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RESEARCH ARTICLE

A REVIEW ON IMMUNOPATHOGENESIS OF VTILIGO

Mahboob Alam[1] and Talat Fatima[2]

1. Assistant Professor, Department Of Dermatology, Narayan Medical College and Hospital, Sasaram, Bihar, India.
2. Senior Resident, Department Of Dermatology, Nalanda Medical College and Hospital, Patna, Bihar, India.

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Abstract

Vitiligo is an acquired depigmenting disease of the skin and mucosa caused due to the destruction of the melanocytes. The white patch leads to social stigma in society and psychological stress in vitiligo patients. The etiopathogenesis of vitiligo is complex interplay of multiple genetic risk factors, environmental factors, neural factors, auto cytotoxicity, oxidative stress by reactive oxygen species, immunological factors, anti-melanocytes and organ-specific auto-antibodies mediated by autoreactive T lymphocytes in a genetically susceptible person. Hence, it is termed polygenic and multifactorial. All these factors, either alone or mixed leads to melanocytes death in a susceptible person leading to formation of depigmented patch. This review is an overview of the different pathomechanism involved in vitiligo.

Introduction:-

Vitiligo is an acquired, idiopathic disorder, usually asymptomatic and progressive, characterized clinically by depigmentedpatch and histologically by degeneration of melanocytes in the involved skin, hair or mucous membrane.[1]Although, a cutaneous pigmentary aberration with no influence on physical capability of the affected individual or on life span, yet it is a dreaded disease of the pigmented race. It puts tremendous stress on the sufferer and adversely affects the social life.Vitiligo affects all races of the world and the overall estimated prevalence of vitiligo worldwide is about 1%. The incidence varies from 0.15% to 8.8% in different countries of the world. The incidence in India is roughly estimated to be 0.25% to 2.5%.[2,3]Vitiligo is mostly seen in skin type III and IV.[4] Onset varies from birth to 81 years of age.[5]Indian studies also showed peak age of onset between 11 to 20 years.[6,7]It affects either sex with heritable constitutional predilection. The inherent instability of the cells of melanocyte system has been postulated in afflicted individuals.[8]Male to female ratio of different Indian studies are 1:1.22[9]and 1:1.6.[10]

It is associated with multifactorial predisposition and triggering factors such as trauma, stress, sunburn and systemic illness. It may be associated with systemic auto-immune and endocrinal diseases.[11,12] Early lesions may have some functional melanocytes and inflammatory infiltrate but late lesions lack functional melanocytes and inflammatory infiltrate.[13]The exact etiology of vitiligo is still uncertain but several hypotheses postulated are the genetic factor, neural, auto-cytotoxic, oxidative stress, immunological, anti-melanocytes and organ-specific auto-antibodies.[14,15]These factors induce the disease in genetically susceptible person.[16]

Corresponding Author:- Mahboob Alam
Address:- Assistant Professor, Department Of Dermatology, Narayan Medical College and Hospital, Sasaram, Bihar, India.

Family History

Family history is present in approximately 20-30% of cases, which possibly indicates the role of genetic factors in the pathogenesis of the disease. The reported data varies from 7.5% to 41% in the world and about 6.25% to 18% in India.[17]The inheritance pattern was thought to be autosomal dominant with variable expression and incomplete penetrance. Monozygotic twins may have similar or dissimilar mode of onset, type, extent and course. The concordance of vitiligo in monozygotic twins is only 23%, indicating that a non-genetic component also plays an important role in vitiligo.[18]A polygenic multifactorial inheritance and a role of acquired factors for its clinical expression are recently observed. The first-degree relative was affected in 13.68%, second-degree 5.66% and third-degree 2.59% of patient.[19]

VitiligoAetiopathogenesis: Hypothesis

The different types of hypothesis suggested for the pathogenesis of vitiligo are:-[20]

