"ESTIMATION OF SERUM S100B LEVELS IN VITILIGO TO DETERMINE WHETHER INCREASED LEVELS ARE A MARKER OF DISEASE ACTIVITY; A PROSPECTIVE CASE-CONTROL STUDY. "

By

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Dissertation submitted to

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Under the guidance of

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I hereby declare that this dissertation entitled "**ESTIMATION OF SERUM S100B LEVELS IN VITILIGO TO DETERMINE WHETHER INCREASED LEVELS ARE A MARKER OF DISEASE ACTIVITY; A PROSPECTIVE CASE-CONTROL STUDY.**" is a bonafide and genuine research work carried out by me under the guidance of Dr. ARUN C INAMADAR , Professor, Department of Dermatology Venereology and Leprosy, at BLDE (Deemed to be University) Shri B.M. Patil Medical College and Research Centre, Vijayapura.

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LIST OF ABBREVIATIONS

S100B - A member of the S100 protein family

VIDA - Vitiligo Disease Activity

VASI - Vitiligo Area Scoring Index

PASI - Psoriasis Area Severity Index

NCC - Neural Crest Cells

TYRP1 - Tyrosinase-Related Protein 1

TYRP2/DCT - Tyrosinase-Related Protein 2/Dopachrome Tautomerase

TYR - Tyrosinase

MITF - Microphthalmia Transcription Factor

bFGF - Basic Fibroblast Growth Factor

NRG1 - Neuregulin 1

SCF - Stem Cell Factor

TGF-β - Transforming Growth Factor Beta

α-MSH - Alpha-Melanocyte Stimulating Hormone

IL-1α - Interleukin-1 Alpha

IL-2 - Interleukin-2

IL-3 - Interleukin-3

IL-6 - Interleukin-6

IL-10 - Interleukin-10

TNF-α - Tumor Necrosis Factor Alpha

IL-8 - Interleukin-8

NO - Nitric Oxide

PAR2 - Proteinase-Activated Receptor-2

KGF - Keratinocyte Growth Factor

IGF-1 - Insulin Growth Factor-1

TYR - Tyrosinase

L-DOPA - L-3,4-Dihydroxyphenylalanine

DOPAquinone - Dihydroxyphenylalanine Quinone

DHI - 5,6-Dihydroxyindole

TYRP2/DCT - Tyrosinase-Related Protein 2/Dopachrome Tautomerase

DHICA - Dihydroxyindole-2-Carboxylic Acid

Bcl-2 - B-Cell Lymphoma-2

RER - Rough Endoplasmic Reticulum

H2O2 - Hydrogen Peroxide

FOXP1 - Forkhead Box Protein-1

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-O-Methyltransferase

CTLA-4 - Cytotoxic T Lymphocyte Antigen-4

ESR - Estrogen Receptor

MBL2 - Mannose-Binding Lectin 2

TYR - Tyrosinase

AIS - Autoimmune Susceptibility

SLEV1 - Systemic Lupus Erythematosus Vitiligo Related Gene 1

miRNAs - MicroRNAs

TLRs - Toll-Like Receptors

miR-125b - MicroRNA-125b

miR-155 - MicroRNA-155

miR-99b - MicroRNA-99b

miR-199a-3p - MicroRNA-199a-3p

miR-145 - MicroRNA-145

SNPs - Single Nucleotide Polymorphisms

CD8+ - Cluster of Differentiation 8 Positive

Melan-A/MART-1 - Melanoma Antigen Recognized by T-cells 1

gp100 - Glycoprotein 100

IFN γ - Interferon Gamma

CXCL10 - C-X-C Motif Chemokine Ligand 10

IL-17 - Interleukin 17

Th17 - T Helper 17

Tregs - Regulatory T-Cells

Ca2+ - Calcium ion

RAGE - Receptor for Advanced Glycation End-products

VIT - Vitiligo

TRP-2 - Tyrosinase-Related Protein 2

SOX10 - SRY-Box 10

SOX9 - SRY-Box 9

APS1 - Autoimmune Polyendocrine Syndrome Type 1

Hsp70iQ435A - Inducible Heat Shock Protein 70, variant Q435A

ROS - Reactive Oxygen Species

ATP - Adenosine Triphosphate

H2O2 - Hydrogen Peroxide

DOPA - Dihydroxyphenylalanine

NSV - Non-Segmental Vitiligo

LXR - Liver X Receptor

LXR-α - Liver X Receptor Alpha

SV - Segmental Vitiligo

VGICC - Vitiligo Global Issues Consensus Conference

UVA - Ultraviolet A

NB-UVB - Narrowband Ultraviolet B

NSV - Non-Segmental Vitiligo

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

A member of the family of S100 proteins, S100B protein is a damage-associated molecular pattern protein that is expressed in melanocytes and has been proposed as a potential marker of melanocyte cytotoxicity.

Aim:

To measure serum levels of S100B in cases of vitiligo and to correlate those levels with the activity and extent of the disease and to those of controls.

Materials and methods:

It is a hospital-based prospective case control study. Patients with characteristic clinical features of vitiligo irrespective of age, gender and on-going or previous treatment were included, whereas patients with any other co-existing chronic inflammatory disorders, any active cutaneous or systemic infections, co-morbidities, history of smoking, history of recent head injury, malignant melanoma, 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 S100B levels were measured

Results:

A moderate, statistically significant correlation was found between VIDA and S100B levels (p=0.001), There was an absolute absence of correlation between VASI and S100B levels (p=0.0708)

There was a statistically significant correlation between active disease (disease activity in past 6 months) and S100B levels (p=0.001) when compared to stable disease. Patients who were subjected to immunosuppressive therapy had lower serum S100B levels relative to patients who received no therapy (p=0.0027).

S100B values in healthy control subjects were not significantly different compared with stable vitiligo patients (p=0.2752), While patients with active disease showed significantly high mean values of S100B compared to controls (p=0.0052).

The patients not on immunosuppressive therapy showed higher mean S100B levels when compared with controls, (p=0.0046), while those on immunosuppressive therapy did not show higher mean S100B levels when compared to controls (p=0.1192).

Conclusion:

The moderate correlation between VIDA and S100B highlights that S100B levels are elevated during the active depigmenting phase and the findings of increased S100B levels amongst patients not on immunosuppressive therapy highlights that not just disease activity but also treatment status of patients should be considered while evaluating S100B levels in such conditions.

Keywords: Vitiligo, VIDA, VASI, S100B, Activity, Extent

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INTRODUCTION

Vitiligo is a common depigmenting skin disorder, with a reported incidence of 0.5-2%. The condition is distinguished by selective melanocyte loss, which results to the formation of characteristic nonscaly, chalky-white macules. This illness is frequently dismissed as a non-essential worry, despite the fact that its consequences can be extremely distressing and cause severe morbidity.[1]

According to studies from India, vitiligo affects between 0.25% and 4% of dermatological outpatients.[2] The most afflicted populations are those of varying ages when vitiligo develops, and some studies report two peaks. Vitiligo often appears by the age of 30, and numerous studies show that almost half of all patients develop it by the age of 20.[1,3] The presence of a family history should be examined in pediatric patients.[3]

Vitiligo is a complicated condition that typically involves the death of functional melanocytes. Several theories have been proposed to explain the loss of melanocytes in vitiligo. These include genetics, autoimmune responses, oxidative stress, inflammatory mediator production, and melanocyte detachment processes. Both innate and adaptive immune mechanisms appear to be at work.[1]

S100B belongs to the S100 protein family. It is a damage-associated molecular pattern protein found in melanocytes and has been considered as a marker of cytotoxicity.[4] Studies show low amount of S100B to be protective for the

melanocyte by inhibiting p53, and subsequently inhibiting apoptosis process, through activation of the PI3K/ AKT pathway.[7]

Melanocytic cell death during the active phases of vitiligo has been theorized to cause higher S100B levels. Previous research on melanoma and brain injury has demonstrated that the pathogenic events that lead to cell death of S100B-positive cells are associated with an increase in S100B serum levels. As a result, it is postulated that melanocytic cell loss during the active periods of vitiligo may result in elevated S100B levels. [4]

The status of disease activity is an important reference for selecting suitable therapeutic options, determining prognosis, and monitoring the patient's response to treatment. However, there is no defined technique for assessing disease activity in vitiligo during a single appointment. Skin abnormalities such as trichrome/hypopigmented lesions, confetti-like lesions, and Koebner's phenomena have been identified as potential indications of disease activity.[10]

A commonly used method that uses 'the latest time point of illness progression' (VIDA) is an outcome measure provided by the patient that incorporates retrospective information (recall). The VIDA Scale is a regularly used instrument for determining when activity (worsening) of the lesions was last detected.[10] It is a six-point measure that assesses the stability of vitiligo throughout time. Patients themselves report illness activity. It helps to track the success of interventions in preventing and reversing the spread of the disease. An active disease is defined as the formation of new lesions or the spread of existing ones.[11]

VASI, a quantitative instrument, is a theoretical derivative of the psoriasis area severity index (PASI) score, which is used to evaluate psoriasis. VASI is calculated by including all body areas (range: 0-100). One hand unit refers to the palm and ventral portion of the fingers, and it represents approximately 1% of the entire surface area of the body. It is used to calculate the reference base percentage of total body area implicated in vitiligo. The body is divided into five distinct parts: Trunk, upper limbs, hands, lower limbs, and feet.[11,12]

AIM OF THE STUDY:

To measure the levels of S100B protein in serum of patients with vitiligo and correlate those levels with the disease activity and extent and compare the levels of patients with those of healthy controls.

OBJECTIVE OF THE STUDY:

To measure the serum levels of S100B protein in patients with vitiligo

To correlate those levels with the disease activity using VIDA

To correlate those levels with the disease extent using VIDA

To compare those levels with that of healthy controls.

REVIEW OF LITERATURE

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

Melanocytes:

Melanocytes originate from neural crest cells, with the exception of retinal pigment cells, which emerge from the forebrain's optic cup. This cell type develops from the embryonic germinal layer known as the ectoderm. The mid section of the embryonic disc develops in the neuroectoderm, which appears as a neural plate in a 4-week-old human embryo. This plate then folds, forming the neural tube, which will eventually become the brain and spinal cord. This process is known as neuralation, in which a group of cells break from the borders of the neural plate and undergo a phenotypic transition from epithelial to mesenchyme before moving out of the neuroepithelium. The neural crest cells (NCC) are a type of neuroectodermal cell that migrates to various parts of the developing organism. Melanoblasts form when neural crest cells commit to the melanogenic lineage, which allows them to migrate to multiple locations and transform into melanogonia, and eventually adult melanocytes. The signals that control melanoblast migration to their final destinations have yet to be characterized.[14,15]

Melanocytes are found in small numbers in the skin, ears, hair matrix, eye, central nervous system, and mucous membranes. Along with the epidermal basal layer they may also be found in the dermis, as well.[14] A few examples of melanocyte-specific proteins that can be used to detect these cells at the molecular level are Tyrosinase-related protein 1 and 2 (TYRP1, TYRP2/DCT), tyrosinase (TYR), melanosomal matrix proteins, and microphthalmia transcription factor (MITF).[15]

Epidermal melanin unit in the skin:

In 1963, Fitzgerald and Breathnach introduced the concept of a melanocyte interacting with keratinocytes, dubbed "the epidermal melanin unit." They proposed a functional unit made up of one melanocyte and 36 keratinocytes. However, this ratio varies depending on the stage of development, from prenatal life to birth to adulthood.13 Skin contains around 1200 melanocytes per square millimetre. These melanocytes' cytoplasm contains specific membrane-bound organelles known as melanosomes, which are responsible for melanin formation.[15]

The basic function of the melanocyte is to provide pigmentation to the skin, which is accomplished through dendrites that aid in the transfer of melanosomes to the keratinocyte[13,14]. It also plays a part in photoprotection. Melanin granules form a cap around the nucleus of keratinocytes.[15] Melanosome features such as number, size, and type determine skin color. The quantity of melanin and the size of melanosomes are determined by the genotype of the melanocyte, not the keratinocyte.[13] The packed melanosomes are encased in the melanocyte plasma membrane and subsequently

released from many melanocyte dendrites into the extracellular space, where they are phagocytosed by keratinocytes and dispersed throughout the perinuclear region.[16]

Melanocytes, keratinocytes, and fibroblasts in the dermis interact via cell-to-cell contact and released factors. Keratinocytes govern melanocyte growth and activity through a paracrine growth factor system that includes basic fibroblast growth factors (bFGF)13 and cell adhesion molecules such as E- and P-cadherins. These cells, notably melanocytes and keratinocytes, also serve as a medium for numerous hormones that regulate melanocyte proliferation, melanogenesis, and dendrite development. Dermal fibroblasts release factors such as neuregulin 1 (NRG1) and stem cell factor (SCF), which play an important role in the maintenance of melanocyte biology.

These cytokines control melanocyte growth and proliferation, as well as their shape, motility, dendriticity, and adhesive properties. Melanocytes release a variety of signal molecules that target keratinocytes as well as cells of the cutaneous immune system. When triggered, they emit 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. Autocrine factors such as IL-1, IL-6, and TNF-α decrease melanogenesis, while eicosanoids and α-MSH increase melanin synthesis.[15]

Follicular melanin unit:

Melanocytes are located in the proximal bulb of the hair follicle. Bulbar melanocyte bodies are located near the apex of the dermal papillae. Melanocyte dendrites enter between keratinocytes in the cortex and medulla. The melanocyte-to-keratinocyte ratio is 1:5, which is denser than in the epidermis. Melanocytes in the follicle, matrix keratinocytes, and dermal papilla fibroblasts all interact structurally and functionally to cause follicular pigmentation. The trilateral complex is referred to as the follicular melanin unit or hair melanin unit. Melanogenesis activity by follicular melanocytes, migration of melanin granules in keratinocytes, and the creation of pigmented hair shafts all appear to be part of the hair pigmentation process.The migration of melanin granules to the proliferating keratinocytes is thought to be identical to the proteinase-activated receptor-2 (PAR2) receptor-mediated phagocytosis of epidermal melanosomes. The difference, however, resides in the deterioration and quality of melanosomes. The melanocytes here are larger and more dendritic than those in the epidermis. Furthermore, while epidermal keratinocytes destroy supplied melanin almost completely, hair cortical keratinocytes only partially digest pigment granules. Products released by adjacent cells, such as keratinocytes, endothelial cells, and fibroblasts, impact melanin formation in hair. The biochemical pathway of pigment generation and regulation, as well as melanosome biogenesis, parallels that seen in the epidermis; however, it is important to note that melanocytes of the hair follicle are more vulnerable to the effects of aging than epidermal melanocytes, resulting in greying of the hair.

