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RESEARCH ARTICLE

ADVANCES IN DIAGNOSTIC TECHNIQUES FOR HEMOGLOBINOPATHIES AND CONTEMPORARY MANAGEMENT STRATEGIES FOR THALASSEMIA-A COMPREHENSIVE REVIEW

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Abstract

Hemoglobinopathies, comprises ofstructural hemoglobin variations and thalassemias, are prevalent single-gene illnesses worldwide which frequently results in notable morbidity and mortality. These disorders are caused by mutations that impact the amount or caliber of hemoglobin produced. Geographically, these disorders are more or less common; in some areas, sickle cell anemia and beta thalassemia are particularly common. The genetic foundation, clinical presentations, and methods for diagnosing hemoglobinopathies are all included in this review. The focus is on improvements in diagnostic techniques, including capillary zone electrophoresis (CZE), high- performance liquid chromatography (HPLC), peripheral blood smear (PBS) studies, and complete blood counts (CBC). The study also discusses modern management techniques, such as novel gene therapies and blood transfusions, giving a thorough picture of the condition of care for patients with these illnesses today. The review also emphasizes how crucial early and precise diagnosis is to reducing the risk of serious illness consequences and enhancing patient outcomes.

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Introduction:-

With very few exceptions, hemoglobinopathies are the most prevalent single-gene disorders globally, and they are inherited in an autosomal recessive manner.[1] These are categorized according to whether or not they have one or more globinchainimpairments, or thalassemias, and/or structurallyaberrant hemoglobin(Hb), such as hemoglobins S, C, D, and E. [2]

Hemoglobinopathies are a group of disorders where abnormal sickle Hgb partly or totally replaces normal adult hemoglobin (HbA). SCA, or sickle cell anemia, is one of these illnesses. Due to a single base change in the DNA, valine instead of glutamic acid is present at position 6 of the hemoglobin molecule's beta chains.[3]

Nearly saturated hemoglobin (Hb), which binds oxygen in the alveoli and carries it to the tissues, is found in red blood cells (RBCs). RBCs absorb carbon dioxide (CO2) released from tissues and digest it biochemically. Low hemoglobin concentrations cause red blood cells' ability to carry oxygen to be diminished, which makes tissues hypoxic and causes symptoms including exertional dyspnea and overall malaise.[4]

Each of the four globin polypeptide chains (2α and 2β) that make up a hemoglobin molecule contains a heme molecule that connects with oxygen. Hemoglobin molecules and red blood cells depend on the structural stability and

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quantitative balance of their globin chains. Hemolytic anemia arises from abnormalities (pathogenic variations) in the genes encoding the globin chains (HBA for α-globins and HBB for β-globins) that alter the amount or structure of globins..

The most prevalent kind of hemoglobinopathy in humans is thalassemia, which is brought on by pathogenic variations that impair protein synthesis, causing a quantitative imbalance in globin chains. [5,6]Hemoglobinopathies like sickle cell disease are caused by additional pathogenic mutations that lead to structural anomalies in globin chains.

Frequencyofhemoglobinopathygenecarriersintheworld'spopulation

Region	GeneCarriers
Africa	5 to30%
Arabnations	5 to40% Upto60%regionally
CentralAsiaandIndiaSouth-EastAsia	10 to20% 5 to40% Upto70%regionally
USAandCentralAmerica	5 to20%
ItalyGreece Turkey	7 to9% 6 to7% 7 to10%
Germany, Great Britain, Portugal,Spain,France, theNetherlands,Belgium, Scandinaviancountries	Amongtotal population:0.5to1% Amongimmigrants:5%
Albania,TheformerYugoslavia, Croatia, Bosnia-Herzegovina, Bulgaria	2 to5%
Russia	Rare
Transcaucasia	Upto5%

Thalassemia

The most prevalent monogenic condition in the world, thalassaemia, is caused by either reduced or absent globin chain production. [7] A person can inherit the genes for thalassemia from their parents, which can result in the disease or characteristic.[8]

Thalassemia comes in two primary forms: beta and alpha. Beta thalassemia is the result of insufficient beta globin chain production in people with alpha thalassemia, while beta thalassemia is caused by insufficient alpha globin chain production. [9]

The etiologyof thalassemia is linked to the quantitative imbalance of α-and β-hemoglobin chains, which causes reactive oxygen species formed by free heme released from the excess hemoglobin chain to peroxide lipids and proteins in red blood cells.[4]

A reduction in the synthesis of globin (β+ and α+) or its absence (β^0 and α^0) can be caused by mutations or deletions (Cunninghan, 2010). More than 95% of cases of α-thalassemia are caused by deletions; these deletions are -α3.7, -α4.2, and -α5.2 and -α 20.5, which typically cause α0-thalassemia. Based on their clinical severity and transfusion requirement, thalassemia syndromes can be classified phenotypically into transfusion-dependent thalassemias (TDTs) and non transfusion-dependent thalassemias (NTDTs).