1. The biochemical hypothesis (Boissy and Manga, 2004)
2. The neural hypothesis (Cucchi*et al.*, 2000; Lerner, 1959)
3. The autoimmune hypothesis (Garbelli*et al*., 2005; Ongenae *et al*., 2003)
4. A complex biochemical imbalance with defective free radical defense, in which redox enzymes play an important role, could interfare with melanin synthesis and content (Dell'Anna and Picardo, 2006; Schallreuter, 2005)
5. A deficiency in unidentified melanocyte growth factors (Moretti*et al*., 2002)
6. An intrinsic defect of melanocytes adhesion (Gauthier *et al*., 2003)
7. Genetic factors (Zhang *et al*., 2005)
8. The convergence theory (Le Poole *et al*., 1993)

Autoimmune mechanisms with an underlying genetic predisposition are the most likely cause of vitiligo. In the sera of vitiligo patient, antibodies to melanocytes have been found by immunoprecipitation. Damage to the melanocytes in cell cultures occurs by the sera of vitiligo patient, suggesting that the antibodies may be involved in the pathogenesis of the disease. Association of autoimmune disorders such as thyroiditis, pernicious anemia, Addison's disease, diabetes, and alopecia areata have been found in the patient.[20]The cellular mechanism responsible for the appearance of depigmented patch is still uncertain, in contrast to easy clinical diagnosis. The possible pathomechanisms includes the genetic, the immune mediated, the auto-cytotoxic and the neuronal ones, all have been considered in the convergence theory.[21-23]A bipolar approach in the pathogenesis of vitiligo has been developed. Immune mediated damage of the melanocytes supported by one pole (Wijngaard*et al*., 2000; Ongenae*et al*., 2003; Le Poole *et al*., 2004). Toxic-mediated damage of the cell supported by the other pole (Maresca*et al*., 1997; Schallreuter*et al*., 1999; Hasse*et al*., 2004; Agrawal *et al*., 2004; Pelle*et al*., 2005).[24]

Le Poole's group by in vitro evidence supports a link and temporal sequence between the two phenomena and suggested the intrinsic damage of the melanocytes leading to an immune response.[25] The fragility of the melanocytes and susceptibility to damage represents the convergence point of the non-immunological mechanism.

Aetiopathogenesis and Immunology

A complex interplay of genetic, immunological, environmental and biochemical factors are involved in the pathogenesis of vitiligo. ROS (reactive oxygen species) and H_2O_2 have been documented in active vitiligo skin. Excess H_2O_2 hampers the tyrosinase activity through oxidation of methionine residues in this key melanogenic enzyme. H_2O_2 inhibit the mechanisms for repairing this oxidant damage. However, immunological phenomena are also involved in vitiligo, particularly in established chronic and progressive disease. Both innate and adaptive immune systems are involved, with a dominant role of T-cells. Sensitized $CD8^+$ T-cells are targeted to melanocyte differentiation antigens and thereby destroy the melanocytes, either as the primary event in vitiligo or as a secondary promotive consequence. There is speculation on the interplay between ROS and the immune system in the pathogenesis of vitiligo, which is still unclear.[26]The theorem describes an autoimmune process occurring in a genetically susceptible host and triggered by a variety of host and environmental factors.[27]

Melanocytes destruction in vitiligo patients are attributed to three major factors:-[28]

1. Firstly vitiligo patients inherit a set of three "vitiligo" genes which causes melanocytes destruction.[29,30]There are many different sets of three genes that can cause vitiligo, so that the same gene is not inherited in every individual.

2. The second factor is the difference in the melanocytes in the vitiligo patient and the normal person. Melanocytes from vitiligo patient require different and more fastidious culture conditions as compared to melanocytes from normal person.[31,32] Melanocytes from vitiligo patient are much more sensitive to phenolic chemicals and undergo apoptosis under such exposure.[33,34]
3. The third factor is the activation or inhibition of the involved genes by an environmental agent, which causes destruction of the susceptible melanocytes. The activated gene causes an immune reaction that induces apoptosis of melanocytes.[35-38]

The cause of non-segmental vitiligo seems to require the following three factors:-[39]
1. Complex of vitiligo susceptibility genes that influence the autoimmune response
2. Genetically abnormal melanocytes
3. Environmental or physiological factors that activates the genetic program for melanocyte destruction.

