"STUDY OF ALLOIMMUNIZATION IN TRANSFUSION DEPENDENT THALASSEMIA PATIENTS AT A TERTIARY CARE HOSPTAL"

By

REG NO: BN0120001

Dissertation

Submitted to the

KLE Academy of Higher Education and Research
Belagavi, Karnataka
In partial fulfilment of the requirements for the degree of

DOCTOR OF MEDICINE
IN
PATHOLOGY

DEPARTMENT OF PATHOLOGY
J. N. MEDICAL COLLEGE, BELAGAVI
KARNATAKA

JUNE/JULY – 2023

KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH

BELAGAVI, KARNATAKA

Endorsement by Head of Department and Principal / Head of the Institution

This is to certify that the dissertation entitled **"STUDY OF ALLOIMMUNIZATION IN TRANSFUSION DEPENDENT THALASSEMIA PATIENTS AT A TERTIARY CARE HOSPTAL"** is a bonafide research work done by **REG NO: BN0120001.**

DR. Ranjit Kangle MD,
Professor and HOD
Department of Pathology,
J. N. Medical College,
Belagavi, Karnataka

Date: 21/1/2023
Place: Belagavi.

DR.(Mrs) N. S. Mahantashetti MD (Paed).
Principal
J. N. Medical College,
Belagavi, Karnataka.

Date:
Place: Belagavi.

ii

UNDERTAKING

1. **Reg. No. BN0120001** hereby declare that the information and the data mentioned in my dissertation entitled **"STUDY OF ALLOIMMUNIZATION IN TRANSFUSION DEPENDENT THALASSEMIA PATIENTS AT A TERTIARY CARE HOSPTAL"** belongs to me and is original. I am aware of the definition of plagiarism as detailed below:

- An act or instance of using or closely imitating the language and thoughts of another author without authorization and the representation of that author's work as one's own, as by not crediting the original author.
- A piece of writing or other work reflecting such unauthorized use or imitation.
- The deliberate or reckless representation of another's words, thoughts or ideas as one's own without attribution in connection with submission of academic work, whether graded or otherwise.

I hereby declare that the dissertation prepared by me is original and does not involve plagiarism anywhere. In case at a later stage, it is found that I have indulged in plagiarism, then I am solely responsible for the same and the institution is at liberty to take any disciplinary action against me including cancellation of dissertation or any other penalties imposed by the university.

Date: 02.01.2023

Place: Belagavi

Reg. No. BN0120001

PLAGIARISM ACCEPTANCE LETTER

KLE ACADEMY OF HIGHER EDUCATION AND RESEARCH

JAWAHARLAL NEHRU MEDICAL COLLEGE

(Recognized by Medical Council of India, New Delhi)

Accredited 'A+' Grade by NAAC (3rd Cycle) **Placed in Category 'A' by MHRD (GoI)**

Nehru Nagar, Belagavi- 590 010, Karnataka, INDIA

0831 - 2471350 0831 - 2470759 www.jnmc.edu principal@jnmc.edu

Ref No: MDC/PG/ Date: 21-12-2022.

ACCEPTANCE LETTER

The softcopy of thesis entitled: **"STUDY OF ALLOIMMUNIZATION IN TRANSFUSION DEPENDENT THALASSEMIA PATIENTS AT A TERTIARY CARE HOSPTAL"** has been submitted for Anti-Plagiarism check through Turnitin software. The scan has been carried out and the scanned output reveals a match percentage of **07%** which is within the acceptable limits of **10%** as per the guidelines given by UGC.

Guide.

KAHER BELAGAVI J. N. MEDICAL COLLEGE

Dr. (Mrs.) N.S. Mahantashetti.
Chairperson-Antiplagiarism Committee &
Principal,
J. N. Medical College, Belagavi.

To,
Reg. No. BN0120001,
Postgraduate Student,
2020-21 Batch,
Department of Pathology,
J. N. Medical College, Belagavi.

ETHICAL CLEARANCE CERTIFICATE

K.L.E. ACADEMY OF HIGHER EDUCATION AND RESEARCH
(Deemed – to- be- University)
Accredited 'A' Grade by NAAC (2nd Cycle) Placed in Category 'A' by MHRD (GoI)
JAWAHARLAL NEHRU MEDICAL COLLEGE,
NEHRU NAGAR, BELAGAVI-590010 (KARNATAKA-INDIA)
Website: http//www.jnmc.edu
E-Mail : dome@jnmc.edu
Phone: (+ 91-(0)831 Office : 2472550
Principal: 2471701
Fax No. +91 (0)831 – 2470759

Ref: MDC/DOME/131 **Date: 25/01/2021**

To,

Reg. No. BN0120001
PG student in Pathology,
J. N. Medical College,
BELAGAVI.

Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"STUDY OF ALLOIMMUNISATION IN TRANSFUSION DEPENDENT THALASSEMIA PATIENTS AT A TERTIARY CARE HOSPITAL",** is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee on Human Subjects Research.

(Dr. Smita Sonoli)
Member Secretary
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

(Dr. Harsha Hegde)
Chairman,
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

134

TITLE

STUDY OF ALLOIMMUNIZATION IN TRANSFUSION DEPENDENT THALASSEMIA PATIENTS AT A TERTIARY CARE HOSPTAL

ABSTRACT

Background: A well-known consequence of thalassemia patients who receive multiple blood transfusions throughout their lives is the development of alloimmunity, or allo-antibodies against donor red blood cells (RBCs). This leads to hemolysis of the donor's red blood cells, making the transfusion less effective and increasing the risk of secondary problems such as iron overload. This study was conducted to examine how common alloimmunization is in patients with thalassemia.

Methods: The study enrolled 205 thalassemia patients with a history of multiple blood transfusions. Ortho workstation cassettes containing the polyclonal anti-human globulin (AHG) for Direct Coombs test (DCT) was used to detect presence of allo-antibodies against RBC surface antigens.

Results: Among the 205 thalassemia patients enrolled in this study, the average age of the participants was 11+/- 6 years. Thalassemia major (76%) was the most frequent diagnosis followed by thalassemia intermedia (22%). Majority of the cases were diagnosed and started on regular blood transfusion therapy between the ages of 1 and 10 years. Most of Thalassemia major patients (62%) and Thalassemia intermedia patients (67%) were born of parental consanguinity. The most frequent therapeutic regimen employed was that of hyper-transfusion to maintain the pre-transfusion hemoglobin between 9 and 10 g/dl. Only 13% of the patients underwent splenectomy among which Thalassemia major cases were the majority. Even though O-Positive

was the most frequent blood type, most of the operated and alloimmunized cases belonged to the B-Positive blood type. Alloimmunization was seen only in 21 out of 205 patients (10%), among which majority (76%) were born of parental consanguinity. Majority of the operated cases of Thalassemia had developed alloimmunization post-splenectomy (52%). There was no association between the type of thalassemia, alloimmunization or splenectomy and the blood group in total study subjects. With a p value <0.0001, there was a strong association between alloimmunization and splenectomy among multi-transfused thalassemia cases. The total number of transfusions, the volume of blood transfused and the age at which the transfusion was started showed significant correlation with both alloimmunization and splenectomy. Parental consanguinity did not seem to have a significant effect on the alloimmunization, splenectomy and the type of thalassemia among these cases.

Conclusions: Our results highlight the importance of erythrocyte screening and antigen typing before the initial transfusion. Thalassemia patients undergoing multiple transfusions often develop alloimmunization against varied erythrocyte antigens which have varying clinical importance. There is a strong relationship between alloimmunization and lack of a spleen.

Key Words: Alloimmunization, thalassemia major, thalassemia intermedia, blood transfusion, splenectomy, hemoglobin, allo-antibodies.

LIST OF ABBREVIATIONS USED

TM	Thalassemia Major
TI	Thalassemia Intermedia
BTT	Beta Thalassemia Trait
Hb	Hemoglobin
RBC	Red Blood Cells
AGT	Antiglobulin Test
AHTR	Alloimmune-Mediated Hemolytic Transfusion Reaction
DAT	Direct Antiglobulin Test
α	Alpha
β	Beta
DFO	Deferoxamine
SNHL	Sensorineural Hearing Loss
DVT	Deep Vein Thrombosis
PTE	Pulmonary Thrombo-Embolism
AHTR	Acute Hemolytic Transfusion Reaction
FNHTR	Febrile Non-Hemolytic Transfusion Reaction
TRALI	Transfusion Related Acute Lung Injury
HIV	Human Immunodeficiency Virus
CMV	Cytomegalovirus
HBsAg	Hepatitis B Surface Antigen
TDT	Transfusion Dependent Thalassemia
NTDT	Non-Transfusion Dependent Thalassemia
CBC	Complete Blood Count

Hct	Hematocrit
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
RDW	RBC Distribution Width
PBS	Peripheral Blood Smear
nRBC	Nucleated Rbcs
BMA	Bone Marrow Aspirate
TIBC	Total Iron Binding Capacity
HBE	Hemoglobin Electrophoresis
CZE	Capillary Zone Electrophoresis
HPLC	High Performance Liquid Chromatography
MLPA	Multiplex Ligation-Dependent Probe Amplification
BMT	Bone Marrow Transplantation
HSCT	Hematopoietic Stem Cell Transplant
HLA	Human Leukocyte Antigens
PRBC	Packed Red Blood Cells
NAT	Nucleic Acid Testing
Hbd	Desired Hemoglobin
Hbc	Current Hemoglobin
Wt	Weight
IV	Intravenous
EPO	Erythropoetin

HU	Hydroxyurea
CVS	Chorionic Villous Sampling
CTLA-4	Cytotoxic T-Lymphocyte-Associated Antigen-4
PD-1	Programmed Death-1
AHG	Anti-Human Globulin
PBM	Partial Better Matching
Ig	Immunoglobin
EDTA	Ethylene-Diamine Tetra Acetic Acid
HDN	Hemolytic Disease Of Newborn
AIHA	Auto-Immune Hemolytic Anemia
SPSS	Statistical Package For Social Sciences
ROC	Receiver Operating Characteristic

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Hemoglobinopathies are an inherited group of diseases that occurs due to faulty hemoglobin synthesis or defective hemoglobin structure[1-15]. They can be broadly classified as either qualitative or quantitative. The term "thalassemia" refers to a class of qualitative hereditary diseases characterized by aberrant hemoglobin structure brought on by the lack of or reduced synthesis of one or more globin chains. Thalassemia can be of two primary types: When one or more genes associated with the alpha globin protein are absent or altered, it results in alpha thalassemia (mutated). Similar gene abnormalities that influence the generation of the beta globin protein result in beta thalassemia[10,15-22]. The prevalence of alpha and beta thalassemia in the Indian subcontinent reflects the overall disease burden. The prevalence of β-thalassemia in India is widespread, with a carrier frequency of 03-04% on average. Every year, approximately 10,000-15,000 infants are born with Thalassemia Major (TM). Patients with symptomatic thalassemia require numerous blood transfusions throughout the course of their lifetimes to maintain a reasonable standard of living. The need for multiple transfusions increases the risk of problems in thalassemia patients, including iron overload, alloimmunization, blood-borne infections, etc.[1,19,23-31].

The immune system of the recipient responds to foreign antigens (iso-antigens) from the donor, which results in the process of alloimmunization (isoimmunization) after many blood transfusions. A thorough review of the literature revealed that anti-E was the most common antibody and that alloimmunization affects 4.8 (3.9–5.7) of every 100 people who have had numerous blood transfusions. Most of the studies done were cross-sectional, which could have led to an underestimation of the true prevalence of alloimmunization[32-39]. Alloimmunization is a well-known

complication in thalassemia patients when performing multiple transfusions, because their immune system recognizes the donor red blood cells (RBC) surface antigens as foreign entity. Hence, the life span of red blood cells (RBC) is shortened, and the patients are clinically dependent on increased (repeated) RBC transfusions. Thus, alloantibodies and/or autoantibodies of RBC that lead to difficulty in cross-matching and delay in obtaining compatible blood for transfusion are a serious problem in patient blood management of thalassemia[32,40-44]. The antiglobulin test (AGT), also known as Coombs test, is an immunological laboratory procedure used to detect antibodies against the body's circulating RBCs, which cause hemolysis. An alloimmune-mediated hemolytic transfusion reaction (AHTR) is brought on by an alloantibody that was recently identified in a post-transfusion sample. It can take as little as two to three days for an alloantibody to form. Alloantibodies develop, possibly accompanied by hemolysis, and result in a positive Direct Antiglobulin Test (DAT) [1,45-49].

Alloimmunization raises the technical challenges, morbidity, and mortality of transfusion therapy while complicating and limiting it. In patients who have received many transfusions, the incidence of alloimmunization has been significantly decreased using phenotypically matched leucodepleted blood[49]. Hence, this study is aimed at estimating the prevalence of alloimmunization in transfusion dependent thalassemia patients at a tertiary care hospital in North Karnataka.

AIMS AND OBJECTIVES

- To study alloimmunization status in transfusion dependent thalassemia patients.
- To find the association between frequency of blood transfusions in thalassemia patients and positive alloimmunization status.
- To find an association of blood group distribution in thalassemia patients and their alloimmunization status, if any

REVIEW OF LITERATURE

HEMOGLOBIN

Hemoglobin is a unique transport protein present inside the red blood cells, which carries oxygen from lungs to the rest of the body for oxidative metabolism[42,50-60]. Four molecules of oxygen can be carried by one molecule of hemoglobin[42,50-60]. The tetrameric hemoglobin has a molecular weight of 66,700 Daltons and consists of:

- four globin chains
- four prosthetic heme group subunits, each of which has a tetrapyrrole ring with a ferrous ion in the middle[42,50-60] (Figure 1).

The composition of the globin chains varies with the developmental stage of the organism. Genes located on chromosomes 11 and 16 are responsible for production of these globin chains[42,50-60] (Figure 2).

The normal types of hemoglobin for each stage of life are given in table 1[56,59].

Figure 1: Tetrameric structure of hemoglobin[58]

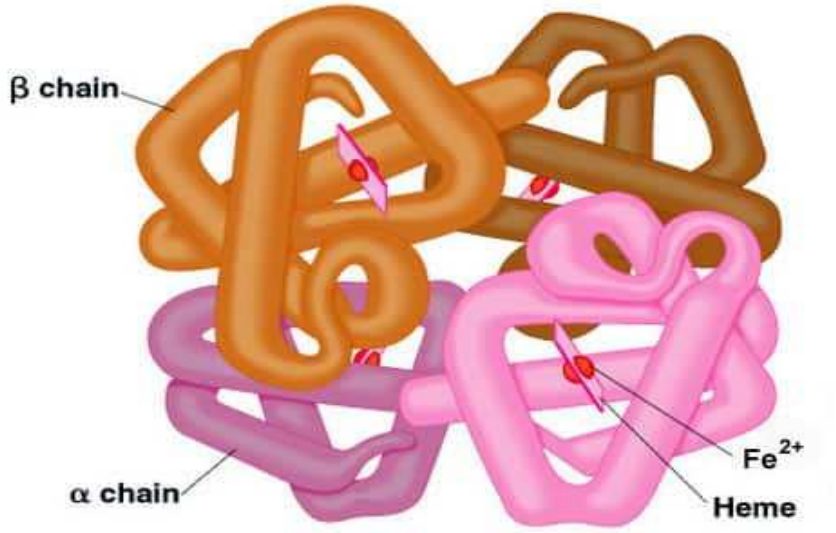


Image taken from: Hemoglobin and Myoglobin. The Medical Biochemistry Page. Updated: September 27, 2022. Retrieved on 19-12-2022, from https://themedicalbiochemistrypage.org/hemoglobin-and-myoglobin/

Table 1: Hemoglobin & Its Variants According to The Developmental Stage[56]

DEVELOPMENTAL STAGE	TYPE	GENOTYPE	REFERENCE INTERVAL
Embryonic	Gower 1	$\zeta_2\varepsilon_2$	-
	Gower 2	$\alpha_2\varepsilon_2$	-
	Portland	$\zeta_2\gamma_2$	-
Fetal (Highest oxygen affinity)	HbF	$\alpha_2\gamma_2$	90-95% before birth 50-85% at birth
	HbA	$\alpha_2\beta_2$	10-40% at birth
	HbA_2	$\alpha_2\delta_2$	<1% at birth
>1 year old	HbF	$\alpha_2\gamma_2$	<2%
	HbA	$\alpha_2\beta_2$	>95%
	HbA_2	$\alpha_2\delta_2$	<3.5%
Adult (Lesser oxygen affinity than HbF)	HbA	$\alpha_2\beta_2$	>95%
	HbA_2	$\alpha_2\delta_2$	1.5-3.7%
	HbF	$\alpha_2\gamma_2$	<2%

Table taken from: Anemia: - Part 5 A – Sickle Cell Anemia, and Sickle Cell Trait, Discussion and Workup. Retrieved on 19-12-2022. From: https://labpedia.net/anemia-part-5-sickle-cell-anemia-and-sickle-cell-trait-discussion-and-workup/

Figure 2: Hemoglobin Chains and Their Chromosomal Locations[59]

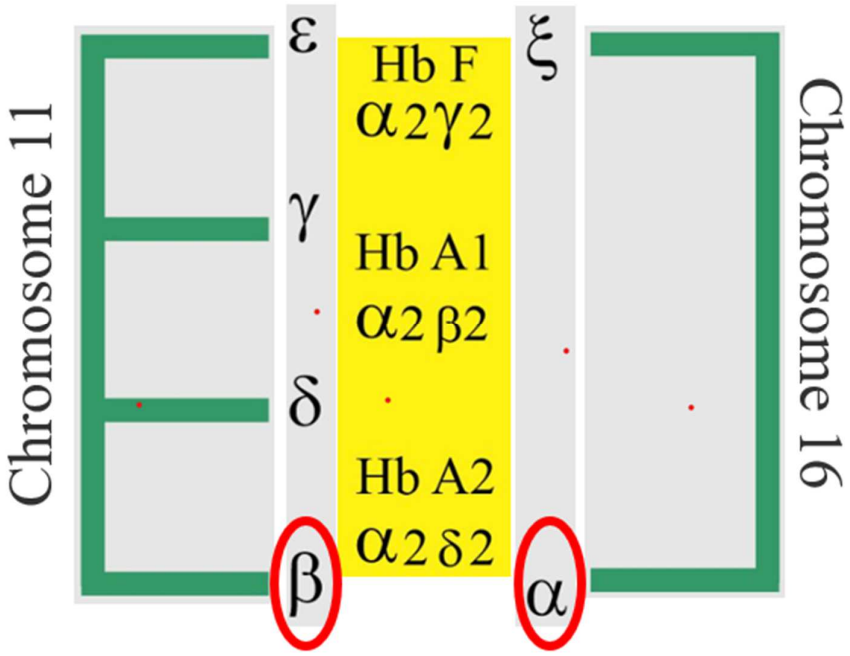


Image taken from: Anemia: - Part 5 A – Sickle Cell Anemia, and Sickle Cell Trait, Discussion and Workup. Retrieved on 19-12-2022. From:

https://labpedia.net/anemia-part-5-sickle-cell-anemia-and-sickle-cell-trait-discussion-and-workup/

HEMOGLOBINOPATHIES

Hemoglobinopathies are inherited diseases occurring due to genetic alterations in the structure and function of the hemoglobin molecule[1,15,24,48,57,61-64].

Around 300,000–500,000 babies are born each year with major hemoglobinopathies, and it is believed that 7% of the world's population carries a defective hemoglobin gene[1,15,24,48,57,61-64].

The abnormality lying within the globin chains can either be qualitative (structural alteration) or quantitative (numerical alteration). The resulting hemoglobin variant may have altered solubility, function, and stability[1,15,24,48,57,61-64].

Sickle cell anaemia, the most common form of qualitative hemoglobinopathy, is caused by deletions or changes in the amino acids that make up the globin chain, and it affects 70% of births worldwide[1,15,24,48,57,61-64].

Thalassemia is a type of quantitative hemoglobinopathy caused by a globin chain mutation that results in a decrease in or absence of the chain. With more than 400 identified mutations affecting the assembly of hemoglobin that result in ineffective erythropoiesis, this group of autosomal recessive illnesses is exceedingly diverse[1,15,24,48,57,61-64].

THALASSEMIA

HISTORY:

Thomas Cooley of Detroit provided the first widely accepted description of this category of illnesses in 1925 after observing numerous paediatric examples of anemia linked to splenomegaly and skeletal deformities, also known as "Cooley's anemia" [23,35,54,63,65,66]. George Hoyt Whipple, an American pathologist and physician, came up with the term "thalassemia". Since the ailment was initially identified in communities residing close to the Mediterranean Sea, its name is taken from the Greek term "Thalassa" which means "the sea" [3,10,15,66-68]. In 1938, J. Caminopetros discovered that this illness is inherited in an autosomal recessive manner[23,66]. The Mediterranean area, Southeast Asia, together with the Indian subcontinent, and West Africa were observed to have the highest rates of this category of anemia[24,35-36,49,65-75]. Several famous individuals have made significant strides in understanding the pathogenesis, the variety of clinical manifestations, the identification of the numerous types, and interconnections of thalassemia syndromes. Many ideas for effective treatment modalities for this illness were created, and these included mandatory monitoring and regular blood transfusions with or without the need for splenectomy. Knowing that frequent transfusions are required in these situations, anonymous safe blood donation evolved into an inexpensive successful solution to lessen the suffering that thalassemia causes for both the patient and the treating physician[63-75].

In due course, the idea of "hyper-transfusion" was developed to cover up the bone marrow's inefficient erythropoiesis and raise hemoglobin levels to almost "supernormal" levels. Iron overload was a side effect of this regimen, and frequent ferritin levels were tracked down and used as a gauge of iron toxicity. Additionally, it was discovered that a hyper-transfusion regimen was not necessary for pre-transfusion

hemoglobin levels of 9 to 10 g/dl. As a result, various other regimens were used, which assisted in lowering the total amount and frequency of blood transfusions. Desferrioxamine, an iron-chelating siderophore that promotes urine elimination of the excess unbound iron, was discovered, which further reduced iron toxicity[3,50,66,76-93].

The wellness and comfort of these patients have improved over time owing to adherence to numerous developed protocols and enhanced thalassemia treatment regimens. Gene therapy research for thalassemia is now promoted and seems to be producing encouraging results, although it has not yet been implemented in clinical settings[10,15,51,54,66,81,82,87,94-97].

HEMOGLOBINOPATHIES VS THALASSEMIAS

The usual amino acid sequence of globin is altered in hemoglobinopathies. The globin subunits that make up the hemoglobin tetramer are normally coordinated, however thalassemias are caused by an imbalance in this process. It is now known that globin gene mutations in the coding area can result in hemoglobinopathy, a structural deficiency, and a biosynthetic defect (thalassemia). It' possible for two different mutations to arise in the same gene, leading to a hemoglobinopathy with thalassemia-like characteristics[3,10,48,51,63,81,82,88,98-104].

The differences between hemoglobinopathies and thalassemia is given in Table 2[63].

Table 2: Hemoglobinopathies Vs Thalassemias[63]

	HEMOGLOBINOPATHY	THALASSEMIA
Characteristics	Qualitative defects in the structure of globin chains resulting in production of abnormal hemoglobin molecules	Quantitative disorders of hemoglobin synthesis that produce reduced amounts of normal hemoglobin
RBC Count	Reduced	Higher than expected for Hb
RBC Indices	Normocytic Normochromic	Microcytic Hypochromic
RBC Morphology	Target cells Sickle cells (in HbS) HbC crystals (in HbC) Others	Target cells Basophilic stippling,
Abnormal Hb	HbS, HbC, HbE etc.	HbH (β^4) Hb Bart's ($¥^4$)
Hb Solubility Test	HbS, Hb Bart's, HbC	-
Reticulocyte Count	Raised more than in thalassemia	Raised

Table taken from: Randolph T R. Thalassemia. In: Clinical laboratory Hematology 3rd edition. Shirlyn B. McKenzie, J. Lynne Williams. Pearson. 2016. p.283-308.

TYPES OF THALASSEMIA AND THEIR GENETIC DEFECTS

The existence of six globin genes (δ, β, ϒ, α, ε & ζ) results in six different versions of thalassemia syndromes[3,55,60-63,96].

The two major types of thalassemia are:

- α-thalassemia

- β-thalassemia

These occur due to impaired synthesis of α globin chains & β globin chains respectively[3,55,60-63,96].

The majority of children with thalassemia major (approximately 1 to 1.5 lakh) and almost 42 million carriers (3-4%) of the β-thalassemia trait are found in India.

Every year, between 10,000 and 15,000 infants are born with thalassemia major[61,105,106]. Certain communities, including Sindhis, Punjabis, Gujaratis, Bengalis, Mahars, Kolis, Saraswats, Lohanas, and Gaurs, have been noted to have higher incidences of the disease[26,30,61,107-110].

According to their severity, thalassemias are clinically classified into[32,63,82]:

1) Thalassemia Major (TM)
2) Thalassemia Intermedia (TI)
3) Thalassemia Minor or Trait[32,63,82].

Thalassemia Major (TM) and the severe form of Thalassemia Intermedia (TI) both require lifelong blood transfusions and iron chelation. They account for most of the disease burden. The aberrant beta thalassemia genes from either one or both carrier parents—or the abnormal beta thalassemia gene from one parent plus an abnormal variant hemoglobin gene (HbE, HbS) from the other parent—are what create the thalassemia syndromes. Beta thalassemia trait (BTT), or thalassemia minor, is the carrier state in which the person is clinically normal[32,63,82].

The rare occurrence of δ-thalassemia and the combinations of gene deletions (e.g.: δβϒ-thalassemia) renders them clinically insignificant.

Thalassemic hemoglobinopathies are structural variants of thalassemia producing the signs and symptoms of thalassemia. These include:

- Hemoglobin Constant Spring: Abnormal length of globin chain
- Hemoglobin E: Point mutation
- Hemoglobin Lepore: Structural abnormality with ineffective synthesis of non-α-globin chains[63].

In some places, the prevalence of HbS among tribal peoples in Southern, Central, and Western Indian states might approach 48%. HbE is widespread in the North-Eastern states and, in some places, has a carrier frequency as high as 50%. West Bengal, Bihar, and Uttar Pradesh in the east are where it is found at lower frequencies, while Punjab has roughly 2% of the population with HbD[1,26,48,61,106,111].

The summary of the types of thalassemia along with their common genetic defects have been given below in Table number 3[63].

Table 3: GENETIC DEFECTS IN THALASSEMIA

TYPE OF THALASSEMIA	MUTATION	EFFECT ON GENE	EFFECT ON GLOBIN CHAIN
Predominantly alpha thalassemia Some beta thalassemia	Deletion (large)	Loss of gene	Absence of production
Predominantly beta thalassemia	Promoter	Impaired transcription	Reduced or absent production
	Nonsense	In frame substitution Frame shift	Amino acid change Amino acid changes distal to shift Longer or shorter globin chains
	Stop codon	Convert stop codon to amino acid codon	Slightly lengthened - Retained - Degraded
	Splice site	Create new splice sites	Slightly shortened - Retained - Degraded
		Loss of splice sites	Slightly lengthened globin chain (retained) Significantly lengthened globin chain (degraded) Unaltered globin chain

PATHOPHYSIOLOGY OF THALASSEMIA

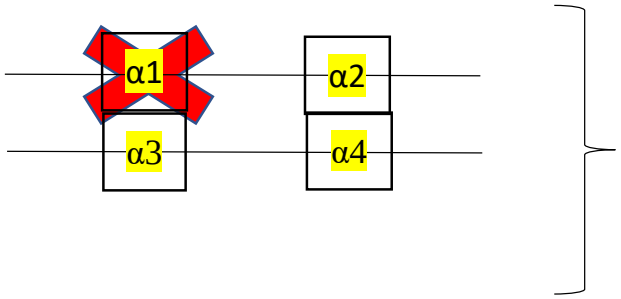
The standard α:β ratio is 1:1. (both produced in equal amounts). An imbalance in this ratio leads to anaemia, ongoing hemolysis, and ineffective erythropoiesis[3,24,55,63].

ALPHA-THALASSEMIA:

Alpha globin (α-globin) is coded by two genes on chromosome 16. Each diploid cell has two copies of chromosome 16, which results in four α-globin alleles. Reduced synthesis of α-globin chains results from deletion mutation of one or more α-globin alleles[63,74,112-114].

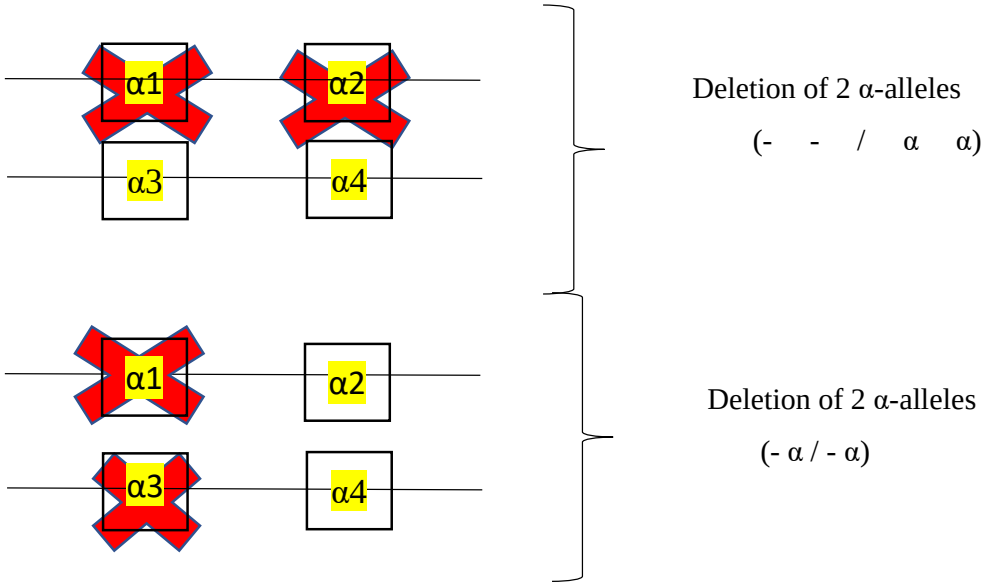
Alpha-thalassemia includes 4 subtypes:

1) Silent carrier



As long as the other three functioning alleles produce enough α-globin, the loss of one α-allele has little effect on the synthesis of β-globin. Typically asymptomatic, these patients have normal RBC indices. These diseases are discovered by molecular testing and family history inquiries[63,112,113].

2) α-thalassemia trait



Deletion of two α-alleles leads to decreased synthesis of α-globin with normal β-globin synthesis[63,112,113].

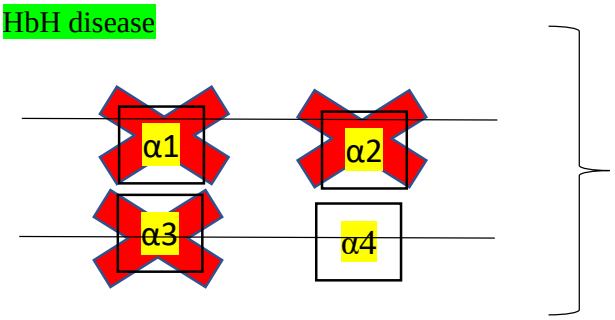
There are two scenarios that have been observed: -

- Decreased HbA resulting in mild anaemia with the existence of microcytic hypochromic RBCs
- No significant surplus β-globin, resulting in a normal Hemoglobin Electrophoresis result and a normal protein chromatography[63,112,113].

These cases are discovered by molecular testing[63,112,113].

On supravital staining, HbH inclusions can be seen with much difficulty inside RBCs[63,112,113].

3) HbH disease



With normal β -globin production, the loss of three α -alleles causes a reduction in -globin synthesis[63,112,113].