α –Thalassemia

A deficiency in the α-globin chain production is the cause of α-thalassemias. They arise from partial (α+) or total (α0) deletions, or less frequently, mutations, of one or more of the four α-globin genes (αα/αα) at the molecular level.[10]

Clinical symptoms are more severe in those with more deleted genes. Related to homozygous α^{0}-thalassemia, fatal hemoglobinBart's hydrops fetalis ischaracterized bysevere hemolytic anemia, hydrops, and ascites in utero (23–38 weeks) or shortly after birth, which can result in fetal death. Preeclampsia and other obstetrical problems are more common in mothers bearing these fetuses.[11]

Thegenes α1andα2arefoundonthe α-globinlocus, whichissituatedonchromosome16, whiletheGγ, Aγ,δ,and βloci are found on chromosome 11. The expression ofglobin genesvaries in step with the developmental stage; for example, the primary hemoglobin tetramer's composition changes from α2 η2 during fetal development to α2β2 in maturity. [12]

BetaThalassemia

Gγ, A γ, δ, and β loci are located on chromosome 11 and make up the β-globin locus. The expression of the globin gene variesaccordingonthe developmental stage, withthe predominant hemoglobintetramer's compositionshiftingfromα2 γ2 duringfetaldevelopmenttoα2β2inmaturity.[13]Amissense mutation(singleaminoacidsubstitution)intheβ-globingeneis the primary cause of β-thalassemia. [14]

The cause of β-thalassemia is either insufficient (β+) or nonexistent (β0) β-globin chain synthesis. Mutations in the β-globin gene are their molecular cause. The majority of patients are from Asia, South-East Europe, the Arab world, and the Mediterranean region. Between the ages of three and six months, hematological changes start to show symptoms. [12,15]

Deletions affecting the entire HBB gene, the HBB and HBD genes, and the HBB gene alone. Rare mutations also exist where the connected β-like globin genes are present and structurally normal, but no expression is produced due to the deletion of the locus control region (LCR), the regulatory section of the HBB locus.[16] According to a WHO report on b- thalassemiainIndia,thecountry'scurrentpopulationof35.6–47.5 millioncarriersofthecondition hasacomparablecarrier frequency of 3-4%.[17]

In India, β-thalassemias and their relationship to hemoglobin E (HbE) and hemoglobin S (HbS) pose a serious health risk and greatly increase morbidity and mortality. Epidemiologically, the identification of these conditions is crucialand population screening can help avoid them. About 250 million people, or 4.5% of the global population, have agene for haemoglobinopathy, which may be pathogenic. Approximately 0.3 million newborns are born with a serious hemoglobinopathy every year. [18]

Accurately identifying different hemoglobin variations, such as β-thalassemia trait, can stop the development of more severe conditions like thalassemia major in infants. [19]

According to Professor J.B. Chatterjee's 1959 report, West Bengal had an incidenceof 3.7% for the beta thalassaemia trait and 3.9% for HbE.[20] Earlier research has already documented the significant prevalence of β- thalassemia and aberrant HbE among Eastern Indian normal populations, including the diverse population of Kolkata. [21]

SickleCell Syndrome

SCD is a monogenic condition in which patients express sickle cell hemoglobin, or HbS, exclusively or mainly. Patients with SCD always carry the HbS allele, which codes for a valine rather than a glutamine acid residue at position 6 of β-globin in homozygous or compound heterozygous individuals. Position 6 of the hydrophobic amino acid valine promotes the polymerization of deoxygenated HbS and the creation of sickle-shaped erythrocytes. Following the hemoglobin transition, individuals experience chronic hemolysis, acute vasoocclusive crises, and a chronic vasculopathy affecting all organs when HbS is replaced by HbS throughout infancy.[22] HbS in its deoxygenated formforms a polymer in red blood cells(RBCs) under hypoxic conditions, causing the cells to obstruct capillaries and potentially form thrombi. Ischemia is brought on by vascular blockage brought on by a thrombus, which results in pain episodes (also known as sickle cell crisis) in the chest and limbs. [23]All indicationsof abnormal HbS levels (percentage of HbS >50%) are referred to as "sickle-cell disease." These comprise a variety of mixed heterozygous hemoglobinopathies (HbS/βthalassemia, HbSC disease, and other combinations) as well as homozygous sickle-cell disease (HbSS). Lack of oxygen causes sickle cells, which induce vascular obliterations and tissue death in practically all organs (skin, liver, spleen, bone, kidneys, retina, CNS). Most of the time, chronic hemolytic anemia is

manageable. [24]After viral infections, aplastic crises with severe anemia are observed. [25]Patients who receive optimal care should expect to live for 50–60 years. Hematological or clinical symptoms do not affect heterozygous HbS gene carriers.[26] Approximately 300 000 to 400 000 newborns are affected by a DNA variant that causes defective haemoglobin synthesis; of these, approximately 300 000 have sickle cell syndromes, and approximately 40 000 have transfusion-dependent β-thalassemia major. This genetic variant is thought to account for 7% of the global population. [27]

ClinicalManifestation

Beta thalassemiadisease occurs between 1 and 2 years of age, and jaundice, cholelithiasis, and splenomegalyareobservedinadditiontoanemia.Increasedbonemarrowhematopoiesisleadstothickening of the skull and prominence of the zygomatic arches, resulting in a so-called thalassemic facial appearance extramedullary hematopoiesis and cranial bone changes.[28]

Homozygotes (HbSS) exhibit serious symptoms, such as anemia, feeding disorders, splenomegaly, and recurrent infections within the first 2 years of life. In addition, vascularocclusion can cause cerebral infarctionthatmaybeassociatedwithdevelopmentaldelay,acutechestsyndrome,legulcers,andaseptic necrosis of bone. [29]

α-globingene.Individualswithgenemoredeletionspresentmoresevereclinicalsymptoms.Fetal hemoglobin Bart's hydrops fetalis. [26]

HBHComplicationsincludecardiacproblems,gallstones,lowerlegulcers,andfolicaciddeficiency.