The role of genetics in the pathogenesis of vitiligo

Several genes affect the immune and the pigment system that predisposes someone to develop vitiligo. A precipitating factor must elicit an interaction between the immune system and the melanocyte, resulting in melanocyte destruction.[40]Vitiligo susceptibility is a complex genetic trait that may involve genes:-
1. Encoding for enzymes playing a role in phenol or catechol metabolism
2. Encoding for enzymes or transcription factors maintaining the redox balance
3. Encoding for T-cell receptors and transcription factors of T-cells for the regulation of autoimmunity, all or none in combination with environmental factors

Biochemical and immunological aberration acts in concert within the same time frame to develop vitiligo. Several genetic markers have been studied in vitiligo though few have been confirmed as being truly associated with the disease.[41]

Table 1:- Genetic influences involved in vitiligo.

Gene	Expression	SNP/Mapping	Effect
CAT[42]	Vitiligo	Exon 9 of the CAT gene/11p13	Quantitative deficiency of catalase activity
COMT[43]	Acrofacialvitiligo	Codon 108/158 of COMT gene/22q11.2	Preventing the formation of toxic *o*-quinones
HLA (LMP/TAP gene)[44]	Vitiligo	G/A in exon 10 of the TAP1 gene (D637 G), G/A exon 3 (R60H) of LMP2 gene, G/T intron 6 marker of LMP7	Transporter associated with antigen-processing (TAP1); subunits of the immunoproteasome (LMP2/LMP7) associated with antigen presentation
CTLA-4 AIS1[45]	Graves, type 1 diabetes, hypothyroidism,generalized vitiligo and Hashimoto thyroiditis	6.1-kb region 3¢/2q33 −/Chromosome 1p31	CTLA-4 is a ligand for B7 molecules (CD80 and CD86) on the surface of antigen-presenting cells (APCs), T-cell regulation.

Abb:-CAT, catalase; COMT, catechol-*O*-methyl transferase; CTLA-4, cytotoxic T-lymphocyte antigen-4; AIS1, autoimmune susceptibility locus 1; TAP1 and TAP2, transporter associated with antigen processing protein 1 and 2; LMP2 and LMP7, low molecular weight polypeptide 2 and 7.

The genetics of generalized vitiligo was reviewed by Spritz.[46]Positive family history is found in about 20% of cases and similar concordance in identical twins. There is strong support for relatively few susceptibility genes, including certain HLA genes, *PTPN22*, *NALP1* and perhaps *CTLA-4* (cytotoxic T-lymphocyte antigen-4). They are associated with a tendency to autoimmunity. HLA molecules present peptides to T-cells and confers more efficient presentation of cognate autoantigen, thereby predisposing to autoimmunity; an example is HLA-DQB1*0301. *NALP1* is involved in the innate immune response to pathogens. The fine-mapping studies showed associations with chromosomes 7 and 9.[47]A recent genome-wide association studies by Jin *et al.*[48]showed significant associations of generalized vitiligo with the following loci, HLA class I and II molecules, *PTPN22*, *LPP*, *IL2RA*, *UBASH3A* and *C1QTNF6*. Two additional immune-related loci identified were *RERE* and *GZMB*. The HLA class I association occurred in the regions between *HLA-A* and *HCG9*, consistent with previous reports of strong associations with the HLA-A*02