The two scenarios seen are:

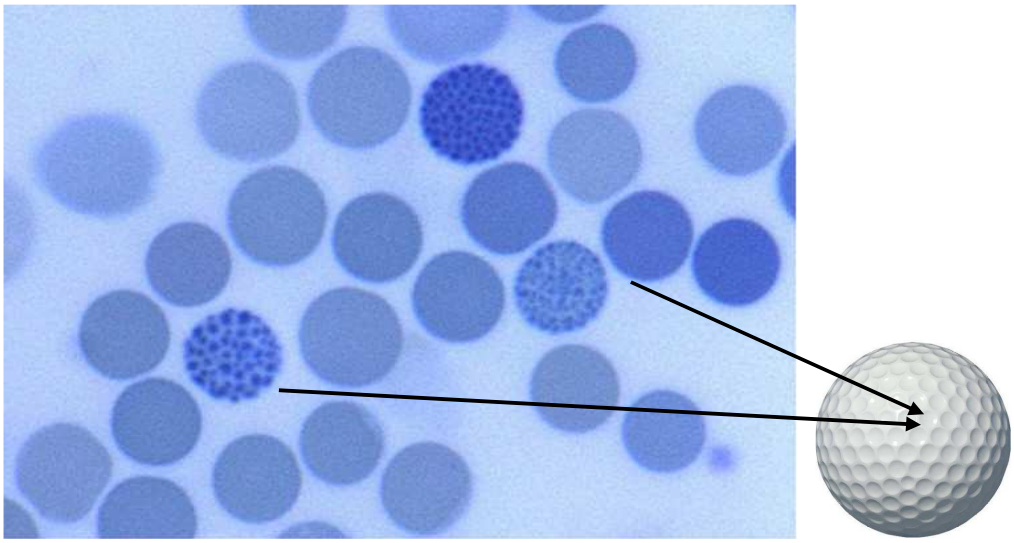
- A decrease in HbA that results in a moderate anaemia and the presence of microcytic hypochromic RBCs

- An excessive number of β-globins that bind to produce unstable HbH (β4). Protein chromatography and Hemoglobin Electrophoresis both reveal HbH[63,112,113].

Supravital staining of RBCs in these cases exhibit inclusions of HbH (golf ball inclusions) [115].

[Figure 3]

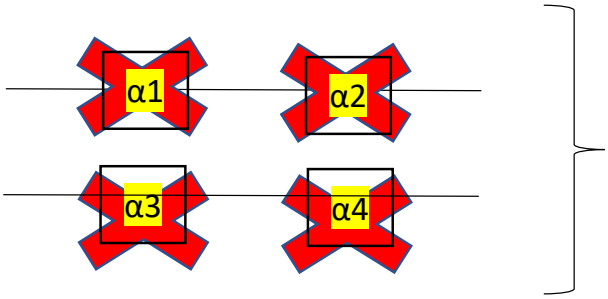
Figure 3: Golf ball inclusions inside RBCs[115]



Stain – Supravital Methylene Blue staining. (100X magnification)

Image taken from: Gibbons R. Alpha thalassaemia-mental retardation, X linked. Orphanet. J. Rare. Dis. 2006; 1(1)15.

4) Hydrops fetalis / α-thalassemia Major



The absence of all four α-alleles results in normal production of ϒ-globin, which binds to itself to form Hb Barts (ϒ4) with a very high affinity for oxygen. This causes tissue hypoxia and significant organ failure in the foetus by preventing the release of oxygen to the tissues. As a result, the foetus dies in-utero and this kind of α-thalassemia is incompatible with life[63,112,113].

Summary of α-thalassemia and its characteristics is given in table 4[63].

Table 4: Characteristics Of Alpha-Thalassemia[63]

ZYGOSITY	GENOTYPE	PHENOTYPE	HEMOGLOBIN ELECTROPHORESIS	SEVERITY
Homozygous alpha thalassemia-1	(--/--) α^0/α^0	Hydrops fetalis	Hb Barts (80-90%) Hb Portland (10-20%)	Fatal
Heterozygous alpha thalassemia-1 Or alpha thalassemia-2	(--/- α) α^0/α^+	Hemoglobin H disease	Birth – Hb Barts Adult – HbH	Chronic, moderately severe hemolytic anemia
Heterozygous alpha thalassemia-1	(--/ α α) α^0/α	Alpha thalassemia minor	Birth – Hb Barts Adult – Normal	Mild to moderate
Homozygous alpha thalassemia-2	(- α-/ α) α^+/α^+			Mild
Heterozygous alpha thalassemia-2	(- α/ α α) α^+/α	Silent carrier	Normal	Normal

Table taken from: Randolph T R. Thalassemia. In: Clinical laboratory Hematology 3rd edition. Shirlyn B. McKenzie, J. Lynne Williams. Pearson. 2016. p.283-308.

BETA-THALASSEMIA

Most cases of this kind of thalassemia are non-deletional and are caused by distinct molecular abnormalities[60,63,70,96,112].

Each of the two beta-globin (β-globin) genes is found on chromosome 11[63,116].

There are two classification systems used:

- Genotypic system: Six types

  Based on zygosity and the degree of alteration in the β-genes.

- Phenotypic system: Four categories

  Based on the severity of clinical presentation[63,112].

Nomenclature[63,112]:

β^+- gene mutation - Partial block in β-chain synthesis (reduced β-globin synthesis)

β^0- gene mutation - Complete absence of β-chain synthesis

β^{SC} - Silent carrier

Only 20 of the more than 200 mutations that have been found are responsible for the bulk of β -thalassemia cases[63,112].

1) β-THALASSEMIA MAJOR

Genotype: $\beta^0\ \beta^0$, $\beta^+\ \beta^+$ } Homozygous

$\beta^0\ \beta^+$ } Double heterozygous

Pathophysiology:

Lack of β-chain synthesis results in -

• Reduced HbA which compromises RBCs ability to carry oxygen

• Compensatory rise in the production of other hemoglobins with a higher affinity for oxygen, such as HbF and HbA2, which results in a reduction in the amount of oxygen delivered to peripheral tissues and tissue hypoxia.

• Chronic hemolysis brought on by the formation of reactive oxygen species and the precipitation of too many free α-chains. The precipitated α-chains in the bone marrow cause a number of apoptotic pathways to be activated, which leads to inefficient erythropoiesis.

• Erythroid hyperplasia in relation to the severity of anaemia. As a result, the following changes may occur:

- Expansion of bone marrow
- Thinning of calcified bone
- Increased absorption of iron that results in iron toxicity

- Extramedullary hematopoiesis in the liver and spleen causes hepatosplenomegaly[63,112]

2) β -THALASSEMIA MINOR

Genotype: $\beta^0\ \beta$] Heterozygous
$\beta^+\ \beta$]

Pathophysiology:

The normal β-gene controls the synthesis of adequate HbA to provide just enough oxygen supply to maintain RBC survival. In heterozygous β+ state, the thalassaemic gene also generates β-chains[63,112].

3) β -THALASSEMIA INTERMEDIA

Genotype: $\beta^0\ \beta^+$ → Double Heterozygous → Severely reduced β-chain
$\beta^0\ \beta$ → Heterozygous → Mild symptoms
$\beta^+\ \beta^+$ → Homozygous

Criteria: These patients have the ability to maintain a hemoglobin level that is just right for a high quality of life without frequently needing blood transfusions[63,112].

4) β-THALASSEMIA MINIMA

Genotype: $\beta^{SC}/\ \beta$

These individuals have only mild laboratory abnormalities and no symptoms with mild imbalance in the synthesis ratio of α-chains to non-α-chains[63,112].

Summary of β-thalassemia and its characteristics is given in table 5[63]

Table 5: Characteristics Of β -Thalassemia[63]

ZYGOSITY	GENOTYPE	PHENOTYPE	Hb A	Hb A2	Hb F	SEVERITY
Homozygous	β^0 β^0	Major	None	Slight Increase	Moderate Increase	Severe
Double Heterozygous	β^0 β^+	Major	Moderate Decrease	Slight Increase	Moderate Increase	Severe
	β^0 β^+	Intermedia	Slight Decrease	Slight Increase	Moderate Increase	Moderate
Homozygous	β^+ β^+	Major	Moderate Decrease	Slight Increase	Moderate Increase	Severe
	β^+ β^+	Intermedia	Slight Decrease	Slight Decrease	Moderate Increase	Moderate
Heterozygous	β^0 β	Intermedia	Slight Decrease	Slight Increase	Slight Increase	Moderate
	β^0 β	Minor	Slight Decrease	Slight Increase	Slight Increase	Mild
Heterozygous	β^+ β	Minor	Slight Decrease	Slight Increase	Slight Increase	Mild
Homozygous	β^{SC} β^{SC}	Mild intermedia	Slight Decrease	Slight Increase	Slight Increase	Mild
Heterozygous	β^{SC}β	Minima	Normal	Normal	Normal	Normal

Table taken from: Randolph T R. Thalassemia. In: Clinical laboratory Hematology 3rd edition. Shirlyn B. McKenzie, J. Lynne Williams. Pearson. 2016. p.283-308.

COMPLICATIONS OF THALASSEMIA

The following categories might be used to group the effects of thalassemia complications:

- Consequences of compensatory mechanisms for anaemia in thalassemia
- As a result of iron overload
- As a result of treatment of iron overload (adverse effects of iron-chelating agents)
- Because of coagulation defects
- As a result of numerous blood transfusions[63,112,117-135]

1) Consequences of compensatory mechanisms for the anemia of thalassemia[63,112-135]

Severe tissue hypoxia in thalassemia

↓

Increased erythropoietin production

↓

Expansion of the dyserythropoietic marrow

↓

Calories required for normal growth and development are diverted towards the development of the RBC precursors (ineffective) ↓ Poor development Muscle wasting Growth retardation	Severe skeletal deformities: o Deformities of skull and face o Porosity in long bones - Pathological fractures - Ineffective drainage Recurrent sinusitis Middle ear infections Extramedullary hematopoietic tumors in extreme cases	Increased erythroid precursors ↓ - Secondary hyperuricemia (Gout) - Severe folate deficiency

2) As a result of iron overload[63,112-135]

By following mechanisms:

(i) Excess free globin chain degradation

↓

Increased production of free iron

↓

Generation of free radicals

↓

- Damage to lipids, proteins
- Transmembrane protein Band-3 assembles inside RBCs and precursor cells

↓

Damage to immune response to new antigens

↓

Destruction of RBCs and their precursors by macrophages

↓ ↓

Ineffective erythropoiesis | Chronic Hemolytic anemia

↓

Hyperbilirubinemia

↓

Gall stones

(ii) Bone marrow hypertrophy

↓

Increased RBC precursors

↓

Decreased production of hepcidin by liver

↓

Increased iron absorption from the intestine (to supply iron for erythropoiesis)

↓

Accumulation of iron

- Liver – Kupffer cells and parenchyma - Hepatomegaly
- Spleen – Macrophages - Splenomegaly

- Skin - Pigmentation
- Pituitary – Hypogonadism, Growth retardation, Adrenal insufficiency
- Parathyroid – Hypoparathyroidism
- Thyroid – Hypothyroidism (2nd most common endocrinopathy in thalassemia major)
- Myocardium – Affects conduction system, Intractable cardiac failure
- Eyes – Retinal pigmentation, Retinal toxicity, cataract
- Pancreas – Diabetes Mellitus

Excess iron → Stimulates production of hepcidin

→ Blocks intestinal absorption of iron

(iii) Multiple regular blood transfusions in thalassemia major

⇩

Excess iron from the transfused RBCs

⇩

Iron overload

Serum ferritin levels can be regularly measured to keep an eye on iron overload and aids in evaluating a patient's prognosis: the lower the ferritin, the lower the risk of heart failure and hypogonadism, and the better the likelihood of survival. T2-MRI is a reliable non-invasive approach for determining the quantitative iron overload in the heart and liver. A reliable but invasive liver biopsy is only carried out prior to a bone marrow transplant.

3) As a result of treatment of iron-overload (adverse effects of iron-chelating agents)

Chelating agents used to treat iron overload are:

- Deferoxamine (DFO) - 25-50 mg/kg/day subcutaneous over 8 to 12 hours
- Deferiprone - 50-100 mg/kg/day in two or three divided oral doses
- Deferasirox - 20-40 mg / kg / day given orally, dispersed in water or juice[63,112-135]

By lowering heart failure rates in thalassemia cases, the use of DFO among them has significantly improved survival. DFO may induce growth retardation, rickets-like bone abnormalities, anaphylaxis, high frequency sensorineural hearing loss (SNHL), reversible retinal and optic nerve damage, and a life-threatening Yersinia infection at doses greater than 40 mg/kg (Yersinia enterocolitica uses DFO as a siderophore) [63,112-135].

Deferiprone can result in neutropenia, agranulocytosis, a rise in liver enzymes, and arthritis in patients with significant iron overload. Deferasirox frequently causes allergic reactions, rashes, gastrointestinal intolerance, an increase in blood transaminases, and a rise in creatinine. Deferoxamine and deferiprone should be used in combination for patients who do not respond to monotherapy at its highest dosage[63,112-135].

4) Because of coagulation defects

Some thalassemia patients have a hypercoagulable state, particularly those who have had splenectomy surgery or have high platelet counts. A thrombo-embolic event is more likely to occur in these people. This is attributed to the procoagulant impact of red blood cells with thalassemia (increased surface phospholipids), which accelerate thrombin production. Deep vein thrombosis (DVT), portal vein thrombosis,

pulmonary thrombo-embolism (PTE), silent strokes due to reduced blood supply to the brain causing infarctions may occur.

5) As a result of numerous blood transfusions

Blood transfusion complications can be categorised as acute (beginning within 24 hours after stopping the transfusion) and delayed (onset more than 24 hours after stopping the transfusion). Additionally, they might be categorised as immunologic and non-immunologic[63,112-136].

The complications are listed in Table 6[63,112-136]

Table 6: Transfusion Complications[112-136]

ACUTE		DELAYED	
IMMUNOLOGIC	NON-IMMUNOLOGIC	IMMUNOLOGIC	NON-IMMUNOLOGIC
- Acute Hemolytic Transfusion Reaction (AHTR) - Allergic reaction - Febrile Non-hemolytic Transfusion Reaction (FNHTR) - Transfusion Related Acute Lung Injury (TRALI)	Volume overload Citrate toxicity Hypothermia Hypocalcaemia	- Delayed Hemolytic Transfusion Reaction - Post-transfusion purpura	Infectious diseases transmission - Malaria - Human Immunodeficiency Virus (HIV) - Hepatitis C Virus (HCV) - Cytomegalovirus (CMV) - Hepatitis B virus antigen (HBsAg)

The summary of complications both common and specific to transfusion dependent thalassemia (TDT) and non-transfusion dependent thalassemia (NTDT) has been listed in table number 7[63,137-138].

Table 7: Complications of Thalassemia[137-138].

<table>
<tr><th>TDT</th><th>NTDT</th><th>COMMON TO BOTH</th></tr>
<tr><td rowspan="3">Pituitary dysfunction
Hypoparathyroidism
Diabetes mellitus</td><td>Ineffective erythropoiesis</td><td>Hypogonadism
Hypothyroidism</td></tr>
<tr><td>Thrombosis – DVT, Leg ulcers, PTE, Pulmonary hypertension, silent strokes</td><td>Skeletal abnormalities, fractures</td></tr>
<tr><td>Acute hemolytic episodes</td><td>Hepatosplenomegaly</td></tr>
<tr><td rowspan="4">Cardiac siderosis (arrhythmias, heart failure)</td><td>Right heart failure</td><td>Growth retardation</td></tr>
<tr><td>Gall stones</td><td>Iron overload</td></tr>
<tr><td>Infections</td><td rowspan="2">Liver fibrosis, cirrhosis
Hepatocellular carcinoma</td></tr>
<tr><td>Folic acid deficiency</td></tr>
</table>

DIAGNOSIS OF THALASSEMIA

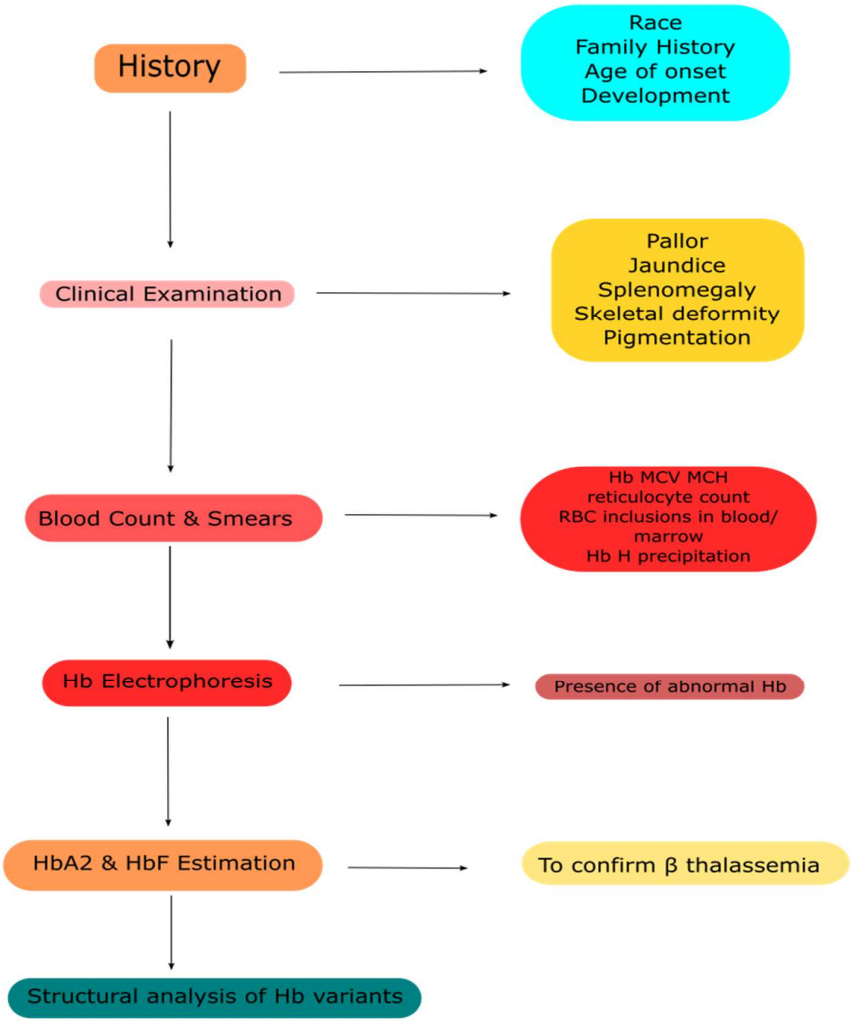
- History: Individual history, Family history, Ethnicity[63,82,112]
- Physical Examination[63,82,112]:

Signs	Causes
Pallor	Anemia
Jaundice	Hemolysis
Splenomegaly	Sequestration of abnormal RBCs
	Extramedullary hematopoiesis,
	Excessive extravascular Hemolysis

Chipmunk facies Enlargement of the maxillcaused by bone marrow expansion

- Laboratory investigations[63,82,112]

The approach to diagnosis of thalassemia syndromes has been given in the flow chart below[112].



Flow chart taken from: Weatherall D J. The Thalassemias: Disorders of Globin Synthesis. In: Williams Hematology. 9th edition. Kenneth Kaushansky, Josef T. Prchal, Oliver W. Press, Marshall A. Lichtman, Marcel Levi, Linda J. Burns, Michael A. Caligiuri. Mc Graw Hill Education. 2016. p. 725-58.

Laboratory features in thalassemias[63,112,139]

1) Complete Blood Count (CBC)

Decreased or Normal	RBC Count
Decreased	Hemoglobin (Hb) Hematocrit (Hct) RBC Indices - Mean corpuscular volume (MCV) - Mean corpuscular hemoglobin (MCH) - Mean corpuscular hemoglobin concentration (MCHC)
Increased	Reticulocyte Count RBC Distribution Width (RDW)
Neutrophilia and thrombocytosis may occur.	

2) Peripheral Blood Smear (PBS) [140,141] [Figure 4 & 5]

Microcytic Hypochromic RBCs

Anisopoikilocytosis – Presence of target cells, nucleated RBCs (nRBCs) and polychromatophils

Basophilic stippling

nRBCs may be dysplastic or show abnormal hemoglobinization.

Figure 4: Peripheral blood film showing microcytic hypochromic RBCs and basophilic stippling[140].

[Stain: Leishman's stain, 400x magnification] (subset showing basophilic stippling)

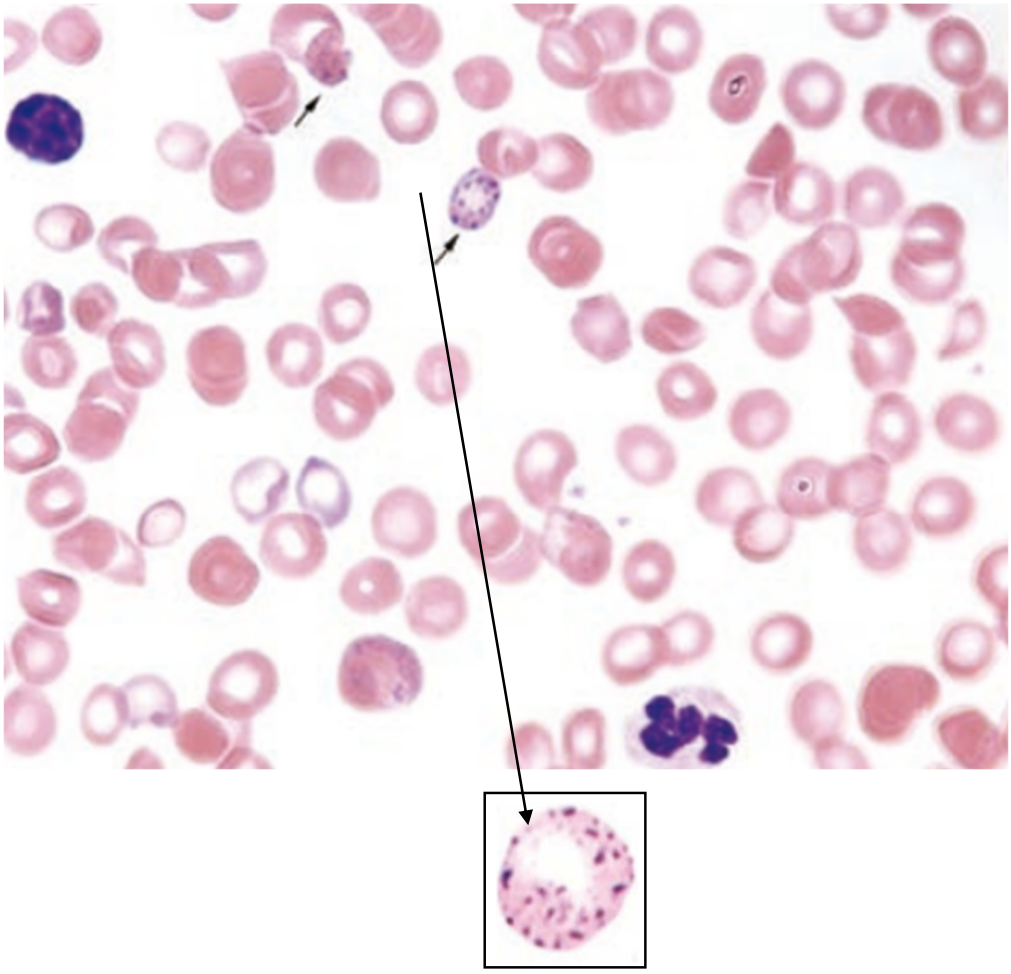


Image taken from: Barth D, Hirschmann J V. Anemia. In: Wintrobe's Atlas of Clinical Hematology. First edition. Douglas C. Tkachuk, Jan V. Hirschmann, editors. Lippincott Williams & Wilkins. 2007. p.18

Figure 5: Peripheral blood smear of beta-thalassemia major[141]

Shows many nucleated RBCs, microcytic hypochromic RBCs with multiple morphologic changes: target cells, teardrop cells, fragments, basophilic stippling, and pappenheimer bodies.

[Stain: Leishman's stain, 400x magnification]

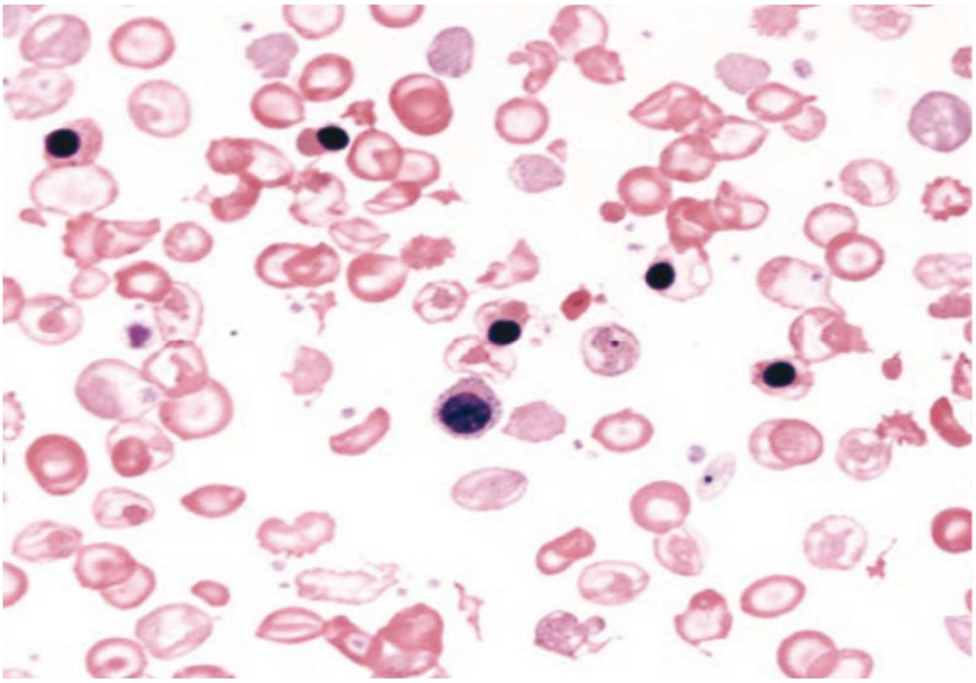


Image taken from: Barth D, Hirschmann J V. Anemia. In: Wintrobe's Atlas of Clinical Hematology. First edition. Douglas C. Tkachuk, Jan V. Hirschmann, editors. Lippincott Williams & Wilkins. 2007. p.15.

3) Bone Marrow (BM) [Figure 6 & 7] [140]

Erythroid hyperplasia

Raised Prussian Blue staining in marrow (raised iron stores)

Figure 6: Bone marrow aspirate (BMA) - Increased numbers of erythroid precursors[140]

[Stain: Leishman's stain, 100x magnification] (subset showing erythroid island)

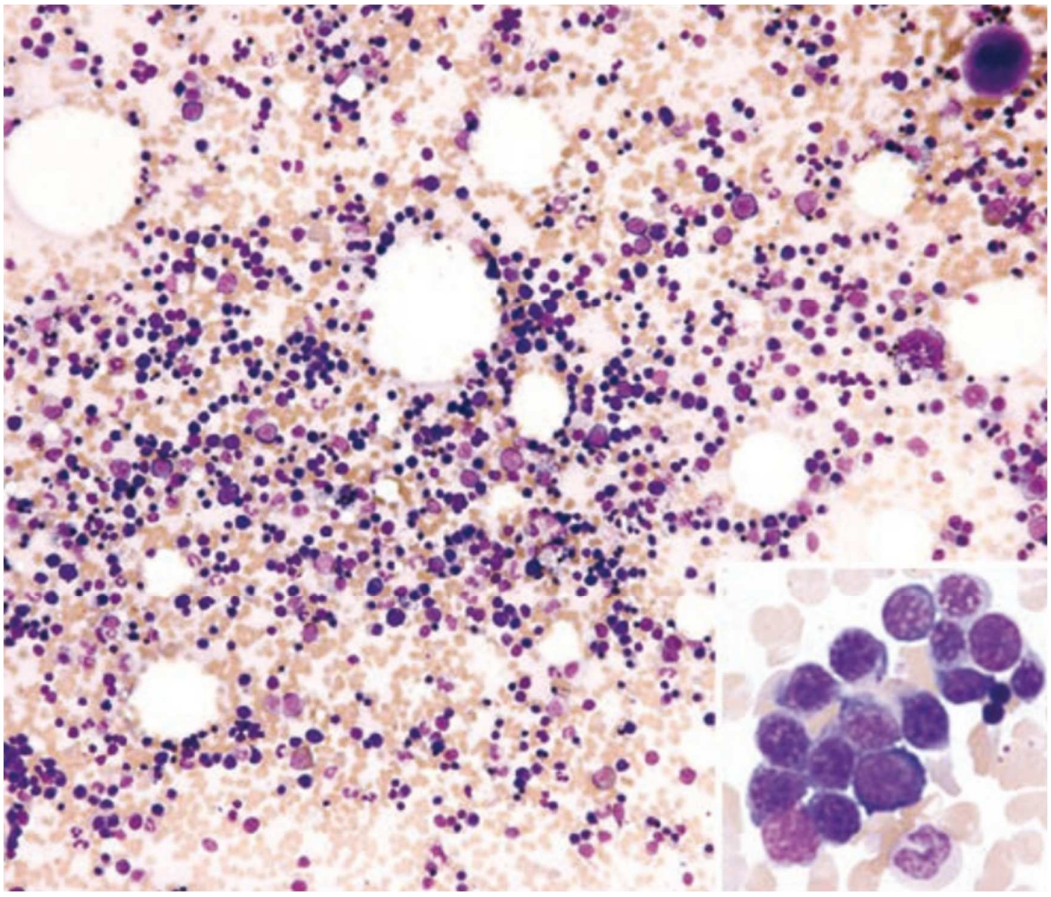


Image taken from: Barth D, Hirschmann J V. Anemia. In: Wintrobe's Atlas of Clinical Hematology. First edition. Douglas C. Tkachuk, Jan V. Hirschmann, editors. Lippincott Williams & Wilkins. 2007. p.18

Figure 7: Bone marrow trephine showing increased numbers of erythroid precursors[140]

[Stain: H&E, 100x magnification]

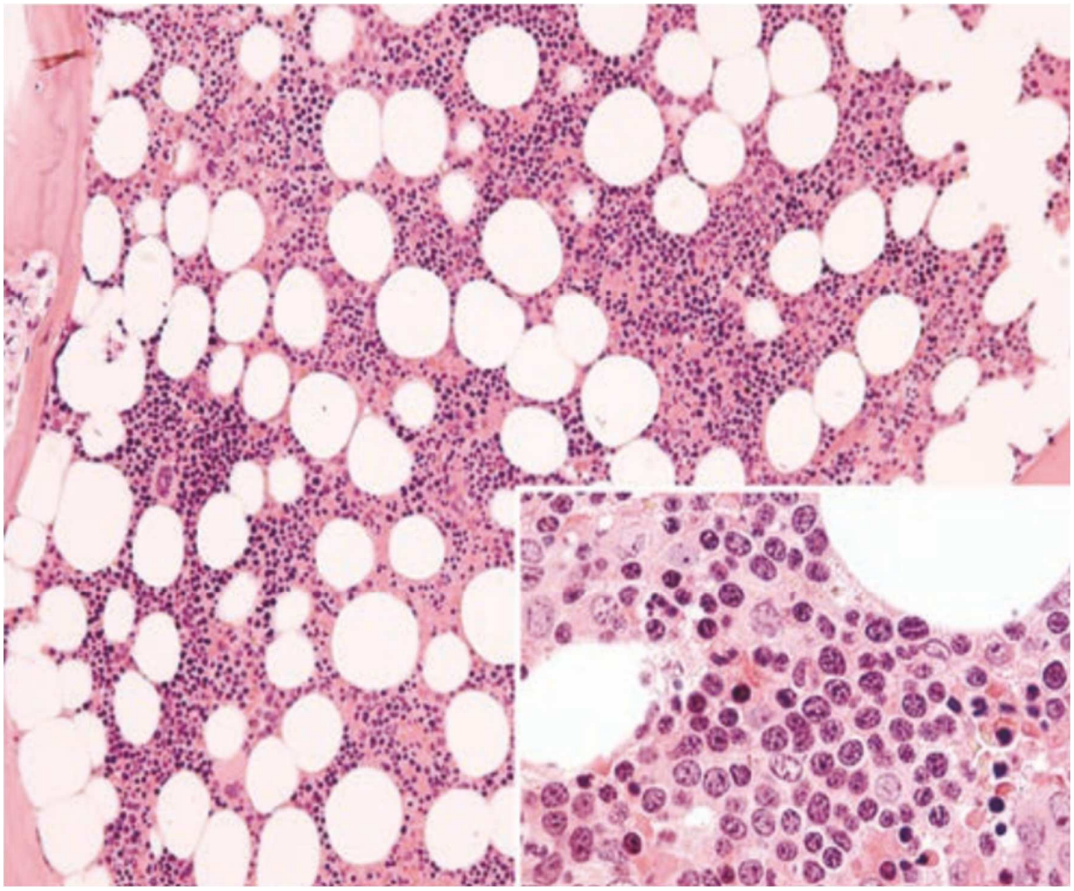


Image taken from: Barth D, Hirschmann J V. Anemia. In: Wintrobe's Atlas of Clinical Hematology. First edition. Douglas C. Tkachuk, Jan V. Hirschmann, editors. Lippincott Williams & Wilkins. 2007. p.18

4) Biochemistry

Raised	Bilirubin Serum Iron Serum Ferritin
Decreased	Total Iron Binding Capacity (TIBC) Haptoglobin

Investigations for quantification, typing & structural analysis of hemoglobin:

1) Hemoglobin Electrophoresis (HBE)
2) Capillary Zone Electrophoresis (CZE)
3) High Performance Liquid Chromatography (HPLC) [52,63,112,114]

Hb electrophoresis is less precise at quantifying hemoglobin A2 (HbA2) than HPLC, which is an automated method of identifying and separating variant hemoglobins. HPLC can extract HbA2 from some hemoglobins, which hemoglobin electrophoresis cannot do[112,142] [Figure 8].