Keratinocyte growth factor (KGF), insulin growth factor (IGF-1), SCF, and noggin, all of which are produced by dermal papilla fibroblasts, appear to play a specialized function in regulating the activity and proliferation of keratinocytes and melanocytes in the hair matrix during hair growth. Epidermal melanocytes survive longer, whereas hair melanocytes are exhausted after around 3-8 years, when the hair cycle stops. Melanogenesis occurs only during the growth phase (anagen stage); pigment development stops in the regressing phase (catagen stage) and is completely absent in the resting phase (telogen stage). Furthermore, it is worth noting that when catagen is taking place, fully differentiated melanocytes in the hair bulb die due to apoptosis, whereas melanoblasts in the hair bulge produce new melanocytes.[15]

Melanogenesis:

Melanogenesis refers to the biological pathway that produces melanin. Melanocytes produce melanosomes, which are specialized organelles in the cytoplasm. There are two types of melanin: pheomelanin and eumelanin. They differ in terms of both synthesis and color. Melanin has several beneficial properties for the body, including free radical scavenging, UV light absorption and scattering, ion storage, and oxidation-reduction reactions. The type of melanin produced is determined by the availability of substrates and the role of melanogenic enzymes.[15]

Tyrosinase (TYR) catalyzes the hydroxylation of tyrosine to L-3,4-dihydroxyphenylalanine (DOPA), which is then quickly oxidized to DOPAquinone. DOPAquinone interacts with cysteine to generate 3- or 5-cysteinylDOPAs, which subsequently oxidize and polymerize, resulting in the creation of yellow-red soluble

melanin, known as pheomelanin. In the absence of thiols (cysteine, glutathione, or thioredoxin), brown-black melanin, also known as eumelanin, is produced. DOPAquinone cyclizes spontaneously into DOPAchrome. DOPAchrome spontaneously loses carboxylic acid, resulting in the formation of 5,6-dihydroxyindole (DHI), which rapidly oxidizes and polymerizes to generate dark brown-black insoluble melanin known as DHI-melanin. Nonetheless, in the presence of (TYRP2/DCT), DOPAchrome generates DHI-2-carboxylic acid (DHICA). Tyrosinase and TYRP1 operate as catalysts for subsequent processes, resulting in DHICA-melanin, a lighter brown color.[15] (Figure 1)

The human skin contains all forms of melanin, and the ratio determines the skin pigmentation. The amount of eumelanin in the skin varies according to race and ethnicity. The skin's hue is determined by the ratio of eumelanin to total melanin. Pheomelanin levels are nearly same in dark and light skin, indicating that pheomelanin does not contribute to skin pigmentation. Hair color is determined by the ratio of eumelanin to pheomelanin. When compared to pheomelanin, eumelanin has superior photoprotective properties, such as increased resistance to degradation and the ability to neutralize reactive oxygen species.[15]

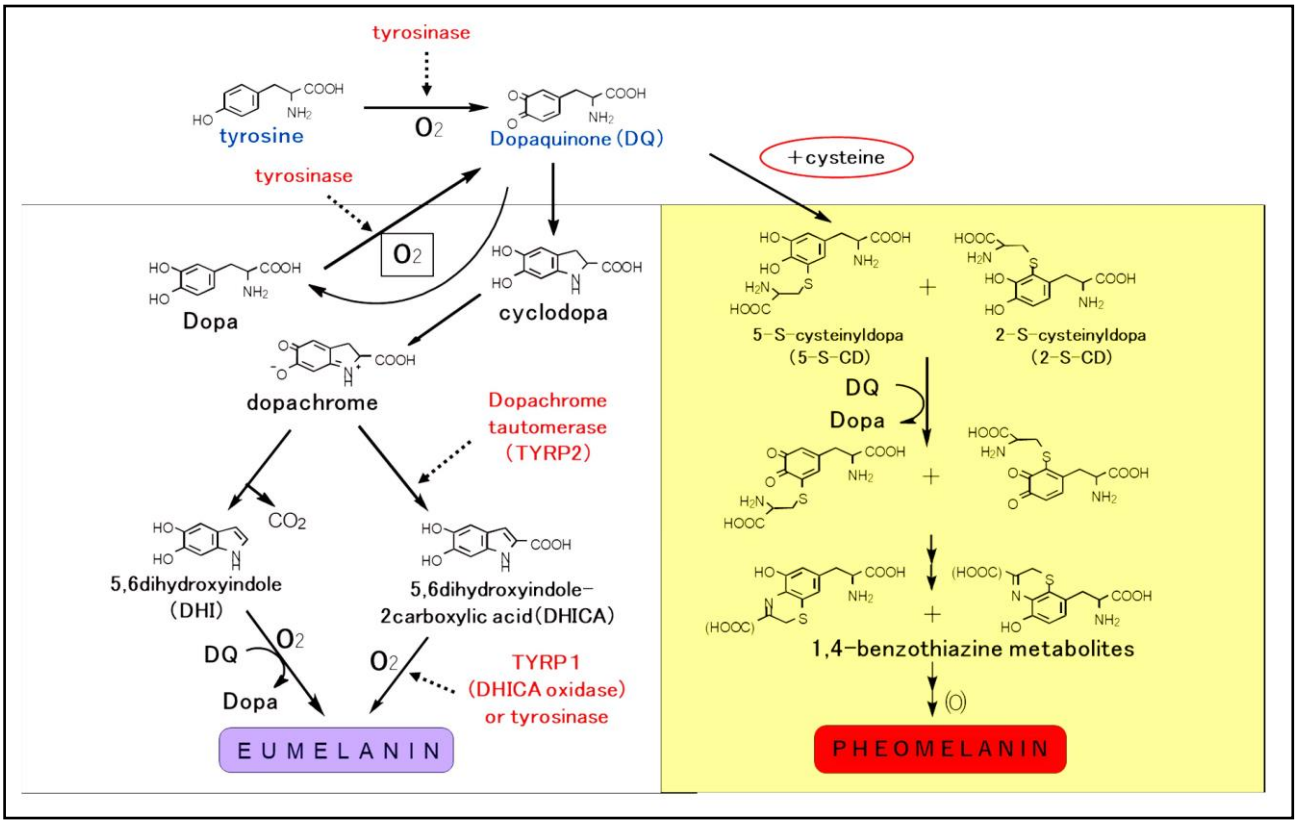
During melanogenesis, cytotoxic chemicals like as quinones and hydrogen peroxide are generated as intermediary metabolites. To protect itself, the melanocyte increases the quantity of the antiapoptotic protein B-cell lymphoma-2 (Bcl-2) and separates the melanogenesis-related areas of its melanosomes. It is still unclear if the endoplasmic reticulum is the creator of melanosomes. TYR, coupled with TYRP1 and TYRP2, is

essential for their formation. Tyrosinase, the most significant of the three enzymes required for melanogenesis, is generated on rough endoplasmic reticulum (RER) ribosomes, transported to the Golgi complex, and glycosylated, which is required for its structural and functional integrity.[15]

Melanosome development has four stages:

- Premelanosomes (Stage I) are amorphous matrix contained in small spherical vesicles.
- Melanosomes in stage II have a well-organized fibrillar matrix primarily composed of glycoprotein100, tyrosinase, and no pigment synthesis.
- Stage III: Melanin development begins, with pigment deposition on protein fibrils.
- In stage IV, pigment accumulates throughout the melanosome.
- When cytoskeletal system elements transmit totally melanized melanosomes to adjacent keratinocytes, their tyrosinase activity is abolished.[15]

Figure 1: Steps in melanin synthesis



Taken from Hida T, Kamiya T, Kawakami A, Ogino J, Sohma H, Uhara H, et al. Elucidation of melanogenesis cascade for identifying pathophysiology and therapeutic approach of pigmentary disorders and melanoma. Int J Mol Sci. 2020;21:6129.[44]

VITILIGO

History of vitiligo:

The first known description of vitiligo comes from the Aushooryan period (2200 B.C.) in Kilăsa. The Ebers Papyrus also contains some information about this illness around 1550 B.C. It has been mentioned in numerous religious texts. Despite its historical knowledge, vitiligo has long been associated with leprosy, leading to a severe stigma for sufferers with the condition. This is a social issue that remains

today in various parts of the world, particularly in rural areas where there is a lack of awareness and medical resources.[2]

Epidemiology:

Global prevalence estimates range from 0.5-2%. It does not vary based on race or ethnicity; nonetheless, there appears to be varying prevalence in different parts of the world.[1-3,17]

Vitiligo prevalence in India is estimated to be between 0.25% and 4% of outpatients, with rates as high as 8.8% in Rajasthan and Gujarat. It affects both males and females with equal frequency, however most accounts indicate a feminine preference.[2,18,19] Some studies have found mixed age groups and two peaks, with onset generally occurring before the age of 30, with the majority indicating onset by the age of 20. The presence of a familial history is significant in cases of early-onset vitiligo seen in children.[3]

Pathophysiology:

A few major hypotheses regarding vitiligo etiology are:

(i) Autoimmune pathogenesis is a long-standing notion.

(ii) The neural theory suggests that nerve endings emit neurochemical substances that can damage melanocytes or reduce melanin synthesis.

(iii) According to biochemical theory, melanocyte damage is caused by hazardous intermediate products of the melanin manufacturing pathway, poor defense against free radicals, and excessive hydrogen peroxide (H2O2) accumulation. Other variables that contribute to depigmentation include genetics, melanocyte structural and functional abnormalities, and a lack of melanocyte growth factors.[1,2]

Genetics:

Given that vitiligo is a multifactorial disorder, many candidate genes 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), NACHThe gene TYR encodes tyrosinase, which acts as a catalyst in the rate-limiting phases of the melanin production pathway. It is the primary autoantigen causing widespread vitiligo.[1]

HLA haplotypes, including HLA-A2, −DR4, −DR7, and −DQB1*0303, are thought to have a role in vitiligo-related autoimmune/autoinflammatory disorders. PTPN22, NALP1, and XBP1 have been identified as the cause of vitiligo. Genome-wide linkage analysis revealed autoimmune susceptibility (AIS) loci related with vitiligo. AIS1 is found on chromosome 1p31.3-p32.2, AIS2 on chromosome 7, and AIS3 on chromosome 8. Whereas AIS1 and AIS2 links were associated with families with vitiligo and other autoimmune illnesses, AIS3 was more common in the non-autoimmune category. Another gene detected in patients with generalized vitiligo,

similar to other autoimmune disorders, is the systemic lupus erythematosus vitiligo related gene (SLEV1), which is located on chromosome 17. Recent study has focused on the role of microRNAs (miRNAs) and toll-like receptors (TLRs) in the pathogenesis of this disease.[2] A study discovered elevated amounts of miR-125b, miR-155, miR-99b, and miR199a-3p in the skin of vitiligo patients, as well as decreased levels of miR-145. Overexpression of miRNAs (miR-155) suppresses melanogenesis-related genes and interferon-regulated gene changes in both melanocytes and keratinocytes. Single nucleotide polymorphisms (SNPs) in TLR7 were discovered to be associated with this depigmenting condition in a study that verified the role of TLRs in disease pathogenesis while also opening the door to future targeted treatments.[2]

Cellular immunity: The CD8+ cytotoxic T-cells are the primary offenders in explaining the concept of cellular immunity.2 Melanocytes are specially targeted by them and destroyed. They exhibit cytotoxic reactivity against melanocytes and express the skin-homing marker cutaneous lymphocyte antigen. Their penetration into the skin has been observed histologically. The majority of cutaneous lymphocyte antigen-positive T cells in the lesional area expressed perforin and granzyme-B. It is a common cause of melanocyte loss and is closely related to disease activity. Melanocytes, whether normal or stressed, produce antigenic proteins such as Melan-A/MART-1, tyrosinase, gp100, and tyrosinase-related proteins 1 and 2.[1]

Interferon γ (IFN γ) has been identified as a "signature cytokine profile" associated with vitiligo. CXCL10 expression increases, synchronizing CD8+ T-cell invasion

into epidermal and follicular regions. IL-17 and Th17 cells are rapidly being recognized as contributing to the elaboration of this cytokine and the development of autoimmunity. Singh et al. discovered increased levels of IL-17 in the blood and tissue samples of vitiligo patients. Recent studies have also offered information on how regulatory T-cells (Tregs) contribute to disease pathogenesis. Their role is to counteract autoimmunity, and it is known that levels of these are low in vitiligo, particularly in lesional and perilesional skin. Besides being less in number, their function is also degraded. Active vitiligo lesions show lower levels of TGF-β and IL-10, which naturally promote Tregs.[2]

Role of S100B:

S100B, a member of the Ca2+-binding S100 protein family, works both inside and outside the cell. Astrocytes are the main intracellular source of S100B. Moreover, S100B is expressed in various neuroectodermal cells, including melanocytes and particular neuronal populations; immune cell subsets, such as dendritic cells and specific lymphocyte subpopulations; endothelial cells; and a few other cell types. Within cells, S100B controls cell proliferation, migration, apoptosis, differentiation, Ca2+ homeostasis, energy consumption, and enzyme activity. S100B is one of the secreted proteins in the S100 family, albeit the mechanism of secretion is unknown. S100B can be secreted passively from damaged tissues. S100B's extracellular actions are mediated by receptors such as RAGE, a multiligand immunoglobulin receptor. In contrast to intracellular S100B, which promotes cell proliferation and prevents

apoptosis , secreted or released S100B has both positive and harmful effects in the brain via paracrine and autocrine mechanisms.

The dual roles have proven dose-dependent; S100B is neurotrophic at low concentrations (nanomolar doses), but neurotoxic at high concentrations (micromolar doses). S100B has also been found to have cytotoxic or proliferative effects in other tissues or cells, with micromolar levels inducing apoptosis in myoblasts.[6]

S100B belongs to the S100 protein family, a multigene group of 21 low-molecular-weight proteinsMany tissues, including as melanocytes, astrocytes, oligodendrocytes, brain progenitor cells, Schwann cells, kidney epithelial cells, adipocytes, skeletal myofibers, Langerhans cells, and a subset of lymphocytes, have been shown to express S100B.[4] Furthermore, in pathological situations, S100B expression can be up-regulated in cell types that do not normally express S100B, as seen in cardiomyocytes.[4]

S100B levels are higher in metastatic melanoma patients and can predict survival after systemic treatment. S100B levels have been found to correlate with chemotherapy cytotoxicity in melanoma patients. However, the involvement of S100B in vitiligo has only been investigated in a handful of studies.

It is shown that Low amount of S100B protects the melanocyte by inhibiting p53, and subsequently the apoptosis process, through stimulation of the PI3K/ AKT pathway.[6,29].

Secretion of Large amounts of S100B within melanocyte, followed by the release of S100B in the extracellular space, stimulates pro-inflammatory cytokinesrelease from macrophages, such as IL-2, IL-6, TNF-a, and IL-1b,[7] this induces reactive oxygen species. The cytotoxic CD8 T-cell skin trafficking and melanocyte destruction is caused by the stimulation of cytokine production in keratinocytes caused by these Reactive oxygen species [7] (Figure 2)

When freeze-thaw cycles were utilised to create cryogenic stress in vitiligo and normal human melanocyte cultures, the result was a large-scale release of S100B from the melanocytes, which increased in proportion to the number of freeze cycles and began in the early stages of melanocyte mortality. This result revealed that along the lesional boundaries of active vitiligo, injured melanocytes can release S100B.[4]

Previous research on melanoma and brain injury has indicated that in the event of cell death of S100B-positive cells coincide with increased blood S100B levels. As a result, it is expected that melanocytic cell loss during the active periods of vitiligo would result in elevated S100B levels.[4]

Figure 2: Hypothetic effects of S100B on vitiligo melanocytes.