DiagnostictechniquesforHemoglobinopathies

Traditional Methods

CBC&PBS Study-

Inthecaseofthalassemiascreening,apositivescreeningresultisusuallydefinedasmeancorpuscularvolume (MCV) of less than 80 fL and/or mean corpuscular hemoglobin (MCH) value of less than 27 pg.[30]

Thenormalpopulation'sMCVandMCHdistributions'twostandarddeviationsareusedtodeterminethesecutoff points. The benefit of using MCV and MCH for thalassemia screening is that automated hematology analyzers canproduce fast, affordable, reliable, and accurate results. Low MCV can, however, also be caused by microcytic anemias, suchasirondeficiencyanemia(IDA);incontrasttoMCH,whichappearstobeconsistentacrossmanyautomated hematology analyzers, variations in MCV from various automated blood cell counters have also been documented.[31] Moreover,a false-negative resultduring thalassemiascreening could result from the combination ofheterozygous α- thalassemia with β-thalassemia phenotype alone or with glucose-6-phosphate dehydrogenase deficiency, whichcould cause a normal MCV.[32,33] As a result, screening for thalassemia with both MCV and MCH would be more appropriate than withMCV alone. It would also be crucial to ascertainthe cutofflevels for each utilizing the automated hematology analyzer that is used in each laboratory.

Theinterpretation of peripheralbloodsmears using bothMCV andMCHwouldbe regardedasa crucial thalassemia screening technique. Thalassemia patients typically have microcytosis, hypochromia, and anisopoikilocytosis intheirRBCmorphology.HypochromicRBCsaredescribedashavinganincreaseinthediameterofthecentralpallorof RBCs, which is more than one-third of their diameter. Microcytes can be assessed by comparing the size of RBC with thoseofthenucleusofsmalllymphocytes.NumerousaberrantRBCmorphologies,suchasschistocytes,microspherocytes, target cells, polychromasia, and nucleated RBCs, can cause anisopoikilocytosis. It is impossibleto identifyaparticularformofthalassemiadiseasebasedsolelyonRBCshape,andthe findingsofperipheralbloodsmears may only indicate specific types of thalassemias from other causes of anemia, such as IDA or anemia of inflammation. Thedegreeofvariationinred cellsizeis measured bythered celldistribution width(RDW),and anincreasein RDW is a characteristic of variouscausesof microcytic anemia, mostnotablyIDA. Althoughallthalassemia syndromes produce homogenousmicrocyticredbloodcellswithoutacorrespondingriseinRDW,somethalassemiasyndromesexhibitlarge increases in RDW, such as Hb H disease and minor beta-thalassemia.[34]As a result, although the RDW is not a helpful stand-alone screening sign, it may offer information that is useful as an adjuvant to diagnosis. Because thalassemia causes a microcytic anemia with an increase in RBC number, the RBC count is also helpful as a diagnostic adjunct.

However,anemiaofchronicillnessandIDAareusuallylinked withadropinRBCnumberthatisproportionatetothe degree of anemia. But you shouldn't rely just on the RBC count to check for hemoglobinopathies and thalassemia.In lightofthis,numerousindicesbasedoncomponentsofthecomplete blood count (CBC) have been established for the purposeofscreening forhemoglobinopathiesand thalassemia;however,noneoftheseindicesoutperformthevalueof the combination of MCV and MCH in identifying cases for further investigation.

ElectrophoresisandHPLC-

Using an electrical field and gel, molecules or compounds can be separated using the electrophoresis technique. Clinical laboratoriescontinueto utilizeitextensivelyforisoenzymedifferentiationandproteinelectrophoresis.In wealthy nations, automated procedures like capillary electrophoresis have replaced the manual fabrication of gel and electrophoresis on a relatively small scale. One common custom electrophoresis method is cellulose acetate electrophoresis. Identification of Hb A, F, S/G/D, C/E, and H as well as additional variants is known to be made possible by it.[35]

For the characterisation of hemoglobin, capillary zone electrophoresis (CZE) and high-pressure liquid chromatography (HPLC) are the two most often employed high-throughout screens.[36]

Using spectrophotometric detection at 415 nm, HPLC and CZE identify haemoglobin species based on their differentialelutionormigration.These parathion processisthemainanalyticaldistinctionbetweenthetwo techniques: CZE separates proteins according to buffer pH, isoelectric point (pI), and endosmotic flow, whereas HPLC separates various haemoglobin variants based on their distinct retention times, enabling quantitative evaluation of each variant. Thesemethods are quite sensitive and can identify variations in hemoglobin that are common or uncommon.[37] Automatedcapillary electrophoresis is a popular technique that has proven useful in identifying certain variants that are difficult to discriminate in numerous automated HPLC systems. [38]However, these techniques have trouble identifying uncommon or complicated variants and necessitate additional testing, which delays diagnosis and treatment commencement.[39] Mass spectrometry and gel electrophoresis are two more laboratory techniques.Any anomalies found utilizing HLPC or CZE can be further characterized or confirmed with the aid ofthe first.[40] As a supplementarytechnique to HPLCor CZE, the second one might increase the sensitivity of Hb analysis. It might also be utilized for the investigation of hemoglobinopathies in large-scale population research. However, a highlevel of knowledge is needed to operate and maintain a massspectrometer. Thus, more research will be required to determine the most effective method for using them in a clinical laboratory.[41]Several companies offer a range of HPLC techniques that uses cation exchange chromatography to separate hemoglobin. The US Food and Drug Administration has approved these techniques for measuring hemoglobin F, hemoglobin A2, and hemoglobin A1c (glycated hemoglobin) in individuals with diabetes inorder to track their treatment.[42] For three different types of laboratories, the International Committee for Standardization in Hematology suggested laboratory testing in 1978.[43]