allele. The HLA class II gene association occurred in the region between *HLA-DRB1* and *HLA-DQA1*, which is associated with the HLA-DRB1*04 allele. With the exception of *PTPN22*, the associations were similar whether patients had vitiligo alone or vitiligo as well as another autoimmune disease. An association with a non-immune-related gene was identified: SNPs (single nucleotide polymorphisms) in the gene encoding tyrosinase, *TYR*. Tyrosinase is a melanocyte enzyme that catalyzes the rate-limiting step in melanin biosynthesis and is a putative target autoantigen in vitiligo.One locus at chromosome 1p31.3-32.2 (labeled as an autoimmunity susceptibility locus - *AIS1*) contains multiple genes. Single nucleotide polymorphisms (SNPs) in one of these candidate genes (Forkhead box D3-*FOXD3*) are co-segregated with vitiligo.[49]A second candidate gene is NACHT-LRR-PYD-containing protein 1 (*NALP1)* on chromosome 17p13.[50] NALP1 protein is a component of the inflammasome, a cytoplasmic multiprotein complex that regulates the innate immune system. It mediates the maturation of proinflammatory cytokines like interleukin-1b and -18, and stimulates cellular apoptosis.[51] This gene could be involved in inducing melanocyte destruction. Variants in protein tyrosine phosphatase non receptor type 22 (*PTPN22)*[52]that putatively functions as a general autoimmunity susceptibility loci, and SPARC-related modular calcium binding protein 2 (*SMOC2)*[53] function unknown, may also be associated with the vitiligo.

A Role of oxidative stress in vitiligo

Melanocytes are susceptible to oxidative stress. In the cell, 4 –*tert*-butylphenol (4-TBP) can undergo enzymatically driven redox-cycling to generate intermediates that are capable of inducing oxidative stress. The enzymatic redox-cycling of phenol results in the formation of phenoxyl radicals which compromise the anti-oxidant defense mechanisms.[54]In melanocytes, tyrosinase causes hydroxylation of mono-phenols (e.g. tyrosine) and *o*-diphenols (e.g. dihydroxyphenylalanine) to *o*-quinones. The unstable *o*-quinones undergo non-enzymatic reactions, cyclization to form aminechrome or *o*-benzoquinones. Tyrosinase mediates the conversion of both 4-TBP and Tyrp1 results in the formation of 4-tertiary butylcatechol, which is converted to a stable t-butyl-*o*-benzoquinone.[55]This reaction requires hydrogen peroxide and results in the generation of peroxides. Consistent with this, is the observation that patients with vitiligo express elevated levels of H_2O_2 in their epidermis.[56]Thus, 4-TBP, a competitive inhibitor of tyrosinase is metabolized in melanocytes and may generate reactive oxygen species capable of damaging these cells and inducing apoptosis. The normal melanocytes cultured in the presence of 4-TBP undergo dose-dependent apoptosis. Hence, the mechanism by which 4-TBP causes melanocyte death may play a role in the pathogenesis of vitiligo. Oxidative stress induced by hydrogen peroxide causes down-regulation of microphthalmia-associated transcription factor (MITF) in melanocytes.[57]MITF stimulates melanin synthesis by up-regulating expression of melanogenic enzymes, tyrosinase-related protein-1 (Tyrp1). MITF, most active when phosphorylated, controls melanocyte proliferation. MITF increases sensitivity of melanocytes to 4-TBP.[58]Cytokine ET-1 increases expression and phosphorylation of MITF, hence increase melanocyte sensitivity to 4-TBP-induced cytotoxicity.[59] Although melanin content did not affect sensitivity to 4-TBP, expression of Tyrp1 significantly increased sensitivity. Melanocytes that express functional Tyrp1 were significantly more sensitive to 4-TBP than Tyrp1-null cells. Thus, melanocytes in vitiligo demonstrate reduced ability to withstand oxidative stress induced by hydrogen peroxide due, partly, to a disruption in MITF regulation of Tyrp1. Thus, MITF is the "master regulator of the melanocyte" controlling expression of the major melanocyte-specific proteins required for melanin synthesis. Evidence of oxidative stress has been demonstrated in both the skin and blood of individuals with active vitiligo.[60]The generation of reactive oxygen species (ROS) is hazardous to the cells, initially causing lipid peroxidation,denature proteins, damages nuclear and mitochondrial DNA, mediate release of proinflammatory cytokines and finally inducing apoptosis.[61]

It has been found that vitiligo melanocytes exhibit:-[62]