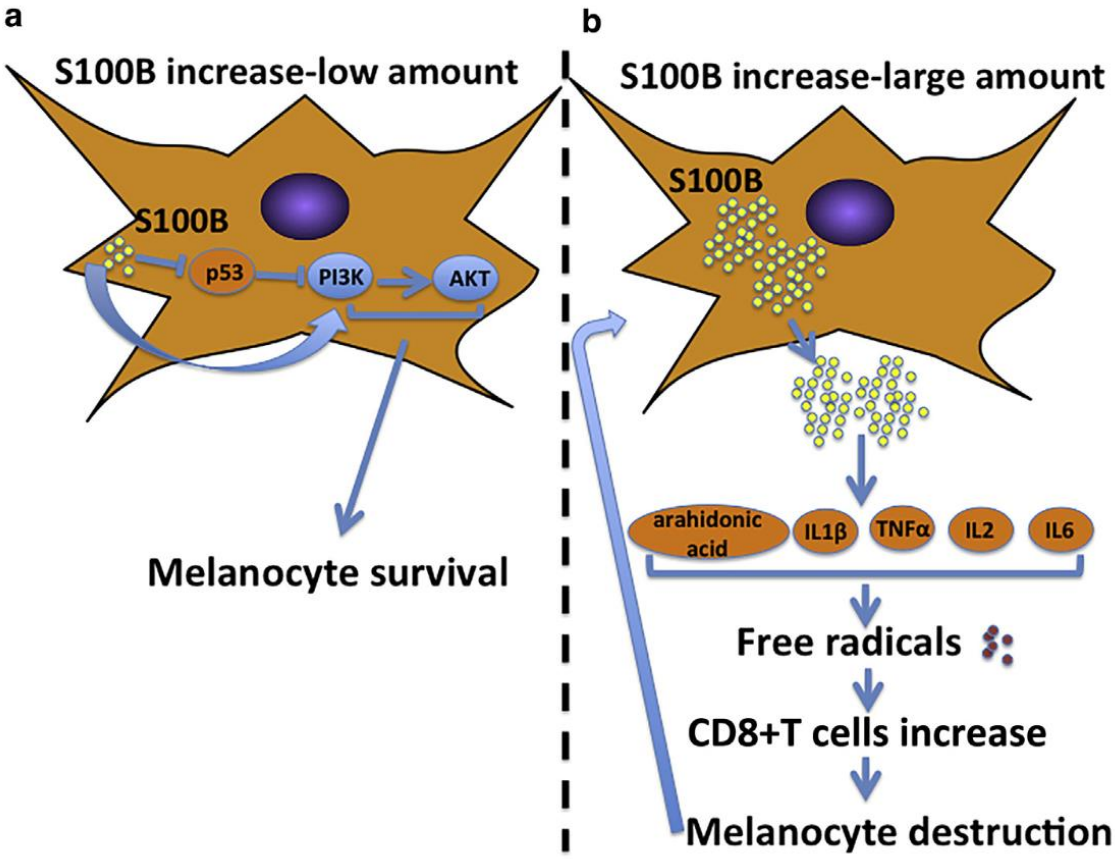


Image Taken from Birlea SA. S100B: Correlation with active Vitiligo Depigmentation. J Invest Dermatol 2017;137:1408–10.[7]

Humoral immunity: In cases with vitiligo, multiple antibody groups have been identified, including those directed against non-pigment cell antigens, cell surface pigment cell antigens and intracellular pigment cell antigens. A collection of antigens known as VIT 40/VIT 75/VIT 90, named for their corresponding weights, are found in 83% of vitiligo sufferers. VIT 75 and VIT 40 are shared by both pigment and non-pigment cells, whereas VIT 90 is limited to pigment cells. There are antibodies in opposition to these non-specific antigens.

Melanocytes are significantly more sensitive to immune-mediated injury than other

cells; even minor damage by non-specific antibodies has the potential to result in significant loss of melanocytes while sparing surrounding cells. In the absence of a concomitant disease Antibodies against tyrosinase, TYRP-1 and TRP-2, SOX10 and SOX9 have also been detected in instances of autoimmune polyendocrine syndrome type 1 (APS1) and vitiligo. Melanocytes' cytoplasm contains a high concentration of anti-melanocyte antibodies. In a separate study, patients with vitiligo were shown to possess antibodies against membrane and cytoplasmic antigens. Protein mass spectrometry verified these membrane antigens as Vimentin X and Lamin A/C. As a result, it is impossible to deny that antibodies against melanocytes play a role in the etiology of this condition.[2] Melanocytes produce exosomes in response to stress, which are then transmitted to the innate immune system. These exosomes express melanocyte-specific antigens, heat shock proteins, miRNAs, and a few other molecular patterns associated with injury. These exosomes are responsible for delivering target antigens to dendritic cells and promoting their development into antigen-presenting cells. Inducible heat shock protein 70 acts as a chaperone for host-specific peptides that inhibit cells from undergoing apoptosis standing out among all of these.

Inducible heat shock protein 70 is known to have an important role in the formation of vitiligo lesions in a mouse model by causing dendritic cells to transfer melanocyte-specific antigens to T lymphocytes in lymphoid organs. Recently, it was shown that an altered form of inducible heat shock protein 70, Hsp70iQ435A, can produce

repigmentation in vitiligo lesions in Sinclair swine, opening the door to a potential treatment for vitiligo sufferers.[1]

Oxidative stress:

Oxidative stress contributes significantly to the onset of vitiligo through melanocyte destruction. Oxidative stress causes redox homeostatic imbalance, which manifests as uncontrolled ROS production and insufficient breakdown. Melanocyte death occurs as a result of ROS activation from both endogenous and external sources. Overproduction of ROS occurs during the melanogenesis process, resulting in a prooxidative milieu that exposes melanocytes to oxidative stress. The contribution of endogenous and exogenous trigger elements results in the uncontrolled generation of ROS.

In terms of exogenous stressors, 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) all cause ROS overproduction to a certain extent, after which ROS generation occurs almost instantly. A succession of inner cues can activate ROS production: (a) genetically defined cellular metabolic processes such as melanogenesis demand more energy; (b) altered energy metabolism in mitochondria eventually leads to cell division, proliferation, and death. ROS are produced throughout the melanogenesis process as a result of the conversion of DOPA to DOPAquinone and then to dopachrome, exposing melanocytes to oxidative injury.

Furthermore, melanogenesis is a process that requires a large amount of energy and hence an increased amount of adenosine triphosphate (ATP). ATP generation, combined with ROS creation in mitochondria, results in a pro-oxidative milieu in the epidermis. Membrane lipid redox mismatch can degrade function and alter structure, affecting intracellular transmission mediated by membrane receptors, mitochondrial energy, and electron transport. All of these events contribute to the melanocyte becoming an epicenter for ROS accumulation. Melanocytes emit ROS in reaction to stress. As a result, the antioxidant system is significantly distorted, with a disparity of elevated oxidative stress indicators (ROS, superoxide dismutase, malondialdehyde) and a significant exhaustion of anti-oxidation mechanisms (catalase, superoxide dismutases thioredoxin reductase and thioredoxin, glutathione peroxidase, glutathione reductase) in the skin and blood. It is claimed that the difference in pro-oxidants and antioxidants is to blame for melanocytes' increased vulnerability to pro-oxidant stresses and, over time, the development of a presenescent state. ROS generation and accumulation can cause DNA damage, protein oxidation and fragmentation, and lipid peroxidation, all of which interfere with cell processes.[1,2]

The introduction of a new theory, haptenation theory, suggests a crucial involvement in vitiligo. According to this notion, high quantities of H2O2 boost the levels and activity of the tyrosinase enzyme. Tyrosinase is an enzyme that can bind to a variety of substrates, including noradrenaline (during moments of high emotional stress), estrogen, and triiodothyronine, to create orthoquinone metabolites. These metabolites convert tyrosinase into a freshly produced antigen, which the immune system identifies as an autoantigen. As a result, an autoimmune response is induced,

resulting in pigmentation loss due to particular damage to melanocytes caused by the autoantigen in the presence of a modified tyrosinase enzyme.[2]

Exogenous stimuli can also produce oxidative byproducts. Monobenzone is the most commonly used drug that causes depigmentation; it increases the discharge of antigen-containing exosomes linked to the melanosome in response to melanocyte ROS overproduction. Oxidative stress reduces melanocyte adhesiveness at the perilesional area, which may explain the Koebner phenomenon. In contrast to desmosomes, which require unique structures, melanocyte-keratinocyte interactions require simple adhesion molecules such as cadherins and integrins. Tenascin, an antiadhesion molecule, is elevated in vitiligo patients' non-lesional skin, but E-cadherin expression is downregulated. Persistent friction in the afflicted skin can activate epithelial cells, which convert mechanical forces into biochemical signals, resulting in cell stress and altered cadherin expression.[1,20]

Melanocytorrhagy:

According to this idea, non-segmental vitiligo causes apoptosis, separation, and transepidermal loss due to altered melanocyte reactions to friction. The occurrence of Koebner's phenomenon is appropriately explained because it suggests that when exposed to minimal friction and/or other stress, separation of weakly anchored melanocytes from the basement membrane, ascend through the epidermis, and are lost, resulting in vitiligo lesions at the sites of trauma. Kumar et al. discovered that

melanocytes adhered weakly to collagen type IV in cases of active vitiligo, although in stable cases this adhesion was quite strong.

An even more significant finding in this study was that in patients with an unstable disease, the perilesional melanocytic dendrites were tiny, retracted, and clubbed, making them unable to cohere melanocytes to the surrounding keratinocytes and the basement membrane, promoting transepidermal loss. It has recently been shown that tenascin, a matrix molecule outside the cell that hinders melanocyte attachment to fibronectin, is higher in lesional skin, which contributes to melanocyte loss. This contributes to the formation of localized gaps and faulty basement membrane growth, which weakens melanocyte adhesion to the basal layer and eventually leads to chronic melanocyte loss known as melanocytorrhagy.

Damaged melanocytes can initiate an immunological response during transepidermal migration, perpetuating vitiligo. Kumar et al. found that melanocytorrhagia in NSV patients is mostly mediated by alterations in the nuclear receptor protein "liver X receptor alpha (LXR)". Additionally, they identified increased expression of LXR-α, an apoptosis promoter, in the skin around NSV lesions. The study found that 22(R)-hydroxycholesterol, an LXR-α agonist, significantly inhibited melanocyte adhesion and proliferation. Researchers discovered that higher LXR-α expression in melanocytes around lesions led to decreased attachment and proliferation, as well as increased cell mortality.[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:

Segmental vitiligo (SV) is a specific distribution of lesions that is segmental in nature. The name "vitiligo" is the suggested nomenclature for all non-segmental subtypes of vitiligo, while "vitiligo"subtype, segmental subtype, and mucosal subtype, while the generalized category is further divided into acrofacial subtype, vulgaris subtype, and universal subtypes. If features from different types are present in combination, it can be referred to as "mixed type." On the basis of whether the lesions of vitiligo are crossing the midline, vitiligo can be divided into segmental type, non-segmental type, and mixed type. A universally accepted classification was created and updated in 2012 at the Vitiligo Global Issues Conference (VGICC), with

the most significant consensus in this classification being the distinction between other types of vitiligo and segmental vitiligo.

Segmental vitiligo (SV) is a specific distribution of lesions that is segmental in nature. The name "vitiligo" is the suggested nomenclature for all non-segmental subtypes of vitiligo, while "vitiligo" or "non-segmental vitiligo" may be used as a temporary designation. It was agreed not to use the name "vulgaris," which means "common," because it had a bad connotation. The mixed subtype, which denotes the coexistence of segmental vitiligo and vitiligo, is included in the category of vitiligo/non-segmental vitiligo. Segmental vitiligo typically occurs prior to vitiligo, whether it is non-segmental or not. In the end, the category of unknown or undefinable sort includes the long-standing variety of focal and mucosal vitiligo. A more accurate classification may be possible after one to two years of follow-up, at which point the focal type of vitiligo is thought to remain unclassified. Clinically, segmental, generalized, or, in rare instances, universal kinds of vitiligo can develop from focal vitiligo.[22]

Non-segmental/ Segmental vitiligo clinical features:

It is distinguished by well-defined circular to oval hypopigmented to depigmented macules or patches that are not connected to bilateral body-wide symptoms. About 85–90% of instances of vitiligo are of the non-segmental kind, which is the most prevalent variety. NSV has been shown to occur 50–90% of the time. Over the face is the most frequent site of beginning (39%), followed by the anterior trunk region (23.6%), the neck region (10.4%), and the posterior neck region (9.1%).[22]

Acrofacial Vitiligo: It is characterized by depigmenting lesions around the mouth and limbs in a circumferential pattern, affecting the hands more than the feet (Figure 1). The body areas nearest to the initial locations usually exhibit the fastest rates of development. However, when the hands are the site of beginning, advancement to the face is most frequently observed. Clinically, this may be explained by the finding that acrofacial lesions accounted for one-third of all individuals with lesions originating in the hands. Moreover, it is stated that the mucosal type of vitiligo may actually be a subtype of this kind due to the correlation between mucosal lesions and acrofacial lesions.[22]

Mucosal vitiligo: The vaginal area and mouth's mucosal membranes are affected by lesions. It is easy to classify mucosal vitiligo instances as non-segmental vitiligo when they are linked to non-segmental vitiligo. On the other hand, mucosal lesions should be included in the category of unclassified vitiligo when they are found alone. Mucosal lesions are often observed near body orifices such as the lips and genitalia. The fact that patients with vitiligo who have mucosae have more frequent progression in their illness is a bad prognostic sign.[22]

Generalized vitiligo Originally identified as vitiligo vulgaris, is the most prevalent subtype of the condition (Figure 2). 1002 (69.8%) of the 1436 vitiligo patients in a previous study that looked at clinical characteristics had vitiligo vulgaris, which is now referred to as the generalized variety of vitiligo.[22]

Universal Vitiligo: It is characterized by loss of pigmentation throughout the body, with entire or near-complete lesions present. However, there are no precise standards for the minimal percentage of body involvement needed to diagnose universal vitiligo.[22]

Mixed Type: This type exhibits both SV and NSV co-occurrence. Segmental vitiligo typically occurs before non-segmental vitiligo (NSV), and it responds to treatment less well than NSV, sometimes exhibiting a delay of up to two years. Segmental lesions are less amenable to treatment than non-segmental ones in the few cases where NSV and SV manifest simultaneously.[22]

Segmental Vitiligo: This type of vitiligo usually appears early in infancy, affects a single segment of the integument, and spreads rapidly within the affected area (Figure 3). Since the segment may consist of several nearby dermatomes, or portions of them, or it may not be related to any dermatome at all, segmental vitiligo can frequently be very difficult to differentiate from focal type of vitiligo until a later stage where lesions are differentiable. Furthermore, segmental vitiligo may not follow any lines at all or have a linear distribution that coincides with Blaschko's line. It is commonly believed that SV may spread by an unknown pathway comprised of a clonal cell population. [22]

Unidentified/Uncategorized Vitiligo

Focal Vitiligo	Small, discrete, isolated patches of vitiligo that may eventually develop into any of the two types but do not fit within the SV or NSV.[23]
Mucosal vitiligo	Isolated involvement of the vaginal and oral mucosae but no skin involvement until two years of follow-up.[23]
Uncommon variations	
Trichrome vitiligo	Three distinct zones are present : normal skin, a small or broad band of intermediate pigmentation in between, and center depigmentation.
Quadrichrome Vitiligo	Four colors of pigmentation are present, which is typically observed in dark individuals. The fourth color is dark brown in places where repigmentation occurs in the perifollicular region.[23]
Pentachrome vitiligo	There are five consecutive pigmentation hues in this condition: white, tan, brown, blue gray, and normal.[23]
Inflammatory vitiligo	Lesions with elevated margins and a burning and itching sensation are indicative of inflammatory vitiligo.[23]

Blue vitiligo	Lesions that cover post-inflammatory hyperpigmented areas. Lesions of this kind have been seen in patients suffering from acquired immunodeficiency syndrome (AIDS).[23]
Contact or occupational vitiligo	Caused due to chemical exposure (aliphatic or aromatic derivatives of catechols and phenols). In these situations, depigmented lesions may mimic NSV and be restricted to the site of contact or they may progressively spread to other body areas.[23]
Vitiligo punctata	It is characterized by well-defined, sharply bordered, punctate-sized (1–1.5 mm) macules at any location on the body.[23]
Minor vitiligo	Lesions that are hypopigmented rather than depigmented, indicating a partial pigmentation deficiency.[23]
Follicular vitiligo	Rare type, the melanocytes in the hair follicles are the primary target. Hair whitening, or leukotrichia, is visible in both vitiliginous and non-vitiliginous regions. Although leukotrichia is less common in NSV lesions, NSV is often detected before to or after to the onset of follicular vitiligo.[23]

Koebner's Phenomena: The appearance of lesions at the traumatized, undamaged skin area. An "isomorphic response" is another name for it. It has been discovered in roughly one-third of cases historically. Patients who experience a higher degree of body surface involvement and a relatively early onset age are at a higher risk of developing Koebner phenomenon.

Diagnosis:

When there are characteristics of acquired, amelanotic, chalky-white macules with well-defined boundaries, no scaling, and presence in a typical distribution, the diagnosis of this disorder is typically determined on a clinical basis.[1,2] Wood's lamp, a UV irradiation instrument that emits UVA, may help with diagnosis. Particularly in cases of pale skin, it helps identify places where lesions might not be visible to the unaided eye and to identify focal melanocyte loss. In the Wood's light, the vitiligo lesions have defined borders and a brilliant blue-white appearance.[1.]

One method for differentiating between vitiligo and other depigmentation conditions is dermoscopy.