Figure 8: High-Performance Liquid Chromatography (HPLC) Sample Demonstrating Increased Hemoglobin A2 (Arrow) In A Case of Beta-Thalassemia Trait[112]

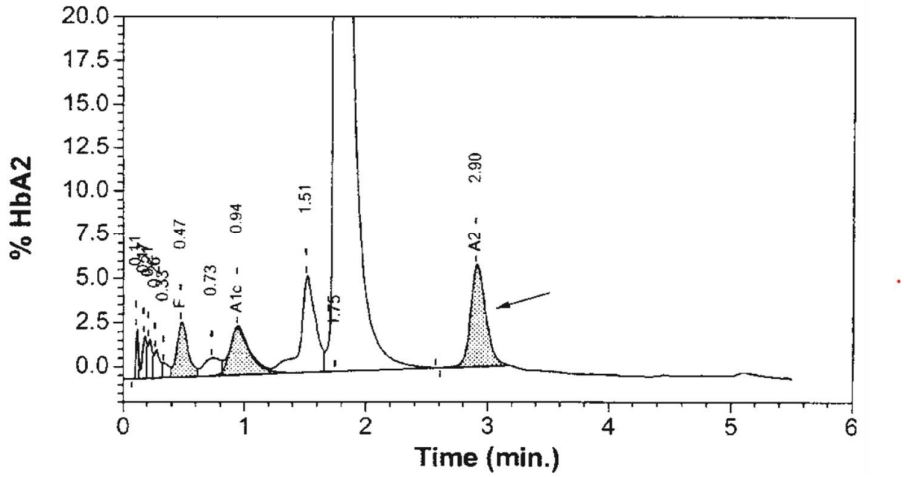


Image taken from: Weatherall D J. The Thalassemias: Disorders of Globin Synthesis. In: Williams Hematology. 9th edition. Kenneth Kaushansky, Josef T. Prchal, Oliver W. Press, Marshall A. Lichtman, Marcel Levi, Linda J. Burns, Michael A. Caligiuri. Mc Graw Hill Education. 2016. p. 725-58.

Figure 9: Hb Electrophoresis Machine

Summary of laboratory diagnosis of thalassemia is given in Table 8[143].

Table 8: Laboratory Diagnosis of Thalassemia[143]

<table>
<tr><td colspan="4">SCREENING TESTS</td></tr>
<tr><td rowspan="2">1.</td><td rowspan="2">CBC</td><td>LOW</td><td>HIGH</td></tr>
<tr><td>HB
HEMATOCRIT
RBC INDICES</td><td>RETIC (slight or moderate)</td></tr>
<tr><td>2.</td><td>PBS</td><td colspan="2">Microcytic hypochromic cells
Varying degrees of anisopoikilocytosis
RBC inclusions
Abnormal RBC morphologies
Presence of nucleated RBCs</td></tr>
<tr><td rowspan="2">3.</td><td rowspan="2">IRON STUDIES
(To rule out iron deficiency)</td><td>S. FERRITIN</td><td>S. IRON</td></tr>
<tr><td colspan="2">Normal to moderately raised</td></tr>
<tr><td colspan="4"></td></tr>
<tr><td colspan="4">PRESUMPTIVE DIAGNOSIS</td></tr>
<tr><td>1.</td><td>SUPRAVITAL STAINING</td><td colspan="2">Alpha-thalassemia: Hb H inclusions</td></tr>
<tr><td>2.</td><td>HEMOGLOBIN FRACTION QUANTIFICATION (HbE, CZE, HPLC)</td><td colspan="2">Quantify and classify types of thalassemia based on types of hemoglobin – HbA, HbA2, HbF, Hb Barts or other mutants</td></tr>
<tr><td colspan="4">DEFINITIVE DIAGNOSIS</td></tr>
<tr><td colspan="4">MOLECULAR GENETIC TESTS
Indications:
- Prenatal diagnosis
- Preconception risk assessment
- Carrier detection in couples
- Diagnosis of rare or complex mutations.</td></tr>
<tr><td>1.</td><td colspan="3">Beta-thalassemia: 280 mutations in HBB</td></tr>
</table>

	Initial DNA sequence analysis OR PCR-based screen for four to six most common mutations (If specific ethnic group known) ⬇ If Negative ⬇ <u>Deletion/duplication analysis</u> MLPA (multiplex ligation-dependent probe amplification) Gene-targeted microarray analysis
2.	Alpha-thalassemia: 110 mutations in HBA1 and/or HBA2
	Initial targeted deletional analysis for five most common deletions ⬇ If Negative ⬇ DNA sequence analysis ⬇ If Negative ⬇ <u>Deletion/duplication analysis</u> MLPA (multiplex ligation-dependent probe amplification) Gene-targeted microarray analysis

Table taken from: Management of Thalassemia Major. Guidelines For Management of Thalassemia and Sickle Cell Disease. In: NHM Guidelines for Prevention of Hemoglobinopathies in India. 2016. p.73-82.

THERAPY

In cases of thalassemia major, the sole curative option is bone marrow transplantation (BMT), specifically "hematopoietic stem cell transplant" (HSCT). However, due to the expense, the scarcity of BMT centres, and the lack of a good HLA (human leukocyte antigens) matched donor, this can only benefit a small number of patients[61-63,112].

In order to remove the significant iron overload caused by the numerous blood transfusions, the mainstay of treatment is a schedule of routine blood transfusions followed by carefully controlled iron chelation therapy. As a result, it is a transfusion-dependent illness that has a significant negative impact on healthcare[61].

Treatment modalities in thalassemia include the following[63,77,112,144]:

- Regular blood transfusions
- Iron chelation therapy to prevent iron overload
- Splenectomy
- Prevention and treatment of infections
- Marrow transplantation in special cases (stem cell transplantation)
- General care

(I) Role of regular blood transfusions in thalassemia –

Patients with β-thalassemia who maintain hemoglobin levels between 9.5 and 14.0 g/dL experience normal growth and development. As long as it does not impede the child's normal developmental progress, pre-transfusion hemoglobin levels can be kept lower than 9.5 g/dl. To prevent wasteful transfusions in situations of intermedia, which are frequently mistaken as major, a thorough transfusion regimen must only be started with severely low hemoglobin readings. The number of transfusions required

varies from every 2-4 weeks based on the child's age, weight, and other variables[8,63,77,79,102,112,144-146].

Prior to storage, all Packed Red Blood Cells (PRBC) are leucodepleted[63,83,112,147,148]. In cases when it is impractical, bedside filtration may be utilised. Red blood cells that have been packed and are ideally no older than two weeks should be used in transfusions[144,150]. Blood must be required to be screened for syphilis, malaria, HIV, hepatitis B, and hepatitis C. Despite being optional, nucleic acid testing (NAT) is advised to reduce the risk of infections being transmitted through blood transfusions, particularly in pregnant women and patients who are anticipated to get a progenitor cell transplant. Typically, leucodepleted blood products are CMV-free[63,95,112,144-150].

Irradiated blood products should only be used in transfusions for immunocompromised patients or those who may have a progenitor cell transplant planned in the near future. Unless the recipient has experienced an urticarial reaction or any transfusion reaction that could be prevented by washed units, saline-washed red cells are not necessary[63,87,112,144,149-153].

Packed Red Blood Cell (PRBC) requirements are determined by the formula[63,112,144,154-157]:

$$\text{PRBC in millilitres (ml)} = (Hbd - Hbc) \times (3 / HCT) \times (Wt)$$

where,

Hbd - Desired hemoglobin

Hbc - Current hemoglobin

3 - Transfusion factor

HCT - Hematocrit of transfused red cells

Wt - weight of the patient in kilograms

Usual dose – 15 ml/kg body weight

Rate of transfusion – 5 ml/kg/hr.

Transporting blood units in controlled, insulated crates that keep their temperature between 2^0C and 8^0C is preferred. Hb increases by 1 g/dl when 3.5 ml/kg of PRBCs with a 60% Hct are transfused. A small volume of packed red blood cells is transfused at a rate of 2 ml/kg per hour with close monitoring in cases of congestive heart failure or a hemoglobin level below 5 g/dl. If the transfused volume is larger than 20 ml/kg, diuretics should be taken into consideration[63,112,144].

Hemovigilance is a term used to describe a set of surveillance procedures that cover the entire transfusion chain, from the collection of blood and its components to the monitoring of its recipients. These procedures are designed to gather data on any unexpected or unfavourable effects that may arise from the therapeutic use of labile blood products and to help prevent their occurrence and recurrence. The main objective of this is to increase the safety of blood transfusions[63,83,112,144,153,158,159].

(II) Role of iron chelation therapy –

One ml of PRBC contains 1.16 mg of iron, i.e., one unit of PRBC contains 200 to 250 mg of iron[63,93,112,160-166].

Iron overload, which ultimately results in cardiac siderosis, is the most prevalent cause of mortality in thalassemia patients who require transfusions. This can be avoided by beginning iron chelation therapy early[63,81,112,164,165].

To determine iron overload, ferritin is employed as an indication. Chelation therapy should be started when the serum ferritin level is greater than 1000 microgram per decilitre (mcg/dL), which typically occurs after the 12^{th} transfusion.

Maintaining a S. Ferritin level of fewer than 1500 mcg/L is the target[8,63,76,79,112,144,146,160-166].

Deferoxamine (Desferrioxamine), given subcutaneously for 8 to 12 hours along the anterior abdominal wall by an infusion pump, was the first iron-chelator with a long-term benefit that had been established. In addition to 100-200 mg of vitamin C (depending on age) administered orally at the beginning of chelation, it is started at a dose of 20 mg/kg for 5 days[3,10,15,48,63,76,81,112,144,158-166].

In situations where there are endocrine or cardiac issues from iron excess, continuous intravenous infusion utilising an intravenous (IV) delivery system is beneficial. The infusion can be made without this medication's negative side effects by including 5 to 10 mg of hydrocortisone. Although local erythema and nodule formation are the most often reported adverse effects of deferoxamine, concerns about neurosensory and ocular toxicity are not insignificant, justifying the necessity for biannual audiometric and ophthalmologic examinations[63,83,112,144,160-166].

It has been established that Deferoxamine is more successful in chelating iron than oral chelating medications like Deferiprone (Ferriprox, L1) and Deferasirox. Due to its capacity to cross membranes, deferiprone (75 mg/kg TID) is used with deferoxamine to remove cardiac iron most effectively. Deferiprone's harmful side effect of agranulocytosis has made it necessary to estimate white cell counts on a weekly basis. Deferasirox's applications, potency, and adverse effects are still being investigated[10,63,66,83,112,144,160-166].

In transfusion-dependent thalassemia, evaluation of cardiac functions and mapping of both hepatic and myocardial iron are of highest importance[112,144,160-166].

(III) Role of splenectomy in thalassemia –

The following situations call for splenectomy in thalassemia patients:

- Hypersplenism – Hypersplenism is the removal of blood cells too quickly and prematurely by an overactive spleen. Thrombocytopenia and neutropenia can complicate these cases by causing bleeding and recurrent bacterial infections respectively. The incidence of hypersplenism can be decreased by maintaining high hemoglobin levels[63,83,112,144,165-168].
- Splenomegaly with abdominal pain or massive splenomegaly with impending rupture[63,83,112,144].
- Patients who receive effective chelation therapy but still have elevated iron storage. The amount of post-transfusional iron loading is decreased by splenectomy[63,83,112,144].
- An increase in the amount of blood needed each year as a result of the development of alloantibodies, recurring infections, and variations in the hematocrit values of transfused blood units[63,83,112,144,165-169].

Surgeries done include:

- Partial splenectomy to preserve some splenic immune function
- Total splenectomy
- Splenic embolization - Less invasive approach to reduce the size of spleen[63,83,112,144,165-169].

Splenectomy is not indicated in children less than 5-years of age due to an increased risk of sepsis below this age. Pneumococcal, meningococcal, and influenza (type B) vaccinations are given two weeks before the surgery, and post-operative prophylactic penicillin is also given (per oral) [83,144,165-169].

(IV) Prevention and treatment of infections in thalassemia –

Annual testing is required for viral illnesses frequently spread through transfusions. These include HIV, CMV, Hepatitis C, and Hepatitis B. Appendicitis must be distinguished from Yersinia enterocolitica infection. Gram negative bacteria like E. coli, Klebsiella, and Pseudomonas aeruginosa can cause life-threatening infections in asplenic patients. Streptococcus pneumoniae (the most frequent), Hemophilus influenzae, and Neisseria meningitidis infections can cause post-splenectomy septicaemia. There are also a few reported cases of post-splenectomy fulminant hemolytic febrile syndrome caused by Babesia (protozoan)[63,95,112,144-150,165-169].

Patients must have a thorough understanding of the disease, its complications, and the benefits and drawbacks of all current treatment options.

(V) Role of stem cell transplantation –

When a person has homozygous beta-thalassemia major or severe HbE-thalassemia, bone marrow transplantation is advised[63,83,112,144].

The hazards of a patient receiving bone marrow or stem cells from a healthy HLA-identical sister as a donor are determined by three factors[63,83,99,112,144]:

- Hepatomegaly
- Irregular iron chelation
- Portal fibrosis

On this basis, the patients can be classified into three classes[144,170] (Table 9) -

Table 9: Classification of Patients for Stem Cell Therapy[144,170]

CLASS	SURVIVAL RATE	DISEASE FREE SURVIVAL RATE	MORTALITY RATE	REJECTION RATE
I LOW RISK Adequate iron chelation with absence of other factors	94%	87%	6%	7%
II INTERMEDIATE Two out of three positive factors	84%	81%	15%	4%
III HIGH All three factors present	80%	56%	18%	33%

The overall survival rate and the disease-free survival rate in all patients receiving stem cell transplants have increased significantly with the use of immunosuppressive medications and alkylating agents. For "conditioning," medications such as busulfan, cyclophosphamide, methotrexate, and cyclosporin are administered prophylactically. However, it is advised that only those individuals who have sustained the least amount of organ damage undergo bone marrow transplantation[63,112,144,171-176].

After-effects of Bone Marrow Transplantation:

- Reduction of severe iron overload - Stem cell transplantation, routine blood draws (phlebotomy), and iron chelation for the first two years following the transplant can all help to lessen the iron laden state.

- Inability to reach puberty was discovered in several studies, particularly in class III instances[144,170-179].

Tried and failed donors for Bone marrow transplant:

- Mismatched family member (sibling, parents, relatives)
- Parental purified haploidentical CD34+ transplantation

Nonmyeloablative transplantation had greater rejection rates but low mortality. Thalassemia relapse can be avoided by additional infusions of lymphocytes from the same donor[80,94,144,170-183].

Studies on transplantation using "Matched Unrelated Donors" have demonstrated a good prognosis and outcome for patients with thalassemia. In comparison to BM transplantation, other research on cord blood transplantation have produced satisfactory but slower haematological recovery outcomes. Instead of waiting until the siblings are older and able to give their agreement for the surgery, the cord blood collected from siblings of the patients enables the retrieval of an adequate amount of stem cells at the moment of the sibling's birth. Thus, aside from class III patients who continue to reject the stem cells, stem cell transplantation is currently the only treatment that can "cure" thalassemia. To solve this problem, modified conditioning programmes are available[63,112,144,170-183].

General care for thalassemia –

Patients with thalassemia need high-level care for the rest of their lives. This includes:

-

- Early infection detection and treatment
- Folate supplementation
- Care for chronic infections brought on by cranial abnormalities, routine dental exams, and follow-up visits with suggested blood transfusion schedules

- Pre-transfusion, transfusion, and post-transfusion care

- Regular testing and monitoring of serum ferritin levels

- Early initiation of iron chelation therapy with ongoing evaluation of the drug's serum levels and side effects

- Symptomatic care for consequences such heart failure, endocrine issues, and metabolic bone diseases

- Annual serological tests for viral infections that are spread through the blood transfusion[63,83,112,144,180-185].

EXPERIMENTAL APPROACHES

(1) Reactivation/Augmentation of fetal hemoglobin (HbF) production

(2) Somatic gene therapy[112,144,180-188]

Numerous studies have been carried out in light of the finding that, whether taken alone or in combination, butyrate analogues and erythropoietin (EPO) may cause an increase in foetal hemoglobin synthesis. When sodium phenylbutyrate and hydroxyurea were administered in combination or hydroxyurea was used alone, HbF production increased in various cases of hemoglobin lepore (Hb Lepore) [homozygous or compound heterozygous] and mutations with deletion of β-globin gene cluster[3,8,51,88,93,94,104,189-192].

In NTDT, hydroxyurea (HU) raises HbF and improves laboratory measures. Antimetabolites, however, are only occasionally used in TDT. Thalidomide (an immunomodulator) in conjunction with HU and Luspatercept (an erythroid maturation agent) have been proven to significantly reduce the need for blood transfusions and associated consequences along with reduction in serum ferritin levels. Both medications have been tested and approved for use in β-TDT with instructions for careful monitoring of any negative side effects. Patients on these

drugs achieve transfusion independency and seem to maintain their response for long periods of time[93,112,144,172-197].

In addition to medications, genes are being targeted to alter HbF production. Somatic gene therapy corrects β-globin gene transcripts by using trans-splicing ribozymes to restore normal splicing and retroviruses as vectors to deliver genes into hematopoietic stem cells[172-197].

In addition, special types of thalassemia require specific treatment protocols. For example,

- Treatment of HbH disease – Splenectomy in severe cases with extreme anemia and splenomegaly.
- Patients with sickle cell thalassemia receive the same care as those with sickle cell anaemia.
- Thalassemia intermedia - It is crucial to closely follow these patients, especially if it appears that their hemoglobin level is stable. However, a regular transfusion schedule must be followed in cases with growth retardation and when anaemia symptoms interfere with everyday activities. In extreme circumstances or if hypersplenism manifests, a splenectomy is required. Due to the increased risk of iron loading in intermedia patients from gastrointestinal iron absorption (3 to 5 mg per day), it is crucial to monitor blood iron and ferritin levels often, as well as to receive a proper and sufficient chelation therapy. Iron supplements are not recommended in these cases since they could increase iron absorption by up to 10 mg per day[3,8,63,83,93,112,144,170-197].

PROGNOSIS

The survival rate and prognosis of patients with thalassemia have significantly improved over time with sufficient blood transfusion, chelation therapy, general care, and monitoring of ferritin levels. Thalassemia is still a global health issue and a financial burden, despite the fact that there are strong facilities for its care and treatment. Some cases have been demonstrated to have a poor prognosis and early mortality. Poor prognostic factors include lack of follow-up, non-compliance with chelation therapy and transfusion regimens, low socioeconomic status, and lack of awareness of the condition[63,112].

PREVENTION

Screening can be divided into two groups on the basis of expected outcomes-

• Screening for early detection of Thalassemia (TM and severe TI) and Sickle Cell Disease to achieve reduction in mortality and morbidity with improvement in quality of life of the affected.

• Screening for detection of carriers of -Thalassemia Trait and Sickle Cell Trait to reduce birth of children affected with Thalassemia or Sickle Cell Disease. Two ways are known to help prevent thalassemia:

- Genetic counselling - Marriage counselling and population screening for disease carriers
- Prenatal diagnosis –

  The pre-requisites of prenatal sampling include:

  • Thalassemia carrier status of the couple under investigation.

  • The mother's blood type to prevent Rh incompatibility if it exists.

  • Pre-test counselling and the couple's fully informed signed consent[55,63-65,83,93,112,144,145].

For prenatal diagnosis, there are three foetal sample techniques available:

1. Chorionic Villous Sampling (CVS) - Conducted between 10 and 12 weeks (first trimester of pregnancy).
2. Amniocentesis - Executed after 16 weeks of gestation in late-arriving patients or when the foetal position makes it impossible to collect the chorionic villi for sampling.
3. Fetal blood sampling: Blood is taken from the hepatic vein, the heart, or by cordocentesis at 18 to 20 weeks of gestation. Either DNA analysis or HPLC are used to process the sample[55,65,93,112,144,198,199].

Mothers are screened during the first prenatal visit, the father is screened if the mothers are carriers, and various options are suggested, including pregnancy termination, if both parents are carriers, which increases the risk that the unborn child will have severe transfusion-dependent thalassemia[112,144,198,199].

Quick DNA sequencing technology allows for the direct detection of mutations in the DNA of the foetus and members of its family, providing a rapid diagnosis with the lowest error rate ever recorded. Fetal DNA is also being extracted from maternal blood and plasma in an effort to diagnose thalassemias before implantation[198-203].

Prenatal diagnosis has several limitations because no test is perfect (2% reported mistakes). Pregnancy termination in cases when a child is affected is stressful for the mother and may be unethical for many individuals. Post-test counselling is crucial for assisting the couple in navigating the emotional strain caused by their bond to their unborn child[198-203].

ALLOIMMUNIZATION

Alloimmunization is the immune response to non-self-antigens following exposure to genetically distinct cells or tissues[16,36,72,100]. RBCs have a variety of antigens on their surface, and each person has a different distribution of these antigens. Before every blood transfusion, only the ABO and Rh D antigens are typically evaluated. Any RBC surface antigen from a donor can cause an immunological reaction in the receiver. This difference may lead to alloimmunization, especially in those who have received numerous frequent blood transfusions. These allo-antibodies do not, however, develop against all or any of the RBC antigens in every person. Individual differences also exist in how these antibodies affect a person's body and how long it takes for an immune response to manifest[35,36,72,204-208].

Patients with thalassemia who require many blood transfusions over the course of their lifespan frequently develop alloimmunization. Immunosuppressive drug therapy must be started in these patients because of the complications these newly produced antibodies can cause. These antibodies can either be of the non-hemolytic type (more prevalent) or the hemolytic kind[200-213].

A complex outstretched phenotype analysis of a patient's RBC surface antigens can help identify the antigen against which the likelihood of developing an antibody would be greatest; for example, a higher alloimmunization rate can be predicted if blood is matched only for a small subset of antigens, and a lower rate can be anticipated if a large antigen panel is tested for match. The variability between donor and recipient RBC antigens should be as low as possible[35,36,72,200-213].

According to one theory, alloimmunization is a reliable indicator of whether HLA alloimmunization may result in harmful clinical effects[209-213].

The following factors are known to contribute to the development of allo-antibodies in thalassemia:

- Disparity between the blood group systems: The development of allo-antibodies may be explained by the existence of 35 blood group systems and more than 330 distinct RBC surface antigens, each of which has a unique distribution pattern in every individual[38,206]. In a study of 319 thalassemia patients receiving routine blood transfusions, Hari Krishan Dhawan et al. (2014) discovered that alloimmunization prevalence was 5.64%. Their study mandated the requirement for RBC antigen typing prior to the first transfusion and the provision of antigen-matched blood to the recipients[214].
- Immune status of the patient: Patients with infections or other immunomodulating diseases may experience more problems because of alloimmunization (increased allo-sensitisation). Additionally, the level of allo-immunization differs from person to person. Despite numerous transfusions from different donors, some persons still fail to build allo-antibodies; nevertheless, when transfused, some people swiftly develop immunity to their least common antigen[215-218]. Rubiraida Molina-Aguilar et al. (2020) revealed how alloimmunization may be associated with immune system imbalances. Randomized antibody screening performed in 1,434 multi-transfused patients using agglutination technique showed a low CD4/CD8 ratio in alloimmunized patients along with increased B lymphocyte count, and lack of regulatory cells in these patients[216]. A study by Mohammad Taher Hojari et al. (2014) concluded that alloimmunization shortens the lifespan of circulating erythrocytes and that identifying RBC phenotypes may help reduce alloimmunization rates. They suggested that DNA technology can help read a patient's blood group profile and solve agglutination problems even though the use

of molecular methods with leukocytes may be unreliable due to contamination of the donor WBC with those of the patients[218].

- Ethnicity: Different ethnic groups have varying degrees of inflammatory immunological responses, which contribute to the development of allo-antibodies[219-221]. African Americans, who are already known to have a greater incidence of several inflammatory disorders, are particularly observed to have higher rates of this. Increased levels of inflammatory cytokines and other stimulatory molecules (such CD80 & CD86) are present in this ethnic group due to a combination of environmental, social, cultural, and economic variables[219-221]. The presence of immunomodulatory genes that favour the synthesis of molecules like cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) and programmed death-1 (PD1) that support a greater immune response may also play a role[21,72,83,215-221].
- Type of thalassemia: More in thalassemia major requiring multiple units and higher frequency of blood transfusion than any other type. Arwa Z. Al-Riyami et al. (2019) studied patients with non-transfusion dependent thalassemia intermedia cases who were more likely to develop alloimmunization. A total of 17 articles were reviewed by them. Alloimmunization rates were found to range from 2.87% to 30% in TDT patients and 6.8% to 19.5% in NTDT patients. The most common antibodies were seen to be against RBC surface antigens K and E. Age at the start of transfusion therapy, gender, history of splenectomy, the number and type of transfused blood units were all seen to be contributory factors[222].
- Race of both the donor and the recipient: A higher rate and level of alloimmunization have been observed in thalassemic recipients who are of a different race from the donor in a small number of studies. For instance, Asians receiving white blood have a larger risk of developing allo-antibodies than Asians

receiving blood from their own ethnicity (homogenicity of red cell antigens) [21,33,36,100,149,223].

- Gender: Previous literature has shown the rate of alloimmunization to be higher in females than in males[214,224,225].
- Type of blood component transfused: Due to changes in immunogenicity, using leucodepleted blood for transfusions has been demonstrated to lower the rate of alloimmunization[226]. Anuja P. Premawardana et al. (2019) examined different forms of thalassemia and assessed the criteria of treatment and the success of ongoing prevention efforts in 1774 patients at 23 different sites. They concluded that providing designated staff and adequate resources would improve the quality of care and help manage patients in multiple small units[227].
- Age of start of transfusion therapy: According to studies, the rate of alloimmunization is lower if the transfusion therapy is initiated earlier. Infants in particular, who are younger age groups, do not develop allo-antibodies to antigens found on blood cells[34]. The rate of alloimmunization can be delayed or decreased by this type of acquired immunological tolerance to the RBC allo-antigens[228]. Arwa Z. Al-Riyami et al. (2018) conducted a retrospective review and found the association between alloimmunization and patient's age and the dose of transfusion units[229].
- Total number and extent of transfusions: The likelihood of developing allo-antibodies against donor RBC antigens increases with blood transfusion volume and frequency. A study done by Nrages Obaidi et al. (2011) found no association between the number of transfused units and alloantibody production in patients with thalassemia, but that it nevertheless plays an important role in alloantibody

production in patients who have received multiple blood transfusions over a long period of time[230].

- Splenectomy status: Patients who have had splenectomy have a higher rate of alloimmunization than patients who have not. This can be related to the RBC membrane's altered shape, which intensifies and amplifies the immunomodulation and raises the risk of allosensitization in splenectomized individuals[49,231,232].
- Absolute number of antigens per RBC and immunogenicity of the antigen: The dose of the antigen in the blood group varies in millions per one RBC. Only a few of them are known to cause the production of antibodies (immunogenicity) [65]. Sumita Mahapatra et al (2019) investigated the incidence, type, and frequency of RBC alloantibodies in 816 patients with thalassemia and sickle cell anemia. They found that multiple transfused individuals need to be phenotyped before transfusion in thalassemia patients[233]. Aveen M. Raouf Abdulqader et al. (2020) examined the prevalence of alloimmunization in 204 multiple-transfused mainline thalassemia patients in Iraq. They used the gel map method to correlate the erythrocyte phenotype of ABO/RhD antigens and performed irregular antibody screen to identify these antibodies. Their study signifies the importance of matching erythrocytes to the Rhesus and Kell lineages to prevent the development of alloantibodies[234].
- Co-existence of autoantibodies in the recipient: Pregnancy or a prior blood transfusion with an unmatched blood type may be to blame. This can delay the acquisition of compatible blood, the initiation of transfusion therapy, and reduce red cell survival following the transfusion. It can also exacerbate and speed up the effects of allo-antibodies and interfere with the results of pre-transfusion compatibility testing[214]. Anjali Handa et al. (2020) performed periodic screening

for red blood cell antibodies at specific time intervals post-transfusion in 100 multiple transfused individuals and concluded its necessity to prevent alloimmunization in these patients[235].

TESTING PRESENCE OF ALLO-ANTIBODIES:

An immunological laboratory test known as the Coombs test or Antiglobulin test is used to do this. Either a direct or indirect technique can be used to identify the presence of antibodies to RBC surface antigens[236].

Principle of coomb's test (Figure 10) –

- Direct coomb's test: This test is used to find antibodies that are firmly bound to antigens on the surface of red blood cells. To separate the recipient's RBCs, the blood sample that was obtained is washed with saline. Additionally, this aids in removing additional unbound antibodies from the patient's blood that can skew test results. The bound antibodies are then identified by adding Coomb's Anti-human Globulin (AHG) monospecific reagent to identify and characterise the antibody type, or Coomb's Anti-human Globulin (AHG) polyspecific reagent to identify both IgG and/or Complement C3[236-240].
- Indirect coomb's test: By removing the native RBCs from the blood sample, it is possible to identify the unbound antibodies that are associated to the RBC surface in the patient's serum. Next, recognised antigenic foreign RBCs are incubated with the isolated serum sample. The incidence of agglutination after adding the Coomb's antiglobulin (AHG) reagent denotes a positive result[236-240].