Accordingtothatrule,alkalineelectrophoresisshouldbeabletobeperformedinascreeninglaboratory.Thereference laboratory was required to conduct extremely challenging tests, such as globin electrophoresis and citrate agarelectrophoresis. Because these electrophoresis procedures involved manual steps for hemoglobin analysis from reagent preparation, electrophoresis, and data interpretation, the effectiveness of the identification process depended on the laboratory professional's skill. New guidelines on hemoglobinopathy and thalassemia have been published as a result of advancements in laboratory techniques and growing understanding of these conditions. Hemoglobin A2 and E can be separated using capillary zone electrophoresis. There are several chromatographic methods that can be used to separate different hemoglobin species. Notably, glycated from non-glycated hemoglobin species can be separated using boronate affinity chromatography, which is helpful for keeping an eye on diabetic patients,including those with hemoglobin variations. [39]

CharacteristicsofRoutineLaboratoryMethods			
CompleteBloodCount(CBC)	**High-Performance Chromatography (HPLC)**	**Haemoglobin Electrophoresis**	**Molecular Genetic Testing**
Thisroutinetestprovidesvaluable Informationaboutthecellular Componentsofblood,includingred Bloodcell(RBC)count,haemoglobin Concentration,haematocrit,mean Corpuscularvolume(MCV),mean Corpuscularhaemoglobin (MCH), and Meancorpuscularhaemoglobin Concentration(MCHC). Anomaliesintheseparameters, Suchasmicrocytosis, Hypochromia,oranisocytosis,mayindicate the presence of haemoglobinopathies	Thisprovides highsensitivity, Accuracy,andabilityto Quantifydifferent Haemoglobinvariants.HPLC Separateshaemoglobin Variantsbasedon their Interactionwith a Chromatographiccolumnand Detectionsystem.Thismethod Allowsfortheidentification Andquantificationofboth Commonandrarehaemoglobinvariants,making Itavaluabletoolfor Diagnosing Haemoglobinopathies	Thistechnique relieson the Principlethathaemoglobin Variantsmigrateat Differentratesunderan Electricfielddue to Variationsintheircharge Andsize.Haemoglobin Variantscommonly Assessedinclude HaemoglobinA(hba), HaemoglobinA2(hba2), Haemoglobin F (hbf), And variousabnormal haemoglobinsassociatedwith haemoglobinopathies	Moleculargenetic Testingplaysacrucial Roleinconfirmingthe Diagnosisof Haemoglobinopathies Andidentifying Specificgenetic Mutationsresponsible Forthedisorder. Techniquessuchas Polymerasechain Reaction(PCR),DNA sequencing, and gene-specific Mutation analysisare Usedtodetectpoint Mutations, Deletions,or Insertionsintheglobin genes. Molecular Genetictesting Provides Valuable Information About the Genotypeofthe Patient,which is Essentialfor Genetic Counsellingand Familyplanning

NewerTechnique

MassSpectrometry-

A method for identifying compounds based on the mass (molecular weight) to charge ratio is massspectrometry. The method's key benefit is that it requires very little particular binding reagent for the target molecules. More precise identification and less interference are made possible by the straightforward analytical idea. Utilizing mass spectrometry to analyze hemoglobin is a complex process that requires a laboratory to possess both sophisticated equipment and specializedknowledge inproteinanalysis. It canidentifyhemoglobin using the molecular weight ofthe complete molecule inadditionto partially analyzing the amino acid sequence. It is helpful for confirming DNA sequencing results and identifying novel variation. [44]

MolecularTechnique-

The goal of molecular diagnostic methods is to identify these typical deletions. Although DNA blotanalysis has been employed in the past, gap-PCR polymerase chain reaction (PCR) techniques are currently the most oftenused.[45] Molecular genetic testing is essential for determining the underlying genetic changes causing hemoglobinopathies. Examples of this testing include DNA sequencing and polymerase chain reaction (PCR). By identifying point mutations, deletions, or insertions intheglobingenes,thesetests

canprovidecomprehensiveinformationonthepatient'sgenotype. Inrecent years,developments in molecular diagnostics, including. [46]

A wide range of molecular methods are employed to identify mutations in the globin gene. The following groups of molecular approaches can be targeted based on the type of mutation: (1) Structural variation detection techniques, such as gene deletion, duplication, or triplication; (2) Sequence variation detection techniques, such as nucleotide substitution, insertion, or short insertion/deletion techniques.

Gap polymerase chain reaction (PCR) particularly designed for the deletion in question can be used to identify known gene deletions. For unknown gene deletions, southern blotting with labeled complementary gene probes may be utilized. The probe amplification method dependent on multiplex ligation. For some ethnic populations, methods like amplification refractory mutation system, reverse dot blotting, denaturing gradient gel electrophoresis, and allele-specific PCR can be used to detect common sequence changes at a reasonable cost. Many labs are now able to sequence the entire globin gene, including the promoter, 3′ UTR, exon-intron boundaries, and deep introns, thanks to the quick advancement and falling cost of sequencing technology. Specifically, targeted genes, exomes, or even entire genomes can be sequenced using massively parallelsequencing technology.