The common hallmarks of vitiligo, telangiectasia and residual perifollicular pigmentation, are absent in other hypopigmentation conditions. More importantly, it can be useful in identifying the disease activity and stage of evolution of vitiligo: perifollicular pigmentation is present in progressive lesions, whereas perifollicular depigmentation is seen in stable or remitting lesions.[1.]

Tests to confirm the diagnosis are not required. Melanocyte absence in a lesion can be evaluated non-invasively via in-vivo confocal microscopy or through a skin sample. Histologically, the core of a vitiligo lesion exhibits both an absence of melanocytes and a complete loss of melanin pigment in the epidermis. Occasional lymphocytes are visible along the lesions' advancing edge.[1.]

Indices used in vitiligo:

VIDA: The Vitiligo Disease Activity Score is a six-point rating system used to evaluate the disease's activity and stability over time. The activity is reported by the patient directly. It helps determine whether a treatment is effective in stopping and reversing the course and severity of the disease. Either the neogenesis of lesions or the extension of the current areas of depigmentation are included in the description of disease activity. The following are the grades: VIDA Score: 0 indicates stability for a year or more; +4 indicates activity lasting six weeks or less; +3 indicates activity lasting six weeks to three months; +2 indicates activity lasting three to six months; +1 indicates activity lasting six to twelve months; and –1 indicates stable with spontaneous repigmentation for a year or more.

A higher VIDA score indicates a more active disease, whereas a lower score suggests a less active or somewhat more stable condition. Currently, a cutaneous examination and the patient's history are used to gauge activity. Typically, photography is utilized to track repigmentation as well as the quantity, grade, and size of lesions during follow-up visits.[11]

VASI: The Vitiligo Area Severity Index (VASI) was proposed by Hamzavi et al. and is based on the PASI (Psoriasis Area and Severity Index) score, which is widely used. The VASI is a standardized and sensitive method that can be used to calculate the percentage and degree of repigmentation. The patient's body is comprised of five different and mutually exclusive zones in the VASI: the hands, upper limbs, trunk, lower limbs, and feet. The axillary region belongs to the upper limbs, whereas the buttocks and inguinal area belong to the lower limbs. While the face and neck can be assessed independently, they are not taken into account in the comprehensive evaluation.

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	

The area of involvement in hand units (1% per unit; rule of palm) and the level of depigmentation that fits in each hand unit-measured lesion (possibilities of 0%, 10%, 25%, 50%, 75%, 90% or 100%) are multiplied to get the VASI for each body area. It includes a subjective element because the doctor must determine the amount of pigment present and the affected area.[11]

Differential diagnoses:

Localized Depigmentation	Localized Hypopigmentation	Widespread Hypopigmentation and Depigmentation
Treponemal diseases like secondary syphilis and late stage of pinta.	Pityriasis alba	Progressive macular hypomelanosis
Postinflammatory depigmentation like atopic dermatitis, lichen planus, psoriasis, pityriasis alba, lichen sclerosus.	Tinea versicolor	Hypopigmented mycosis fungoides (MF)
Tuberous sclerosus	Tuberculoid leprosy	Achromic pityriasis lichenoides chronica
Chronic or severe inflammation seen in cases of atopic dermatitis and contact dermatitis	Sarcoidosis	Oculocutaneous albinism[24]
Lichen sclerosus	Naevus depigmentosus	Hypomelanosis of Ito[25]
Scleroderma	Naevus anemicus	
Systemic sclerosis can show a presence of depigmented lesions dotted with pigmentation in the perifollicular area ("salt and pepper" pattern)	Idiopathic guttate hypomelanosis[24]	
Melanoma or as an adverse event due to immunotherapy with inhibitors of interleukin-2, interferon-alpha and immune checkpoint.		
Chemical leucoderma		
Piebaldism		

Treatment:

Medical therapy:

The most recent developments in medicine include the use of UV light in conjunction with topical immunosuppresant treatment using calcineurin antagonists, pseudocatalase, and vitamin D analogs, as well as narrowband ultraviolet B (NB-UVB) treatment and targeted ultraviolet B. Excimer laser therapy is also an option.[2]

NON-PHARMACOLOGICAL
NB-UVB therapy:
First line of treatment for patients with vitiligo vulgaris (patchy vitiligo) and generalized vitiligo. It uses UV lamps with a peak emission of approximately 311 nm.[2] It works by causing localized immunosuppression and promoting melanocyte proliferation in the outer sheath of hair roots as well as the skin.
Laser Therapy
Excimer laser, which works by the use of Xenon-Chlorine (gas) and produces monochromatic laser light with a wavelength of 308 nm. The laser can be used alone or in conjunction with PUVA-sol treatment or topical immunosuppressants.[25]
Micropigmentation

This technique uses cosmetic tattooing to conceal the skin's depigmented spots. It may work well to improve the skin's appearance, but it is only a temporary fix and may need follow up treatments.
PHARMACOLOGICAL
Systemic immunomodulator therapy
systemic steroid therapy has been the most widely utilized treatment. However, because of the high rate of adverse effects, it should be used cautiously, especially in the pediatric population, which is the age group most impacted. To get around this restriction, steroids are therefore prescribed in pulse or even mini-pulse forms.2 It is used twice a week on consecutive days as part of oral minipulse (OMP) therapy.[26]
Topical analogs of vitamin D
Topical calcipotriol has been used to treat vitiligo either alone or in conjunction with topical steroids. Vitamin D3 affects the development and differentiation of keratinocytes in addition to melanocytes. Their efficacy has been demonstrated to vary when paired with topical prednisone and UV light therapy.[2].
Topical Immunomodulators

Topical medications such as tacrolimus and pimecrolimus have had positive results in the treatment of vitiligo in the latter stages of the condition. Due to their outstanding safety profile, they have been employed continuously for the past few years. These have certain benefits over topical steroids, such as not causing atrophy or telangiectasia, which makes them a safe therapy option for young children. In contrast to the use of strong topical steroids, there is also no chance of suppression of the hypothalamus pituitary-adrenal (HPA) axis.

Since the 1950s, topical corticosteroids (TCS) have also been used for their immunomodulatory and anti-inflammatory properties.1

In order to minimize side effects, guidelines recommend topical application of a strong or extremely strong corticosteroid once daily as the first-line therapy, avoiding the periocular area. [25]

Pseudocatalase

The use of this in vitiligo patients is supported by research indicating elevated levels of H2O2 and oxidative stress in the afflicted location. It is put over the lesion, and then the damaged skin or the entire body is exposed to UVB rays. The mixture is supposed to address the oxidative stress that melanocytes experience in vitiligo, which causes repigmentation.[2.]

Topical 5-Fluorouracil

Topical 5-fluorouracil causes repigmentation by overstimulating melanocytes in the follicle that migrate to the epidermal layer during the epithelialization process. Spot

dermabrasion of the affected area may be used with this kind of topical treatment to improve the repigmentation response.

Surgical therapies:

Choosing the right patient is the first and most crucial stage in surgical care. The selection of appropriate individuals for surgery is essential because not all patients benefit from a surgical modality. The immunologic response of patients appears to be the cause of the diversity in therapeutic improvement. Generally speaking, a stable illness or a condition without an immunological cause responds better to surgical intervention. Surgery is typically the first line of treatment for patients with focal or segmental vitiligo because of its promising response to it. In contrast, surgeries are less successful in treating other subtypes of vitiligo and should only be considered in cases where medical therapy has failed and the patient's disease has stabilized.28

Keloidal tendencies, significant bleeding disorders and blood borne infections are few contradindications to surgical procedures. [28]

Tissue grafting technique:

Acellular grafting techniques:
Suction blister grafting
At the donor site, suction blisters are produced by applying sufficient negative pressure to the skin. From these blisters, thin epidermal grafts are removed and placed on recipient locations that have undergone dermabrasion. This leads to repigmentation, and the cosmetic matching is excellent.[2]
Split thickness grafting
Graft is taken from a donor area and placed on the recipient areas that have undergone dermabrasion using a Silver's knife, a dermatome, a Humby's knife, or just a razor blade. For faster and more effective results, NB-UVB therapy can be initiated or sustained after suction blister grafting and partial thickness skin grafting.[2]
Miniature punch grafting
Punch grafts with a diameter of 1.0 to 2.0 mm that are full-thickness are taken from an appropriate donor site and placed on recipient sites with beds that resemble punches. The recipient location is treated with PUVA/PUVA-sol or topical corticosteroids to encourage the transplanted punches' pigment to spread to the surrounding areas. The entire recipient region develops repigmentation over time.[2]
Follicular unit grafting

donor site is used to extract individual hair follicular units, much like in hair transplantation. Cut above the follicular bulb, these follicular units are then transplanted into lesions of vitiligo. Transferring melanocytes from the follicular unit to the lesional skin, which subsequently serves as a source of pigment at the recipient site, is the fundamental idea behind this approach.[2]
Cellular grafting techniques:
Smash grafting
A partial thickness graft is taken and "smashed," or mashed into tiny pieces, with a surgical blade against a hard surface, such as a glass slide. Once the recipient area has been dermabraded, this "smashed" tissue is transplanted and, in order to keep the graft from being disturbed, is covered with a specific powder.[2]
Uncultured epidermal suspensions
A split-thickness graft is taken from a donor site and left to incubate for the entire night. The following day, the cells are manually separated using a trypsin-EDTA solution, and a suspension is created by centrifuging the mixture. After dermabrading the recipient area, this suspension is applied to the afflicted areas, a collagen dressing is put on top.[2]
Melanocyte culture transplantation
Involves the harvesting of a split-thickness graft from a donor site, followed by incubation in a culture medium to facilitate the formation of melanocytes or keratinocytes-melanocytes in vitro. The application of these cultivated cells is done on the lesional location that has been manually or laser abraded.[2]

SOURCE OF DATA:

Patients of clinically diagnosed vitiligo presenting to Out Patient 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, will be enrolled for the study.

Period of study:

The study was conducted from August 2022 to february 2024.

Study design:

Cross sectional case control study .

Sample size

With anticipated Sensitivity and specificity of S100B Protein 80% and 60% respectively, considering the prevalence of Generalised Vitiligo 89% [4], at precision of 10% and 98%confidence ,the required sample size is 70.

Formula used is— **(Formula reference:** Buderer, N. M. F. (1996). Statistical methodology: I. Incorporating the prevalence of disease into the sample size calculation for sensitivity and specificity. Academic Emergency Medicine, 3(9), 895-900.)

$$N=\frac{Z^2P(1-p)}{\Delta^2}$$

N ill be (a+c) if we use sensitivity as p

N= (a+c)/Prevalence

METHOD OF COLLECTION OF DATA:

Inclusion criteria:

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

Exclusion criteria:

- Patients with any chronic and systemic diseases.
- Patient with recent head injury/ trauma/ cerebrovascular accidents.
- Patients with melanoma.
- Patients with any history of smoking.
- Patients with congenital (e.g., piebaldism, Wardenberg syndrome, albinism, etc.) and acquired (e.g., post-inflammatory depigmentation, contact leucoderma, etc.) causes of depigmentation disorders.0pp

Methods:

All patients enrolled in the study signed an informed consent. Details of the their current illness which included the timing of onset, duration of the disease in each case, past history of any diseases and family history were documented as per the proforma (ANNEXURE VII).

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

Methodology:

- Initial clinical examination was done and signs and symptoms of the lesions were recorded in the proforma.
- · The Vitiligo Disease Activity Score was used to calculate the disease activity: +4 indicated a duration of 6 weeks or less, +3 indicated a duration of 6 weeks to 3 months, +2 indicated a duration of 3 to 6 months, +1 indicated a duration of 6 to 12 months, 0 indicated a stability of 1 year or more, and -1 indicated a stability with spontaneous repigmentation since 1 year or more.
- The Vitiligo Area Severity Score was used to determine the extent of the disease: 100% indicates complete depigmentation, meaning no pigment is present; 90% indicates pigment specks are present; 75% indicates that the depigmented and pigmented areas are equal; 25% indicates that the pigmented area exceeds the depigmented area; and 10% indicates only specks of depigmentation are present.
- After obtaining consent, a blood sample of 5 ml was collected in a plain tube and the sample was allowed to clot at room temperature and centrifuged, 2 mL of serum was refrigerated in a plastic vial.
- Assessment of serum level of Human (S100B) was done by sandwich ELISA technique. [Human S100B (S100 Calcium Binding Protein B) ELISA Kit Make: Elabscience, HSN Code: 3822]

- After generation of the proper, data was entered in Microsoft Excel (Microsoft Corp., Redmond, Washington, United States) and analysis was done through SPSS for Windows.
- These values are represented in a table and their values are correlated with the activity and extent of vitiligo.

INVESTIGATIONS:

Following investigations will be done:

1. Serum S100B protein levels.

STATISTICAL ANALYSIS

- The data obtained was documented in a Microsoft Excel sheet, and statistical analysis was performed using the software JMP®, Version 16. SAS Institute Inc., Cary, NC, 1989-2021.
- Results are presented as Mean)±SD, Median and Inter quartile range, frequency, percentages and diagrams.
- The normally distributed continuous variables between two groups were compared using Independent t test. For not normally distributed variables Mann Whitney U test was.
- Categorical variables are compared using Chi square test.
- Personn OR Spearman correlation analysis is done for vitiligo disease activity
- $p<0.05$ will be considered statistically significant. All statistical tests are performed 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 August 2022 to February 2024. A total of 58 patients with a clinical diagnosis of vitiligo and 30 healthy controls 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 S100B was estimated by ELISA.

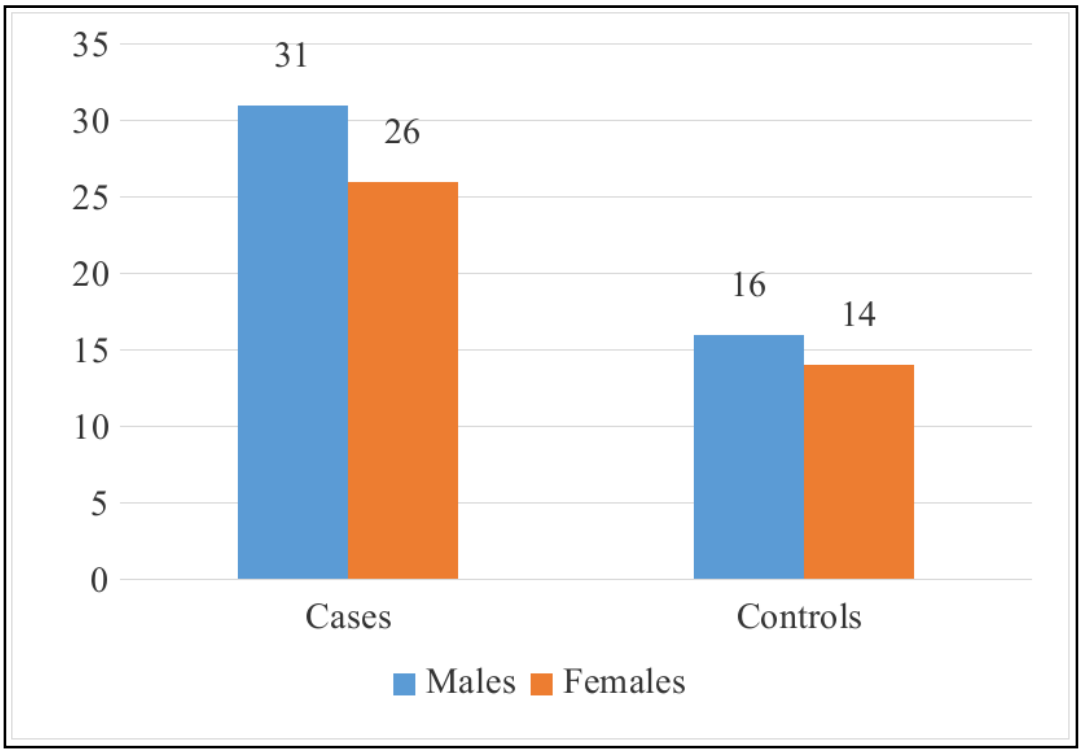
Gender distribution

Amongst the 58 cases enrolled, 31(53%) were females and 27(47%) were males (Table 1, Figure 3). Females outnumbered males in both the cases and controls.