Figure 10: Coomb's Test[237]

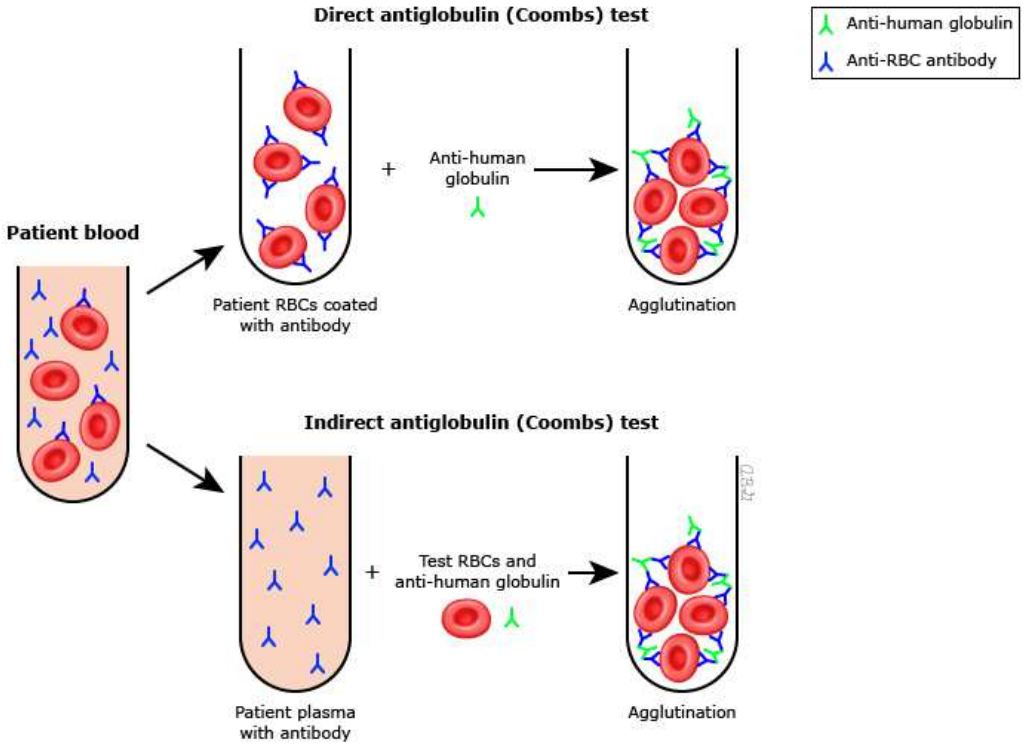


Image taken from: Direct and indirect antiglobulin (Coombs) testing [Online] Retrieved on 20-12-2022. From:

https://www.uptodate.com/contents/image?imageKey=HEME%2F100639

The patient's welfare should always come first when trying to prevent alloimmunization. But making sure that the donor and recipient are a perfect match takes time and money. Cost-benefit analysis recommends providing "Partial Better Matching" (PBM), where the antigens frequently causing alloimmunization are carefully examined. This is because the majority of alloimmunizations are non-hemolytic in nature. The antigens Rh, Cc, Ee, K, Fy, and Jk are among them[147,220,225,241,242].

The danger of alloimmunization should be decreased theoretically by using leucodepleted blood in addition to PBM. To detect delayed alloimmunization reactions, recipients must be closely monitored. Patients getting multiple and frequent transfusions should be rigorously monitored and educated about their condition[241,242].

DIRECT COOMB'S TEST [DIRECT ANTI-GLOBULIN TEST (DAT)]

USE:

To demonstrate the coating/sensitisation of RBCs in-vivo with immune antibodies [immunoglobulin G (IgG)] of the complement[236-248].

PRINCIPLE:

Anti-human globulin (AHG) reagent, which binds to the Ig G antibodies present on the RBC surface, is added to a sample of antibody-coated RBCs to determine the presence of agglutination[236-240,243-248].

AHG reagents are used to directly screen washed RBCs from a patient or a donor to look for cell sensitization brought on by blood transfusions or even hemolysis brought on by incompatibility and mismatched organ transplants[236-240,243-248].

It can be done simultaneously, although it is typically done initially with only polyspecific reagents and then repeated with monospecific reagents in case of a positive polyspecific DAT[236-240,243-248].

SAMPLE:

4 to 7 ml of patient's blood in Ethylenediamine tetraacetic acid [EDTA tube (lavender top)] is preferred. EDTA is used to chelate serum calcium to prevent in-vitro fixation of complement factor C3 that would otherwise lead to a falsely negative result[244].

REAGENTS, EQUIPMENTS AND SUPPLIES:

- 12 x 75 mm tubes
- Polyspecific AHG
- Monospecific AHG
- Dispo pipettes
- Anti-IgG
- Anti-C3
- Inedible marking pens
- Isotonic saline
- Coomb's control cells
- Serofuge
- Lighted agglutination viewer[244]

PROCEDURE[236-240,243-247]:

Wash the patient's RBCs 3-6 times with normal saline

⬇

Decant the supernatant saline from the last wash

⬇

Make 5% suspension of washed red cells

⬇

Add one drop of 5% washed cells to a labelled tube

⬇

Add one drop of AHG reagent

⬇

Mix well and centrifuge at 1000 RPM x 1 min

⬇

Note the results as per the reaction grading chart

Table 10: Reactions Grading Chart[236] [Refer to Figure 11]

REACTIONS	PATTERN
4+	Agglutinated RBCs form a band at the top of the bead column
3+	Most agglutinated RBCs are retained or trapped in the upper half of the bead column
2+	Agglutinated RBCs are observed throughout the length of the bead column A small button of cell may be visible at the bottom of the bead column
1+	Most agglutinated RBCs are retained or trapped in the lower half of the bead column A small button of cell may be visible at the bottom of the bead column
0.5+	Most agglutinated RBCs pass through and form a disrupted button at the bottom of the bead column
0 Negative	All RBCs pass through and form a smooth button at the bottom of the bead column (No agglutination)

Table taken from: Tholpady A. Antibody screening. Retrieved on 20-12-2022. From: https://emedicine.medscape.com/article/1731232-overview#:~:text=The%20antibody%20screening%20test%20performed,that%20are%20considered%20clinically%20significant.

Figure 11 – DCT Results – Reaction Grading Chart[236]

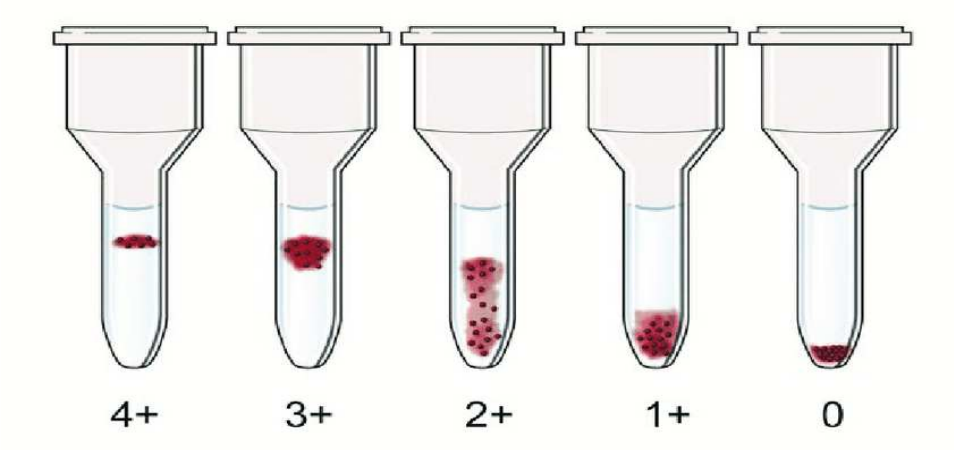


Image taken from: Tholpady A. Antibody screening. Retrieved on 20-12-2022. From: https://emedicine.medscape.com/article/1731232-overview#:~:text=The%20antibody%20screening%20test%20performed,that%20are%20considered%20clinically%20significant.

APPLICATIONS OF DCT:

- Diagnosis of hemolytic disease of the new-born (HDN)
- Diagnosis of AIHA (Auto-Immune Hemolytic Anemia)
- Diagnosis of drug induced immune hemolytic anemia
- Investigation of hemolytic transfusion reactions due to allo- or auto-antibodies[247]

Sources of errors in DCT are given in Table 11[247].

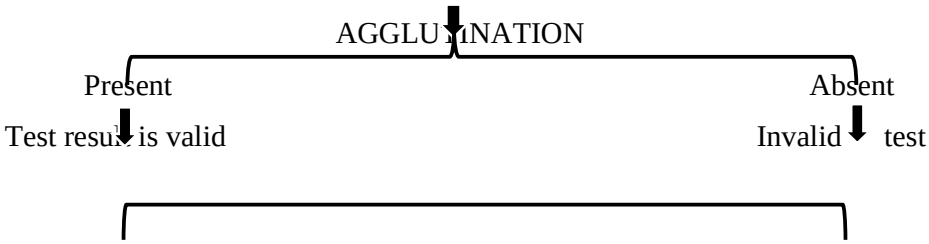
Table 11: Sources of Errors in DCT[247]

FALSE NEGATIVE	FALSE POSITIVE
Inadequate washing of red cells	Autoagglutination or poly-agglutination of RBCs
Test is interrupted or delayed	Improper washing of glassware
Problems with AHG reagent - Bacterial contamination of reagent - Improper storage - Failure to add AHG reagent - Decreased reactivity of AHG reagent	Over centrifugation
	Presence of other antibodies in the AHG reagent
	Contaminated reagents
	Saline contaminated by heavy metals or colloidal silica

Table taken from: Coombs test. Retrieved on 20-12-2022. From: https://my.clevelandclinic.org/health/diagnostics/22978-coombs-test#:~:text=Direct%20Coombs%20test%20(sometimes%20called,such%20as%20autoimmune%20hemolytic%20anemia.

VALIDATION OF NEGATIVE DCT[243-248]:

Add IgG sensitized red cells (check cells) to negative coombs test



LIMITATIONS:

- Observer variation of the interpretation of the results may affect the accuracy of the test result.
- Proper use and handling of the product should be strictly followed as per instructions[243-248].

MATERIALS AND METHODS

STUDY DESIGN: One year hospital based prospective cross-sectional study.

STUDY PERIOD: January 01, 2021 to December 31, 2021

STUDY POPULATION: All transfusion dependent thalassemia patients admitted under thalassemia day care unit at KLE's Dr. Prabhakar Kore Charitable hospital and KLE's Dr PK Hospital and MRC, Belagavi, from 1st January 2021 to 31st December 2021.

INCLUSION CRITERIA:

- All transfusion dependent thalassemia patients of either sex of all age groups.

EXCLUSION CRITERIA:

- Refusal of consent to participate in the study
- Thalassemia patients with additional hemoglobinopathies.
- Immunocompromised patients
- Thalassemia patients on treatment with immunosuppressants
- Pregnant thalassemia patients

METHODOLOGY:

A total of 205 patients of Thalassemia registered for regular blood transfusion management at KLE's Dr. Prabhakar Kore Charitable hospital and KLE's Dr PK Hospital and MRC, Belagavi, Karnataka were selected for the study. An informed written consent (from patients >18-years of age) or assent (from parents/guardians of patients <18-years of age) was taken in all the cases.

The information about the clinical parameters was obtained from the patient's records from Medical Records Dept, Blood bank and Department of Pathology, KLE's Dr. Prabhakar Kore Charitable hospital and KLE's Dr PK Hospital & MRC, Belagavi.

The data was collected using a predesigned proforma consisting of the following information:

- Age of patient
- Gender
- Blood group
- Consanguinity
- Siblings with thalassemia
- Diagnosis via Hb-Electrophoresis
- Age at diagnosis and the first blood transfusion
- Transfusion regimen
- Total number of transfusions till date
- Type of blood component used for transfusion (standard, washed, leucodepleted)
- Total volume of transfusion received till date
- Hb before the last transfusion
- Hb after the last transfusion

- Rate of fall of hemoglobin per week
- Splenectomy status
- Alloimmunization status

After obtaining the above information, 4 to 6 ml of venous blood was collected from each patient in EDTA tube (lavender top).

Step 1 – Preparation of the 4% red blood cell suspension.

0.5 ml of the anticoagulated blood was added to 4 ml of normal saline in a test-tube. This was mixed and centrifuged at 1000 rpm for 5 min. The supernatant was removed and discarded. 50 microlitre of the settled (packed) RBCs was then mixed with 1 ml of normal saline in a separate test-tube to prepare the 4% RBC suspension.

Step 2 – Performing Direct Coomb's test

The wells of the Ortho workstation cassettes containing the polyclonal anti-human globulin (AHG) are opened. 6 samples can be tested at a time. To this, 10 microlitre of the above prepared cell suspension is added and then centrifuged for 5 minutes at 1500 rpm. The results are read as per the grading chart (Table 12, Figure 12).

Table 12: Reactions Grading Chart

REACTIONS	PATTERN
4+	Agglutinated RBCs form a band at the top of the bead column
3+	Most agglutinated RBCs are retained or trapped in the upper half of the bead column
2+	Agglutinated RBCs are observed throughout the length of the bead column A small button of cell may be visible at the bottom of the bead column
1+	Most agglutinated RBCs are retained or trapped in the lower half of the bead column A small button of cell may be visible at the bottom of the bead column
0.5+	Most agglutinated RBCs pass through and form a disrupted button at the bottom of the bead column
0 Negative	All RBCs pass through and form a smooth button at the bottom of the bead column (No agglutination)

Figure 12: Anti-Human Globulin (AHG) Coombs Test Card

STATISTICAL ANALYSIS

The presentation of the Categorical variables was done in the form of number and percentage (%). On the other hand, the quantitative data were presented as the means ± SD and as median with 25th and 75th percentiles (interquartile range). The data normality was checked by using Kolmogorov-Smirnov test. The cases in which the data was not normal, we used non-parametric tests. The following statistical tests were applied for the results:

1. The association of the variables which were quantitative and not normally distributed in nature were analysed using Mann-Whitney Test (for two groups) and variables which were quantitative and normally distributed in nature were analysed using ANOVA (for more than two groups) and Independent t test (for two groups).
2. The association of the variables which were qualitative in nature were analysed using Chi-Square test. If any cell had an expected value of less than 5 then Fisher's exact test was used.
3. Receiver operating characteristic curve was used to find cut off point of volume of blood transfused (mL) for predicting splenectomy, positive alloimmunization.

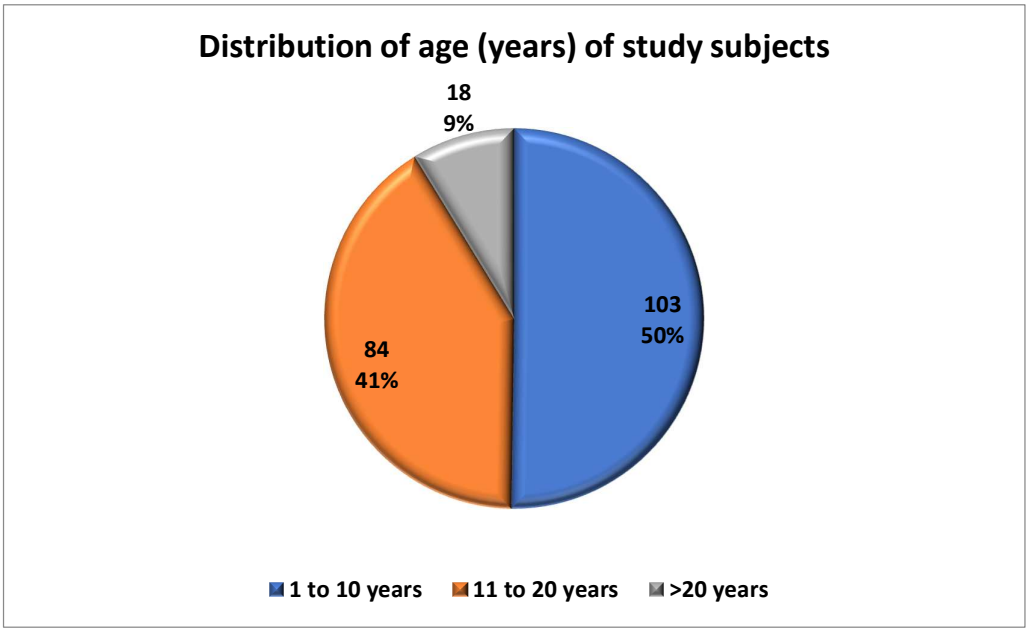
The data entry was done in the Microsoft EXCEL spreadsheet and the final analysis was done with the use of Statistical Package for Social Sciences (SPSS) software, IBM manufacturer, Chicago, USA, version 25.0.

For statistical significance, p value of less than 0.05 was considered statistically significant.

RESULTS AND OBSERVATIONS

The study was conducted at KLE's Dr. Prabhakar Kore Charitable hospital and KLE's Dr PK Hospital and MRC, Belagavi. 205 transfusion dependent Thalassemia patients of either sex of all age groups were included in the study apart from the patients meeting the exclusion criteria. Phlebotomy was performed under strict aseptic precautions and 4-6 mL of venous blood was collected in EDTA test tubes. Antibody screening via Direct Coomb's Test (DCT) was performed in all collected samples and results are as follows.

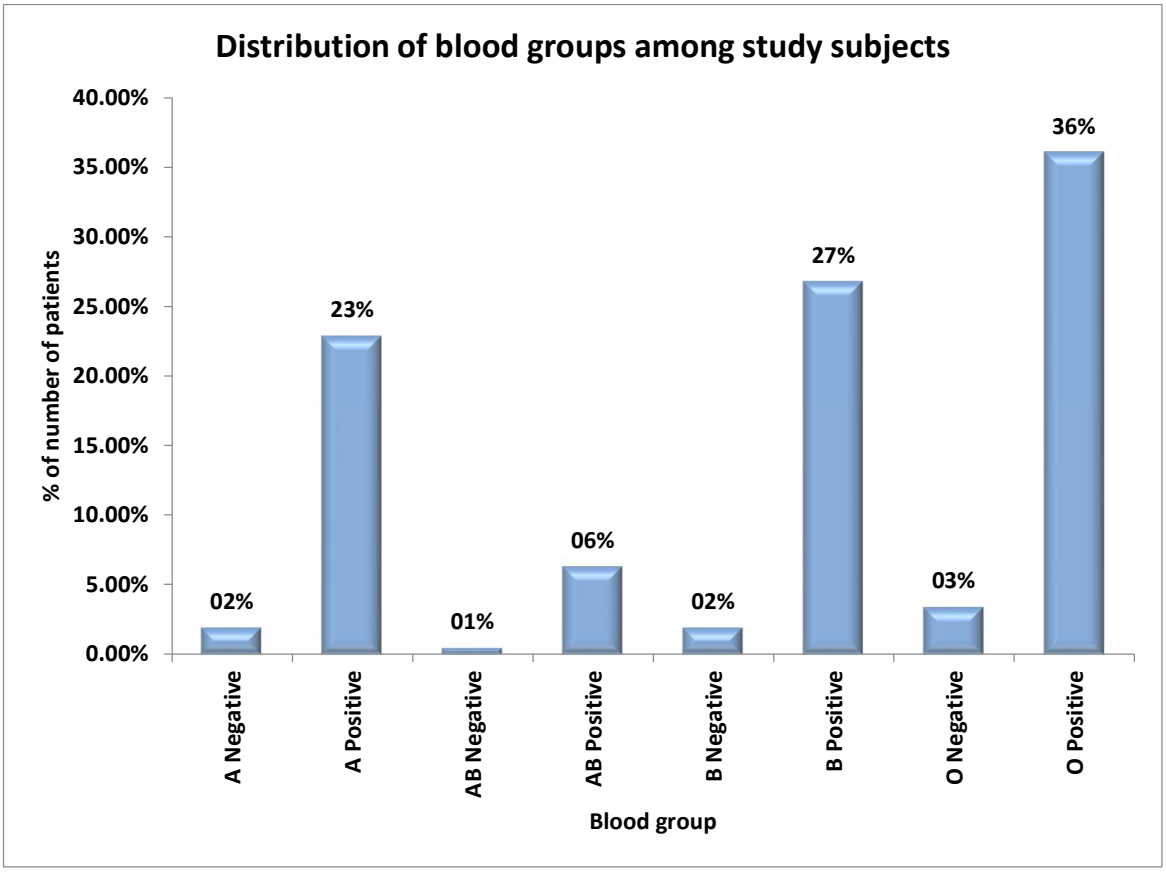
Figure 13: Distribution of age (years) of study subjects



Patients between the ages of 1 and 10 made up 50% (103), while those between the ages of 11 and 20 made up 41% (84) of the cases. Only 09% (18) were in the >20-year-old age group [Figure 13].

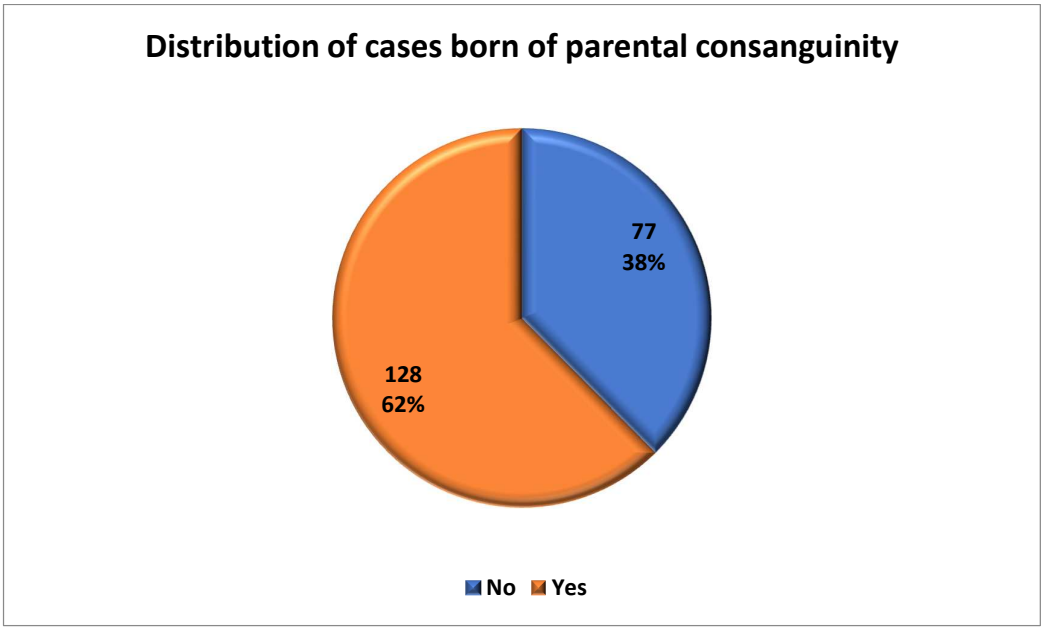
The average age of the study participants was 11 +/- 06, while the median age (25th-75th percentile) was 10 (07-15). Of the total number of patients, 43% (88) were female and 57% (117) patients were male.

Figure 14: Distribution of blood groups among study subjects



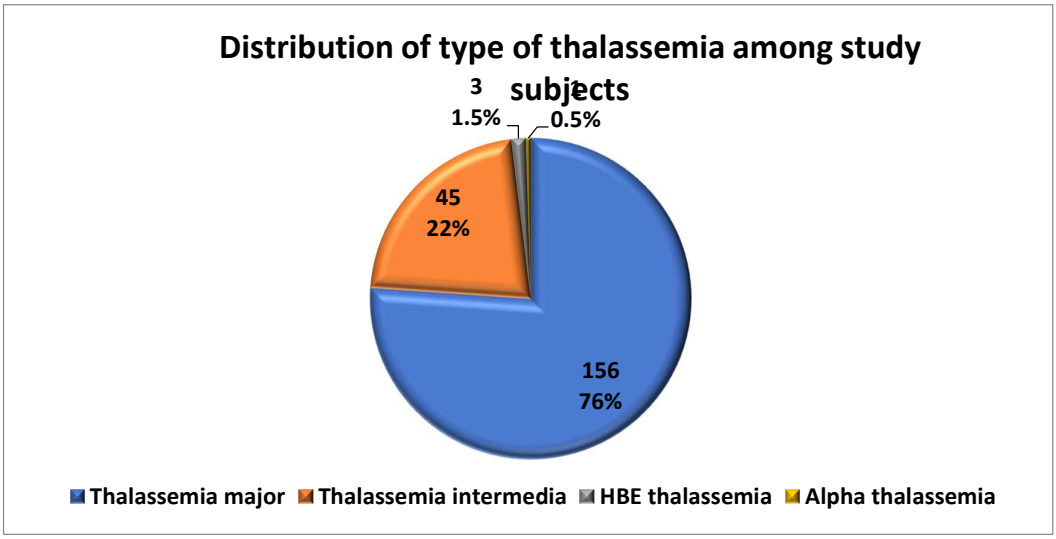
O-Positive made up most patient blood types (74, or 36%), followed by B-Positive (55, or 27%), A-Positive (47, or 23%), AB-Positive (13, or 06%), O-Negative (07, or 03%), A-Negative (04, or 02%), and B-Negative (04, or 02%). Only one patient (01%) out of 205 had an AB-Negative blood group. [Figure 14]

Figure 15: Distribution of cases born of parental consanguinity



Majority of the thalassemia patients (128, or 62%) were born of parental consanguinity [Figure 15]. Among them 41 (20%) had a family history of hemoglobinopathies and 39 (19%) cases had a sibling with diagnosed thalassemia.

Figure 16: Distribution of type of thalassemia among study subjects



Hemoglobin electrophoresis was used to determine the type of thalassemia. Thalassemia major, followed by thalassemia intermedia, was the most frequently diagnosed condition - 156 (76%) and 45 (22%) respectively. Only 3 cases of HB-E thalassemia (1.5%) and 1 case of alpha-thalassemia (0.5%), were observed. [Figure 16]

Table 13: Distribution of age at diagnosis and age at first blood transfusion (years) among the study subjects.

Age at diagnosis Age at first transfusion (years)	Frequency	Percentage
1 to 10 years	128	**62%**
11 to 20 years	67	**33%**
>20 years	09	**04%**
Unknown	01	**01%**
Mean ± SD	9 ± 5	
Median (25th-75th percentile)	8.5 (6-13)	
Range	0.5-28	

A total of 128 patients (62%) received their diagnoses and started transfusion therapy between the ages of 1 and 10 years, 67 patients (33%) were diagnosed and treated from the ages of 11 and 20. Only 09 out of 205 patients (04%) with a diagnosis and transfusion therapy started when they were older than 20, and in 01 out of 205 patients (01%) this history was unknown. [Table 13]

Majority of Thalassemia major patients (98/157 (62%)) and Thalassemia intermedia patients (30/45 (67%)) were born of parental consanguinity.

Twenty six out of 157 patients of Thalassemia major (16.5%) and 11 out of 45 Thalassemia intermedia patients (24%) had a sibling with Thalassemia.

Table 14: Distribution of transfusion regimen among study subjects.

Transfusion regimen	Pre-transfusion hemoglobin to be maintained (g/dl or g%)	Frequency	Percentage
Super-transfusion	11 to 12	04	**02%**
Hyper-transfusion	09 to 10	163	**79.5%**
Hypo-transfusion	07 to 08	38	**18.5%**
Total		205	**100%**

The majority (163 (80%) of our patients were on hyper-transfusion regimen to maintain the pre-transfusion hemoglobin between 09 and 10 g/dl, followed by hypo-transfusion regimen (38 (19%) to maintain pre-transfusion hemoglobin between 07 and 08 g%, and only 04 out of 205 patients (02%) received super-transfusion regimen to maintain pre-transfusion hemoglobin between 11 and 12 g% [Table 14].

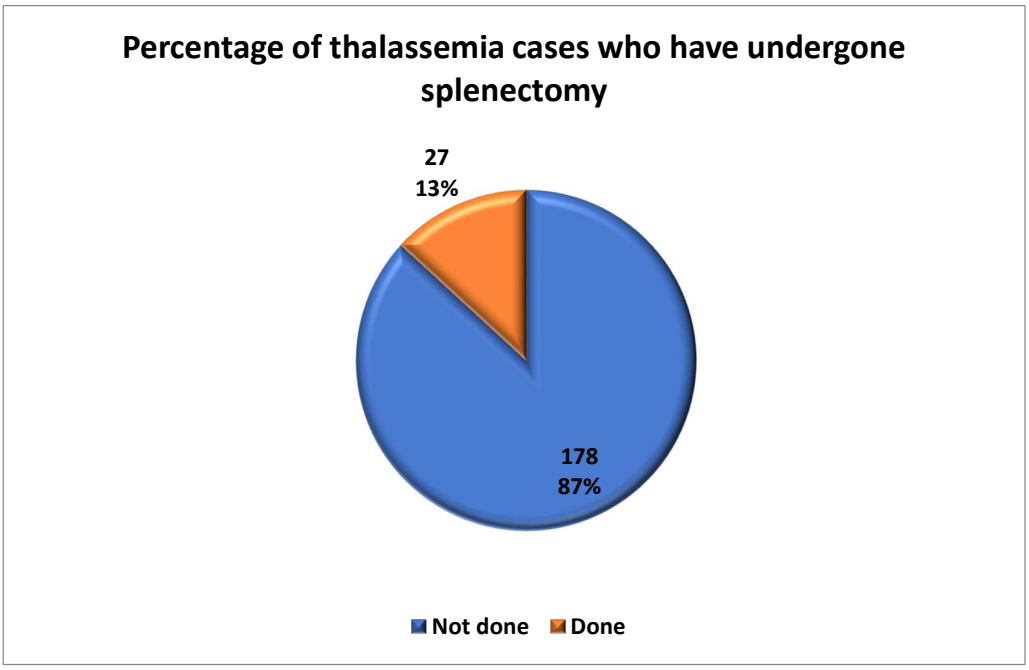
Majority of the cases (192, or 94%) were receiving matched leucodepleted packed red blood cells (PRBC), 08 (04%) patients receiving triple saline-washed PRBC (contains 10-20% less RBCs than original unit) and 05 (02%) patients are receiving Donor Type ‘O’ PRBC.

Table 15: Descriptive statistics of number of transfusions of study subjects

Variable	Mean ± SD	Median (25th-75th percentile)	Range
Number of transfusions	118 ± 78	105 (60-168)	4-412

Mean value of number of transfusions of study subjects was 118 ± 78 with median (25th-75th percentile) of 105 (60-168). [Table 15]

Figure 17: Percentage of thalassemia cases who have undergone splenectomy



Only 27 patients (13%) out of 205 underwent splenectomy [Figure 17].

Table 16: Type of thalassemia among the operated cases.