1. More reactive oxygen species
2. Membrane peroxidation
3. Impaired mitochondrial electron transport chain complex 1
4. More readily induced apoptosis

Reactive Oxygen Species (ROS) in vitiligo

Ahsan *et al.*[63]has reviewed the potential role of oxygen free radicals in human autoimmune disease. Free radicals are atoms or molecules having one or more unpaired electrons. They are reactive and unstable. Free radicals derived from oxygen include four moieties. They are superoxide anion radical, singlet oxygen, hydroxyl radical and perhydroxyl radical. These are termed ROS, which are routinely generated during cellular, biochemical and metabolic reactions.ROS can denature proteins, alter apoptotic pathways, damage nuclear and mitochondrial DNA and mediate release of proinflammatory cytokines.[64]Generation of a reactive *o*-quinone from MBEH via tyrosinase was confirmed recently *in vitro*, with isolation of several by-products with potential toxicity to melanocyte.[65]

Cytotoxic experiments have also confirmed that both 4-TBP and MBEH induce melanocyte death, but by different pathways. 4-TBP activates the caspase cascade and causes DNA fragmentation with apoptosis but MBEH induces release of Mobility Group Box-1 protein, which causes necrosis. Confirmation has been done by ultrastructural studies of MBEH-treated melanocytes.[66]Many drug are potential exogenous sources of ROS, especially when metabolized by CYP enzyme, as they produce reactive quinones and semiquinones.[67] Proton pump inhibitors were recently shown to reactivate vitiligo, probably through generation of free radicals or pH changes relating to melanogenic enzymes.[68]

Immunological mechanisms in vitiligo

Table 2:- T-cell changes in vitiligo.[26]

Phenotype	**Level**
Peripheral	
Total T-cell	n/↓
$CD4^+/CD8^+$	n/↓/↑
$CD45RA^+$	↓
$CD45RO^+$	↑
Total NK T-cell	↓
Tissue	
Total T-cell	↑
$CD8^+$	↑
$CD45RO^+$	↑
IL-2 receptor	↑
CLA^+	↑
Cytokine	
Peripheral	
IL-1	↑
IL-6	↑
IL-8	n/↑
TNF-α	n/↑/↓
IFN-γ	↓
TGF-β	↓
IL-2 receptor	↑/↓
TNF receptor	n
Tissue	
IL-2 receptor	↑
IL-6	↑
TNF-α	↑
IFN-γ	↑
IL-10	↑

T cells expressing a cutaneous lymphocyte-associated antigen, typical of skin-homing T cells are found at the edge of depigmented patches. This finding is consistent with a hypothesis that, lesional T cells, rather than circulating anti-melanocytic antibodies may be responsible for the patchy destruction of cutaneous melanocytes.Melanocytes seem to be uniquely fragile in people with a tendency to vitiligo. Numerous endogenous and exogenous sources of H_2O_2 and other ROS have been described in vitiligo, which are deleterious to a variety of melanocytic cellular processes, particularly in the context of impaired cellular antioxidant defense. The resultant protein and lipid damage could be sufficient, on its own to initiate melanocyte failure, but another effect of oxidization could be to initiate melanocyte failure and apoptosis leading to uptake by Langerhans cells. If these Langerhans cells become activated, they may trigger a melanocyte-reactive immune response that can eradicate melanocytes in the skin, leading to depigmentation. This immune response principally involves cytotoxic T-cells.Vitiligo is occasionally associated with other autoimmune diseases, which suggests an immunological pathomechanisms. There is detection of serum autoantibodies to melanocytes in active disease, especially against melanosomal proteins such as the tyrosinase family of enzymes.[69]

Conclusion:-

Vitiligo is multifactorial in origin. The best supported hypothesis is the autoimmune pathology in genetically susceptible person. Another one is the toxin mediated damage of the melanocytes by multiple environmental factors. The different pathomechanism may contribute to formation of different clinical varieties of vitiligo.

Conflicts of Interest

The authors stated that they had no conflicts of interest.

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