Table 1: Distribution of cases and controls according to sex

		GROUPS		Total	Chi square	P-Value
		Cases	Controls			
Sex	F	31	16	47	0.009	0.925
		54.4%	53.3%	54.0%		
	M	26	14	40		
		45.6%	46.7%	46.0%		
Total		57	30	87		
		100.0%	100.0%	100.0%		
Statistically Insignificant						

Figure 3: Distribution of cases and controls according to sex



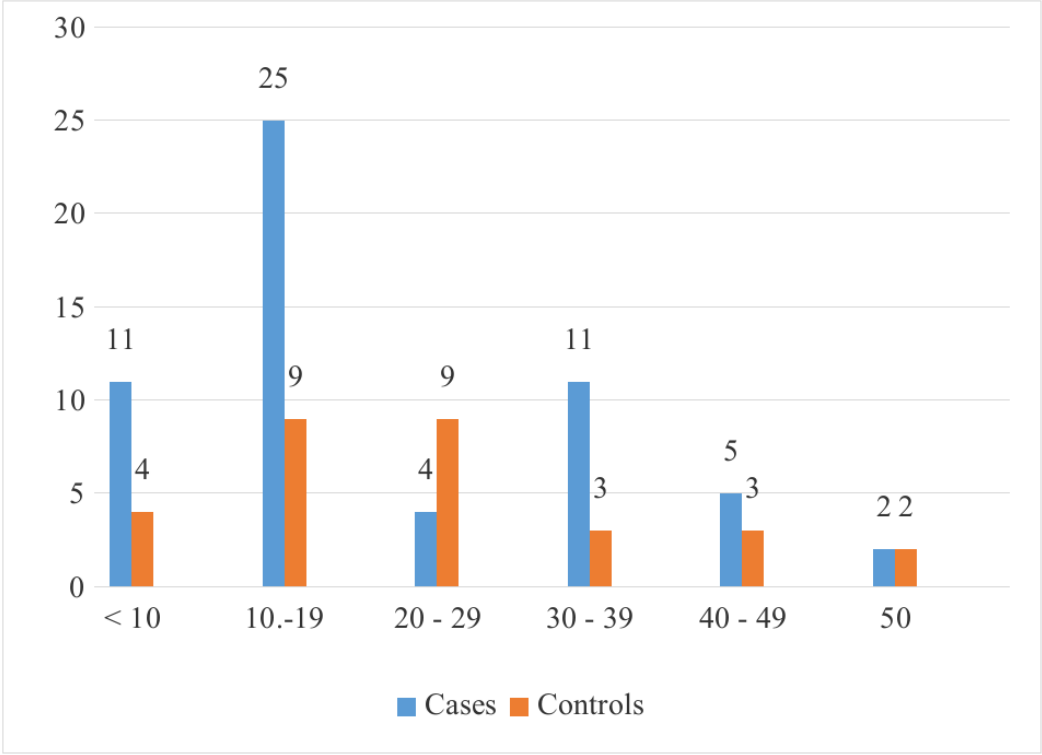
Age group

The age group of patients included in the study ranged from 3 years to 56 years. Most patients in the study belonged to the 2nd decade (25 patients – 43.1%) (Table 2, Figure 4).

Table 2: Distribution of cases and controls according to age

Age(Years)	Cases	Controls	Total	Chi square test	Significant value
< 10	11	4	15		
	19.0%	13.3%	17.0%		
10 - 19	25	9	34		
	43.1%	30.0%	38.6%		
20 - 29	4	9	13		
	6.9%	30.0%	14.8%		
30 - 39	11	3	14	9.882	P=0.079
	19.0%	10.0%	15.9%		
40 - 49	5	3	8		
	8.6%	10.0%	9.1%		
50	2	2	4		
	3.4%	6.7%	4.5%		
Total	58	30	88		
	100.0%	100.0%	100.0%		
Statistically Insignificant					

Figure 4: Distribution of cases and controls according to age



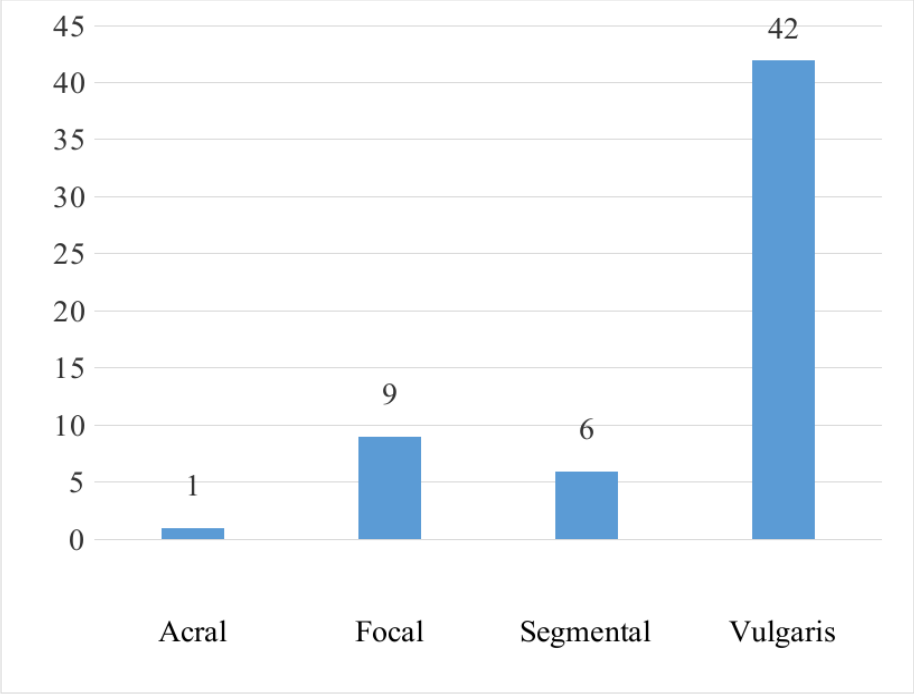
Types of Vitiligo:

Types of vitiligo encountered in our patients were vitiligo vulgaris, segmental, focal, segmental and acral. 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%). (Table 3, Figure 5)

Table 3: Distribution of cases according to type of vitiligo

Type Of Vitiligo		
Final diagnosis	Acral	1
		1.7%
	Focal	9
		15.5%
	Segmental	6
		10.3%
	Vulgaris	42
		72.4%
Total		58
		100.0%

Figure 5: Distribution of cases according to type of vitiligo



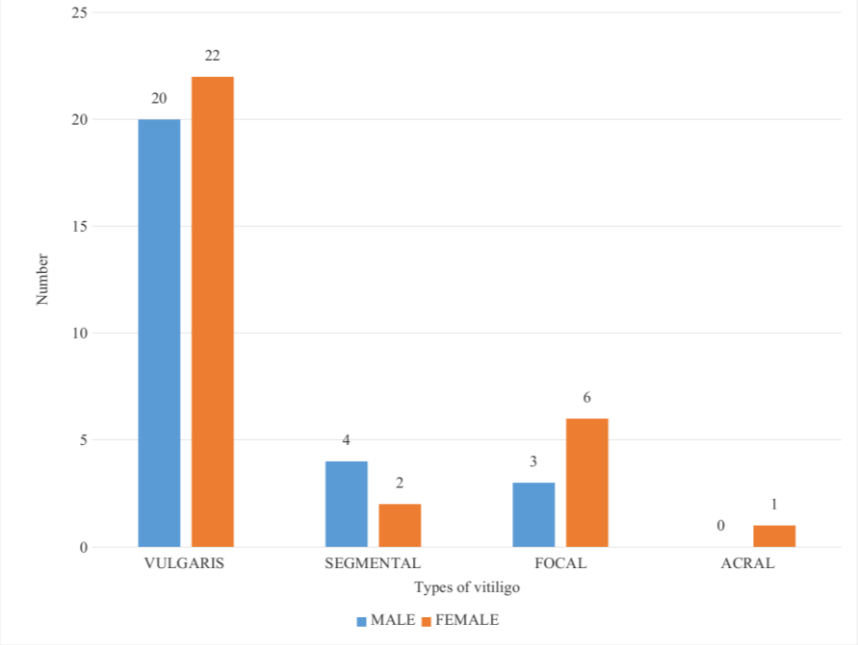
Relation between gender and types of vitiligo:

About similar percent of males (20 out of 27, 74%) and females (22 out of 31, 70.9%) had vitiligo vulgaris, segmental vitiligo comprised of 4 out of 31 (12%) female patients, 2 out of 27 (7.4%) male patients, Focal vitiligo being seen in about 3 out of 27 (11.1%) males and 6 out of 31 (19.3%) females and Acral vitiligo was seen in 1 out of 31 (3.2%) female patient and no male patient (Table 4, Figure 6).

Table 4: Distribution of types of vitiligo within gender groups

GENDER	Type of Vitiligo			
	Vulgaris	Segmental	Focal	Acral
Male	20	4	3	0
Female	22	2	6	1
TOTAL	42	6	9	1

Figure 6: Distribution of types of vitiligo within gender groups



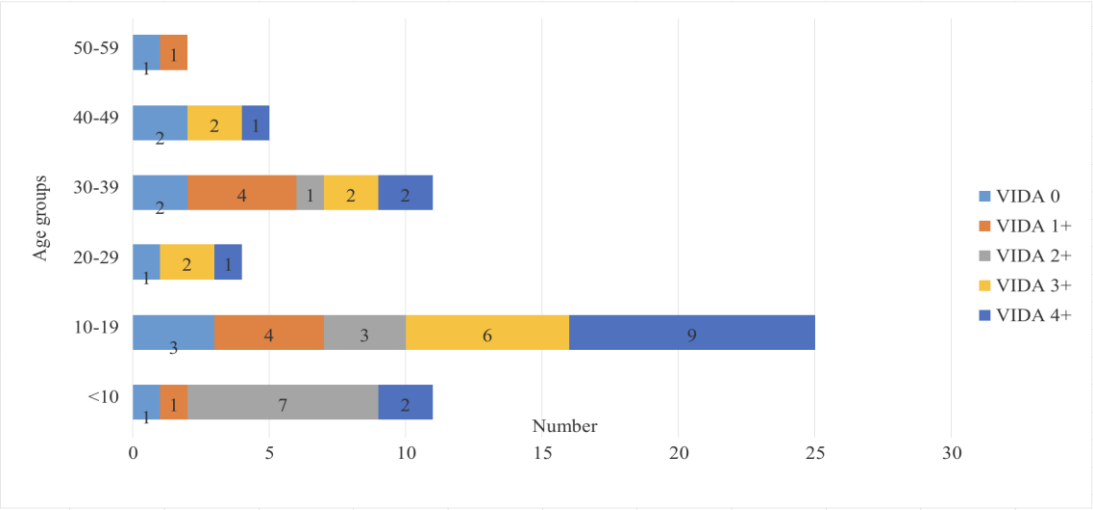
Relation between age and VIDA:

Age group between 10-19 years showed the highest number of patients with VIDA score of 4+ (9 out of 27, 33.3%) while the age group that showed maximum number of patients having a stable disease compared to other age groups was 40 to 49 years (2 out of 5, 40%) (Table 5, Figure 7).

Table 5: Distribution of VIDA within age groups

Age groups (in years)	VIDA				
	VIDA 0	VIDA 1+	VIDA 2+	VIDA 3+	VIDA 4+
<10	1	1	7	0	2
10-19	3	4	3	6	9
20-29	1	0	0	2	1
30-39	2	4	1	2	2
40-49	2	0	0	2	1
50-59	1	1	0	0	0
TOTAL	10	10	11	12	15

Figure 7: Distribution of VIDA within age groups



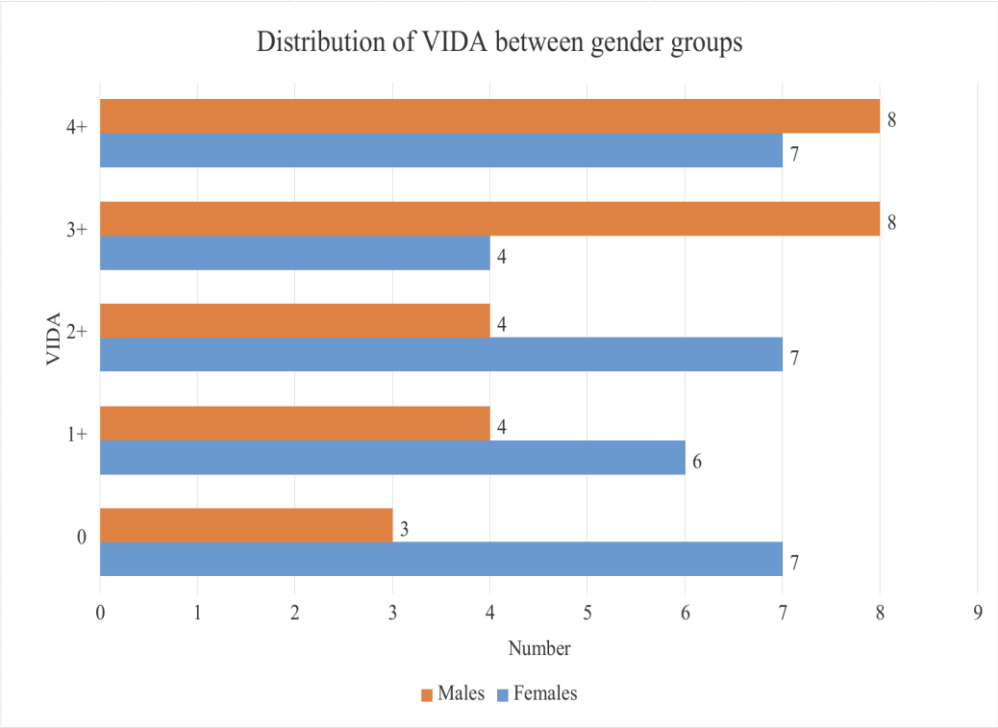
Relation between gender and VIDA:

Out of a total of 58 patients, 15 out of 58, 25.8% patients showed a highly active disease (VIDA 4+). 7 out a total of 31 female patients (22.5%) showed a VIDA score of 4+, while VIDA score of 4+ was noted in 8 out of 27 male patients (29.6%) (Table 6, Figure 8).

Table 6: Distribution of VIDA between gender groups

Gender	VIDA				
	0	1	2	3	4
Females	7	6	7	4	7
Males	3	4	4	8	8
Total	10	10	11	12	15

Figure 8: Distribution of VIDA between gender groups



Relation between gender and VASI:

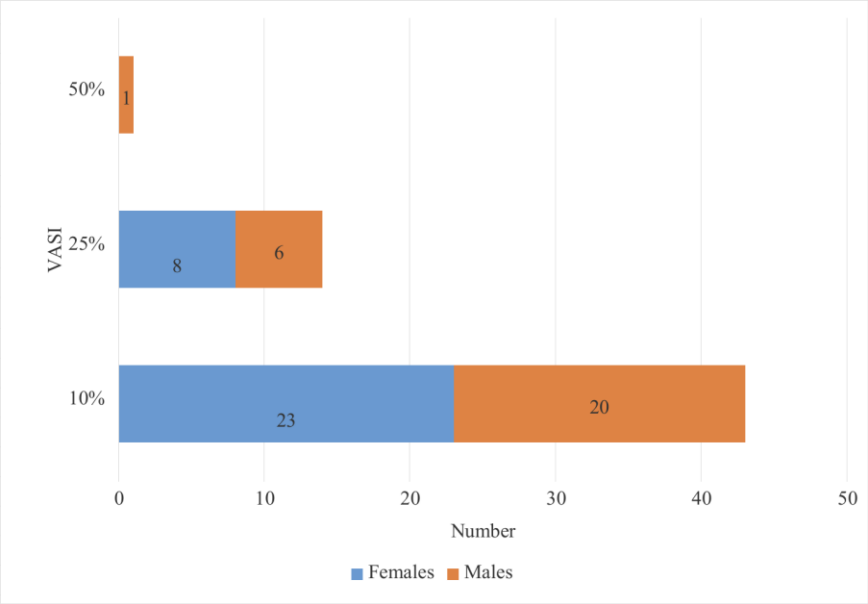
Amongst all 58 patients, 43 (74.1%) patients showed a VASI of 10%. This group had 23 out of all 31 females (74.1%) and 20 out of 27 males (74%).