Multiplex ligation-dependent probe amplification is an alternative method for characterizing deletions (MLPA) This method detects gene deletions based on a decrease in gene dosage by quantifying the number of copies of a gene. In MPLA, the chromosomal region of interest is covered at appropriate intervals by a sequence of oligonucleotideprobe pairs that anneal to DNA in nearby locations. DNA ligase will covalently connect the probes that anneal at adjacent places when the oligonucleotides anneal to their target sequences. The resulting ligated sequences are then amplified by PCR. MLPA can identify novel deletions that are either not covered by primer sets used by a particular laboratory because of its patient population, or that have not been previously reported. While MLPA had trouble consistently detecting trans HBA deletions,this approach has been utilized successfully to characterize known and novel deletions of the HBA locus that cause α- thalassemia.[47] Gene deletions that are known or unknown can befoundusingthe(MLPA)approach.BecauseMLPAissimpletouse,extremelysensitive,andcapableofdetecting many types of deletions, it is commonly employed.

During the last few years, advancements in molecular diagnostics, particularly next-generation sequencing (NGS), have revolutionized genetic testing, shifting to a rapid transition from a research setting to a clinical application and turning into the preferred technique in a lot of clinical genetics labs. Comprehensive study of haemoglobin gene mutations, including uncommon and novel variants, may be made possible by NGS-based techniques. This would not only help with accurate diagnosis but also offer insightful information about the prognosis of the disease and the relationship between genotype and phenotype. [48]

Sangersequencing-

Since the globin genes are very small (1.2 kb for HBA1 and HBA2 and 1.8 kb for HBB), direct sequencing of the globin genes covers not only all exons but also all introns and the 5¨ and 3´ untranslated regions, in contrast to most hereditary disorders. A two-stage amplification process is necessary for direct sequencing of the highly homologous duplicated HBA genes. The first step of the amplification process amplifies the HBA1 or HBA2 gene. Each HBA gene can produce two overlapping fragments from the second amplification utilizing nested primers that are short enough to be sequenced in a single sequencing reaction and cover the promoter all the way to the 3´UT. A fully automated work-flow for the direct sequencing reaction can be facilitated by adding common sequence tags to the 5´ end of the allele-specific amplification primers. These primers are complementary to a set of Universal Sequencing primers (21M13-F and M13REV), which can then include all amplicons across all globin genes in a single reaction. The HBB gene was sequenced using the same method. There are four PCR fragments that overlap in the HBB gene ddNTPs labeled with fluorescent dyes in four colorsare used as bait for automatic sequencing equipment (such as the ABI310, 3700, 3730 [Life Technologies, Applied Biosystems, CA, USA]), preferablywithcapillarychemistryseparation(suchas the ABI BigDye® Terminator [Life Technologies, UK]. Analyzingdata is done using any suitable data-analysis program (like the Seqscape suite). Direct sequencing employing life technologies can identify both known and unknown point mutations, minor deletions and insertions that cause Hb variants and alpha and beta thalassemia. [49]

DNAarrays-
DNAmicroarrays,sometimesreferredtoasDNAchips,arehigh-throughputtechnologiesthatallowforthesimultaneousdetection of many genetic variants. This method is very useful for determining globin gene mutations linked to hemoglobinopathies. It enables the simultaneous study of several DNA sequences, offering thorough screening for known mutations. When typical hematological and biochemical testing produce unclear results or when numerous mutations may be present, this approach is helpful for detecting difficult cases. [50]
Numerous mutationsincludingthosethatresultinthalassemiaand other hemoglobinopathies,canbefound usingDNAarrays. Thismethodiseffective forlarge-scalescreeningssinceitcanexamine several samplesat once.Itoffercomprehensive details on the particular mutations that are present, which is essential for precise diagnosis and genetic counseling.

1. SNPArrays(SingleNucleotidePolymorphismArrays):
Appliedtofindvariationsinasinglenucleotidethroughoutthegenome.
Effectiveinidentifyingcertainmutations,likepointmutationsintheHBBgene,associatedwith hemoglobinopathies.
2. CGHArrays(ComparativeGenomicHybridizationArrays):
Detectvitaldeletionsorduplicationsthatcancausealphaorbetathalassemia.
Detectscopynumberchanges,includingdeletionsandduplications,acrossthegenome.
3. ExpressionArrays:
Although less common in hemoglobinopathy diagnosis, theycan offer insights into the expression patterns of globin genes in different conditions.

Measuresgeneexpressionlevelsthroughoutthegenome.

There are about 2.67 million markers on the arrayCGH cytoscan HD array (Affymetrix, Thermo Fisher Scientific, Santa Clara, CA), of which approximately 743000 are SNP probes and about 19550000 are copy number probes (average spacing: one probe every 1.1 kb). The cytogenetic lab uses ArrayCGH technology as a tool to visualize deletions that would otherwise be difficult or impossible to find throughFISH analysisor microscopic examination. In the genetic lab, array CGH has essentially taken the place of these methods. It is possible to distinguish between significant deletions in maternal or paternal chromosomes by examining SNPs on the array.[51]

POCT-
Clinicallaboratorytestingcarriedoutincloseproximitytothepatientcaresite,wherecareortreatmentis administered, is known as "point-of-care" testing (POCT).[52]