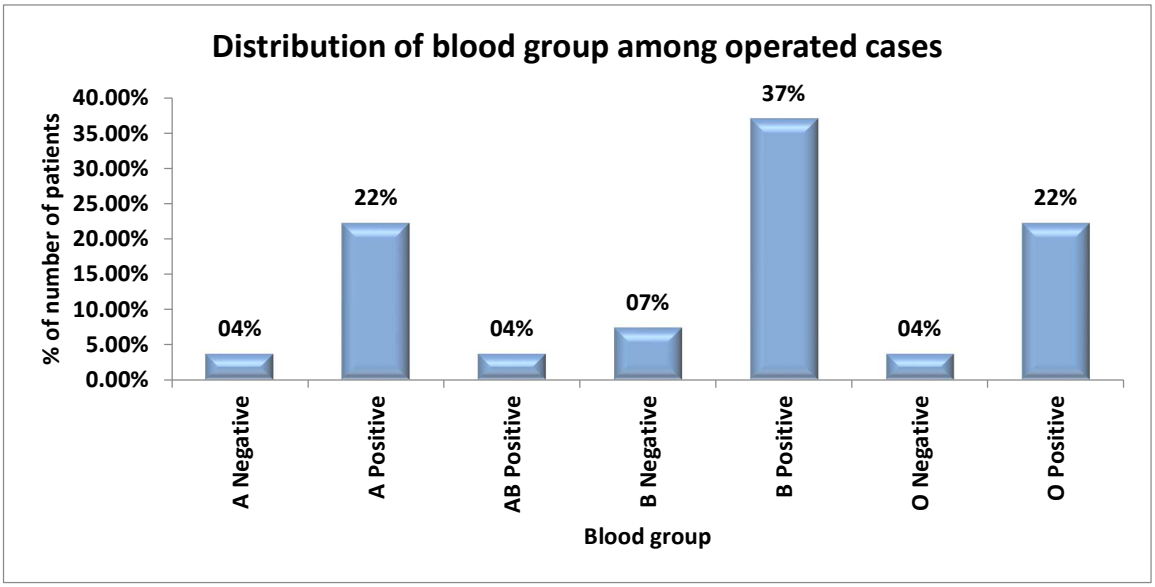
Type of thalassemia	Frequency	Percentage
Thalassemia major	14	52%
Thalassemia intermedia	12	44%
Hb-E Thalassemia	01	04%
Total operated cases	27	100%

Majority of the operated patients were that of Thalassemia major (14 (52%)) followed by Thalassemia intermedia (12 (44%)) and 1 case (04%) of Hb-E Thalassemia. [Table 16]

Only 7 operated (26%) cases had a sibling with Thalassemia.

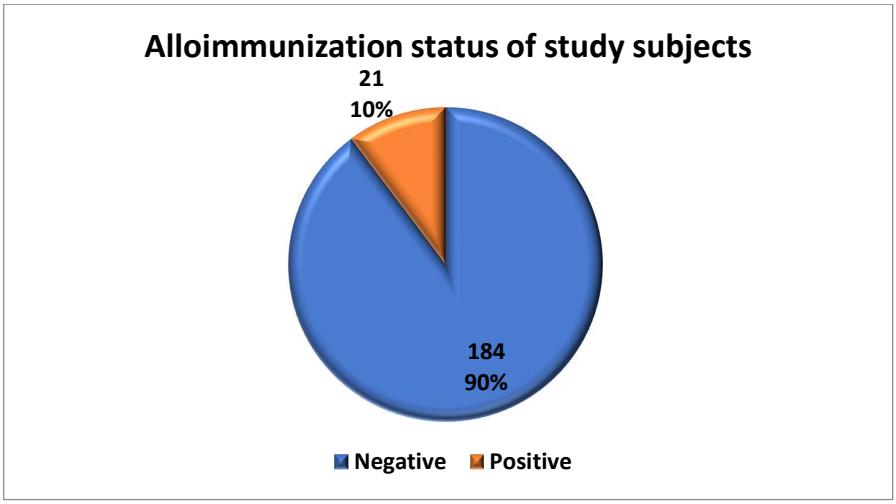
Majority (17 (63%)) of the operated cases were born of parental consanguinity.

Figure 18: Distribution of blood group among operated thalassemia cases.



Majority of the operated cases of Thalassemia belonged to the blood group B-Positive (10 (37%)) followed by A-Positive (6 (22%)), O-Positive (6 (22%)) and B-Negative (02 (07%)). Only 1 out of 27 operated patients (4%) each belonged to the blood groups A-Negative, AB-Positive and O-Negative. [Figure 18]

Figure 19: Distribution of alloimmunization status of study subjects.

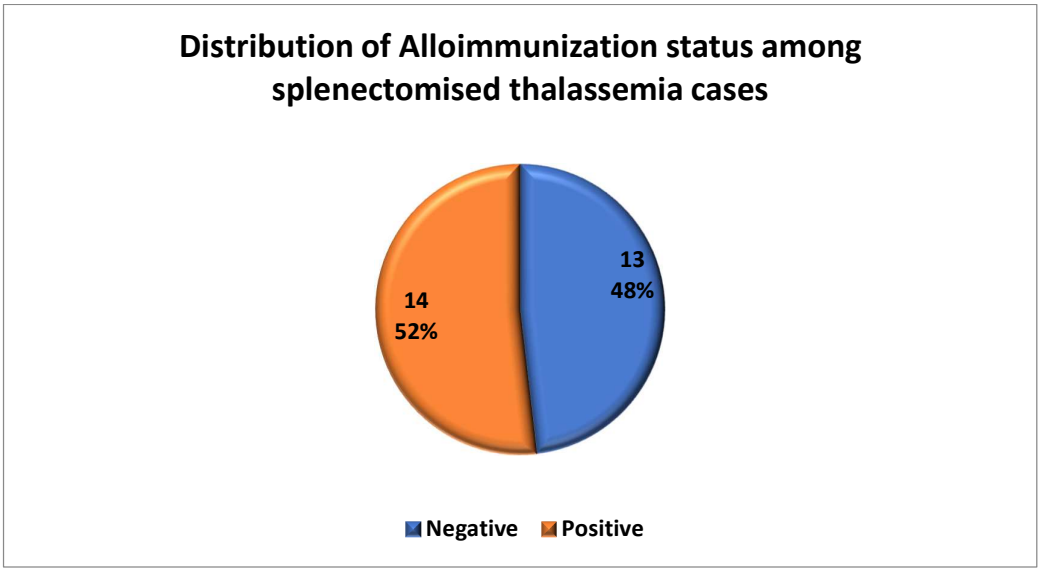


Only 21 out of 205 thalassemia patients (10%) showed presence of alloimmunization. [Figure 19]. Thalassemia major outnumbered Thalassemia intermedia among the alloimmunized cases.

Out of 41 cases with family history of Thalassemia, majority (37 (90%)) developed Alloimmunization. Only 04 out of 21 cases with positive Alloimmunization (19%) had sibling with Thalassemia.

Majority (16 (76%)) of the alloimmunized cases were born of parental consanguinity.

Figure 20: Distribution of alloimmunization status among splenectomised patients



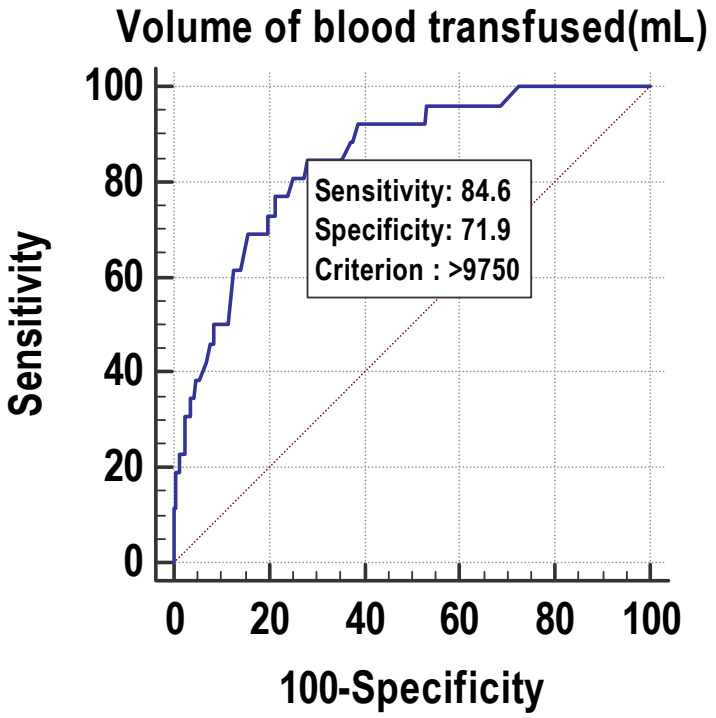
Among the operated thalassemia cases, majority [52% (8 major and 6 intermedia)] of them developed Alloimmunization post-splenectomy. [Figure 20]

Table 17: Distribution of blood groups among the alloimmunized cases

Blood group	Frequency	Percentage (%)
A-Positive	05	**24**
B-Negative	01	**05**
B-Positive	08	**38**
O-Positive	07	**33**
Total cases with positive Alloimmunization	21	**100**

Most of the alloimmunized cases belonged to the blood group B-Positive (8 (38%)), followed by O-Positive (7 (33%)) and A-Positive (5 (24%)) with only 1 case (5%) belonging to the blood group B-Negative. [Table 17]

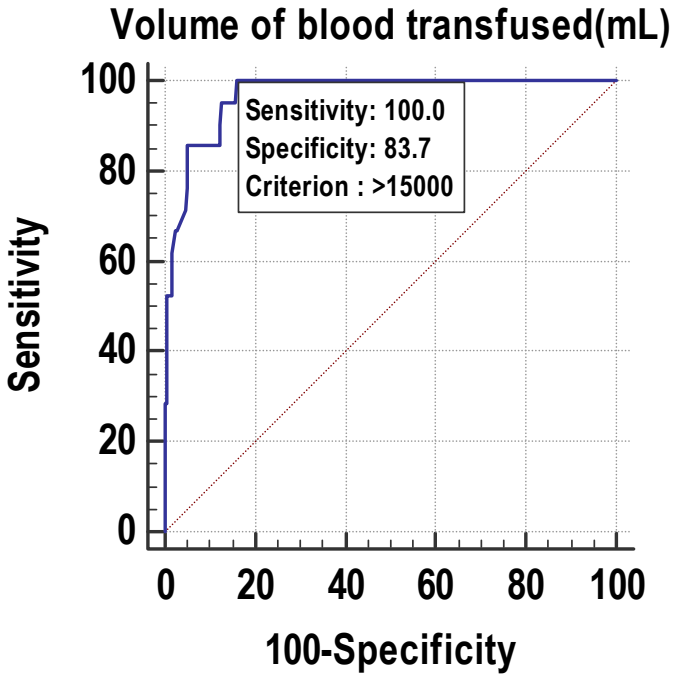
Figure 21: Receiver operating characteristic (roc) curve of volume of blood transfused (ml) for predicting splenectomy.



ROC curves above the diagonal line are considered to have reasonable discriminating ability to predict splenectomy. Discriminatory power of volume of blood transfused (mL) (AUC 0.848; 95% CI: 0.789 to 0.896) was excellent. Volume of blood transfused was the significant predictor of splenectomy at cut off point of >9750 ml with area under curve of 0.848 for correctly predicting splenectomy. [Figure 21]

The above table shows that the patients who had undergone splenectomy, 84.62% of patients had volume of blood transfused >9750 ml. If volume of blood transfused >9750 ml, then there was 31.90% probability of splenectomy and if volume of blood transfused <= 9750 ml, then 96.80% chances of no splenectomy. Among patients who had not undergone splenectomy, 71.86% of patients had volume of blood transfused <=9750 ml.

Figure 22: Receiver operating characteristic curve of volume of blood transfused (ml) for predicting positive alloimmunization.



ROC curves above the diagonal line are considered to have reasonable discriminating ability to predict positive Alloimmunization. Interpretation of the area under the ROC curve showed that the performance of volume of blood transfused (mL) (AUC 0.967; 95% CI: 0.932 to 0.988) was outstanding. Volume of blood transfused was the significant predictor of positive Alloimmunization at cut off point of >15000 ml with area under curve of 0.967 for correctly predicting positive Alloimmunization. [Figure 22]

The above table shows that the patients who had positive Alloimmunization, 100% of patients had volume of blood transfused >15000 ml. If volume of blood transfused >15000 ml, then there was 42.90% probability of positive Alloimmunization and if volume of blood transfused <=15000 ml, then 100% chances of negative Alloimmunization. Among patients who had negative Alloimmunization, 83.72% of patients had volume of blood transfused <=15000 ml.

Table 18: Association of blood group with type of thalassemia in total study subjects.

Blood group	Thalassemia major (n=156)	Thalassemia intermedia (n=45)	Others (n=4)	Total	p value
A-Negative	03 (**02%**)	01 (**02%**)	0 (**0%**)	04 (**01%**)	0.54*
A-Positive	34 (**22%**)	12 (**27%**)	01 (**25%**)	47 (**25%**)	
AB-Negative	01 (**01%**)	0 (**0%**)	0 (**0%**)	01 (**0.3%**)	
AB-Positive	09 (**06%**)	04 (**09%**)	0 (**0%**)	13 (**05%**)	
B-Negative	03 (**02%**)	01 (**02%**)	0 (**0%**)	04 (**01%**)	
B-Positive	44 (**28%**)	08 (**18%**)	03 (**75%**)	55 (**27%**)	
O-Negative	04 (**2%**)	03 (**07%**)	0 (**0%**)	07 (**2%**)	
O-Positive	58 (**37%**)	16 (**35%**)	0 (**0%**)	74 (**36%**)	
Total cases of Thalassemia	156 (**100%**)	45 (**100%**)	04 (**100%**)	205 (**100%**)	

* Fisher's exact test

With p value=0.54, there was no association between the type of thalassemia and blood group in total study subjects. [Table 18]

Table 19: Association of alloimmunization status with blood group in total study subjects.

Alloimmunization status	A- (n=04)	A+ (n=47)	AB- (n=01)	AB+ (n=13)	B- (n=04)	B+ (n=55)	O- (n=07)	O+ (n=74)	Total	p value
Negative	04 (**100%**)	42 (**89%**)	01 (**100%**)	13 (**100%**)	03 (**75%**)	47 (**85%**)	07 (**100%**)	67 (**90.5%**)	184 (**90%**)	0.649*
Positive	0 (**0%**)	05 (**11%**)	0 (**0%**)	0 (**0%**)	01 (**25%**)	08 (**14.5%**)	0 (**0%**)	07 (**9%**)	21 (**10%**)	
Total	04 (**100%**)	47 (**100%**)	01 (**100%**)	13 (**100%**)	04 (**100%**)	55 (**100%**)	07 (**100%**)	74 (**100%**)	205 (**100%**)	

* Fisher's exact test

With p value=0.649, there was no association between alloimmunization and blood group in total study subjects. [Table 19]

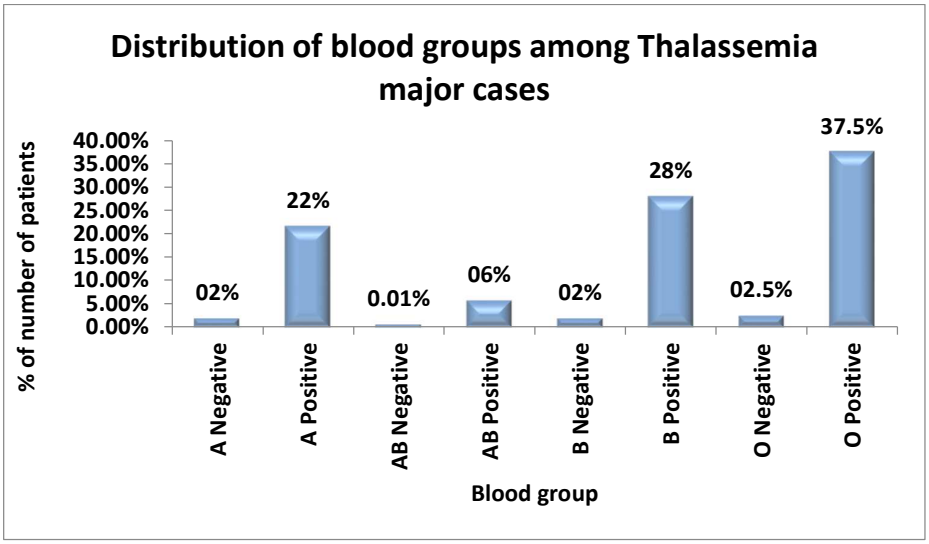
Table 20: Association of alloimmunization with splenectomy in total study subjects.

Alloimmunization	Splenectomy not done (n=178)	Splenectomy done (n=27)	Total	p value
Negative	171 (**96%**)	13 (**48%**)	184 (**90%**)	<0.0001†
Positive	07 (**04%**)	14 (**52%**)	21 (**10%**)	
Total	178 (**100%**)	27 (**100%**)	205 (**100%**)	

† Chi square test

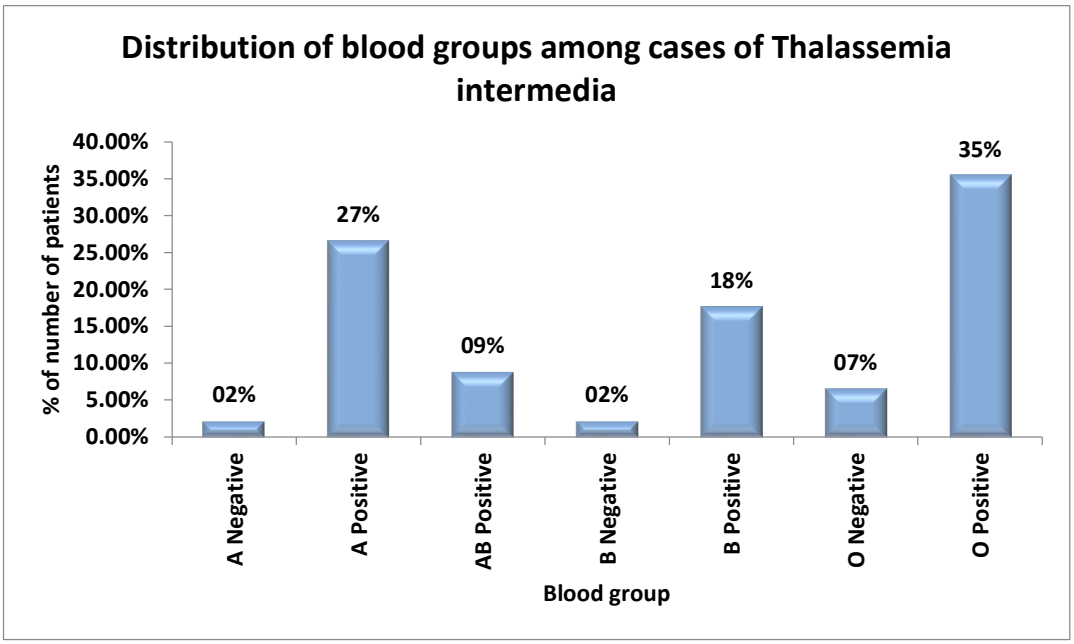
Proportion of patients with positive Alloimmunization was significantly higher among cases that underwent splenectomy (p value <0.0001). There was a strong association between alloimmunization and splenectomy among thalassemia cases. [Table 20]

Figure 23: Distribution of blood groups among thalassemia major cases



Majority of the Thalassemia major cases belonged to the blood group O-Positive (59 (37.5%)), followed by B-Positive (44 (28%)), A-Positive (34 (22%)), AB-Positive (09 (06%)), O-Negative (04 (02.5%)), A-Negative (03 (02%)) and B-Negative (03 (02%)). Only one case of Thalassemia major belonged to the blood group AB-Negative. [Figure 23]

Figure 24: Distribution of blood groups among cases of thalassemia intermedia.



Most of the cases of Thalassemia intermedia belonged to the blood group O-Positive (16 (35%)), followed by A-Positive (12 (27%)), B-Positive (08 (18%)), AB-Positive

(04 (09%)) and O-Negative (03 (07%)). Only 1 out of 45 intermedia patients (02%) belonged to the blood groups A-Negative and B-Negative. [Figure 24]

Majority of the cases with positive alloimmunization were undergoing hyper-transfusion regimen (12 (57%)) to maintain the hemoglobin of 09 to 10 g%. Only 09 out of 21 patients (43%) were undergoing hypo-transfusion regimen to maintain the hemoglobin between 07 to 08 g%.

Majority of the splenectomised cases were undergoing hyper-transfusion regimen (16 (59%)) to maintain the hemoglobin of 09 to 10 g%. Only 11 out of 27 patients (41%) were undergoing hypo-transfusion regimen to maintain the hemoglobin between 07 to 08 g%.

Table 21: Association of alloimmunization with type of thalassemia.

Alloimmunization	Thalassemia major (n=156)	Thalassemia intermedia (n=45)	Others (n=4)	Total	p value
Negative	142 (**91%**)	38 (**84%**)	4 (**100%**)	184 (**90%**)	0.397*
Positive	14 (**09%**)	7 (**16%**)	0 (**0%**)	21 (**10%**)	
Total	156 (**100%**)	45 (**100%**)	4 (**100%**)	205 (**100%**)	

* Fisher's exact test

With p value=0.397, there was no association between alloimmunization and diagnosis. [Table 21]

Table 22: Association of splenectomy with type of thalassemia.

Splenectomy	Thalassemia major (n=156)	Thalassemia intermedia (n=45)	Others (n=04)	Total	p value
Not done	142 (**91%**)	33 (**73%**)	03 (**75%**)	178 (**87%**)	0.006*
Done	14 (**09%**)	12 (**27%**)	01 (**25%**)	27 (**13%**)	
Total	156 (**100%**)	45 (**100%**)	04 (**100%**)	205 (**100%**)	

* Fisher's exact test

With P value=0.006, there was no association between splenectomy and type of thalassemia (table 22).

Table 23: Association of blood group with operated thalassemia cases.

Blood group	Splenectomy not done (n=178)	Splenectomised (n=27)	Total	p value
A-Negative	03 (**02%**)	1 (**04%**)	4 (**03%**)	0.201*
A-Positive	41 (**23%**)	6 (**22%**)	47 (**22.5%**)	
AB-Negative	1 (**0.5%**)	0 (**0%**)	1 (**0.2%**)	
AB-Positive	12 (**07%**)	1 (**04%**)	13 (**06.5%**)	
B-Negative	2 (**01.5%**)	2 (**07%**)	4 (**02%**)	
B-Positive	45 (**25%**)	10 (**37%**)	55 (**27%**)	
O-Negative	6 (**03%**)	1 (**04%**)	7 (**03%**)	
O-Positive	68 (**38%**)	6 (**22%**)	74 (**36%**)	
Total	178 (**100%**)	27 (**100%**)	205 (**100%**)	

* Fisher's exact test

With p value=0.201, there was no connection between blood type and splenectomy in Thalassemia patients irrespective of the type of thalassemia. [Table 23]

Table 24: Association of age at diagnosis and 1st transfusion (years) with alloimmunization.

Age at diagnosis and 1st transfusion (years)	Negative (n=183)	Positive (n=21)	Total	p value
1 to 10 years	126 (**69%**)	02 (**09.5%**)	128 (**63%**)	<0.0001*
11 to 20 years	53 (**29%**)	14 (**67%**)	67 (**33%**)	0.0005†
>20 years	04 (**02%**)	05 (**24%**)	09 (**04%**)	0.0007*
Mean ± SD	8.57 ± 4.76	16.72 ± 5.1	9.41 ± 5.39	<0.0001¶
Median (25th-75th percentile)	8.1 (5.25-11.35)	15.8 (13.3-19.3)	8.5 (5.6-13.1)	
Range	0.5-28	7.2-27.8	0.4-28.1	

¶ Independent t test, * Fisher's exact test, † Chi square test

With p value <0.0001, the age at diagnosis and 1st transfusion (years) was significantly associated with the development of alloimmunization. [Table 24]

Table 25: Association of number of blood transfusions with alloimmunization.

Number of blood transfusions	Negative (n=184)	Positive (n=21)	Total	P value
Mean ± SD	106.91 ± 71.73	216.76 ± 56.63	118.16 ± 77.75	<0.0001‡
Median (25th-75th percentile)	98.5 (53-149.75)	190 (174-263)	105 (60-168)	
Range	4-412	102-319	4-412	

‡ Mann Whitney test

With p value <0.0001, the total number of transfusions was significantly associated with the development of alloimmunization in thalassemia cases. [Table 25]

Table 26: Association of parental consanguinity with alloimmunization among thalassemia.

Parental Consanguinity	Negative (n=184)	Positive (n=21)	Total	p value
No	72 (**39%**)	05 (**24%**)	77 (**38%**)	0.17†
Yes	112 (**61%**)	16 (**76%**)	128 (**62%**)	
Total	184 (**100%**)	21 (**100%**)	205 (**100%**)	

† Chi square test

Parental consanguinity did not seem to have a significant effect on the alloimmunization status among thalassemia cases (p value=0.17). [Table 26]

Table 27: Association of volume of blood transfused (ml) with alloimmunization.

Volume of blood transfused (mL)	Negative (n=172)	Positive (n=21)	Total	p value
Mean ± SD	6919.84 ± 7937.88	32197.62 ± 10714.06	9670.27 ± 11417.46	<.0001‡
Median (25th-75th percentile)	3750 (475-10562.5)	31200 (24000-43200)	5750 (600-16750)	
Range	40-40000	16750-52250	40-52250	

‡ Mann Whitney test

With p value <0.0001 the volume of blood transfused (mL) was seen to significantly affect the alloimmunization status in thalassemia patients. [Table 27]

Table 28: Association of age at diagnosis and 1st transfusion (years) with splenectomy.

Age at diagnosis and 1st transfusion (years)	Not done (n=177)	Done (n=27)	Total	p value
1 to 10 years	122 (**69%**)	06 (**22%**)	128 (**63%**)	<0.0001†
11 to 20 years	50 (**28%**)	17 (**63%**)	67 (**33%**)	0.0003†
>20 years	05 (**03%**)	04 (**15%**)	09 (**04%**)	0.019*
Mean ± SD	8.6 ± 5.01	14.67 ± 4.88	9.41 ± 5.39	<0.0001¶
Median (25th-75th percentile)	8.1 (5-11.3)	14.1 (13.1-17.15)	8.5 (5.6-13.1)	
Range	0.5-28	6.8-25.5	0.5-28	

¶ Independent t test, * Fisher's exact test, † Chi square test

With p value <0.0001, the age at which the transfusion was started was seen to significantly impact the need for splenectomy in thalassemia patients. [Table 28]

Table 29: Association of number of transfusions with splenectomy.

Number of transfusions	Not done (n=178)	Done (n=27)	Total	P value
Mean ± SD	109.56 ± 75.59	174.85 ± 68.41	118.16 ± 77.75	<0.0001‡
Median (25th-75th percentile)	98.5 (53-156.25)	174 (139.5-197.5)	105 (60-168)	
Range	4-412	29-319	4-412	

‡ Mann Whitney test

The total number of transfusions was seen to significantly affect the need for splenectomy among thalassemia cases (p value <0.0001). [Table 29]

Table 30: Association of parental consanguinity with splenectomy.

Parental Consanguinity	Not done (n=178)	Done (n=27)	Total	p value
No	67 (**38%**)	10 (**37%**)	77 (**38%**)	0.952†
Yes	111 (**62%**)	17 (**63%**)	128 (**62%**)	
Total	178 (**100%**)	27 (**100%**)	205 (**100%**)	

† Chi square test

There was no association between parental consanguinity and need for splenectomy among thalassemia cases (p value=0.952). [Table 30]

Table 31: Association of volume of blood transfused (ml) with splenectomy.

Volume of blood transfused (mL)	Not done (n=167)	Done (n=26)	Total	p value
Mean ± SD	7466.25 ± 9127.78	23826.92 ± 14389.4	9670.27 ± 11417.46	<0.0001‡
Median (25th-75th percentile)	3500 (400-11250)	20475 (13500-31425)	5750 (600-16750)	
Range	40-45000	600-52250	40-52250	

‡ Mann Whitney test

The volume of blood transfused (mL) was seen to significant affect the need for splenectomy among thalassemia cases (p value <0.0001). [Table 31]

DISCUSSION

Patients with hereditary thalassaemia syndromes are among the most common users of blood transfusions across the globe. This population is more prone to transfusion-related problems like blood-borne infections, iron overload, and production of allo-antibodies (IgG) against minor blood group systems such the Kidd, Kell, and Rh systems (E, C, e), which may aggravate the hemolytic reaction[234].

Alloimmunization, an immunological problem brought on by frequent transfusions of red blood cells, can make it challenging to find appropriate donor blood. Blood banks that have fewer donor resources and technical restrictions must take this into account when planning long-term transfusion support for cases that depend on transfusions[16,36,72,100].

This study evaluated the alloimmunization status in 205 cases of thalassemia. Association between the number of blood transfusions thalassemia patients received and their positive alloimmunization status as well as the distribution of blood groups among thalassemia patients and their alloimmunization status was also investigated. Anti-red cell alloantibodies were present in 10% of our patients.

Table 32: Number of study subjects and gender distribution in various studies outside India

Study	Total number of Subjects	Males (%)	Females (%)
Obeidi et al.[230]	90	**43**	**57**
El-Beshlawy et al.[225]	200	**47**	**53**
Hassan et al.[249]	75	**64**	**36**
Singer et al.[215]	64	**42**	**58**
Ameen et al.[21]	190	**51**	**49**
Obaid et al.[100]	40	**42**	**58**
El-Danasoury et al.[16]	235	**54**	**46**
Ghasemi et al.[250]	110	**56**	**44**
Ahmed AM et al.[36]	501	**56**	**44**
Saied DA et al.[73]	95	**49**	**51**
Sadeghian et al.[35]	313	**60**	**40**
Hussein et al.[33]	272	**49**	**51**
Pazgal et al.[251]	40	**52**	**48**
Present Study	205	**57**	**43**

Table 33: Number of study subjects and gender distribution in various studies in India

Study	Total number of Subjects	Males (%)	Females (%)
Gupta et al.[38]	100	**56**	**44**
Dhawan et al.[214]	319	**74**	**26**
Present Study	205	**57**	**43**

The number of participants in the current study was comparable to that in El-Beshlawy et al. Males make up the majority of the cases in our study, as they do in the majority of the studies described above as shown in Table 32. Given that thalassemia is observed to be equally prevalent in both men and women, this gender disparity in studies may be owing to more male patients being enrolled for treatment than female patients, or it may be related to gender differences in thalassemia prevalence across various geographic regions [Table 32 & 33].

Table 34: Mean age of study subjects in various studies outside of India

Study	Mean age (years)
Obeidi et al.[100]	18
El-Beshlawy et al.[16]	23
Hassan et al.[249]	18
Singer et al.[215]	15
Ameen et al.[21]	13
Davoudi-Kiakalayeh A et al.[208]	26
El Danasoury et al.[16]	12
Ghasemi et al.[250]	23
Sadeghian et al.[35]	14
Ho et al[223]	18
Saied DA et al[73]	17
Present Study	11

Table 35: Mean age of study subjects in various studies in India

Study	Mean age (years)
Gupta et al.[38]	26
Dhawan et al.[214]	15
Present Study	11

The mean age in the current study was comparable to that reported by Davoudi-Kiakalayeh A et al. and Ameen et al [Table 34]. Numerous studies show diverse age ranges due to variances in thalassemia prevalence among various age groups of diverse populations (some places may have a higher population of the paediatric age group with thalassemia while other areas may have a higher adult population). [Table 34 & 35]

Table 36: Most frequent blood group among the study subjects in various studies outside India

Study	Blood Group
Marbut et al.[252]	O Positive
Laghari et al.[253]	O Positive
Patidar et al.[254]	O Positive
Davoudi-Kiakalayeh A et al.[208]	O Positive
Sadeghian et al[35]	O Positive
El-Beshlawy, A. et al.[16]	A Positive
Saied DA et al[73]	A Positive
Hussein et al[33]	A Positive
Ghasemi A et al.[250]	O Negative
Present Study	O Positive

Table 37: Most frequent blood group among the study subjects in various studies in India

Study	Blood Group
Sinha et al.[30]	O Positive
Mondal et al.[1]	O Positive
Solanki et al.[24]	O Positive
Gupta et al.[38]	A Positive
Present Study	O Positive

Subjects in the current study and the majority of the research stated above are O-Positive blood type carriers (shown in Table 36 & 37). This may be because people with this blood group are more likely to have thalassemia major which is the most prevalent among all thalassemia syndromes.