14 of 58 total patients (24.1%) showed a VASI of 25%. 8 out of 31 females (25.8%) and 6 out of 27 males (22%) were found to be in this group. And 1 male patient (1.7%) showed a VASI of 50% (Table 7, Figure 9).

Table 7: Distribution of VASI between gender groups

Gender	VASI		
	10%	25%	50%
Females	23	8	0
Males	20	6	1
Total	43	14	1

Figure9: Distribution of VASI between gender group



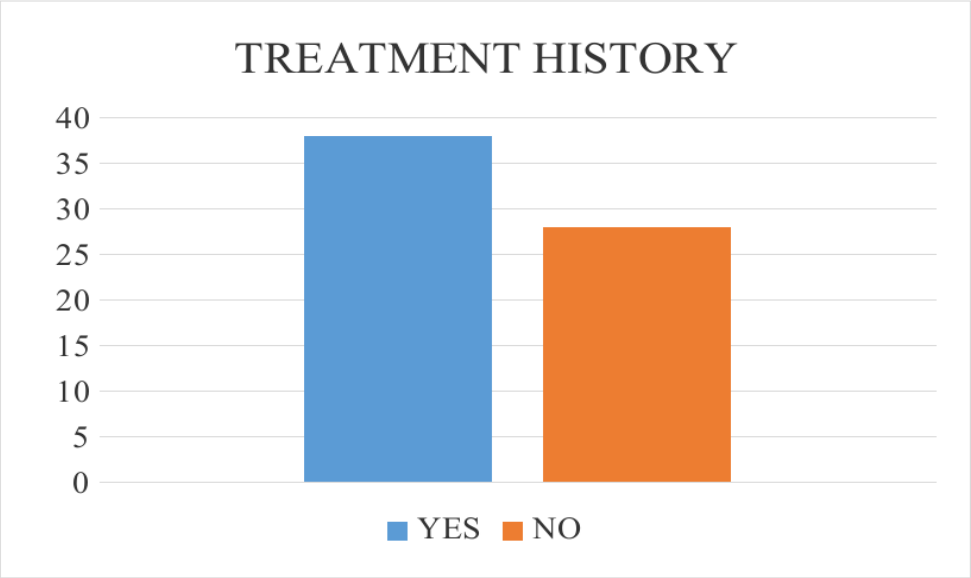
Treatment History among patients:

Out of a total of 58 patients, 38 out of 58, 65.5 % patients had taken treatment for the disease and 20 out of 58 (34.5%) had no history of taking treatment for vitiligo. (Table 8, Figure 10).

Table 8: History of taking treatment amongst vitiligo patients.

			Mann-whitney U test value	P-Value
Treatment history	NO	20	196	0.0027
		34.5%		
	YES			
		65.5%		
Total		58		
		100.0%		
Statistically significant				

Figure 10: History of taking treatment amongst vitiligo patients.

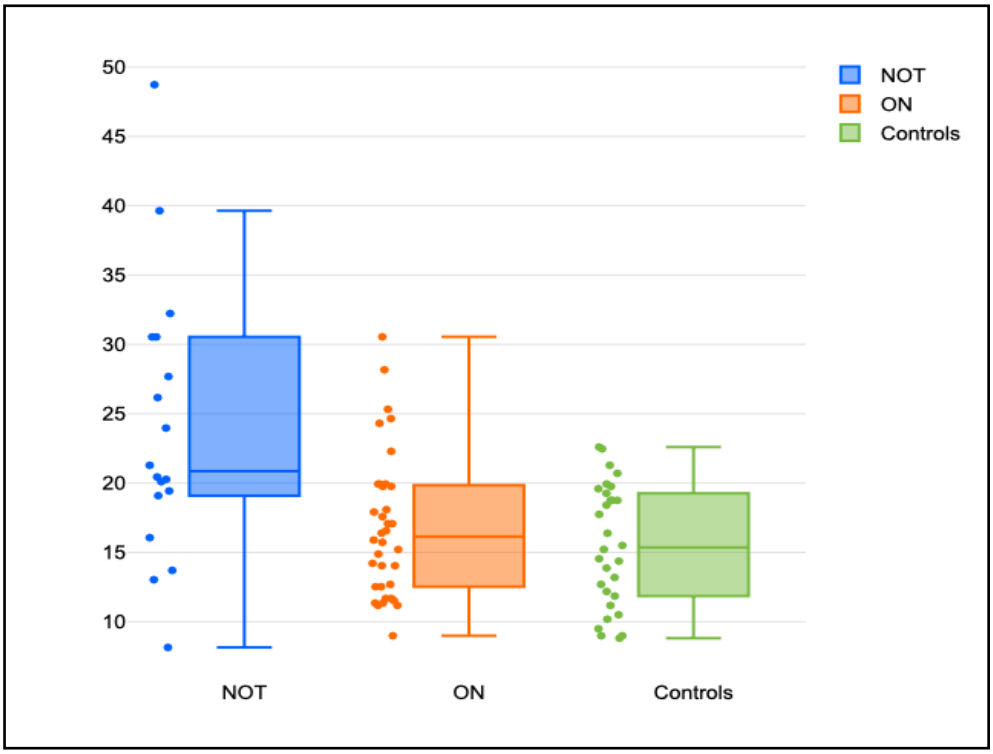


The mean values of S100B levels were found to be significantly higher in the subgroup not on immunosuppressive treatment as compared to the subgroup on immunosuppressants which was statistically significant with a p-value of 0.0027 and S100B values of patients on immunosuppressive therapy were comparable to that of controls. (Table 9, Figure 11)

Table 9: Median and range of Serum level of S100B among patients who are on treatment and those not on treatment compared to those of controls

Groups:	NOT	ON	Controls
Number	20	38	30
Minimum:	8.1463	8.988	8.81
Maximum:	155.006	77.029	22.6
Q1:	19.26	12.525	11.85
Median:	22.625	16.475	15.3595
Q3:	31.385	19.93	19.261
IQR	12.125	7.405	7.411
Mean ($\bar{x}$):	33.9117	19.0596	15.5312
Outliers:	155.006, 92.18	41.32, 77.029	

Figure 11: Box plot showing median and range of Serum level of S100B among patients who having have received treatment and those with no history of treatment compared to those of controls



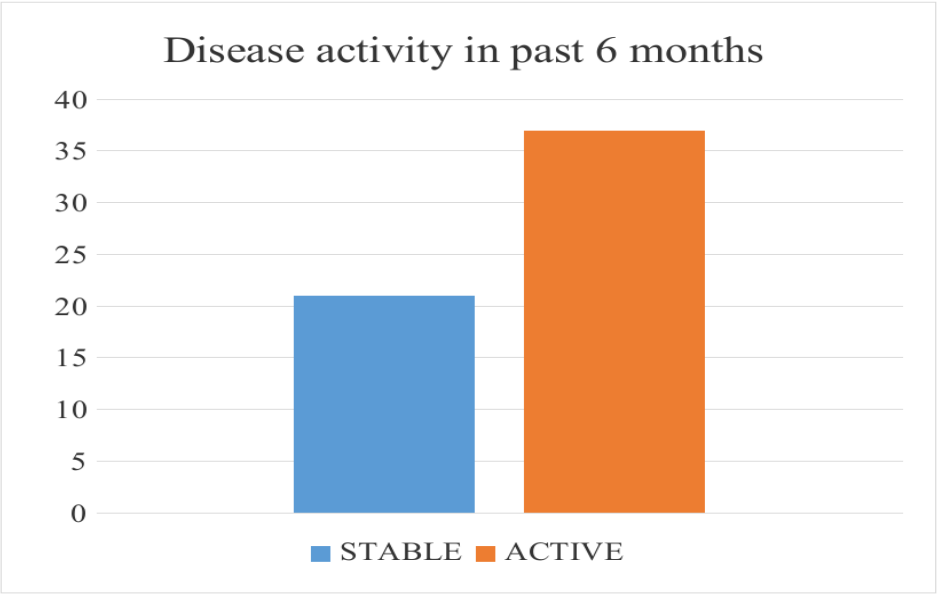
Disease activity in past 6 months:

Out of a total of 58 patients, 21 out of 58, (36.2%) patients had stable disease at the time of presentation (No disease activity in past 6 months) and 37 out of 58 (63.8%) patients had active disease (Disease activity in past 6 months) . (Table 10, Figure 12).

Table 10: Disease activity in past 6 months:

			Mann-whitney U test value	P-Value
Disease activity in past 6 months	Stable	21	111.5	< .00001
		36.2%		
	Active	37		
		63.8%		
Total		58		
		100%		

Figure 12: Disease activity in past 6 months

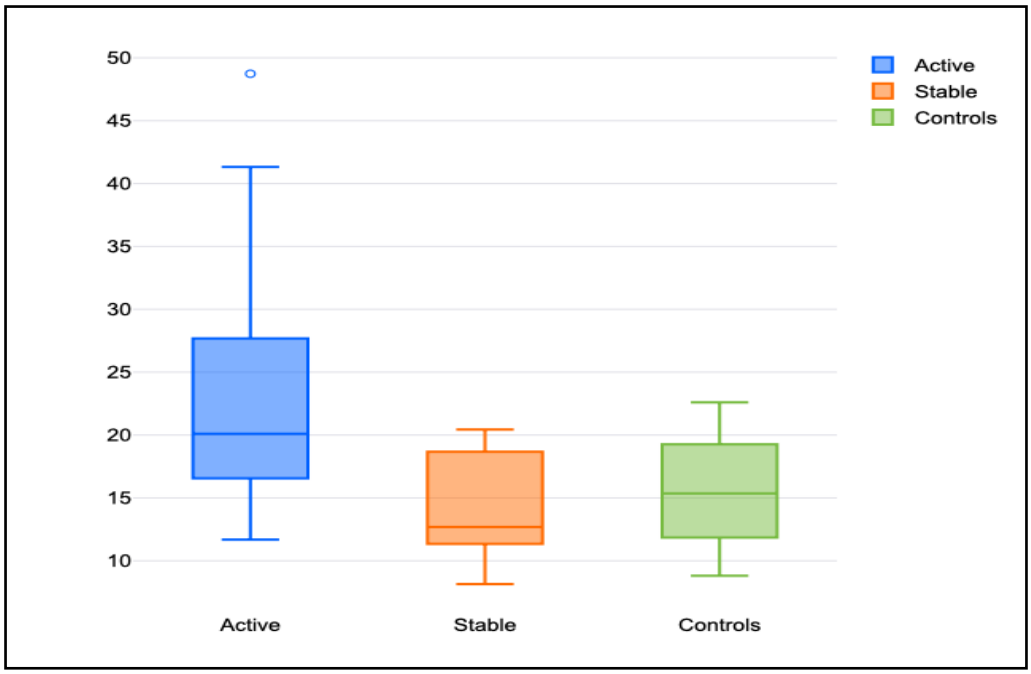


The mean values of S100B levels were found to be higher in the subgroup with active disease (disease activity in past 6 months) as compared to the stable group (No disease activity in past 6 months) which was statistically significant with a p-value of 0.001 and S100B values of patients with stable disease was comparable to that of controls. (Table 11, Figure 13)

Table 11: Showing median and range of Serum level of S100B among patients who having active and stable disease compared to those of controls.

Groups:	Active	Stable	Controls
Number	37	21	30
Minimum:	11.683	8.1463	8.81
Maximum:	155.006	20.44	22.6
Q1:	16.944	11.346	11.85
Median:	21.28	12.689	15.3595
Q3:	30.54	18.29	19.261
IQR	13.596	6.944	7.411
Mean ($\bar{x}$):	29.8372	14.2153	15.5312
Outliers:	155.006, 92.18, 77.029		

Figure 13: Box plot showing median and range of Serum level of S100B among patients who having active and stable disease compared to those of controls



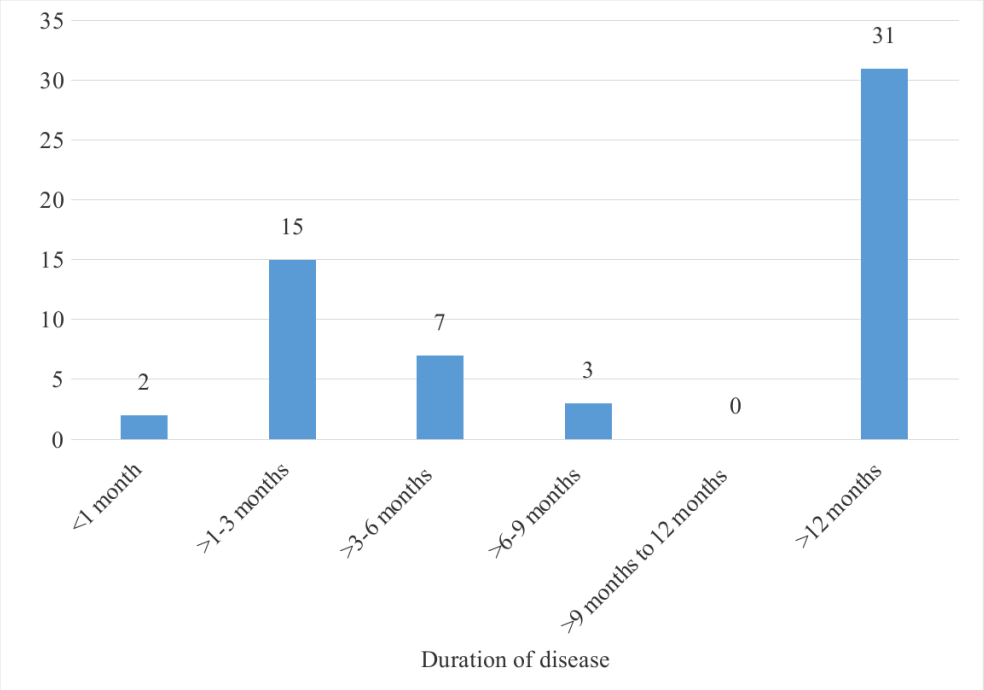
Duration of the disease:

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 12, Figure 14).

Table 12: Distribution of cases based on the duration of disease.

Duration (In months)	Number
<1 month	2
>1-3 months	15
>3-6 months	7
>6-9 months	3
>9 months to 12 months	0
>12 months	31
Total	58

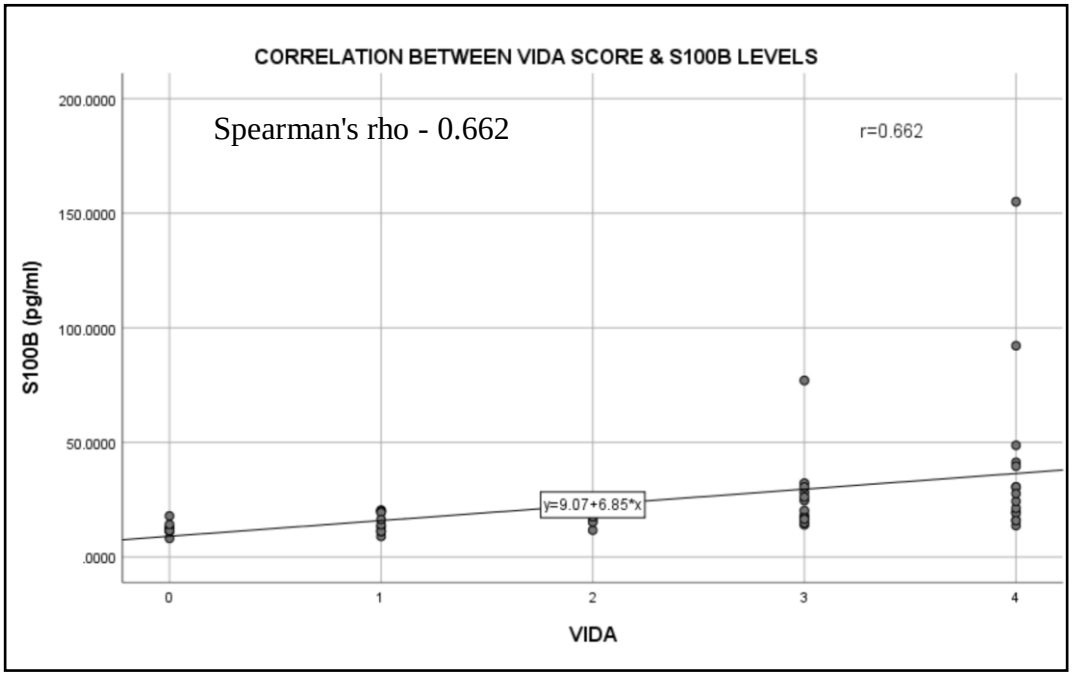
Figure 14: Distribution of cases based on the duration of disease.