Compared to laboratory testing, POCT offers short test result turnaround, with the potential to get a result rapidly so that the proper therapy can be adopted, leading to improved clinical or economic outcomes. [53]

One of the most important factors in the quick and easy identification of hemoglobinopathies is point-of-care testing (POCT). Hemoglobinopathies are genetic illnesses that impact the hemoglobin molecule in red blood cells. Theseconditions include illnesses like sickle cell disease and several types of thalassemia. POCT has a number of benefits for the diagnosis and treatment of various illnesses, particularly in situations where quick judgment is crucial.[54]Several PCOT devices usemicrochip electrophoresis for quick screening in the diagnosis of hemoglobinopathies.Traditionally a lab-based method, portable point-of-care HPLC systems are being developed to provide detailed hemoglobin profiles.Immunoassay Certain hemoglobin variations, such asHbS (sickle hemoglobin) or HbA2, which are indicators for particular hemoglobinopathies, are detected by these tests using antibodies..[55]Diagnostics using molecules Methods such as Polymerase Chain Reaction (PCR)areutilizedtoidentifyparticulargeneticalterationslinkedto hemoglobinopathies.Theuse ofportablePCRequipment forfield diagnosticsisgrowingindemand.Whenitcomestoearlyhemoglobinopathydetectioninnewbornscreeningprograms, POCT is quite helpful. Timely actions that result from early diagnosis can greatly improve outcomes. Fast testing can help determine thebestcourseofactionforearlytreatmentwhentherearacuteissues,likeasicklecellcrisis. Blood transfusions may be necessary as part of the treatment for chronic illnesses like thalassemia, which can bemanaged with regularmonitoring of hemoglobin types and levels. In contrast to standard laboratory testing, which can take hours or days, POCT yields results in minutes. POCT devices can have a high initial cost, but by averting complications, they may lower overall healthcare expenses.[56]

PrenatalDiagnosis
When there is a possibility that the unborn child may be impacted, pregnant women are offered the opportunity to discuss whether or not they want to have a screening test for the unborn child. This test determines the type of thalassaemia and whether the unborn child truly has it.[57]

Earlydetectionofnewbornswithdrepanocyticsyndromes(SCD) maybepossiblethroughneonatal screening.Itcan be carried out on a blood sample taken from cord blood or metabolic screening filter papers using capillary electrophoresis or HPLC. Early detection of hemoglobin variations (HbE, for example) and hemoglobin H illness through neonatal screening could guarantee appropriate family counseling. Certain disorders, such being a healthy carrier of alpha and beta thalassemia, cannot be identified by newborn screening, while others can be suspected based on the lack of HbA. .[58]

Because samples can be acquired more easily postnatally and aberrant hematologic results can be discovered, DNA analysis isparticularlyusefulinprenataldiagnosis.Thediagnosisof hemoglobinopathiesand complex thalassemias seen in ethnically varied populations has been made easier by developments in molecular testing. Canada and several European nations have implemented universal screening programs to identify carriers and provide prenatal diagnosis in pregnancies at risk for thalassemia.

Parentalscreeningcanbecarriedout withoutputtingthe fetusat greaterriskbecauseit isnon-invasive.Theadvent of noninvasive prenatal diagnostic testing that uses fetal DNA extracted from mother plasma without the need for invasive procedures has made it possible to actively investigate the genetic analysis of the fetus.[59]

Many techniques, such as mass spectrometry, next-generation sequencing, and genotyping test, have been used to detect fetal hemoglobinopathies . Since the methods are still difficult, further research is required to improve and validate them in order to eventually provide a noninvasive, accurate, and dependable prenatal diagnosis of thalassemia and hemoglobinopathies.

Instead of conducting a sequential test, screening both partners at the same time can help identify at-risk couples early on. If the couple has never had an investigation done before, the request for screening tests should be made duringthe first obstetric appointment. Earlydetectionofalpha thalassemia and its potential linkto fetal hydropsisis essentialforproperpregnancymonitoring,intrauterinetransfusion,andpreventionofwidespreadedema,Ballantyne syndrome, and mirror syndrome in mothers. [60]

VariousTechniques UsedfortheDiagnosisofHemoglobin Disorders

Methods	Advantages	Limitations
Celluloseacetateelectrophoresisalkalineph	Superblaboratoryexperienceat Alowcost.	Lowresolution,which is rarely utilized in contemporary labs, might overlook β-thalassemiaduringtheneonatalstage.
Citrateagarelectrophoresisacidicph	Lowcost,abletodifferentiaterare variations from hemoglobin S or hemoglobin C	High-performance,low- resolution liquid chromatography can typically be used as a Replacement
High-performanceliquidchromatography	Quantitative,quickly,withhigh resolution,andfrequentlyused	Unable to identify some variations withcertainty, insensitive to α-thalassemia,andproneto missing β-thalassemia Duringtheneonatalstage
Capillaryzone electrophoresis	Resolve hemoglobin A2 from hemoglobinEwithhighresolution and quantitative	Unable to identify some variations withcertainty, insensitive to α-thalassemia,andproneto missing β-thalassemia Duringtheneonatalstage
Massspectrometry	Helpfulforquicklydescribing variations of hemoglobin	Notapplicableforthe Thalassemia diagnosis