Table 38: Most Frequent Diagnosis Among Various Studies

Study	Thalassemia Major (%)	Thalassemia Intermedia (%)	Others (%)
El-Beshlawy, A.[16]	**74**	**26**	**0**
Saied DA et al[73]	**78**	**22**	**0**
Obaid et al[100]	**65**	**35**	**0**
Ho et al.[223]	**96**	**0**	HbE/beta thalassemia heterozygocity syndrome - **4**
Ahmed AM et al.[36]	**78**	**12**	Sickle cell-Beta-Thalassemia syndrome – **11**
Thompson AA et al.[149]	**76**	**8**	HbE – **12** Alpha- thalassemia – **1** HbH or HbH/Constant Spring - **2** Other variants - **1**
Present Study	**76**	**22**	HbE – **2** Alpha-Thalassemia – **1**

Thalassemia major is the most frequent diagnosis in the present analysis, which is consistent to practically all of the earlier studies examined as shown in Table 38. This might be the case since Thalassemia major is the most common form of thalassemia syndromes worldwide.

Table 39: Frequency of Alloimmunization Among Thalassemia Cases in Various Studies outside India

Study	Frequency of alloimmunization (%)
Obeidi et al.[230]	**10**
Bilwani et al.[255]	**9.2**
Hassan K et al.[249]	**22.7**
Hussein et al.[33]	**22.8**
Seferi et al.[259]	**11.8**
Zaidi et al.[108]	**8.6**
Saifeldeen et al.[257]	**23.1**
Qidwai et al.[258]	**10.75**
Abdulqader et al.[234]	**5.8**
Vichinsky E et al.[153]	**19**
Davoudi-Kiakalayeh A et al.	**24.7**
Ahmed AM et al.	**11.3**
Saied DA et al.	**28.4**
Gader AGMA et al.	**22.06**
Ho et al.[223]	**7.4**
El Danasoury et al.[16]	**19.5**
Abdelrazik AM et al[259]	**7.98**
Ameen R et al[21]	**30**
Azarkeivan A et al.[260]	**12.1**
Bahatti et al.[261]	**4.97**
Pazgal I et al[251]	**42.5**

Thompson AA et al[149]	**16.5**
Cheng CK et al.[262]	**29.8**
M Vaziri et al.[263]	**4.0**
El-Beshlawy Amal et al[16]	**18**
Amin M et al.[20]	**17.9**
Obaid JMAS et al[100]	**42.5**
El Kababi Set al[242]	**8.75**
Jansuwan S et al.[49]	**17.5**
Hussein E et al[33]	**22.8**
Present study	**10**

Table 40: Frequency of Alloimmunization Among Thalassemia Cases in Various Studies in India

Study	Frequency of alloimmunization (%)
Datta SS et al.[109]	**5.6**
Dhawan HK et al[214]	**5.64**
Jeengar RK[43]	**6.67**
Gupta JK et al[38]	**7**
Present study	**10**

With a 10% alloimmunization frequency, the current study was comparable to past studies by Obeidi et al., Bilwani et al., Bashawari et al., and Qidwai as shown in Table 39. This could be explained by the following factors:

- Giving blood to participants in the various research populations from donors with similar ethnic backgrounds and homogeneous red blood cell antigens.
- There is a good supply of leucodepleted PRBCs in these areas.
- There is agreement between the sample sizes and the most prevalent type of thalassemia in these studies.

The prevalence of alloimmunization differs greatly between studies as shown in Table 39 & 40. If the studies only included adult populations who had already received several blood transfusions, included cases of thalassemia major in their entirety or those comprising mainly of splenectomised thalassemia cases, and those who were diagnosed at a very early age, alloimmunization would be much higher than the others. There may also be a role for variations in immunological tolerance between populations, sample sizes employed in various studies, and a deficiency in leucodepleted PRBC in certain regions.

Table 41: Most Frequent Blood Group in Cases with Positive Alloimmunization Status

Study	Most frequent Blood group with alloimmunization	Prevalence of Alloimmunization among them (%)	Association
Saied DA et al[73]	O-Positive	**38**	Not significant
Sadeghian et al[35]	A-Positive	**67**	Significant
Hussein et al[33]	A-Positive	**21**	-
Gupta et al[38]	O-Positive	**57**	-
Gader AGMA et al.[72]	O-Positive	**44**	-
Present study	B Positive	**38**	Not significant

In most research, O-Positive blood groups exhibited the highest prevalence of alloimmunization; however, this was not the case in the current study, where B-Positive blood groups exhibited the highest prevalence. This might be as a result of the population included in that research having a high prevalence of that specific blood group. Literature on the impact of blood type on alloimmunization is scarce. Although B-Positive blood groups were the most often alloimmunized blood groups in our analysis, the correlation was found to be inconsequential, which is consistent with a study by Saied DA et al. that revealed no correlation between O-Positive blood groups and alloimmunization. [Table 41]

Table 42: Age at first blood transfusion (years) among the study subjects with alloimmunization.

Study	Mean age at first blood transfusion (years)	Association: Age at first transfusion and development of alloimmunization
Jeengar RK et al.[43]	2.3	Significant
Dhawan HK et al.[214]	2	Significant
Abdelrazik AMR et al.[259]	2.8	Significant
Saifeldeen ER et al.[257]	8	Significant
Obeidi N et al.[100]	2.14 ± 2.40	Not significant
Pazgal I et al.[251]	1	Significant
Hussein E et al.[33]	<12	Not significant
El-Beshlawy A et al.[225]	1 – 10	Significant
Our study	9.41 ± 5.39	Significant

The majority of studies agree with our present study in that they discovered a correlation between age at the start of the transfusion and the presence of alloimmunization. According to certain research, there is a statistically significant difference in the mean age at first transfusion between patients who are alloimmunized and those who are not i.e., the likelihood of developing alloimmunization increases with the age at which transfusion therapy is initiated. Patients who started transfusion therapy at a young age are likely to have a lesser risk of developing alloimmunization since their immune systems are still developing and may have developed some sort of immunological tolerance to allogeneic RBC antigens. [Table 42]

Table 43: Associations Between Splenectomy and Alloimmunization (i & ii)

Table 43 (i): Associations Between Splenectomy and Alloimmunization

Study	Splenectomised cases (%)	Prevalence of Alloimmunization among them (%)	Association
Jansuwan S et al.[49]	**23.8**	**29.4**	Significant
Thompson AA et al.[149]	**49.4**	**21**	Significant
El-Beshlawy et al.[225]	**59**	**-**	Not significant
Pujani M et al.[147]	**7**	**6.25**	Not significant
Our study	**13**	**52**	Significant

Table 43 (ii): Associations Between Splenectomy and Alloimmunization

Study	Frequency of Alloimmunization (%)	Splenectomised cases with alloimmunization (%)	Association
Qidwai A et al.[258]	**40**	**0.3**	Not significant
Zaidi U et al.[108]	**8.6**	**7**	Not significant
Our study	**10.24**	**67**	Significant

The impact of splenectomy on alloimmunization status is subject to conflicting findings as seen in Table 43 (i & ii). Although there is a higher prevalence of alloimmunization among splenectomized thalassemia cases, the association between the two varies depending on the sample size, age groups taken, the number of blood transfusions the study population received, and the number of splenectomized cases taken in those studies.

The elevated rates of alloantibody formation in the operated cases were due to the spleen's inability to remove antigens and damaged cells from the blood stream. In thalassemia patients, it also results in altered immunological tolerance and increased serum IgG and IgA concentrations.

The malformed RBCs in operated cases show novel antigens that trigger an immune response. However, this discovery may simply be the product of a larger transfusion burden in those instances.

Table 44: Association Between Gender and Alloimmunization

Study	Gender	Association between gender and alloimmunization
Thompson AA et al[149]	Females > Male	Not significant
El-Beshlawy et al.[225]	Male > Female	Not significant
Qidwai A et al.[258]	Females > Male	Not significant
Sadeghian MH et al[35]	Females > Male	Not significant
Pazgal I et al.[251]	Male > Female	Not significant
Our study	Male > Female	Not significant

According to the minimal literature that is currently available (as seen in Table 44), there is no correlation between gender and alloimmunization. This result was consistent with our current research.

Table 45: Association Between Number of Blood Transfusions and Alloimmunization in various studies outside India

Study	Association between number of blood transfusions and alloimmunization
Jansuwan S et al.[49]	Significant
El-Beshlawy et al.[225]	Significant
Schonewille H et al.[264]	Not significant
El Danasoury A S et al.[16]	Significant
Pazgal I et al.[251]	Not significant
Hussein E et al.[33]	Significant
Saied DA et al.[73]	Not significant
Ahmed et al[36]	Not significant
Our study	Significant

Table 46: Association Between Number of Blood Transfusions and Alloimmunization in various studies in India

Study	Association between number of blood transfusions and alloimmunization
Pahuja Set al[34].	Not significant
Patel AS et al.[83]	Not significant
Our study	Significant

It has been shown that thalassemia patients who get more blood transfusions both in terms of volume and quantity have an earlier onset of alloimmunization [Table 45 & 46]. However, most of the literature reviewed viewed this correlation as being insignificant. According to the present study and the studies conducted by Jansuwan S. et al., El-Beshlawy et al., El Danasoury A. S. et al., and Hussein E. et al., there was a significant correlation between the two variables. The prevalence of non-transfusion dependent thalassemia in the different study groups, the sample size, and the type of blood component used for transfusion could all be factors in this varying observation.

SUMMARY

This study was a descriptive observational study of 205 cases from January 01, 2021, to December 31, 2021 and was performed in was performed in Pathology Department of KAHER's Jawaharlal Nehru Medical College and Dr Prabhakar Kore Hospital & Research Centre, Belagavi.

The study aimed at estimating the prevalence of alloimmunization in transfusion dependent thalassemia patients at a tertiary care hospital in North Karnataka. Other objectives included finding an association between frequency of blood transfusions and a positive alloimmunization status and to find any association between blood group distribution in thalassemia patients and their relationship with alloimmunization status.

Significant findings in this study are as follows:

- The greatest number of patients belonged to the age group 1-10 years (**50%**).
- There were more males than females (**57% males, 43% females**) .
- O-positive was the most frequently observed blood group among thalassemia cases (**36%**).
- Majority (**76%**)) of our patients consisted of Thalassemia major diagnosed by Hemoglobin electrophoresis.
- Majority of the operated cases of Thalassemia and the cases with alloimmunization belonged to the blood group B-Positive [**37 % and 38%** respectively].
- Majority (**79.5%**) of our patients were receiving blood transfusion according to the hyper-transfusion protocol.

- Only **13%** of the patients in our study underwent splenectomy and majority of them had Thalassemia intermedia who were born of parental consanguinity.
- Prevalence of alloimmunization was found to be **10%** in our study among which **67%** were Thalassemia major and only **33%** were Thalassemia intermedia cases. Majority of them were also found to have already undergone splenectomy and were born of parental consanguinity.
- Out of 41 cases with family history of Thalassemia, **90%** developed Alloimmunization.
- B-Positive was the most prevalent blood group among the alloimmunized thalassemia cases.
- Volume of blood transfused was the significant predictor of splenectomy at cut off point of >9750 ml with area under curve of 0.848 for correctly predicting splenectomy.
- Volume of blood transfused was the significant predictor of positive Alloimmunization at cut off point of >15000 ml with area under curve of 0.967 for correctly predicting positive Alloimmunization.
- Alloimmunization and splenectomy had a significant correlation. The age at which the transfusion was started, which acts as a barometer for how long the transfusion therapy had been ongoing, was also demonstrated to have a strong correlation with both.
- The frequency, volume, and total number of blood transfusions have a big impact on the development of alloantibodies and needing splenectomy.
- The type of thalassemia, gender, parental consanguinity, and blood groups had no impact on either alloimmunization or splenectomy.

CONCLUSION

Patients with multiple transfusions for thalassemia frequently develop alloimmunization. According to our findings, 10% of the multi-transfused thalassemia patients have alloimmunization.

In this study, it was discovered that alloantibodies developed in the majority of splenectomized patients, particularly in those with thalassemia major. While the majority of the cases in our study were O-positive, blood group B-positive individuals were those who developed alloimmunization.

Parental consanguinity was a common factor in patients who showed positive alloimmunization or had to undergo splenectomy or were diagnosed with thalassemia major.

The amount of blood transfused over the course of a person's lifetime is discovered to be a significant contributor to the development of alloimmunization and the requirement for splenectomy. It was observed that a higher volume of blood transfusion is needed for cases to develop alloimmunization than those who develop transfusion complications and ultimately need splenectomy.

Alloimmunization can be prevented by extended matched phenotyping. Since this is financially and logistically demanding the need for better transfusion policies are necessary. The results of this study can help with patient interventions, health education, and future research projects aimed at the therapeutic transfusion problems in thalassemia patients.

LIMITATION

- The frequency of type of allo-antibody produced in each patient could not be carried out due to financial limitations.
- Some study components and their causes could not be compared or evaluated due to lack of relevant literature. For instance, why O-Positive is the most prevalent blood type among people with thalassemia and why some blood types are more likely to develop alloimmunization.

BIBLIOGRAPHY

1) Mondal B, Maiti S, Biswas BK, Ghosh D, Paul S. Prevalence of hemoglobinopathy, ABO and rhesus blood groups in rural areas of West Bengal, India. J. Res. Med. Sci. 2012; 17:772-6.
2) Rodwell VW. Harper's Biochemistry. In: Murray RK, Granner DK, Mayes PA, Rodwell VW, editors. 25th ed. Stamford: Appleto and Lange; 2000. p. 63-73.
3) Haddad A, Tyan P, Radwan A, Mallat N, Taher A. β-Thalassemia Intermedia: A Bird's-Eye View. Turk. J. Hematol. 2014; 31:5-16.
4) Ingram VM, Stretton AO. Genetic basis of the thalassaemia diseases. Nature. 1959; 184:1903-9.
5) Weatherall DJ. Thalassemia: the long road from the bedside through the laboratory to the community. Transfus. Med. 2011 ;21:218-23.
6) Weatherall DJ, Clegg JB, Naughton MA. Globin synthesis in thalassaemia: an in vitro study. Nature. 1965; 208:1061-5.
7) Galanello R, Cao A. Relationship between genotype and phenotype. Thalassemia intermedia. Ann N Y Acad Sci. 1998; 850:325-33.
8) Bhurani D, Kapoor J, Yadav N, Khushoo V, Agrawal N, Ahmed R, et al. Experience with combination of hydroxyurea and low-dose thalidomide in transfusion-dependent beta thalassemia patients. Ann. Hematol. 2021; 100:1417–27.
9) Modell B, Darlison M. Global epidemiology of haemoglobin disorders and derived service indicators. Bull. World Health Organ. 2008; 86(6):480–7.
10) Saxena R, Banerjee T, Aniyery RB. Thalassemia and its Management during Pregnancy. World J. Anemia. 2017;1(1):5-17.

11) Marengo-Rowe AJ. Haemoglobinopathies. Br. J. Hosp. Med. 1971; 6:617-30.

12) Wood WG. Increased HbF in adult life. Baillieres Clin. Haematol. 1993 Mar;6(1):177-213.

13) Clegg JB, Weatherall DJ, Na-Nakorn S, Wasi P. Haemoglobin synthesis in beta-thalassaemia. Nature. 1968 Nov 16; 220(5168):664-8.

14) Schroeder WA, Huisman TH, Shelton JR, Shelton JB, Kleihauer EF, Dozy AM, Robberson B. Evidence for multiple structural genes for the gamma chain of human fetal hemoglobin. Proc. Natl. Acad. Sci. USA 1968 Jun; 60(2):537-44.

15) Marengo-Rowe A J. The thalassemias and related disorders. Proc. (Bayl. Univ. Med. Cent.) 2007; 20:27–31.

16) El-Danasoury A S, Eissa D G, Abdo R M, Elalfy M S. Red blood cell alloimmunization in transfusion-dependent Egyptian patients with thalassemia in a limited donor exposure program. Transfusion. 2012; 52:43-7.

17) Wang LY, Liang DC, Liu HC, Chang FC, Wang CL, Chan YS, et al. Alloimmunization among patients with transfusion-dependent thalassemia in Taiwan. Transfus. Med. 2006; 16:200-3.

18) Rivella S. Ineffective erythropoiesis and thalassemias. Curr. Opin. Hematol. 2009; 16:187–94.

19) Saleha H A, Alkhateepa Y M, Mohammed A N. Role of splenectomy in thalassemic patients. Menoufia Med. J. 2018; 31:118–25.

20) Amin M, Gholamhossein T, Majid N, Marziyeh H, Narges S, et al. Prevalence of Alloimmunization against RBC Antigens in Thalassemia Major Patients in South-East Of Iran. J. Blood Disorders. Transf. 2013; 4:147.

21) Ameen R, Al-Shemmari S, Al-Humood S, Chowdhury RI, Al-Eyaadi O, et al. RBC alloimmunization and autoimmunization among transfusion-dependent Arab thalassemia patients. Transfusion. 2003; 43:1604-10.

22) Kattamis C, Touliatos N, Haidas S, Matsaniotis N. Growth of children with thalassaemia: effect of different transfusion regimens. Arch. Dis. Childh. 1970; 45:502-5.

23) Chernoff AI. The distribution of the thalassemia gene: a historical review. Blood. 1959 Aug; 14(8):899-912.

24) Solanki S D, Amin R, Mehta N C. A study to find the distribution of ABO blood group in beta-thalassemia. Int. J. Sci. Res. 2020; 9(9). PRINT ISSN No. 2277 – 8179.

25) Madan N, Sharma S, Sood S K, Colah R, Bhatia L H. Frequency of β-thalassemia trait and other hemoglobinopathies in northern and western India. Indian J. Hum. Genet. 2010 Jan;16(1):16-25.

26) Mohanty D, Colah RB, Gorakshakar AC, Patel RZ, Master DC, Mahanta J, Sharma SK, Chaudhari U, Ghosh M, Das S, Britt RP, Singh S, Ross C, Jagannathan L, Kaul R, Shukla DK, Muthuswamy V. Prevalence of β-thalassemia and other haemoglobinopathies in six cities in India: a multicentre study. J. Community Genet. 2013 Jan; 4(1):33-42.

27) WHO/TIF Meeting on the Management of Haemoglobin Disorders (2007: Nicosia, Cyprus), World Health Organization & Thalassaemia International Federation. (2008). Management of haemoglobin disorders: report of a joint WHO-TIF meeting, Nicosia, Cyprus, 16-18 November 2007. World Health Organization. https://apps.who.int/iris/handle/10665/43969

28) Ninth plenary meeting, 27 May 2006 – Committee A, fourth report). WHO resolution on Sickle Cell Disease (WHA59.20) and Thalassemia (EB118.R1), 29 May 2006. Retrieved on 20-12-2022. From : https://apps.who.int/gb/ebwha/pdf_files/WHA59/A59_R20-en.pdf

29) Giordano P C, Harteveld C L, Bakker E. Genetic Epidemiology and Preventive healthcare in Multiethnic Societies: Hemoglobinopathies. Int. J. Environ. Res. Public Health. 2014; 11(6)6136-46.

30) Sinha S, Black M L, Agarwal S, Colah R, Das R, Ryan K, et al. Profiling β-thalassaemia mutations in India at state and regional levels: implications for genetic education, screening and counselling programmes. Hugo J. 2009; 3:51–62.

31) Balgir R S. Genetic epidemiology of the three predominant abnormal hemoglobins in India. J. Assoc. Physicians of India. 1996; 44(1)25-8.

32) Shastry S, Chenna D, Basavarajegowda A, Das S, Chaudhary R K. Red blood cell alloimmunization among recipients of blood transfusion in India: A systematic review and meta-analysis. Vox. Sang. 2022 Sep; 117(9):1057-69.

33) Hussein E, Desoukey N, Rihan A, Kamal A. Predictors of Red Cell Alloimmunization in Multitransfused Egyptian Patients With β-Thalassemia. Arch. Pathol. Lab. Med. 2014; 138.684-8.

34) Pahuja S, Pujani M, Gupta SK, Chandra J, Jain M. Alloimmunization and red cell autoimmunization in multitransfused thalassemics of Indian origin. Hematology. 2010 Jun; 15(3):174-7.

35) Sadeghian M H, Keramati M R, Badiei Z, Ravarian M, Ayatollahi H, Rafatpanah H, Daluei M K. Alloimmunization among transfusion-dependent thalassemia patients. Asian J. Transfus. Sci. 2009 Jul; 3(2):95-8.

36) Ahmed A M, Hasan N S, Ragab S H, Habib S A, Emara N A, Aly A. Red cell alloimmunization and autoantibodies in Egyptian transfusion-dependent thalassaemia patients. Arch. Med. Sci. 2010 Aug 30; 6(4):592-8.

37) Koçyiğit C, Eliaçık K, Kanık A, Atabay B, Türker M. Frequency of red cell allo- and autoimmunization in patients with transfusion-dependent beta thalassemia and affecting factors. Turk. J. Pediatr. 2014 Sep-Oct; 56(5):487-92.

38) Gupta N, Riaz Mehdi S, Zaidi N, Ahmad S. Alloimmunization in Patients And Donors Visiting Blood Bank of A Tertiary Care Hospital. Natl. J. Integr. Res. Med. 2018; 9(4): 49-54.

39) Sarihi R, Oodi A, Dadkhah Tehrani R, Jalali SF, Mardani F, Azarkeivan A, et al. Blood group genotyping in alloimmunized multi-transfused thalassemia patients from Iran. Mol. Genet. Genomic Med. 2021 Jul; 9(7): e1701.

40) Ameen R, Al-Shemmari S, Al-Humood S, Chowdhury RI, Al-Eyaadi O, Al-Bashir A. RBC alloimmunization and autoimmunization among transfusion-dependent Arab thalassemia patients. Transfusion. 2003 Nov; 43(11):1604-10.

41) Beguin Y, Clemons GK, Pootrakul P, Fillet G. Quantitative assessment of erythropoiesis and functional classification of anemia based on measurements of serum transferrin receptor and erythropoietin. Blood. 1993 Feb 15; 81(4):1067-76.

42) Ahmed MH, Ghatge MS, Safo MK. Hemoglobin: Structure, Function and Allostery. Subcell. Biochem. 2020; 94:345-82.

43) Jeengar R K, Upadhyaya A, Agarwal N, Mehta A. Red cell alloimmunization in repeatedly transfused children with beta thalassemia major. Int. J. Contemp. Pediatr. 2017; 4(3)775-9.

44) Seferi I, Xhetani M, Face M, Burazeri G, Nastas E, Vyshka G. Frequency and specificity of red cell antibodies in thalassemia patients in Albania. Int. J. Lab. Hematol. 2015 Aug; 37(4):569-74.

45) Patterson S, Singleton A, Branscomb J, Nsonwu V, Spratling R. Transfusion Complications in Thalassemia: Patient Knowledge and Perspectives. Front. Med. (Lausanne). 2022 Mar; 1(9):772886.

46) Ness PM, Shirey RS, Thoman SK, Buck SA. The differentiation of delayed serologic and delayed hemolytic transfusion reactions: incidence, long-term serologic findings, and clinical significance. Transfusion. 1990 Oct; 30(8):688-93.

47) Al-Dughaishi T, Al Harrasi Y, Al-Duhli M, Al-Rubkhi I, Al-Riyami N, Al Riyami A, et al. Red Cell Alloimmunization to Rhesus Antigen Among Pregnant Women Attending a Tertiary Care Hospital in Oman. Oman Med. J. 2016 Jan; 31(1):77-80.

48) Sinha PA, Mulkutkar SH, Bhavani JB. Study of distribution of ABO blood groups in ß-thalassemia patients. Int. J. Res. Med. Sci. 2017; 5:3479-83.

49) Jansuwan S, Tangvarasittichai O, Tangvarasittichai S. Alloimmunization to Red Cells and the Association of Alloantibodies Formation with Splenectomy Among Transfusion-Dependent β-Thalassemia Major/HbE Patients. Indian J. Clin. Biochem. 2015 Apr; 30(2):198-203.

50) Adamson J W, Finch C A. Hemoglobin function, oxygen affinity, and erythropoietin. Annu. Rev. Physiol. 1975; 37:351-69.

51) Schechter AN. Hemoglobin research and the origins of molecular medicine. Blood. 2008 Nov 15; 112(10):3927-38.

52) Marengo-Rowe AJ. Structure-function relations of human hemoglobins. Proc. (Bayl. Univ. Med. Cent.). 2006 Jul; 19(3):239-45.

53) Kosmachevskaia OV, Topunov AF. Hemoglobins: diversity of structures and functions. Prikl. Biokhim. Mikrobiol. 2009 Nov-Dec; 45(6):627-53.

54) Shafique F, Ali S, Almansouri T, Van Eeden F, Shafi N, Khalid M, et al. Thalassemia, a human blood disorder. Braz J Biol. 2021 Sep 3; 83:e246062.

55) Guidelines For Prevention of Hemoglobinopathies (Thalassemias And Variant Hemoglobin Disorders) In: NHM Guidelines for Prevention of Hemoglobinopathies in India. 2016. p.35-7.

56) McKenzie S B. Hemoglobin. In: Clinical laboratory Hematology 3rd edition. Shirlyn B. McKenzie, J. Lynne Williams. Pearson. 2016. p.109-28.

57) Hemoglobinopathies: Current Practices for Screening, Confirmation and Follow-up. Association Of Public Health Laboratories. 2015.p.5-9.

58) Hemoglobin and Myoglobin. The Medical Biochemistry Page. **Updated: September 27, 2022.** Retrieved on 19-12-2022. From: https://themedicalbiochemistrypage.org/hemoglobin-and-myoglobin/

59) Anemia:- Part 5 A – Sickle Cell Anemia, and Sickle Cell Trait, Discussion and Workup. Retrieved on 19-12-2022. From: https://labpedia.net/anemia-part-5-sickle-cell-anemia-and-sickle-cell-trait-discussion-and-workup/

60) Viprakasit V, Origa R. Genetic basis, pathophysiology and diagnosis. In: Guidelines for The Management Of Transfusion Dependent Thalassaemia (TDT). 3rd edition. Thalassaemia international federation TIF publication no. 20. Cappellini MD, Cohen A, Porter J, Taher A, Viprakasit V, editors. 2014. p.14-27.

61) Burden of Hemoglobinopathies in India. In: NHM Policy For Prevention and Control of Hemoglobinopathies In India. p.20.

62) Laudicina R J. Hemoglobinopathies: Qualitative Defects. In: Clinical laboratory Hematology 3rd edition. Shirlyn B. McKenzie, J. Lynne Williams. Pearson. 2016. p.263-82.

63) Randolph T R. Thalassemia. In: Clinical laboratory Hematology 3rd edition. Shirlyn B. McKenzie, J. Lynne Williams. Pearson. 2016. p.283-308.

64) Chou ST, Liem RI, Thompson AA. Challenges of alloimmunization in patients with haemoglobinopathies. Br. J. Haematol. 2012 Nov; 159(4):394-404.

65) Banton A H. A genetic study of Mediterranean anaemia in Cyprus. Am. J. Hum. Genet. 1951 Mar; 3(1):47-64.

66) Loukopoulos D. Milestones in the History of Thalassemia and Sickle Cell Disease. Thalassemia Reports. 2014; 4:4866.

67) Whipple GH, Bradford WL. Mediterranean disease—"thalassemia" (erythroblastic anemia of Cooley); associated pigment abnormalities simulating hemochromatosis. J. Pediatr. 1936; 9:279–311.

68) Weatherall DJ. In Wintrobe MM, ed. Blood, Pure and Eloquent: A Story of Discovery, of People, and of Ideas. New York: McGraw-Hill, 1980; 378.

69) Talk: Thalassemia. In: Wikipedia, the free encyclopedia. Edited on 23-09-2022. Retrieved on 19-12-2022. From: https://en.wikipedia.org/wiki/Talk%3AThalassemia.

70) De Sanctis V, Kattamis C, Canatan D, Soliman A. T, Elsedfy H, Karimi M, et al. β-thalassemia distribution in the old world: an ancient disease seen from a historical standpoint. Mediterr. J. Hematol. Infect. Dis. 2017; 9(1):e2017018.

71) Al-Riyami AZ, Daar S. Red cell alloimmunization in transfusion-dependent and transfusion-independent beta thalassemia: A review from the Eastern Mediterranean Region (EMRO). Transfus. Apher. Sci. 2019 Dec; 58(6):102678.

72) Gader AG, Al Ghumlas AK, Al-Momen AK. Transfusion medicine in a developing country - Alloantibodies to red blood cells in multi-transfused patients in Saudi Arabia. Transfus. Apher. Sci. 2008 Dec; 39(3):199-204.

73) Saied DA, Kaddah AM, Badr Eldin RM, Mohaseb SS. Alloimmunization and erythrocyte autoimmunization in transfusion-dependent Egyptian thalassemic patients. J. Pediatr. Hematol. Oncol. 2011 Aug; 33(6):409-14.

74) Harteveld CL, Higgs DR. Alpha-thalassaemia. Orphanet. J. Rare Dis. 2010 May 28; 5:13.

75) Old JM. Screening and genetic diagnosis of haemoglobin disorders. Blood Rev. 2003 Mar; 17(1):43-53.

76) Cazzola M, Borgna-Pignatti C, Locatelli F, Ponchio L, Beguin Y, De Stefano P. A moderate transfusion regimen may reduce iron loading in beta-thalassemia major without producing excessive expansion of erythropoiesis. Transfusion. 1997 Feb; 37(2):135-40.

77) Propper RD, Button LN, Nathan DG. New approaches to the transfusion management of thalassemia. Blood. 1980 Jan; 55(1):55-60.

78) Piomelli S, Karpatkin MH, Arzanian M, Zamani M, Becker MH, Geneiser N, et al. Hypertransfusion regimen in patients with Cooley's anemia. Ann. N. Y. Acad. Sci. 1974; 232(0):186-92.

79) Pootrakul P, Kitcharoen K, Yansukon P, Wasi P, Fucharoen S, Charoenlarp P, et al. The effect of erythroid hyperplasia on iron balance. Blood. 1988 Apr; 71(4):1124-9.

80) Issaragrisil S. Stem cell transplantation for thalassemia. Int. J. Hematol. 2002 Aug; 76 Suppl 1:307-9.

81) Borgna-Pignatti C, Gamberini MR. Complications of thalassemia major and their treatment. Expert Rev. Hematol. 2011 Jun; 4(3):353-66.

82) Sharma D C, Arya A, Kishor P, Woike P, Bindal J. Overview on Thalassemias: A Review Article. Med. Res. Chronicles. 2017; 4(3)325-37.

83) Patel AS, Gamit S, Gohil M. Role of RBC's alloimmunization in multiple transfused thalassaemia patients. Int. J. Res. Med. Sci. 2016; 4:822-8.

84) Piomelli S, Danoff SJ, Becker MH, Lipera M J, Travis S F. Prevention of bone malformations and cardiomegaly in Cooley's anemia by early hypertransfusion regimen. Ann. N. Y. Acad. Sci. 1969; 165:427-36.

85) Schorr J B, Radel E. Transfusion therapy and its complications in patients with Cooley's anemia. Ann. N. Y. Acad. Sci.1964; 119:703-8.

86) Wolman I J. Transfusion therapy in Cooley's anemia: growth and health as related to long-range hemoglobin levels. A progress report. Ann. N. Y. Acad. Sci. 1964; 119:736-47.

87) Goss, C. Giardina, P. Degtyaryova D, Kleinert D, Sheth S, Cushing M. Red blood cell transfusions for thalassemia: results of a survey assessing current practice and proposal of evidence-based guidelines. *Transfusion. 2014; 54*.1773-81.