Relation between VIDA and S100B:

There was a moderate positive correlation found between VIDA and S100B levels which was statistically significant (Spearman's rho- 0.662, Moderate correlation). (Figure 15).

Figure 15: Scatter diagram showing a correlation of VIDA and S100B

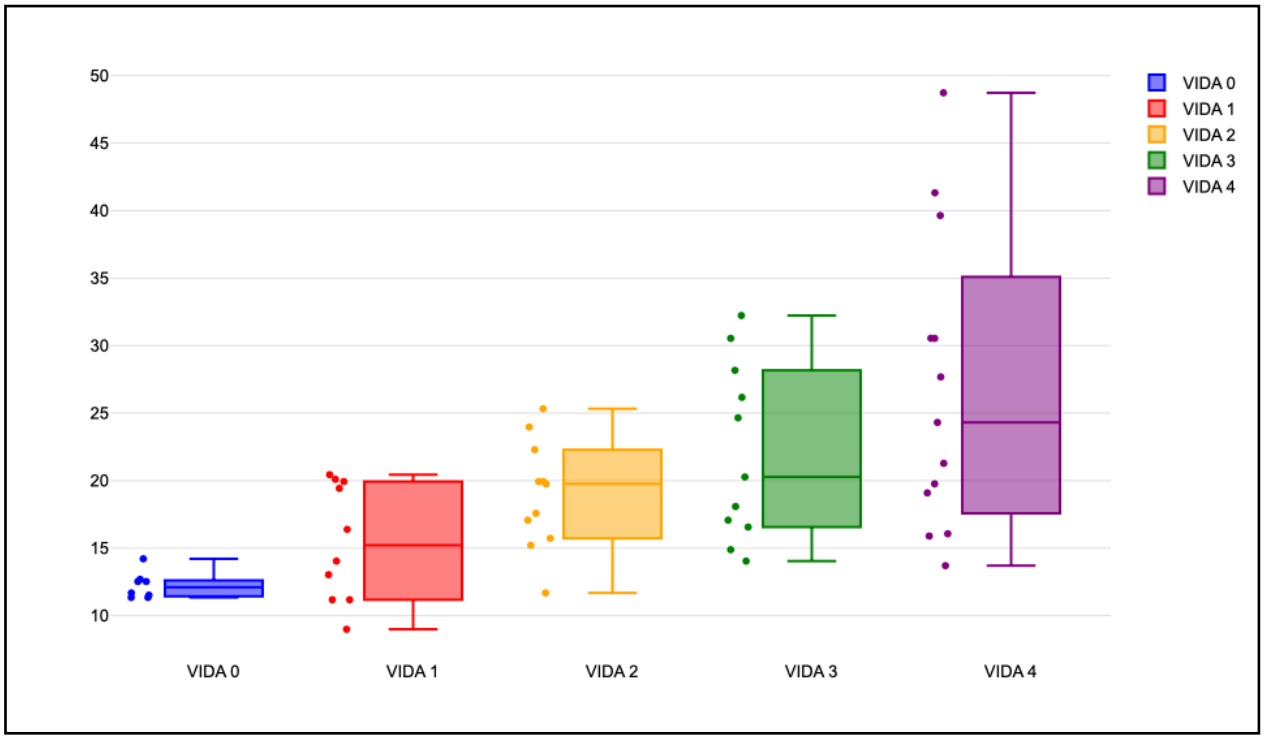


The mean values of S100B levels were found to increase proportionately with increase in disease activity, being lowest for VIDA 0 and highest for VIDA 4 which is depicted in the box plot (Table 13, figure 16).

Table 13 : Median and range of Serum level of S100B according to VIDA score among patients.

Groups:	VIDA 0	VIDA 1	VIDA 2	VIDA 3	VIDA 4
Number	10	10	11	12	15
Minimum:	8.1463	8.988	11.683	14.04	13.702
Maximum:	17.91	20.44	25.324	77.029	155.006
Q1:	11.346	11.177	16.0617	16.816	19.2575
Median:	12.1025	15.215	19.76	22.46	27.682
Q3:	12.689	19.93	21.7	29.355	40.9
IQR	1.343	8.753	5.6382	12.539	21.6425
Mean ($\bar{x}$):	12.389	15.4702	18.9519	26.641	39.7161
Outliers:	8.1463, 17.91			77.029	155.006, 92.18

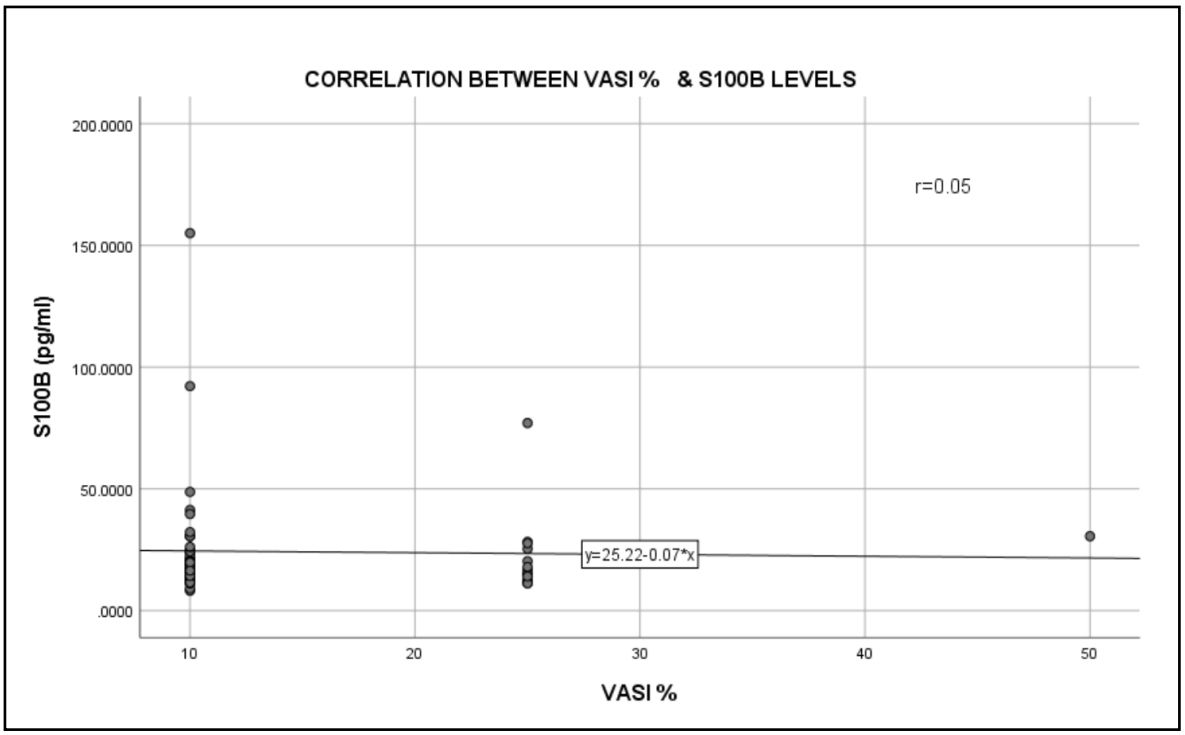
Figure 16: Box plot depicting median and range of Serum level of S100B according to VIDA score among patients.



Relation between VASI and S100B:

There was absolute absence of any correlation between VASI and S100B levels (Spearman's rho: -.050) (Figure 17).

Figure 17: Scatter diagram showing a correlation of VASI and S100B



The mean values of S100B levels showed no correlation to the area involved or the extent of the disease in patients, (Table 14, figure 18).

Table 14 : Median and range of Serum level of S100B according to VASI score among patients compared to those of controls.

Groups:	VASI 10	VASI 25	VASI 50
Number	43	14	1
Minimum:	8.1463	11.177	30.54
Maximum:	155.006	77.029	30.54
Q1:	14.0822	12.525	NaN
Median:	19.43	15.885	30.54
Q3:	24.225	25.324	NaN
IQR	10.1428	12.799	NaN
Mean (x̄):	24.8407	21.7004	30.54
Outliers:	155.006, 41.32, 92.18, 39.64, 48.73	77.029	

Figure 18 : Median and range of Serum level of S100B according to VASI score among patients compared to those of controls.

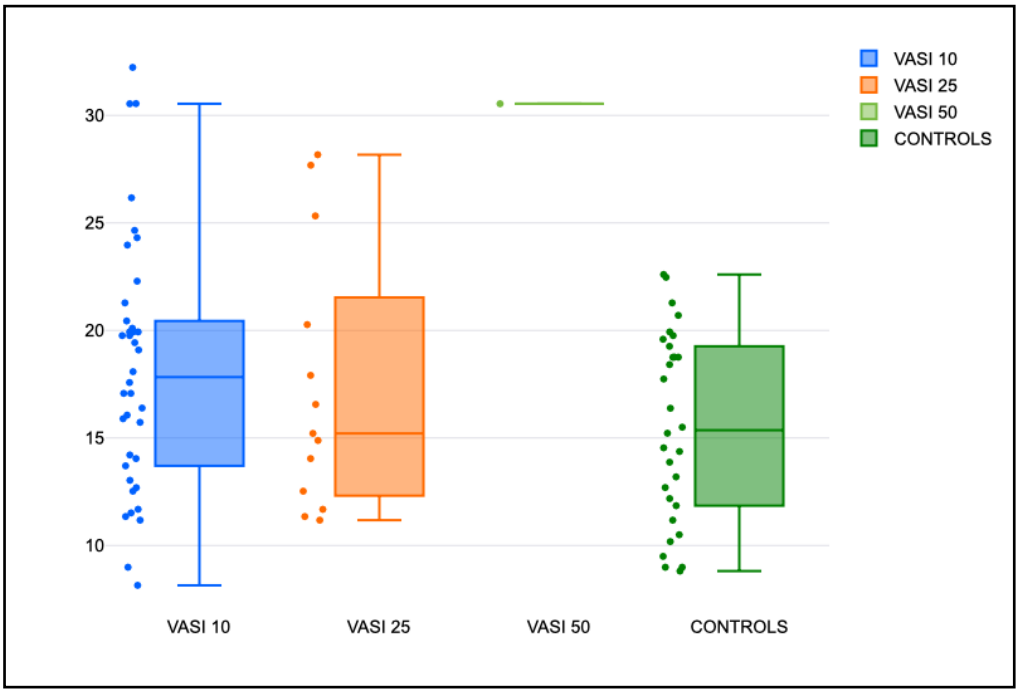


Table 15: Non-parametric correlations

Variable	Spearman's rho correlation coefficient	p-value
VIDA	0.662	0.001
VASI	-0.050	0.708

Correlation of S100B of vitiligo patients with other parameters:

There was no significant correlation between serum S100B levels with the age, gender of the patient or duration of onset. (Table 16)

Table 16: Correlation between S100B, age, gender, duration of diseases. (*n*=58)

Variable	r	p-value
Age	-0.056	0.676
Gender	-0.164	0.2186
Duration of onset	-0.168	0.207
Statistically insignificant at $p < .05$		

Comparison of S100B levels based on disease activity in patients with control group:

When compared with Controls, the patients with active disease showed significantly high mean values of S100B , (t-2.892, p=0.005) which was statistically significant, while patients with inactive disease did not show higher mean values of S100B when compared to controls (t-1.1034,p=0.275)

Table 17: Comparison of S100B levels based on disease activity in patients with control group

Variable	N	Mean	SD	t-test	p-value
Active disease	37	29	26.7	2.892	0.0052
Control	30	15	4.3		
Statistically significant at p < .05					
Stable disease	21	14.21	3.91	1.1034	0.2752
Control	30	15.53	4.37		
Statistically insignificant at p < .05					

Comparison of S100B levels based on treatment history in patients with the control group:

When compared with controls, the patients not on immunosuppressive therapy showed higher mean S100B levels (t-2.9628, p=0.004),which was statistically significant, while those on immunosuppressive therapy did not show higher mean S100B levels when compared to controls (t-1.578, p=0.1192)

Table 18:

Variable	N	Mean	SD	t-test	p-value
Not On Immunosuppressants	20	33.9	33.64	2.9628	0.0046
Control	30	15.5	4.37		
Statistically significant at p < .05					
On Immunosuppressants	38	19.05	11.59	1.578	0.1192
Control	30	15.53	4.37		
Statistically insignificant at p < .05					

DISCUSSION:

Vitiligo, a frequent acquired depigmenting condition, is characterized by selective melanocytic loss, which results in the emergence of well-defined, non-scaly, hypopigmented to depigmented macules and/or patches on virtually any portion of the body, as well as leukotrichia. It is linked to a number of different autoimmune disorders and has a detrimental psychological effect on the patients.[1–5.]

The etiopathogenesis of this condition has been explained by a number of distinct theories. One of these is cellular immunity, which postulates the existence of antibodies to the cytoplasmic and surface antigens of melanocytes, which causes complement-mediated lysis and antibody-mediated cytotoxicity, ultimately resulting in the death of melanocytes. In patients with damaged skin, CD8+ T lymphocytes have been detected in both the dermis and the epidermis. These T cells have the ability to produce different cytokines. .. S100 B is thought to affect melanocytes in two ways, and these actions are dependent on concentration. At lower concentrations, S100 B protects melanocytes, but at greater concentrations, it causes inflammation.10 It has been suggested that the serum level of S100B serves as a marker for melanocyte cytotoxicity.[4]

Over time, a number of indices have been employed to evaluate the dynamic changes in the disease, including activity, severity, extent, etc. VIDA is one such index; a lower score indicates less active/more stable disease. It is based on the patient's recollection of the most recent seen disease worsening, which includes the emergence of new lesions or an increase in size of the pre-existing lesions. Its accuracy is called

into question because of potential mistakes in identifying gradual changes over time, variations in skin tone, etc. Even so, it's one of the most widely utilized tools available today.[10, 11]

VASI is a measure of illness severity based on the extent of involvement. The body is divided into five distinct sections, and the impacted area % is computed. Once more, it involves subjectivity because the doctor must evaluate the level of involvement.[11, 12]

This study was undertaken to assess the serum levels of serum S100B protein in vitiligo patients and correlate these values with VIDA and VASI. and compare those values to those of healthy controls

Amongst the 58 cases enrolled, 31(53.4%) were females and 27(46.5%) were males. Females outnumbered males in the study with a ratio of 1.14:1. 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 female dominance.

The age group of the patients in this study ranged from 3 years to 56 years with a mean of 21.17 years and a predominance of patients in the age group of 10-19 years. A predominance of patients in the second decade was seen in other studies done by Namazi et al.[8], Shah et al.[19], Abdallah et al.[29], Ranjkesh et al.[30]

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 21.5 months (1.8 years). A maximum number

of patients had a rather acute onset of vitiligo, that is less than 6 months (around 26 weeks) duration. A study done by Dave et al.[31] revealed the mean duration of disease to be 3.5 years.

Age group of 10-19 showed the highest number of patients with VIDA score of 4+ (9 out of 25, 36%) while the age group that showed maximum number of patients having a stable disease (VIDA 0) compared to other age groups was 40 to 49 years (2 out of 5, 40%). Ali et al.[32] and Sheth et al.[33] discovered comparable results of increased 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, because 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.

