33	Focal	ON
51	32	M	6	2	10	2.8	3.86	Vulgaris	NOT
52	23	M	1.5	4	10	2.1	2.06	Vulgaris	ON
53	59	F	12	4	25	11.5	23.58	Vulgaris	NOT
54	48	M	24	4	10	4.3	1.77	Vulgaris	ON
55	12	M	2	4	10	2.5	0.1	Segmental	ON

56	6	M	36	3	10	1.4	0.11	Vulgaris	NOT
57	22	F	6	4	10	3.7	3.22	Vulgaris	ON
58	6	F	10	4	10	36.8	9.6	Vulgaris	NOT