88) Nienhuis AW, Nathan DG. Pathophysiology and Clinical Manifestations of the β-Thalassemias. Cold Spring Harb Perspect Med. 2012 Dec 1; 2(12):a011726.

89) Taher A, Hershko C, Cappellini MD. Iron overload in thalassaemia intermedia: reassessment of iron chelation strategies. Br. J. Haematol. 2009; 147:634-40.

90) Origa R, Galanello R, Ganz T, Giagu N, Maccioni L, Faa G, et al. Liver iron concentrations and urinary hepcidin in β-thalassemia. Haematologica. 2007; 92:583-8.

91) Hershko C. Pathogenesis and management of iron toxicity in thalassemia. Ann. N. Y. Acad. Sci. 2010; 1202:1-9.

92) Musallam KM, Cappellini MD, Wood JC, Motta I, Graziadei G, Tamim H, et al. Elevated liver iron concentration is a marker of increased morbidity in patients with β thalassemia intermedia. Haematologica. 2011; 96:1605-12.

93) Guidelines For Management of Hemoglobinopathies. In: NHM Guidelines for Prevention of Hemoglobinopathies in India. 2016. p.73-82

94) Chen JM, Zhu WJ, Liu J, Wang GZ, Chen XQ, Tan Y, et al. Safety and efficacy of thalidomide in patients with transfusion-dependent β-thalassemia: a randomized clinical trial. Signal. Transduct. Target. Ther. 2021 Nov 18; 6(1):405.

95) Tayyab M, Shahzad AH, Khan AA, Hanif F, Afzal I, Sajed AN, et al. Transfusion Associated Diseases and Complications of Blood Transfusion in Thalassaemia Patients. Europasian J. Med. Sci. 2020; 2(Covid-19 Special Issue):1-11.

96) Thein SL. Molecular basis of β thalassemia and potential therapeutic targets. Blood Cells Mol. Dis. 2018 May; 70:54-65.

97) Isa M, Thayeb A, Yani A, Hutagalung M B Z. Post total splenectomy outcome in thalassemia patients. Bali Med. J. 2019; 8(3): 764-7.

98) MLAB 1415: Hematology Keri Brophy-Martinez. Published by Nathaniel Evans. From: https://slideplayer.com/slide/8196385/

99) Kohne E. Hemoglobinopathies: clinical manifestations, diagnosis, and treatment. Dtsch. Arztebl. Int. 2011 Aug; 108(31-32):532-40.

100) Obaid JM, Abo El-Nazar SY, Ghanem AM, El-Hadidi AS, Mersal BH. Red blood cells alloimmunization and autoimmunization among transfusion-dependent beta-thalassemia patients in Alexandria province, Egypt. Transfus. Apher. Sci. 2015 Aug; 53(1):52-7.

101) Musallam KM, Taher AT, Rachmilewitz EA. β-thalassemia intermedia: a clinical perspective. Cold Spring Harb. Perspect. Med. 2012 Jul; 2(7):a013482.

102) Karimi M, Cohan N, De Sanctis V, Mallat NS, Taher A. Guidelines for diagnosis and management of Beta-thalassemia intermedia. Pediatr. Hematol. Oncol. 2014 Oct; 31(7):583-96.

103) Correia JG, Moreira N, Costa Almeida CE, Reis LS. Partial Splenectomy in the treatment of an adult with β thalassemia intermedia: A case report. Int. J. Surg. Case. Rep. 2017 Nov 11; 41:446-9.

104) Li X, Hu S, Liu Y, Huang J, Hong W, Xu L, et al Efficacy of Thalidomide Treatment in Children with Transfusion Dependent β-Thalassemia: A Retrospective Clinical Study. Front. Pharmacol. 2021 Aug 12; 12:722502.

105) Old JM. Screening and genetic diagnosis of haemoglobin disorders. Blood Rev. 2003 Mar; 17(1):43-53.

106) Sinha S, Seth T, Colah RB, Bittles AH. Haemoglobinopathies in India: estimates of blood requirements and treatment costs for the decade 2017-2026. J Community Genet. 2020 Jan; 11(1):39-45.

107) Tambse M P, Vasaikar M S, Chavan S. Patterns and Demographic Distribution of Hemoglobinopathies in North Maharashtra. Annals of Applied Biosciences. 2016; 3(2)184-8.

108) Zaidi U, Borhany M, Ansari S, Parveen S, Boota S, Shamim I, et al. Red cell alloimmunisation in regularly transfused beta thalassemia patients in Pakistan. Transfus. Med. 2015 Apr; 25(2):106-10.

109) Datta SS, Mukherjee S, Talukder B, Bhattacharya P, Mukherjee K. Frequency of Red Cell Alloimmunization and Autoimmunization in Thalassemia Patients: A Report from Eastern India. Adv. Hematol. 2015; 2015:610931.

110) Bhushan R, Shukla S, Singh D, Trivedi S, Sharma S. Reliability of Different RBC Indices To Differentiate Between Beta Thalassemia Trait And Iron Deficiency Anemia During Antenatal Screening. 2018; 10. ISSN-2277-3460.

111) Mondal SK, Mandal S. Prevalence of thalassemia and hemoglobinopathy in eastern India: A 10-year high-performance liquid chromatography study of 119,336 cases. Asian J. Transfus. Sci. 2016; 10:105-10.

112) Weatherall D J. The Thalassemias: Disorders Of Globin Synthesis. In: Williams Hematology. 9th edition. Kenneth Kaushansky, Josef T. Prchal, Oliver W. Press, Marshall A. Lichtman, Marcel Levi, Linda J. Burns, Michael A. Caligiuri. Mc Graw Hill Education. 2016. p. 725-58.

113) Yang Liu S. Alpha thalassemia: Pathogenesis and clinical presentations. In: The Calgary guide to understanding diseases. Retrieved on 21-12-2022. From https://calgaryguide.ucalgary.ca/alpha-thalassemia-pathogenesis-and-clinical-presentations/

114) Baysal E, Huisman TH. Detection of common deletional alpha-thalassemia-2 determinants by PCR. Am. J. Hematol. 1994 Jul; 46(3):208-13.

115) Gibbons R. Alpha thalassaemia-mental retardation, X linked. Orphanet. J. Rare. Dis. 2006; 1(1)15.

116) Akhtar IK, Ashraf M, Khalid IU, Hussain M. Surgical outcome of spelenectomy in Thalassemia major in children. Pak. J. Med. Sci. 2016 Mar-Apr; 32(2):305-8.

117) Weatherall DJ, Clegg JB: The Thalassaemia Syndromes, 4th ed. Blackwell, Oxford, 2001.

118) Weatherall DJ, Clegg JB, Higgs DR, Wood WG: The hemoglobinopathies, in The Metabolic and Molecular Bases of Inherited Disease, 8th ed, edited by Scriver CR, Beauder AL, Sly WS, Valle D, McGraw-Hill, New York, 2001. p. 4571.

119) Rund D, Rachmilewitz E: Advances in the pathophysiology and treatment of thalassemia. Crit. Rev. Oncol. Hematol.1995; 20:237.

120) Schrier SL: Pathobiology of thalassemic erythrocytes. Curr. Opin. Hematol. 1997; 4:75.

121) Fibach E, Rachmilewitz E: The role of oxidative stress in hemolytic anemia. Curr. Mol. Med. 2008; 8:609.

122) O'Donnell A, Premawardhena A, Arambepola M, et al: Age-related changes in adaptation to severe anemia in childhood in developing countries. Proc. Natl. Acad. Sci. U S A. 2007; 104:9440.

123) Multicentre study on prevalence of endocrine complications in thalassemia major. Italian Working Group on Endocrine Complications in Non-endocrine Diseases. Clin. Endocrinol. (Oxf). 1995; 42:581.

124) Wood JC, Enriquez C, Ghugre N, Otto-Duessel M, Aguilar M, Nelson MD, et al. Physiology and pathophysiology of iron cardiomyopathy in thalassemia. Ann. N. Y. Acad. Sci. 2005; 1054:386-95.

125) Olivieri NF, Brittenham GM: Iron-chelating therapy and the treatment of thalassemia. Blood. 1997; 89:739.

126) Singer ST, Ataga KI: Hypercoagulability in sickle cell disease and beta-thalassemia. Curr. Mol. Med. 2008; 8:639.

127) Morris CR, Kuypers FA, Kato GJ, Lavrisha L, Larkin S, Singer T, et al. Hemolysis-associated pulmonary hypertension in thalassemia. Ann. N. Y. Acad. Sci. 2005;1054:481-5.

128) Weatherall DJ: Phenotype-genotype relationships in monogenic disease: Lessons from the thalassaemias. Nat. Rev. Genet. 2001; 2:245.

129) Weatherall DJ, Pressley L, Wood WG, Higgs DR, Clegg JB. Molecular basis for mild forms of homozygous beta-thalassaemia. Lancet. 1981 Mar 7; 1(8219):527-9.

130) Wainscoat JS, Old JM, Weatherall DJ, Orkin SH: The molecular basis for the clinical diversity of beta thalassaemia in Cypriots. Lancet. 1983; 1:1235.

131) Labie D, Pagnier J, Lapoumeroulie C, Rouabhi F, Dunda-Belkhodja O, Chardin P, et al. Common haplotype dependency of high G gamma-globin gene expression and high Hb F levels in beta-thalassemia and sickle cell anemia patients. Proc. Natl. Acad. Sci. U S A. 1985 Apr; 82(7):2111-4.

132) Thein SL, Wainscoat JS, Sampietro M, Old JM, Cappellini D, Fiorelli G, et al. Association of thalassaemia intermedia with a beta-globin gene haplotype. Br. J. Haematol. 1987 Mar; 65(3):367-73.

133) Thein SL, Hesketh C, Wallace RB, Weatherall DJ: The molecular basis of thalassaemia major and thalassaemia intermedia in Asian Indians: Application to prenatal diagnosis. Br. J. Haematol. 1988; 70:225.

134) Ho PJ, Hall GW, Luo LY, Weatherall DJ, Thein SL. Beta-thalassaemia intermedia: is it possible consistently to predict phenotype from genotype? Br. J. Haematol. 1998 Jan; 100(1):70-8.

135) Adewoyin A, Olayinka A. Oyewale. Complications of Allogeneic Blood Transfusion: Current Approach to Diagnosis and Management. Int. *blood* res. rev. 2015 May;135-51.

136) Taher AT, Radwan A, Viprakasit V. When to consider transfusion therapy for patients with non-transfusion-dependent thalassaemia. Vox Sanguinis. 2014;108(1).

137) Pinto VM, Forni GL. Management of Iron Overload in Beta-Thalassemia Patients: Clinical Practice Update Based on Case Series. Int. J. Mol. Sci. 2020; 21(22):8771.

138) Brancaleoni V, Di Pierro E, Motta I, Cappellini MD. Laboratory diagnosis of thalassemia. Int. J. Lab. Hematol. 2016 May; 38 Suppl 1:32-40.

139) Barth D, Hirschmann J V. Anemia. In: Wintrobe's Atlas of Clinical Hematology. First edition. Douglas C. Tkachuk, Jan V. Hirschmann, editors. Lippincott Williams & Wilkins. 2007. p.14.

140) Barth D, Hirschmann J V. Anemia. In: Wintrobe's Atlas of Clinical Hematology. First edition. Douglas C. Tkachuk, Jan V. Hirschmann, editors. Lippincott Williams & Wilkins. 2007. p.18.

141) Barth D, Hirschmann J V. Anemia. In: Wintrobe's Atlas of Clinical Hematology. First edition. Douglas C. Tkachuk, Jan V. Hirschmann, editors. Lippincott Williams & Wilkins. 2007. p.15.

142) Guidelines For Prevention Of Hemoglobinopathies (Thalassemias And Variant Hemoglobin Disorders) In: NHM Guidelines for Prevention of Hemoglobinopathies in India. 2016. p.49.

143) Management Of Thalassemia Major. Guidelines For Management Of Thalassemia And Sickle Cell Disease. In: NHM Guidelines For Prevention Of Hemoglobinopathies In India. 2016. p.73-82.

144) Modell B. Total management of thalassaemia major. Arch. Dis. Child. 1977 Jun; 52(6):489-500.

145) Piomelli S. Management of Cooley's anaemia. Baillieres Clin Haematol. 1993 Mar; 6(1):287-98.

146) Pujani M, Pahuja S, Dhingra B, Chandra J, Jain M. Alloimmunisation in thalassaemics: a comparison between recipients of usual matched and partial better matched blood. An evaluation at a tertiary care centre in India. Blood Transfus. 2014 Jan; 12 Suppl 1(Suppl 1):s100-4.

147) Aydinok Y. Infections. In: guidelines for the management of transfusion dependent thalassaemia (TDT). 3rd edition. Thalassaemia international federation TIF publication no. 20. Cappellini MD, Cohen A, Porter J, Taher A, Viprakasit V, editors. 2014. p.134-45.

148) Thompson AA, Cunningham MJ, Singer ST, Neufeld EJ, Vichinsky E, Yamashita R, et al. Thalassemia Clinical Research Network Investigators. Red cell alloimmunization in a diverse population of transfused patients with thalassaemia. Br. J Haematol. 2011 Apr; 153(1):121-8.

149) Trompeter S, Cohen A. Blood Transfusion. In: guidelines for the management of transfusion dependent thalassaemia (TDT). 3rd edition. Thalassaemia international federation TIF publication no. 20. Cappellini MD, Cohen A, Porter J, Taher A, Viprakasit V, editors.2014. p.28-40.

150) Chaudhari CN. Red Cell Alloantibodies in Multiple Transfused Thalassaemia Patients. Med. J. Armed Forces India. 2011 Jan; 67(1):34-7.

151) Dibyajyoti S. Transfusion Support and Red Cell Alloimmunisation in Thalassemia Patients. *Int. J. Hematol*. 2018; 2(1):000123.

152) Vichinsky E, Neumayr L, Trimble S, Giardina PJ, Cohen AR, Coates T, et al. Transfusion complications in thalassemia patients: a report from the Centers for Disease Control and Prevention (CME). Transfusion. 2014 Apr; 54(4):972-81; quiz 97.

153) Short J L, Diehl S, Seshadri R, Serrano S. Accuracy of formulas used to predict post-transfusion packed cell volume rise in anemic dogs. J. Vet. Emerg. Crit. Care (San Antonio). 2012 Aug; 22(4):428-34.

154) Müller M, Geisen C, Zacharowski K, Tonn T, Seifried E. Transfusion of Packed Red Cells: Indications, Triggers and Adverse Events. Dtsch. Arztebl. Int. 2015 Jul 20; 112(29-30):507-17; quiz 518.

155) Liumbruno G, Bennardello F, Lattanzio A, Piccoli P, Rossetti G. Recommendations for the transfusion of red blood cells. Blood. Transfus. 2009 Jan; 7(1):49-64.

156) Davies P, Robertson S, Hegde S, Greenwood R, Massey E, Davis P. Calculating the required transfusion volume in children. Transfusion. 2007 Feb; 47(2):212-6.

157) Shah FT, Sayani F, Trompeter S, Drasar E, Piga A. Challenges of blood transfusions in β-thalassemia. Blood Rev. 2019 Sep; 37:100588.

158) Kasraian L, Karimi MH. The Incidence Rate of Acute Transfusion Reactions in Thalassemia Patients Referred to the Shiraz Thalassemia Centre, Shiraz, Iran, Before and After the Establishment of the Hemovigilance System. Hemoglobin. 2015; 39(4):274-80.

159) Porter J, Viprakasit V. Iron Overload and Chelation. In: Guidelines For The Management Of Transfusion Dependent Thalassaemia (TDT). 3rd edition. Thalassaemia international federation TIF publication no. 20. Cappellini MD, Cohen A, Porter J, Taher A, Viprakasit V, editors. 2014. p.42-96.

160) Propper RD, Cooper B, Rufo RR, Nienhuis AW, Anderson WF, Bunn HF, Rosenthal A, Nathan DG. Continuous subcutaenous administration of deferoxamine in patients with iron overload. N. Engl. J. Med. 1977 Aug 25; 297(8):418-23.

161) Propper RD, Shurin SB, Nathan DG. Reassessment of the use of desferrioxamine B in iron overload. N. Engl. J. Med. 1976 Jun 24; 294(26):1421-3.

162) Barry M, Flynn DM, Letsky EA, Risdon RA. Long-term chelation therapy in thalassaemia major: effect on liver iron concentration, liver histology, and clinical progress. Br. Med. J. 1974 Apr 6; 2(5909):16-20.

163) Ammar S A, Elsayh K I, Zahran A M, Embaby M. Splenectomy for patients with β-thalassemia major: long-term outcomes. *Egyptian J. Surgery. 2014; 33:232–6.*

164) Taher A, Tyan P I. The Spleen. In: Guidelines For The Management Of Transfusion Dependent Thalassaemia (TDT). 3rd edition. Thalassaemia international federation TIF publication no. 20. Cappellini MD, Cohen A, Porter J, Taher A, Viprakasit V, editors.2014. p.126-32.

165) Rebulla P, Modell B. Transfusion requirements and effects in patients with thalassaemia major. The Lancet. 1991 Feb 2; 337(8736):277-80.

166) Daar S, Taher A. Splenomegaly and splenectomy. In: 2021 Guidelines for The Management Of Transfusion Dependent Thalassaemia (TDT). 4th edition. Thalassaemia international federation TIF. Cappellini M D, Farmakis D, Porter J, Taher A. p.200-210.

167) Carl H. Smith, Marion E. Erlandson, Gertrude Stern, Irving Schulman; The Role of Splenectomy In The Management Of Thalassemia. *Blood* 1960; 15 (2): 197–211.

168) Saleh H A, Alkhateep Y M, Mohammed A N. Role of splenectomy in thalassemic patients. Menoufia Med J. 2018;13(1)118-25.

169) Strocchio L, Locatelli F. Hematopoietic Stem Cell Transplantation in Thalassemia. Hematol Oncol Clin North Am. 2018 Apr;32(2):317-28.

170) Mathews V, Savani BN. Conditioning regimens in allo-SCT for thalassemia major. Bone Marrow Transplant. 2014 May;49(5):607-10.

171) Angellucci E, Srivastava A, Usai S. Haemopoietic Stem Cell Transplantation. In: Guidelines For The Management Of Transfusion Dependent Thalassaemia (TDT).

3rd edition. Thalassaemia international federation TIF publication no. 20. Cappellini MD, Cohen A, Porter J, Taher A, Viprakasit V, editors.2014. p.186-91.

172) Sodani P, Oevermann L. Haematopoietic Stem Cell Transplantation for Thalassaemia. In: 2021 Guidelines for The Management of Transfusion Dependent Thalassaemia (TDT). 4th edition. Thalassaemia international federation TIF. Cappellini M D, Farmakis D, Porter J, Taher A. p.266-76.

173) Lucarelli G, Clift RA, Galimberti M, Angelucci E, Giardini C, Baronciani D, Polchi P, Andreani M, Gaziev D, Erer B, Ciaroni A, D'Adamo F, Albertini F, Muretto P. Bone marrow transplantation in adult thalassemic patients. Blood. 1999 Feb 15;93(4):1164-7.

174) Mathews V, George B, Viswabandya A, Abraham A, Ahmed R, Ganapule A, Sindhuvi E, Lakshmi KM, Srivastava A. Improved clinical outcomes of high risk β thalassemia major patients undergoing a HLA matched related allogeneic stem cell transplant with a treosulfan based conditioning regimen and peripheral blood stem cell grafts. PLoS One. 2013 Apr 26;8(4):e61637.

175) Matthes-Martin S, Pötschger U, Barr R, Martin M, Boztug H, Klingebiel T, Attarbaschi A, Eibler W, Mann G. Costs and cost-effectiveness of allogeneic stem cell transplantation in children are predictable. Biol Blood Marrow Transplant. 2012 Oct;18(10):1533-9.

176) Lucarelli G, Isgrò A, Sodani P, Gaziev J. Hematopoietic stem cell transplantation in thalassemia and sickle cell anemia. Cold Spring Harb Perspect Med. 2012 May;2(5):a011825.

177) Gaziev J, Sodani P, Polchi P, Andreani M, Lucarelli G. Bone marrow transplantation in adults with thalassemia: Treatment and long-term follow-up. Ann N Y Acad Sci. 2005;1054:196-205.

178) Inamoto Y, Lee SJ. Late effects of blood and marrow transplantation. Haematologica. 2017 Apr;102(4):614-25.

179) Peffault de Latour R. Transplantation for bone marrow failure: current issues. Hematology Am Soc Hematol Educ Program. 2016 Dec 2;2016(1):90-8.

180) Gaziev D, Galimberti M, Lucarelli G, Polchi P, Giardini C, Angelucci E, Baronciani D, Sodani P, Erer B, Biagi MD, Andreani M, Agostinelli F, Donati M, Nesci S, Talevi N. Bone marrow transplantation from alternative donors for thalassemia: HLA-phenotypically identical relative and HLA-nonidentical sibling or parent transplants. Bone Marrow Transplant. 2000 Apr;25(8):815-21.

181) Angelucci E, Pilo F, Targhetta C, Pettinau M, Depau C, Cogoni C, Usai S, Pani M, Dessì L, Baronciani D. Hematopietic stem cell transplantation in thalassemia and related disorders. Mediterr J Hematol Infect Dis. 2009 Dec 3;1(1):e2009015.

182) Wilber A, Hargrove PW, Kim YS, Riberdy JM, Sankaran VG, Papanikolaou E, Georgomanoli M, Anagnou NP, Orkin SH, Nienhuis AW, Persons DA. Therapeutic levels of fetal hemoglobin in erythroid progeny of β-thalassemic CD34+ cells after lentiviral vector-mediated gene transfer. Blood. 2011 Mar 10;117(10):2817-26.

183) Angastiniotis M, Lobitz S. Thalassemias: An Overview. Int J Neonatal Screen. 2019 Mar 20;5(1):16.

184) Angastiniotis M, Lobitz S. Thalassemias: An Overview. Int J Neonatal Screen. 2019 Mar 20;5(1):17.

185) Olivieri NF, Brittenham GM. Management of the thalassemias. Cold Spring Harb Perspect Med. 2013 Jun 1;3(6):a011767.

186) Raja JV, Rachchh MA, Gokani RH. Recent advances in gene therapy for thalassemia. J Pharm Bioallied Sci. 2012 Jul;4(3):194-201.

187) Ali S, Mumtaz S, Shakir HA, Khan M, Tahir HM, Mumtaz S, Mughal TA, Hassan A, Kazmi SAR, Sadia, Irfan M, Khan MA. Current status of beta-thalassemia and its treatment strategies. Mol Genet Genomic Med. 2021 Dec;9(12):e1788.

188) Motta I, Bou-Fakhredin R, Taher AT, Cappellini MD. Beta Thalassemia: New Therapeutic Options Beyond Transfusion and Iron Chelation. Drugs. 2020 Jul;80(11):1053-1063.

189) Telen MJ. Beyond hydroxyurea: new and old drugs in the pipeline for sickle cell disease. Blood. 2016 Feb 18;127(7):810-9.

190) Karimi M, Darzi H, Yavarian M. Hematologic and clinical responses of thalassemia intermedia patients to hydroxyurea during 6 years of therapy in Iran. J Pediatr Hematol Oncol 2005;27:380-5.

191) Galanello R, Barella S, Turco MP, Giagu N, Cao A, Dore F, Liberato NL, Guarnone R, Barosi G. Serum erythropoietin and erythropoiesis in high- and low-fetal hemoglobin beta-thalassemia intermedia patients. Blood. 1994 Jan 15;83(2):561-5.

192) Yang K., Wu Y., Zhou Y., Long B., Lu Q., Zhou T., Wang L., Geng Z., Yin X. Thalidomide for patients with β-thalassemia: A multicenter experience. Mediterr J Hematol Infect Dis 2020, 12(1): e2020021,

193) Karponi G, Zogas N. Gene Therapy For Beta-Thalassemia: Updated Perspectives. Appl Clin Genet. 2019 Sep 23;12:167-80.

194) Nienhuis AW, Persons DA. Development of gene therapy for thalassemia. Cold Spring Harb Perspect Med. 2012 Nov 1;2(11):a011833.

195) Rattananon P, Anurathapan U, Bhukhai K, Hongeng S. The Future of Gene Therapy for Transfusion-Dependent Beta-Thalassemia: The Power of the

Lentiviral Vector for Genetically Modified Hematopoietic Stem Cells. Front Pharmacol. 2021 Oct 1;12:730873.

196) Thompson AA, Walters MC, Kwiatkowski J, Rasko JEJ, Ribeil JA, Hongeng S, et al. Gene Therapy in Patients with Transfusion-Dependent β-Thalassemia. N Engl J Med. 2018 Apr 19;378(16):1479-93.

197) Soni S. Gene therapies for transfusion dependent β-thalassemia: Current status and critical criteria for success. Am J Hematol. 2020 Sep;95(9):1099-112.

198) Aziz MA, Khan WA, Banu B, Das SA, Sadiya S, Begum S. Prenatal Diagnosis and Screening of Thalassemia Mutations in Bangladesh: Presence of Rare Mutations. Hemoglobin. 2020 Nov;44(6):397-401.

199) Rosatelli MC, Saba L. Prenatal Diagnosis of β-Thalassemias and Hemoglobinopathies. Mediterr J Hematol Infect Dis. 2009 Nov 15;1(1):e2009011.

200) Harano K, Harano T. [Simple and rapid analysis of beta-thalassemia mutation by sequence-specific amplification]. Rinsho Byori. 2013 Mar;61(3):217-23.

201) Achour A, Koopmann TT, Baas F, Harteveld CL. The Evolving Role of Next-Generation Sequencing in Screening and Diagnosis of Hemoglobinopathies. Front Physiol. 2021 Jul 27;12:686689.

202) He J, Song W, Yang J, Lu S, Yuan Y, Guo J, Zhang J, Ye K, Yang F, Long F, Peng Z, Yu H, Cheng L, Zhu B. Next-generation sequencing improves thalassemia carrier screening among premarital adults in a high prevalence population: the Dai nationality, China. Genet Med. 2017 Sep;19(9):1022-1031.

203) Munkongdee T, Chen P, Winichagoon P, Fucharoen S, Paiboonsukwong K. Update in Laboratory Diagnosis of Thalassemia. Front Mol Biosci. 2020 May 27

204) Hauck-Dlimi B, Achenbach S, Strobel J, Eckstein R, Zimmermann R. Prevention and management of transfusion-induced alloimmunization: current perspectives. International Journal of Clinical Transfusion Medicine. 2014;2:59-63.

205) Poncelet P, Carayon P. Cytofluorometric quantification of cell-surface antigens by indirect immunofluorescence using monoclonal antibodies. J Immunol Methods 1985;85:65-74.

206) Dholakiya SK, Bharadva S, Vachhani JH, Upadhyay BS. Red cell alloimmunization among antenatal women attending tertiary care center in Jamnagar, Gujarat, India. Asian J Transfus Sci 2021;15:52-6.

207) Cabrera G, Cot M, Migot-Nabias F, Kremsner PG, Deloron P, Luty AJ. The sickle cell trait is associated with enhanced immunoglobulin G antibody responses to Plasmodium falciparum variant surface antigens. J Infect Dis. 2005;191:1631–8.

208) Davoudi-Kiakalayeh A, Mohammadi R, Pourfathollah AA, Siery Z, Davoudi-Kiakalayeh S. Alloimmunization in thalassemia patients: New insight for healthcare. Int J Prev Med 2017;8:101.

209) Philip J, Jain N. Resolution of alloimmunization and refractory autoimmune hemolytic anemia in a multi-transfused beta-thalassemia major patient. Asian J Transfus Sci. 2014 Jul;8(2):128-30.

210) Tormey CA, Hendrickson JE. Transfusion-related red blood cell alloantibodies: induction and consequences. Blood. 2019 Apr 25;133(17):1821-1830.

211) Valle Neto OGD, Alves VM, Pereira GA, Moraes-Souza H, Martins PRJ. Clinical and epidemiological profile of alloimmunized and autoimmunized multi-transfused patients against red blood cell antigens in a blood center of Minas Gerais. Hematol Transfus Cell Ther. 2018 Apr-Jun;40(2):107-11.

212) McPherson ME, Anderson AR, Castillejo MI, Hillyer CD, Bray RA, Gebel HM, Josephson CD. HLA alloimmunization is associated with RBC antibodies in multiply transfused patients with sickle cell disease. Pediatr Blood Cancer. 2010 Apr;54(4):552-8.

213) Yazdanbakhsh K, Ware RE, Noizat-Pirenne F. Red blood cell alloimmunization in sickle cell disease: pathophysiology, risk factors, and transfusion management. Blood. 2012 Jul 19;120(3):528-37.

214) Dhawan HK, Kumawat V, Marwaha N, Sharma RR, Sachdev S, Bansal D, et al. Alloimmunization and autoimmunization in transfusion dependent thalassemia major patients: Study on 319 patients. Asian J Transfus Sci 2014;8:84-8.

215) Singer ST, Wu V, Mignacca R, Kuypers FA, Morel P, Vichinsky EP. Alloimmunization and erythrocyte autoimmunization in transfusion-dependent thalassemia patients of predominantly asian descent. Blood. 2000 Nov 15;96(10):3369-73.

216) Gharehbaghian A, Ghezelbash B, Aghazade S, Hojjati MT. Evaluation of Alloimmunization Rate and Necessity of Blood Type and Screening Test among Patients Candidate for Elective Surgery. Int J Hematol Oncol Stem Cell Res. 2014;8(1):1-4.

217) Molina-Aguilar R, Gómez-Ruiz S, Vela-Ojeda J, Montiel-Cervantes LA, Reyes-Maldonado E. Pathophysiology of Alloimmunization. Transfus Med Hemother. 2020 Apr;47(2):152-9.

218) Hojati MT, Mahdavi MR. A Review on Incidence of Alloimmunization with incompatible Rh Blood Group Transfusion and Role of Molecular Methods in Finding Compatible Blood Units in Multi Transfused Patients. Journal of Mazandaran University of Medical Sciences. 2014 Aug 10;24(115):192-202.

219) van der Merwe PA, Bodian DL, Daenke S, Linsley P, Davis SJ. CD80 (B7-1) binds both CD28 and CTLA-4 with a low affinity and very fast kinetics. J Exp Med. 1997 Feb 3;185(3):393-403.

220) Tatari-Calderone Z, Luban NL, Vukmanovic S. Genetics of transfusion recipient alloimmunization: can clues from susceptibility to autoimmunity pave the way? Transfus Med Hemother. 2014 Nov;41(6):436-45.

221) Essa ES, El-Hawy MA, Ahmedy IA, Elmaghraby AS. Red-blood cell alloimmunization in a cohort of multitransfused β-thalassemic patients in Menoufia Governorate, Egypt. Menoufia Med J 2022;35:34-40.

222) Al-Riyami AZ, Al-Mahrooqi S, Al-Hinai S, Al-Hosni S, Al-Madhani A, Daar S. Transfusion therapy and alloimmunization in Thalassemia Intermedia: a 10 year experience at a tertiary care university hospital. Transfus Apher Sci. 2014 Aug;51(1):42-6.

223) Ho HK, Ha SY, Lam CK, Chan GC, Lee TL, Chiang AK, Lau YL. Alloimmunization in Hong Kong southern Chinese transfusion-dependent thalassemia patients. Blood. 2001 Jun 15;97(12):3999-4000.

224) Verduin EP, Brand A, Schonewille H. Is female sex a risk factor for red blood cell alloimmunization after transfusion? A systematic review. Transfus Med Rev. 2012 Oct;26(4):342-53, 353.e1-5.