Managementstrategiesforthalassemia

Blood transfusion therapy

Deliveringasafeandsuccessful transfusionregimenand reducingtheimpact oftransfusiontherapyondailylife are the goals of blood transfusion for thalassaemia patients. Good growth and development, healthy energy levels, and adequate suppression of intra- and extramedullary haematopoiesis are the outcomes of an efficient transfusion regimen. [60] A safe transfusion regimen will include the use of a product that is obtained, examined, chosen, issued, and administered in accordance with established quality and safety guidelines and regulations; it will also involve informed patient consent; it will be carried out in a system with an effective hemovigilance structure; and it will be administered by staff who have received training in blood transfusion. [61] Before first transfusion, extended red cell antigen typing including at least C, c, E, e, and Kell and if available a full red cell phenotype or genotype should be performed. ABO, Rh(D) compatibleblood should beadministered ateachtransfusion. Screening for newantibodies and an IAT cross-match should be performed before each transfusion. The red cells should be stored in CPDA within one week of collection. Transfusions should be performed every 2–4 weeks, maintaining pretransfusion hemoglobin level of 9 to 10.5 g/dL or 11 to 12 g/dL for cardiac patients. Packed red blood cells (pRBCs) are the component of choice for the transfusion. Leucodepleted pRBCs are used and preferably pre-storage leucodepletionis recommended with mandatory screening of blood for HIV, hepatitis B, hepatitis C, malaria and syphilis Depending upon the body weight of the patient, 15 ml/kg pRBCs at the rate of 5ml/kg/hour should be administered and 3.5ml/kg of pRBCs are to be transfused to raise haemoglobin by 1gm/dL**.**

Ironoverload

Depending upon the severity of anemia, in case of thalassemia major and thalassemia intermedia, additionally iron absorptionfromthe intestine increases to as muchas 3 to 5mgper daywhichresultsinanadditional 1-2 gmofiron loading per year, and hence they are contraindicated.

Each milliliter of pRBCs contains 1.16 mg of iron. On an average, each unit of pRBCs cells contains 200 to 250 mg of iron**.** Iron overload occurs when iron intake is increased over a sustained period of time resulting from either red blood cell transfusions or increased absorptionof ironthrough the gastro-intestinal tract. These conditions occur in thalassemia, where blood transfusion therapy is the major cause of iron overload in transfusion dependent thalassemia (TDT) and increased GI absorption being more important in non- transfusion dependent thalassemia (NTDT). [62]

In TDT patients, the body lacks the mechanism to excrete the excess iron and so iron overload is inevitable as they receive regular blood transfusions. Iron accumulation is toxic to many tissues, causing heart failure, cirrhosis, liver cancer, growth retardation, and multiple endocrine abnormalities. [63]

Evaluationof ironoverload

1. Serum ferritin level is measured at least every 3 months (1–3 months). The value is currently between 500 and 1000 µg/L.
2. Liver iron concentration (LIC) are measured by magnetic resonance imaging (MRI) based methods. [64,65] Normal LIC values are up to 1.8mg/g dry weight. High LIC above 15mg/g dry weight are associated with worse prognosis, liver fibrosis progression, or liver function abnormalities. [66]
3. MyocardialironcanbeassessedbyT2*cardiacMRI.Whencardiacironishigh(eg,T2*<10 ms) there is a high risk of deteriorating function and heart failure and urgent chelation of iron is required.

Therearesomeotherparametersofironoverloadthatincludes24hoursurinaryironestimation,Non- transferrinbound plasma iron and labile plasma iron.

Toguidechelationtherapyitisimportantto record ageofonsetofregulartransfusionsandironchelation therapygiven to each patient, maintenance of each iron loading and annual record of blood usage.

Ironchelation therapy

Chelation of iron is an effective treatment which helps to improve survival, decrease therisk of cardiac failure,and also decreases morbidities from transfusion-induced iron overload.If Chelation therapy at the correct doses and frequencyis given, it can balance iron excretion with iron accumulation from blood transfusions.Over chelation increases side effectsso therefore the doses should be decreased as decrease in serum ferritin or liver iron levels is observed.

The serum ferritin levels should be checked after 10 to 15 transfusions and chelation therapy should be initiated when the serum ferritin value is more than 1000µg/L. To achieve iron chelation, chelating drugs like Desferrioxamine (DF) and Deferiprone are commonly used. [67]

Desferrioxamine

In the early 1960s Desferrioxamine Mesylate was first introduced. The drug is mostly administered subcutaneously(40-60 mg/kg, over 8-12 hours in the period of 5 days per week). Thedesferrioxamine should never be added directly into the blood bag. Oral Vitamin C (50-200 mg) together withdesferrioxamine should begivenafter starting theinfusion.Inpatients witha lowserum ferritin level, high doses of this drug may lead to visual and auditory side effects. Desferrioxamine also increases the susceptibility to Yersinia enterocolitica and Yersinia pseudo tuberculosis infections.[67]

Deferiprone

This was the first oral iron chelator which was introduced in 1995. Deferiprone (1, 2 dimethyl-3- hydroxypyrid- 4-one, L1) is said to have the potential to change the prognosis of all transfusionaliron-loaded patients.[67] It is more effective in chelating iron from the heart as compared to desferrioxamine due to its low molecularweight.

Deferasirox

Deferasirox is a new oral iron chelator introduced in India in 2008. It is administered at a dose of14- 28 mg/kg/day once and lower doses in cases of Non-transfusion dependent Thalassemia.

Noveltherapiesinthetreatmentof thalassemia

Based on the improved understanding of pathophysiology of β-thalassemia, emerging therapies includes hematopoietic stem cell transplantation (HSCT) or gene therapy for the correction of the α or β globin chain imbalance and targeting ineffective erythropoiesis or iron dysregulation by novel agents such as luspatercept.