Out of the total number of patients, one quarter (25.8%) patients showed a highly active disease (VIDA 4+). Out of this, the proportion of females (46%) and males (44%) having active disease was almost equal. In context with VASI, the majority of patients had a VASI score of 10 (74%) and only 1 (1.7%) patient had a VASI of 50.

The commonest type of vitiligo encountered was vitiligo vulgaris (72.4%) followed by segmental (10.3%), focal (15.5%) and acral (1.7%). This finding was similar to that found by Silverberg et al.[3], Shah et al.[19] and Dave et al.[31]

Segmental vitiligo was exclusively found in the paediatric age group ranging from 7 to 17 years. A solitary case of acral variety was found in a 21-year-old female patient.

Similar findings were reported by Palit et al.[34] and Silverberg et al.[35], who found that the occurrence of segmental type is higher in children vitiligo, while acral/acrofacial kinds are rarer than adults.

There was Moderate, statistically significant correlation found between VIDA and S100B levels in patients with active disease compared to those of controls. Patient with stable disease did not show significant correlation compared to controls. Similar results were seen in by Speeckaert R et al.[4] and Shoieb MA et al.[8]

There was absolutely no correlation found between VASI and S100B levels in this study. Speeckaert R et al.[4] found that S100B serum levels correlated with the vitiligo-affected body surface area. Only patients with active vitiligo exhibited a link between the impacted BSA and circulating S100B levels; no correlation was observed between BSA and S100B serum levels in patients with non-progressive vitiligo.

There was no statistically significant correlation of S100B levels with other parameters which included age of the patient, gender and duration of illness.

A further division of patients was done into two groups, that is patients who were on at least one type of immunosuppressive therapy before being investigated and those who were not on any immunosuppressants. The mean levels of S100B were noted to be higher in the group which was not on immunosuppressants as compared to the one

that was, this difference was statistically significant. The S100B levels of patients on immunosuppressants were comparable to that of healthy controls.

This suggests that immunosuppressive medication may have a role in lowering these markers in vitiligo sufferers. Immunosuppressive treatment (corticosteroids or steroid sparing immunosuppressive agents) halts and control disease progression, This possibly explains why levels of S100B showed a decline in patients on immunosuppressive therapy.

This also suggests that early initiation of immunosuppressive therapy in vitiligo patients may reduce inflammatory mediators, further halting progression of the disease.

When compared with Controls, the patients with active disease showed significantly high mean values of S100B , while patients with inactive disease did not show higher mean values of S100B when compared to controls.

When compared with controls, the patients not on immunosuppressive therapy showed higher mean S100B levels, while those on immunosuppressive therapy did not show higher mean S100B levels when compared to controls.

The limitations of the study includes the small sample size, the study only assessed the level of S100B at one time point and, couldn’t examine progression in levels at different time points. The lack of large population studies is a limiting factor for the standardization of S100B levels. History of other autoimmune disease was not elicited in patients as other autoimmune diseases can lead to elevated S100B levels. Futher correlation with Immunohistochemistry can enhance the accuracy of the test.

CONCLUSION

The study was undertaken to estimate the levels of S100B in serum of patients with vitiligo and correlate their values with the disease activity and extent using indices namely VIDA and VASI. These values were then compared with that of healthy individuals. A total of 58 patients and 30 healthy controls were included in the study with a male to female ratio of 1:14:1, and the age of patients ranged from 3 to 56 years with majority being in the 2nd decade. The mean duration of disease was 21.7 months with the range being 2 weeks to 20 years. There was no statistical difference in the age and gender distribution between the cases and healthy controls.

There was almost equal incidence of active disease amongst male and females in the study, in which majority of patients with VIDA 4+ belonged to the 10 to 19 years group while those with stable disease predominated in the 40-49 years group. This increased incidence of vitiligo in younger age group can be attributed to the autoimmune causality of vitiligo and a higher association of other autoimmune diseases among this population.

The commonest type of vitiligo was the vulgaris subtype followed by focal, segmental and acral vitiligo in that order. Majority of the segmental vitiligo cases were children whereas one middle age female patient had the acral subtype.

There was statistically significant positive correlation of VIDA with S100B levels. Moderate statistically significant increase in serum S100B levels was found in patients with active disease in the last 6 months compared with patients with stable

disease in the last 6 months. This might be explained by the fact that S100B is secreted from actively depigmenting cells and stable disease does not have elevated S100B levels.

The trend of the results showed that there was proportional increase in S100B levels with increasing VIDA score.

There was absence of statistically significant correlation of VASI scores with S100B levels indicating that levels of S100B are independent of disease extent, this absence of correlation may also be due to the fact that many patients were on immunosuppressive at time of sampling and it may imply that immunosuppressive agents have a role in reducing the levels of S100B, possibly hinting towards a targeted therapy to reduce these levels to prevent further downstream effects of S100B induced signaling pathways in inducing depigmentation.

When compared to healthy controls there was significantly higher mean values of S100B among the patients who had active disease and those who had not received any treatment in contrast to low mean values in those with stable disease and on immunosuppressant therapy.

Therefore this study underscores the possible role of measuring the levels S100B in patients with vitiligo to determine disease activity and opens further scope for future research on S100B targeted therapy in vitiligo.

SUMMARY

Vitiligo is an acquired immune mediated depigmenting disorder characterized by selective loss of melanocytes, resulting in well-defined, non-scaly, hypopigmented to depigmented macules and patches, and associated with various autoimmune conditions. It has a significant psychological impact on affected individuals. The etiopathogenesis of vitiligo is multifactorial, with cellular immunity playing a key role through antibodies targeting melanocyte antigens, leading to melanocyte destruction.

This study aimed to assess serum S100B levels in vitiligo patients, correlate these levels with VIDA and VASI scores, and compare them to healthy controls. Key findings include:

1. **Demographics**: Of the 58 cases, 53.4% were females and 46.5% were males, with a mean age of 21.17 years, predominantly in the 10-19 years age group.

2. **Disease Duration**: Duration of disease ranged from 0.5 months to 240 months, with a mean duration of 21.5 months. Majority of the patients had a disease duration of less than 6 months.

3. **VIDA Scores**: The highest VIDA scores (4+) were observed in the 10-19 years age group, indicating high disease activity among patients in this age group. Patients aged 40-49 years had the most stable disease. Approximately 25.8% of patients had highly active disease, with no significant gender difference.

4. **VASI Scores**: The majority of patients had a VASI score of 10, indicating a lower Body surface area involvement.

5. **Types of Vitiligo**: The most common type was vitiligo vulgaris (72.4%), followed by focal, segmental and acral types. Segmental vitiligo was more prevalent in children.

6. **S100B Levels and VIDA**: A moderate, statistically significant correlation was found between VIDA scores and S100B levels in patients with active disease, indicating rising S100B levels with increasing disease activity.
   No significant correlation was found between S100B levels of patients with stable vitiligo which were comparable to that of healthy controls.

7. **S100B levels and VASI**: No correlation was found between VASI scores and S100B levels. Patients on immunosuppressive therapy had lower S100B levels comparable to healthy controls, suggesting the potential benefit of early immunosuppressive treatment in reducing disease progression.

8. **Comparison with controls**: Mean values of S100B levels among patients with active disease and those not on immunosuppressive therapy were significantly elevated in comparison to healthy controls.

The study highlights the possible role of measuring S100B levels in patients with vitiligo to be used as a marker of disease activity, although large scale data is still required for standardization of S100B levels.

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BLDE
(DEEMED TO BE UNIVERSITY)
Declared as Deemed to be University u/s 3 of UGC Act, 1956
Accredited with 'A' Grade by NAAC (Cycle-2)
The Constituent College
SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

BLDE (DU)/IEC/ 699/2022-23 30/8/2022

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this University met on **Friday, 26th August, 2022 at 3.30 p.m. in the Department of Pharmacology** scrutinizes the Synopsis of Post Graduate Student of BLDE (DU)'s Shri B.M.Patil Medical College Hospital & Research Centre from ethical clearance point of view. After scrutiny, the following original/ corrected and revised version synopsis of the thesis/ research projects has been accorded ethical clearance.

TITLE: "ESTIMATION OF SERUM S100B LEVELS IN VITILIGO TO DETERMINE WHETHER INCREASED LEVELS ARE A MARKER OF DISEASE ACTIVITY: A PROSPECTIVE CASE-CONTROL STUDY".

NAME OF THE STUDENT/PRINCIPAL INVESTIGATOR: Dr. Mohd. Salman Hyder

NAME OF THE GUIDE: Dr. Arun C. Inamadar, Professor & HoD, Dept. of Dermatology.

Dr. Santoshkumar Jeevangi
Chairperson
IEC, BLDE (DU),
VIJAYAPURA

Chairman,
Institutional Ethical Committee,
BLDE (Deemed to be University)
Vijayapura

Dr.Akram A. Naikwadi
Member Secretary
IEC, BLDE (DU),
VIJAYAPURA

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

Following documents were placed before Ethical Committee for Scrutinization

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

APPENDIX – VI

B.L.D.E.U's SHRI B M PATIL

MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTRE,

VIJAYAPURA-586 103

RESEARCH INFORMED CONSENT FORM

TITLE OF THE PROJECT: -	ESTIMATION OF SERUM S100B LEVELS IN VITILIGO TO DETERMINE WHETHER INCREASED LEVELS ARE A MARKER OF DISEASE ACTIVITY; A PROSPECTIVE CASE-CONTROL STUDY.
PG GUIDE: -	DR. ARUN C INAMADAR
PG STUDENT: -	DR. MOHAMMED SALMAN HYDER

PURPOSE OF RESEARCH:

I have been informed that this project will determine the serum levels of S100B Protein 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 S100B 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 additional questions about the study at any time. Concerned. Dr. Mohammed Salman Hyder is available to answer my questions or queries. I understand that I will be informed about 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. Mohammed Salman Hyder 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 promptly reported, 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, 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

APPENDIX – VII

B.L.D.E.U'S SHRI B. M. PATIL MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTRE, VIJAYAPURA.

Department of Dermatology, Venereology and Leprosy.

SCHEME OF CASE TAKING

GENERAL INFORMATION

Sl. No.: Date:

Name: I.P/O.P No:

Age: Hospital:

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

-Commorbidities:

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

GENERAL PHYSICAL EXAMINATION

- Built: Well/Moderate/Poor
- Nourishment: Well/Moderate/Poor
- Others: Pallor/Icterus/Cyanosis/Clubbing/Oedema/Lymphadenopathy

- Vital signs: Pulse rate: R.R:
  B.P: Temperature:

CUTANEOUS EXAMINATION

- Lesions: Hypopigmented/Depigmented/Both
- Sites:
- Borders: Regular/Irregular
- Side: Unilateral/Bilateral

- Body surface area (According to the rule of palm):
- Examination of mucous membranes:

  Oral

  Genital
- Others: Hair

  Genitals Nails
- 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	S100B:

FINAL DIAGNOSIS:

KEY TO MASTERCHART

y - years

m - months

F - female

M - male

ON - On immunosuppressants

NOT- Not on immunosuppressants

YES - Disease activity present in past 6 months

NO - No disease activity in past 6 months

pg - picograms

ml - millilitre

MASTERCHART
CASES

Sl no	Age (y)	Sex	Duration (m)	Immunosuppressants	VIDA	VASI %	Type	Disease activity	S100B (pg/ml)
1	9	F	1	ON	4	10	Vulgaris	YES	19.76
2	18	F	2	ON	3	10	Vulgaris	YES	18.082
3	48	F	240	ON	0	25	Vulgaris	NO	11.346
4	5	M	12	NOT	1	10	Vulgaris	NO	13.03
5	7	F	48	NOT	0	10	Segmental	NO	8.1463
6	45	F	36	ON	0	25	Vulgaris	NO	12.525
7	55	M	48	ON	0	25	Vulgaris	NO	11.68
8	17	M	4	ON	3	10	Vulgaris	YES	24.65
9	10	M	12	ON	0	10	Vulgaris	NO	12.689
10	13	M	1	NOT	4	10	Vulgaris	YES	155.006
11	12	F	1	NOT	4	10	Focal	YES	16.061
12	17	M	0.5	NOT	4	10	Segmental	YES	13.702
13	12	F	1	NOT	4	10	Segmental	YES	19.09
14	35	F	7	ON	1	10	Vulgaris	NO	8.988
15	18	M	1	NOT	4	10	Vulgaris	YES	30.54
16	9	M	12	ON	4	10	Vulgaris	YES	41.32
17	30	M	1	ON	4	10	Vulgaris	YES	15.893
18	19	M	6	ON	1	25	Vulgaris	NO	11.177
19	3	F	1	ON	2	10	Vulgaris	YES	17.072
20	35	F	24	ON	4	10	Vulgaris	YES	30.548
21	30	M	9	ON	3	25	Vulgaris	YES	28.17
22	14	M	1	ON	3	10	Segmental	YES	14.04
23	32	F	6	ON	2	10	Vulgaris	YES	15.725
24	32	F	12	ON	1	10	Vulgaris	NO	11.177
25	12	M	4	NOT	2	10	Segmental	YES	23.97
26	20	F	12	NOT	3	25	Vulgaris	YES	20.27
27	38	M	2	ON	3	25	Vulgaris	YES	14.883

28	25	M	0.5	NOT	4	10	Vulgaris	YES	21.28
29	5	M	18	ON	2	10	Vulgaris	YES	11.683
30	28	M	120	ON	3	10	Vulgaris	YES	17.072
31	48	M	4	NOT	3	10	Focal	YES	32.23
32	44	F	12	ON	3	25	Vulgaris	YES	16.56
33	12	M	3	ON	2	25	Vulgaris	YES	15.21
34	8	M	6	ON	2	10	Segmental	YES	22.29
35	12	M	12	ON	4	10	Vulgaris	YES	24.31
36	11	M	12	NOT	3	50	Vulgaris	YES	30.54
37	40	F	18	NOT	4	10	Vulgaris	YES	92.18
38	32	F	60	NOT	1	10	Vulgaris	NO	20.44
39	38	M	12	NOT	1	10	Vulgaris	NO	20.1
40	15	F	12	ON	1	10	Focal	NO	19.93
41	18	F	72	NOT	3	10	Vulgaris	YES	26.166
42	7	F	12	ON	2	25	Vulgaris	YES	25.324
43	13	F	6	NOT	4	25	Vulgaris	YES	27.682
44	15	F	2	NOT	4	10	Vulgaris	YES	39.64
45	56	F	9	NOT	1	10	Vulgaris	NO	19.43
46	6	F	3	ON	2	10	Focal	YES	17.577
47	14	F	12	ON	1	25	Vulgaris	NO	14.04
48	10	M	24	ON	0	10	Focal	NO	12.525
49	36	F	84	ON	0	10	Vulgaris	NO	11.346
50	20	F	24	ON	0	10	Focal	NO	11.514
51	12	F	48	ON	0	10	Focal	NO	14.209
52	15	F	24	ON	2	10	Focal	YES	19.93
53	12	M	3	ON	3	25	Vulgaris	YES	77.029
54	8	F	24	ON	2	10	Vulgaris	NO	19.93
55	8	F	12	ON	2	10	Vulgaris	YES	19.76
56	18	M	2	NOT	4	10	Focal	YES	48.73
57	16	M	36	ON	1	10	Vulgaris	NO	16.39
58	31	F	60	ON	0	25	Acral	NO	17.91

MASTERCHART
CONTROLS

Sl no	Age (y)	Sex								S100B (pg/ml)
1	13	M								22.469
2	50	M								20.7
3	40	F								16.38
4	41	M								18.41
5	24	M								13.19
6	26	M								15.5
7	22	M								11.85
8	16	M								19.93
9	24	F								21.28
10	25	F								10.18
11	23	F								12.18
12	23	M								14.54
13	6	F								19.59
14	10	M								19.76
15	36	F								22.6
16	55	F								13.872
17	17	M								12.693
18	12	F								11.177
19	31	F								18.76
20	40	F								18.756
21	9	M								8.988
22	15	F								19.261
23	24	F								9.493
24	4	F								17.74
25	32	M								18.75
26	22	F								15.219
27	16	M								14.37
28	19	F								8.988
29	17	M								8.81
30	5	F								10.5