Hematopoieticstemcelltransplantation

Allogeneic hematopoietic stem cell transplantation (HSCT) is the only and curative treatment for thalassemia major, with more than 3000 HSCTs performed worldwide [68-70] and HLA matched sibling transplants is a well- established and accepted therapy, available in India. Cord blood can also be used. Following HSCT there is a risk of bone marrow graft rejection, graft versus host disease, and veno-occlusive disease (VOD). [71] In recent years, factors including improved prevention of graft versus host disease and more effective antimicrobial treatment resulted in a significant improvement of outcomes for HSCT with a cure of thalassemia has been achieved.

Genetherapy

With the advent recombinant DNA-technologies and thecloning of β- globininthemid 70sandfrom today's outcome, premature attempts for gene therapy of thalassemia have been made.Gene therapy aims to provide curefor thalassemia through the manipulation of the genome of hematopoietic stem cells, and so it compensates the inadequate or inappropriate function of mutated genes. This can be done by gene addition or gene editing. Gene addition is done by using viral vectors. While gene editing is repairing the gene in situ orby using site specific nucleases. [72,73]

Amongthenovelgenetherapies,themostmatureinterventionisthelentiviralvectorgenetherapy which provides clinical efficacy and safety.[74] Viral vectors are derived from the human immunodeficiency virus (HIV) by deleting accessory virulence factors and regulatory genes from the genome. Patients treated in trials using lentiviral vectors, no malignancy related to lentiviral insertional mutagenesis has so far been diagnosed.[75,76]

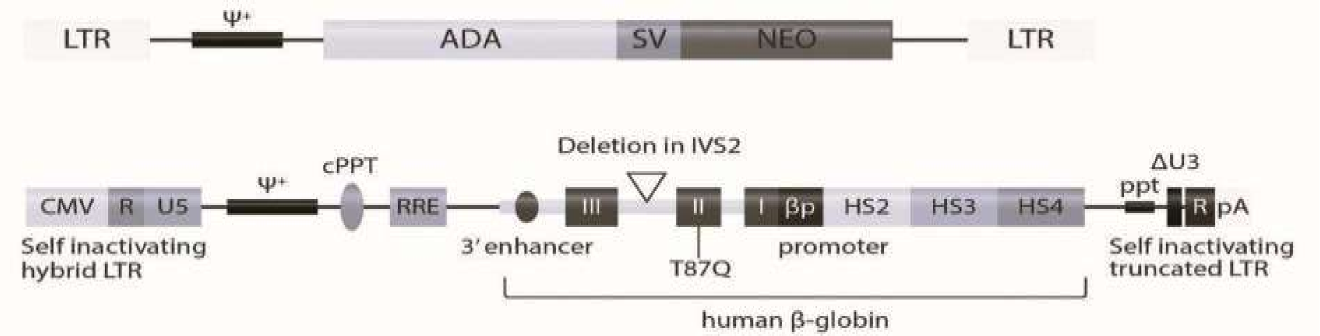

Fig.:- Geneaddition vectors.

Lifestyleandstandardofliving

There are a number of things to think about before pushing thalassaemia major women to become pregnant. These include the extent of liver failure and cardiac impairment that already existed, as well as the potential for vertical viral transmission. Recognising the importance of social integration and leading a normal life is part of a comprehensiveapproachtopatientcare.Allpatientsshouldtakecalciumandvitamin Dsupplements,2000IUdaily, and have their vitamin D levels checked every six months. Additionally advised is a dietcalcium rich diet. All patients with low haemoglobin levels require folic acid supplements, up to 1 mg per day. Since there is little chance of toxicityand a potential reduction in thrombosis, supplementation for all patients maybe taken into consideration.60

Psychologicalsupport

Patients experience stigmatisation, discrimination, low self-esteem, unwarranted anxiety, andprivacy invasion. Overall, a plethora of case controlled analytical studies and large cohorts of randomised, controlled trials with patients with thalassaemia suggests that psychological well-being influences adherence to treatment for chronic diseases in general, despite the general lack of such evidence. [80]Patients with thalassemiaexperience vulnerable psychological challenges. Numerous strategies have been proposed to highlight the advantages of psychological support, that includes focussing on modifications to institutional organisation procedures, patient group sessions, family therapyand patient chelation camps.If well-designed, multicenter, interventional studies utilising common standardised measureswereconductedtoassesstheefficacyofpsychologicalandpsychosocialsupport ontreatment adherence, funding for clinical psychological support services may be more broadly attained. This type of research, which employ well-established behavioural and social science methodologies, must determine which activeelements of "psychological support" are most beneficial to thalassaemia patients.

Conclusion:-

Hemoglobinopathies, such as sickle cell disease and thalassemias, are common and have a substantial influence on morbidity and death, making them major worldwide health concerns. Modern diagnostic methods, like high-performance liquid chromatography (HPLC) and capillary zone electrophoresis (CZE), have increased diagnosis accuracy and speed, facilitating better management and treatment planning. Gene therapy and routine blood transfusions are two examples of modern treatment techniques that have greatly improved patient outcomes. For affected persons to have a higher quality of life and avoid serious problems, early and accurate diagnosis is still essential. In the future, there is hope for more potent treatments and possibly even a cure, which will allow hemoglobinopathies to have a much less negative impact.

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