225) El-Beshlawy A, Salama AA, El-Masry MR, El Husseiny NM, Abdelhameed AM. A study of red blood cell alloimmunization and autoimmunization among 200 multitransfused Egyptian β thalassemia patients. Sci Rep. 2020 Dec 3;10(1):21079.

226) Hendrickson JE, Tormey CA. Understanding red blood cell alloimmunization triggers. Hematology Am Soc Hematol Educ Program. 2016 Dec 2;2016(1):446-51.

227) Premawardhana AP, Mudiyanse R, De Silva ST, Jiffry N, Nelumdeniya U, de Silva U, et al. A nationwide survey of hospital-based thalassemia patients and standards of care and a preliminary assessment of the national prevention program in Sri Lanka. PLoS One. 2019 Aug 16;14(8):e0220852.

228) Stack G, Tormey CA. Detection rate of blood group alloimmunization based on real-world testing practices and kinetics of antibody induction and evanescence. Transfusion. 2016 Nov;56(11):2662-7.

229) Al-Riyami AZ, Al-Muqbali A, Al-Sudiri S, Murthi Panchatcharam S, Zacharia M, Al-Mahrooqi S, et al. Risks of red blood cell alloimmunization in transfusion-dependent β-thalassemia in Oman: a 25-year experience of a university tertiary care reference center and a literature review. Transfusion. 2018 Apr;58(4):871-8.

230) Obeidi N, Mankhian A, Hatami G, Emami H. Antibody Screening in Patients With Thalassemia Major. Lab. Med. 2011.42(10)618-21.

231) Evers D, van der Bom JG, Tijmensen J, de Haas M, Middelburg RA, de Vooght KMK, van de Kerkhof D, Visser O, Péquériaux NCV, Hudig F, Zwaginga JJ. Absence of the spleen and the occurrence of primary red cell alloimmunization in humans. Haematologica. 2017 Aug;102(8):289-92.

232) Das S S, Biswas R N, Safi M, Zaman R. Alloimmunization to Erythrocyte Antigens in Patients Receiving Multiple Blood Transfusions: Clinico-immunohematological and Demographic Risk Factors and impact of Extended Red Cell Phenotyping. Glob. J. Transfus. Med. 2022.6(2)171-7.

233) Mahapatra S, Panda B. Study of alloimmunization against red blood cell antigens in multi-transfused patients. Int J Med Sci Curr Res. 2019;2:535–42.

234) Abdulqader A, Mohammed A, Mohammed N. Red Cell Alloimmunization and Autoimmunization in Multi-Transfused Thalassemia Patients in Sulaymaniyah Province-Iraq. "Korean J Clin Lab Sci". 2020;52:98-104.

235) Handa A, Kukar N, Maharishi RN, Syal N, Arora H. Analysis of red cell alloimmunization in multi transfused patients at a Tertiary care teaching hospital. J Family Med Prim Care. 2020 Jun 30;9(6):2907-11.

236) Tholpady A. Antibody screening. Retrieved on 20-12-2022. From: https://emedicine.medscape.com/article/1731232-overview#:~:text=The%20antibody%20screening%20test%20performed,that%20are%20considered%20clinically%20significant.

237) Direct and indirect antiglobulin (Coombs) testing [Online] Retrieved on 20-12-2022. From: https://www.uptodate.com/contents/image?imageKey=HEME%2F100639

238) Khan S. Clinical utility of the Coombs test. CMAJ. 2006 Oct 10;175(8):919.

239) Coombs test.[Online] Retrieved on 20-12-2022. From: https://en.wikipedia.org/wiki/Coombs_test.

240) Rath L. What Is a Coombs Test? [Online]. Retrieved on 20-12-2022. From: https://www.webmd.com/a-to-z-guides/antibody-coombs-test

241) Tahhan HR, Holbrook CT, Braddy LR, Brewer LD, Christie JD. Antigen-matched donor blood in the transfusion management of patients with sickle cell disease. Transfusion. 1994 Jul;34(7):562-9.

242) El Kababi S, Benajiba M, El Khalfi B, Hachim J, Soukri A. Red blood cell alloimmunizations in beta-thalassemia patients in Casablanca/Morocco: Prevalence and risk factors. Transfus Clin Biol. 2019 Nov;26(4):240-8.

243) Anti-Human Globulin (Formulated for Automated Testing) [Online] Retrieved on 20-12-2022. From: https://www.fda.gov/vaccines-blood-biologics/anti-human-globulin-formulated-automated-testing.

244) Direct and Indirect Antiglobulin Test (Direct and Indirect Coombs) [Online]. Retrieved on 20-12-2022. From: https://www.spectrumhealthlakeland.org/lab-tests/labs-d/coombs-test-direct-and-indirect

245) Aryal S. Coombs Test- Principle, Types, Procedure and Result Interpretation. Last updated: August 10, 2022. Retrieved on 20-12-2022. From: https://microbiologyinfo.com/coombs-test-principle-types-procedure-and-result-interpretation/.

246) Theis S R, Hashmi M F. Coombs Test. Last updated on September 12, 2022. Retrieved on 20-12-2022. From: https://www.ncbi.nlm.nih.gov/books/NBK547707/.

247) Coombs test. Retrieved on 20-12-2022. From: https://my.clevelandclinic.org/health/diagnostics/22978-coombs-test#:~:text=Direct%20Coombs%20test%20(sometimes%20called,such%20as%20autoimmune%20hemolytic%20anemia.

248) Bruce M, Watt AH, Hare W, Blue A, Mitchell R. A serious source of error in antiglobulin testing. Transfusion. 1986 Mar-Apr;26(2):177-81.

249) Hassan K, Younus M, Ikram N, Naseem L, Zaheer H. Red Cell Alloimmunization in Repeatedly Transfused Thalassemia Major Patients. Int J Pathol Clin Res. 2004;2(1)16-9.

250) Ghasemi A, Abbasian S, Ghaffari K, Salmanpour Z. Prevalence of Alloantibodies and Autoantibodies in Transfusion Dependent Thalassemia Patients. IJBC 2016; 8(3): 80-5.

251) Pazgal I, Yahalom V, Shalev B, Raanani P, Stark P. Alloimmunization and autoimmunization in adult transfusion-dependent thalassemia patients: a report from a comprehensive center in Israel. Ann Hematol. 2020 May;99:2731–6.

252) Sarah M. Mahmoud Marbut, S M, Hamdi M A, M.Jumaa A, Salman B A. Distribution of ABO blood groups in beta thalassemia patients dependent on blood transfusion In Bagdad city. JMAUC. 2018Jan;10(2):1-11.

253) Laghari Z A, Baig N M, Charan T R, Lashari K H, Suhag R. Distribution of ABO Blood Groups And Rhesus Factor In ß-Thalassemia Patients At Thalassemia Care Center Nawabshah, Pakistan. SindhUniv. Res. Jour. (Sci. Ser.). 2018;50(1)123-8.

254) Patidar G.K, Dhiman Y. Distribution of ABO and Rh (D) Blood groups in India: A systematic review. VOXS, 2021;16:37-48

255) Bilwani F, Kakepoto GN, Adil SN, Usman M, Hassan F, Khurshid M. Frequency of irregular red cell alloantibodies in patients with thalassemia major: a bicenter study. J Pak Med Assoc. 2005;55(12):563–5.

256) Seferi I, Xhetani M, Face M, Burazeri G, Nastas E, Vyshka G. Frequency and specificity of red cell antibodies in thalassemia patients in Albania. Int J Lab Hematol. 2015 Aug;37(4):569-74.

257) Saifeldeena E R, Awada M A, El-Tonbaryb Y A, Aladlea D A, Elghannam D M. Risk for red cell immunization among thalassemic patients. Egypt J Haematol. 2017;42:58–63.

258) Qidwai A, Mansoor N, Syeda A, Maheen H. Mohammad I. Malik F. Trends of red cell alloimmunization in β-thalassemia major patients: A single center retrospective study in Karachi. J Blood Disord Treat. 2018;1(1):3-5.

259) Abdelrazik AM, Elshafie SM, El Said MN, Ezzat Ahmed GM, Al-Gamil AK, El Nahhas MG, Sady AA. Study of red blood cell alloimmunization risk factors in

multiply transfused thalassemia patients: role in improving thalassemia transfusion practice in Fayoum, Egypt. Transfusion. 2016 Sep;56(9):2303-7.

260) Azarkeivan A, Ansari S, Ahmadi MH, Hajibeigy B, Maghsudlu M, Nasizadeh S, Shaigan M, Toolabi A, Salahmand M. Blood transfusion and alloimmunization in patients with thalassemia: multicenter study. Pediatr Hematol Oncol. 2011 Sep;28(6):479-85.

261) Bahatti FA, Salamat N, Nadeem A, Shabbir N. Red cell immunization in beta thalassemia major. J Coll Physicians Surg Pak. 2004;14:657–60.

262) Cheng CK, Lee CK, Lin CK. Clinically significant red blood cell antibodies in chronically transfused patients: a survey of Chinese thalassemia major patients and literature review. Transfusion. 2012 Oct;52(10):2220-4.

263) Vaziri M, JavadzadehShahshahani H, Moghaddam M, Taghvaee N. Prevalence and specificities of red cell alloantibodies in transfusion-dependent beta thalassemia patients in Yazd. Iran J Ped Hematol Oncol. 2015;5(2):93-9.

264) Schonewille H, van de Watering LM, Loomans DS, Brand A. Red blood cell alloantibodies after transfusion: factors influencing incidence and specificity. Transfusion. 2006 Feb;46(2):250-6.

ANNEXURE I
INFORMED CONSENT

STUDY OF ALLOIMMUNIZATION IN TRANSFUSION DEPENDENT THALASSEMIA PATIENTS AT A TERTIARY CARE HOSPTAL

Principal Investigator: Reg. No. BN0120001

Guide: DR_____

Purpose of the study: You are being asked to enroll as per the eligibility criteria for participation in this study.

The purpose of this study is to detect alloimmunization in transfusion dependent thalassemia patients.

Procedure: During this study, your blood group will be tested, and Direct Coomb's Test (DCT) will be done to detect your alloimmunization status. The principal investigator of the study is Reg. No. BN0120001 under the guidance of Dr. _____.

If you agree to enroll yourself in this study, your DCT report along with your blood group report will be used for research purpose.

Risks and benefits: There are no risks involved in taking part in this study. The benefit would be to know a better way to predict alloimmunization status in thalassemia patients and the importance of advocating specific transfusion guidelines to prevent complications due to alloantibodies produced against the donor RBCs in thalassemia patients undergoing multiple blood transfusions.

Alternatives: Taking part in this study is voluntary. You may choose not to take part in this study or if you decide to take part now, you can later change your mind and

withdraw from the study. The study doctor or sponsor may terminate your participation in this study anytime.

Privacy and confidentiality: All information collected about you during this study will be kept confidential to the extent permitted by law. The code numbers will identify you in this research record. Information from this study will be published but your identity will be confidential in any publication. No information about you or information provided by you during research will be disclosed to other without your written permission except:

1. In emergency to protect your rights and welfare.
2. If required by law.

Financial incentives for participation: You will not be paid / offered any gift / incentives for participating in this study.

Authorization to publish results: The results of this study would be forwarded to the KAHER, Belagavi as a part of requirement towards the completion of MD degree, review, and publishing.

Questions: In case you have any questions related to the study in future you can contact:

1. Reg. No. BN0120001, Department of Pathology, J.N. Medical College.
2. Dr. _____, Department of Pathology, J.N. Medical College.
3. If you have any queries about your rights as a study subject, you may call Dr. Roopa Bellad, Professor, Department of Paediatrics, Chairman of J.N. Medical College Institutional Ethical Committee of Human Subjects Research, Ph No- 9448113403, at J.N. Medical College, Belagavi

CONSENT STATEMENT

I voluntarily agree to take part in this study by signing below. I may withdraw at any time. I am not giving up any legal rights by signing this form. My signature below indicates that I have read, or it has been read to me, this entire consent form and have had all my questions answered.

In case of the queries during the study or in future you may contact following person.

Principal Investigator:

Guide :

Name of the participant:

(signature/thumbprint)

Name of the witness:

(signature/thumbprint)

Name of the investigator:

(signature/thumbprint)

Date:

Address:

Phone no:

ತಿಳುವಳಿಕೆಯುಳ್ಳ ಒಪ್ಪಿಗೆ

ವರ್ಗಾವಣೆಯಲ್ಲಿ ಅವಲಂಬಿತ ಥಲಸ್ಸೆಮಿಯಾ ರೋಗಿಗಳಲ್ಲಿ ತೃತೀಯ ಆರೈಕೆ ಆಸ್ಪತ್ರೆಯಲ್ಲಿ ಅಲೋಇಮ್ಯುನೈಸೇಶನ್ ಅಧ್ಯಯನ.

ಪ್ರಧಾನ ತನಿಖಾಧಿಕಾರಿ: Reg. No. BN0120001

ಮಾರ್ಗದರ್ಶಿ: ಡಾ. _____

ಅಧ್ಯಯನದ ಉದ್ದೇಶ:

ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಅರ್ಹತಾ ಮಾನದಂಡಗಳ ಪ್ರಕಾರ ದಾಖಲಾತಿ ಪಡೆಯಲು ನಿಮ್ಮನ್ನು ಕೇಳಲಾಗುತ್ತಿದೆ.

ವರ್ಗಾವಣೆ ಅವಲಂಬಿತ ಥಲಸ್ಸೆಮಿಯಾ ರೋಗಿಗಳಲ್ಲಿ ಅಲೋಇಮ್ಯುನೈಸೇಶನ್ ಅನ್ನು ಕಂಡುಹಿಡಿಯುವುದು ಈ ಅಧ್ಯಯನದ ಉದ್ದೇಶವಾಗಿದೆ.

ವಿಧಾನ:

ಈ ಅಧ್ಯಯನದ ಸಮಯದಲ್ಲಿ, ನಿಮ್ಮ ರಕ್ತ ಗುಂಪನ್ನು ಪರೀಕ್ಷಿಸಲಾಗುತ್ತದೆ ಮತ್ತು ನಿಮ್ಮ ಮಿಶ್ರಲೋಹೀಕರಣ ಸ್ಥಿತಿಯನ್ನು ಕಂಡುಹಿಡಿಯಲು ಡೈರೆಕ್ಟ್ ಕೂಂಬ್ಸ್ ಟೆಸ್ಟ್ (ಡಿಸಿಟಿ) ಮಾಡಲಾಗುತ್ತದೆ. ಡಾ._____ (ಮಾರ್ಗದರ್ಶಿ) ಅವರ ಮಾರ್ಗದರ್ಶನದಲ್ಲಿ ಅಧ್ಯಯನದ ಪ್ರಮುಖ ತನಿಖಾಧಿಕಾರಿ Reg. No. BN0120001. (ಪಿ.ಜಿ). ಈ ಅಧ್ಯಯನದಲ್ಲಿ ನಿಮ್ಮನ್ನು ದಾಖಲಿಸಲು ನೀವು ಒಪ್ಪಿದರೆ, ನಿಮ್ಮ ರಕ್ತ ಗುಂಪು ವರದಿಯೊಂದಿಗೆ ನಿಮ್ಮ ಡಿಸಿಟಿ ವರದಿಯನ್ನು ಸಂಶೋಧನಾ ಉದ್ದೇಶಕ್ಕಾಗಿ ಬಳಸಲಾಗುತ್ತದೆ.

ಅಪಾಯ ಮತ್ತು ಪ್ರಯೋಜನಗಳು

ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸುವುದರಿಂದ ಯಾವುದೇ ಅಪಾಯಗಳಿಲ್ಲ. ಥಲಸ್ಸೆಮಿಯಾ ರೋಗಿಗಳಲ್ಲಿ ಅಲೋಇಮ್ಯುನೈಸೇಶನ್ ಸ್ಥಿತಿಯನ್ನು ಉಹಿಸಲು ಉತ್ತಮ ಮಾರ್ಗವನ್ನು ತಿಳಿದುಕೊಳ್ಳುವುದು ಮತ್ತು ಅನೇಕ ರಕ್ತ ವರ್ಗಾವಣೆಗೆ ಒಳಗಾಗುವ ಥಲಸ್ಸೆಮಿಯಾ ರೋಗಿಗಳಲ್ಲಿ ದಾನಿ ಆರ್‌ಬಿಸಿಗಳ ವಿರುದ್ಧ ಉತ್ಪತ್ತಿಯಾಗುವ ಅಲೋಆಂಟಿಬಾಡಿಗಳಿಂದ ಉಂಟಾಗುವ ತೊಂದರೆಗಳನ್ನು ತಡೆಗಟ್ಟಲು ನಿರ್ದಿಷ್ಟ ವರ್ಗಾವಣೆ ಮಾರ್ಗಸೂಚಿಗಳನ್ನು ಪ್ರತಿಪಾದಿಸುವ ಪ್ರಾಮುಖ್ಯತೆ.

ಪರ್ಯಾಯಗಳು:

ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸುವುದು ಸ್ವಯಂಪ್ರೇರಿತವಾಗಿದೆ. ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸದಿರಲು ನೀವು ಆಯ್ಕೆ ಮಾಡಬಹುದು ಅಥವಾ ನೀವು ಈಗ ಭಾಗವಹಿಸಲು ನಿರ್ಧರಿಸಿದರೆ, ನೀವು ನಂತರ ನಿಮ್ಮ ಮನಸ್ಸನ್ನು ಬದಲಾಯಿಸಬಹುದು ಮತ್ತು ಅಧ್ಯಯನದಿಂದ ಹಿಂದೆ ಸರಿಯಬಹುದು. ಅಧ್ಯಯನದ ವೈದ್ಯರು ಅಥವಾ ಪ್ರಾಯೋಜಕರು ಈ ಅಧ್ಯಯನದಲ್ಲಿ ನಿಮ್ಮ ಭಾಗವಹಿಸುವಿಕೆಯನ್ನು ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಕೊನೆಗೊಳಿಸಬಹುದು.

ಗೌಪ್ಯತೆ

ಈ ಅಧ್ಯಯನದ ಸಮಯದಲ್ಲಿ ನಿಮ್ಮ ಬಗ್ಗೆ ಸಂಗ್ರಹಿಸಲಾದ ಎಲ್ಲಾ ಮಾಹಿತಿಯನ್ನು ಕಾನೂನಿನಿಂದ ಅನುಮತಿಸುವ ಮಟ್ಟಿಗೆ ಗೌಪ್ಯವಾಗಿಡಲಾಗುತ್ತದೆ. ಈ ಸಂಶೋಧನಾ ದಾಖಲೆಯಲ್ಲಿ ಕೋಡ್ ಸಂಖ್ಯೆಗಳು ನಿಮ್ಮನ್ನು ಗುರುತಿಸುತ್ತವೆ. ಈ ಅಧ್ಯಯನದ ಮಾಹಿತಿಯನ್ನು ಪ್ರಕಟಿಸಲಾಗುವುದು ಆದರೆ ಯಾವುದೇ ಪ್ರಕಟಣೆಯಲ್ಲಿ ನಿಮ್ಮ ಗುರುತು ಗೌಪ್ಯವಾಗಿರುತ್ತದೆ. ನಿಮ್ಮ ಲಿಖಿತ ಅನುಮತಿಯಿಲ್ಲದೆ ನಿಮ್ಮ ಬಗ್ಗೆ ಯಾವುದೇ ಮಾಹಿತಿ ಅಥವಾ ಸಂಶೋಧನೆಯ ಸಮಯದಲ್ಲಿ ನೀವು ಒದಗಿಸಿದ ಮಾಹಿತಿಯನ್ನು ಇತರರಿಗೆ ಬಹಿರಂಗಪಡಿಸಲಾಗುವುದಿಲ್ಲ:

1. ನಿಮ್ಮ ಹಕ್ಕುಗಳು ಮತ್ತು ಕಲ್ಯಾಣವನ್ನು ರಕ್ಷಿಸಲು ತುರ್ತು ಪರಿಸ್ಥಿತಿಯಲ್ಲಿ.

2. ಕಾನೂನಿನ ಪ್ರಕಾರ ಅಗತ್ಯವಿದ್ದರೆ.

ಭಾಗವಹಿಸುವಿಕೆಗೆ ಆರ್ಥಿಕ ಪ್ರೋತ್ಸಾಹ:

ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ನಿಮಗೆ ಯಾವುದೇ ಉಡುಗೊರೆ / ಪ್ರೋತ್ಸಾಹ ಧನ ನೀಡಲಾಗುವುದಿಲ್ಲ.

ಫಲಿತಾಂಶಗಳನ್ನು ಪ್ರಕಟಿಸಲು ಅಧಿಕಾರ:

ಈ ಅಧ್ಯಯನದ ಫಲಿತಾಂಶಗಳನ್ನು ಎಂಡಿ ಪದವಿ, ವಿಮರ್ಶೆ ಮತ್ತು ಪ್ರಕಟಣೆಯ ಪೂರ್ಣಗೊಳಿಸುವಿಕೆಯ ಅವಶ್ಯಕತೆಯ ಭಾಗವಾಗಿ ಬೆಳಗವಿಯ ಕಾಹೇರ್‌ಗೆ ರವಾನಿಸಲಾಗುತ್ತದೆ.

ಪ್ರಶ್ನೆಗಳು

ಭವಿಷ್ಯದಲ್ಲಿ ನೀವು ಅಧ್ಯಯನಕ್ಕೆ ಸಂಬಂಧಿಸಿದ ಯಾವುದೇ ಪ್ರಶ್ನೆಗಳನ್ನು ಹೊಂದಿದ್ದರೆ ನೀವು ಸಂಪರ್ಕಿಸಬಹುದು:

1. Reg. No. BN0120001, ರೋಗಶಾಸ್ತ್ರ ವಿಭಾಗ, ಜೆ.ಎನ್. ವೈದ್ಯಕೀಯ ಕಾಲೇಜು.

2. ಡಾ. _____, ರೋಗಶಾಸ್ತ್ರ ವಿಭಾಗ, ಜೆ.ಎನ್. ವೈದ್ಯಕೀಯ ಕಾಲೇಜು.

3. ಅಧ್ಯಯನದ ವಿಷ್ಯವಾಗಿ ನಮ್ಮ ಹಕ್ುಂಗಳ ಬಗೆಿ ನೀವು ರ್ಾವುದೇ ಪರಶ್ಿಿಗಳನುಿ ಹೆಂದಿದದರೆ, ನೀವು ರ್ಷಾ ಹೆರ್ಿಿ

ಅಧ್ುಕ್ಷರಯ, ಜೆಎನ್ ಎಂಸ್ಥ ಐಇಸ್ಥ ಮ್ತುಯಿ ವಿಜ್ಞಗನಿ ಡಿಐಸ್ಥಎಂಆರ, ನಗುಷ್ನಲ್ ಇನಿಿಿಟ್ ುಟ್ ಆಫ್ ಟಿಿಡಿಶನಲ್ ಮೆಡಿಸ್ಥನ್

ಬೆಳಗ್ಗವಿ -590010, ಅವರನುಿ ಕ್ರೆ ಮಾಡಬಹುದು, ಪ್ಪಎಚ್ ಸಂಖ್ಿಯ- 9480422500, ಜೆ.ಎನ್. ವೈೆದಯಕೀೀಯ ಕಾಲೇೀಜು, ಬೆಳಗವಿ

ಒಪ್ಪಿಗೆಯ ಹೇಳಿಕೆ

ಕೆಳಗೆ ಸಹಿ ಮಾಡುವ ಮೂಲಕ ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ನಾನು ಸ್ವಯಂಪ್ರೇರಣೆಯಿಂದ ಒಪ್ಪುತ್ತೇನೆ. ನಾನು ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಹಿಂತೆಗೆದುಕೊಳ್ಳಬಹುದು. ಈ ಫಾರ್ಮ್ಗೆ ಸಹಿ ಮಾಡುವ ಮೂಲಕ ನಾನು ಯಾವುದೇ ಕಾನೂನು ಹಕ್ಕುಗಳನ್ನು ಬಿಟ್ಟುಕೊಡುತ್ತಿಲ್ಲ. ಕೆಳಗಿನ ನನ್ನ ಸಹಿ ನಾನು ಓದಿದ್ದೇನೆ, ಅಥವಾ ಅದನ್ನು ನನಗೆ ಓದಿದೆ, ಈ ಸಂಪೂರ್ಣ ಒಪ್ಪಿಗೆಯ ರೂಪ ಮತ್ತು ನನ್ನ ಎಲ್ಲಾ ಪ್ರಶ್ನೆಗಳಿಗೆ ಉತ್ತರಿಸಿದೆ ಎಂದು ಸೂಚಿಸುತ್ತದೆ

ಪ್ರಧಾನ ತನಿಖಾಧಿಕಾರಿ:

ಮಾರ್ಗದರ್ಶಿ:

ಭಾಗವಹಿಸುವವರ ಹೆಸರು: (ಸಹಿ / ಹೆಬ್ಬೆರಳು)

ಸಾಕ್ಷಿಯ ಹೆಸರು: (ಸಹಿ / ಹೆಬ್ಬೆರಳು)

ತನಿಖಾಧಿಕಾರಿಯ ಹೆಸರು: (ಸಹಿ)

ದಿನಾಂಕ:

ವಿಳಾಸ: ದೂರವಾಣಿ ಸಂಖ್ಯೆ:

माहितीपूर्ण संमती

टेरिटरी केअर रुग्णालय येथे रक्तसंक्रमण अवलंबित थॅलेसीमिया रुग्णांमध्ये एलोईम्यूनायझेशनचा अभ्यास.

प्रधान अन्वेषक: Reg. No. BN0120001

मार्गदर्शक: डॉ._____

अभ्यासाचा उद्देश:

या अभ्यासामध्ये भाग घेण्यासाठी पात्रतेच्या निशर्कशनुसार आपल्याला नोंदणी करण्यास सांगितले जाते.

रक्तसंक्रमण अवलंबून असलेल्या थॅलेसीमियाच्या रुग्णांमध्ये एलोईम्यूनायझेशन शोधणे हा या अभ्यासाचा हेतू आहे.

प्रक्रियाः

या अभ्यासादरम्यान, आपल्या रक्तगटाची चाचणी केली जाईल आणि आपली एलोईम्यूनायझेशन स्थिती शोधण्यासाठी डायरेक्ट कोम्बची चाचणी (डीसीटी) केली जाईल. डॉ. _____ (मार्गदर्शक) यांच्या मार्गदर्शनाखाली Reg. No. BN0120001 (पीजी) या अभ्यासाचे मुख्य तपासनीस आहेत. आपण या अभ्यासामध्ये आपली नावनोंदणी करण्यास सहमती दर्शविल्यास आपल्या रक्तगटाच्या अहवालासह आपला डीसीटी अहवाल संशोधनाच्या उद्देशाने वापरला जाईल.

जोखीम आणि फायदे

या अभ्यासामध्ये भाग घेण्यात कोणतीही जोखीम नाही. थॅलेसीमियाच्या रुग्णांमध्ये एलोईम्यूनायझेशन स्थितीचा अंदाज लावण्याचा अधिक चांगला मार्ग आणि थॅलेसीमियाच्या रूग्णांमध्ये रक्तदात्यांकडून रक्तदात्या आरबीसीविरूद्ध रक्तवाहिन्या तयार केल्या जाणाऱ्या गुंतागुंत रोखण्यासाठी विशिष्ट रक्तसंक्रमण मार्गदर्शक तत्त्वांचे समर्थन करण्याचे महत्त्व जाणून घेण्याचा फायदा होईल.

विकल्पः

या अभ्यासामध्ये भाग घेणे ऐच्छिक आहे. आपण या अभ्यासामध्ये भाग न घेण्याची निवड करू शकता किंवा आपण आता भाग घेण्याचा निर्णय घेतल्यास आपण नंतर आपले मत बदलू आणि अभ्यासापासून दूर जाऊ शकता. अभ्यास डॉक्टर किंवा प्रायोजक या अभ्यासामधील आपला सहभाग कधीही रद्द करू शकतात.

गोपनीयता

या अभ्यासाच्या दरम्यान आपल्याबद्दल संकलित केलेली सर्व माहिती कायद्याद्वारे परवानगी असलेल्या मर्यादेपर्यंत गोपनीय ठेवली जाईल. कोड नंबर आपल्याला या संशोधन रेकॉर्डमध्ये ओळखतील. या अभ्यासाची माहिती प्रकाशित केली जाईल परंतु आपली ओळख कोणत्याही प्रकाशनात गोपनीय असेल. आपल्याबद्दल किंवा संशोधनादरम्यान प्रदान केलेली माहिती किंवा □तर माहिती आपल्या लिखित परवानगीशिवाय □तरांना उघड केली जाणार नाहीः

1. आपत्कालीन परिस्थितीत आपले हक्क आणि कल्याण संरक्षित करण्यासाठी.

2. कायद्याने आवश्यक असल्यास.

सहभागासाठी आर्थिक प्रोत्साहनः

या अभ्यासामध्ये भाग घेण्यासाठी आपल्याला कोणतीही भेट / प्रोत्साहन दिले जाणार नाही.

परिणाम प्रकाशित करण्यासाठी अधिकृततताः

या अभ्यासाचे निकाल एमएडी पदवी, आढावा आणि प्रकाशन पूर्ण करण्याच्या आवश्यकतेचा भाग म्हणून काहेर, बेलागावीकडे पाठविला जाईल.

प्रशनावळी:

भविष्यात अभ्यासाशी संबंधित काही प्रश्न असल्यास आपण संपर्क साधू शकताः

1. डॉ. ऐश्वर्या एम एस, पॅथॉलॉजी विभाग, जे.एन. मेडिकल कॉलेज, फोन नंबर- 7483884149 / 8861399064

2. डॉ. सुनीता वाय पाटील , पॅथॉलॉजी विभाग, जे.एन. मेडिकल कॉलेज, फोन नंबर- 9845284106

3. आपल्याकडे अभ्यासाचा विषय म्हणून आपल्या हक्कांबद्दल काही शंका असल्यास आपण डॉ. रूपा बेल्लद, प्रोफेसर, बाल रोगशास्त्र विभाग, मानवी विषय संशोधनाची संस्था नैतिक समिती, अध्यक्ष जे.एन. मेडिकल कॉलेज बेलागावी. कॉल करू शकता. फोन नंबर 9448113403,

संमती विधान

मी खाली स्वाक्षरी करून या अभ्यासात भाग घेण्यास स्वेच्छेने सहमत आहे. मी कधीही माघार घेऊ शकतो. या फॉर्मवर सही करून मी कोणतेही कायदेशीर हक्क सोडत नाही. खाली माझी स्वाक्षरी सूचित करते की मी हा संपूर्ण संमती फॉर्म वाचला आहे किंवा माझ्या कडून वाचविला गेला आहे आणि माझ्या सर्व प्रश्नांची उत्तरे दिली आहेत.

प्रधान अन्वेषक:

मार्गदर्शक:

सहभागीचे नाव: (स्वाक्षरी / अंगठाचा ठसा)

साक्षीदाराचे नाव: (सही / अंगठाचा ठसा)

चौकशीचे नाव: (स्वाक्षरी)

तारीख:

पत्ता: फोन नंबरः

ANNEXURE II

PROFORMA

STUDY OF ALLOIMMUNISATION IN TRANSFUSION DEPENDENT THALASSEMIA PATIENTS AT A TERTIARY CARE HOSPITAL. Thalassemia patients who are registered at KLE's Dr. Prabhakar Kore Charitable hospital and KLE's Dr PK Hospital and MRC, Belagavi are enrolled in this study. This study will include 205 cases of thalassemia receiving regular transfusions. The study is a prospective cross-sectional study. The data collected will comprise of age, sex, age at first transfusion, transfusion frequency, number of transfusions till date and splenectomy status as a part of their treatment. Phlebotomy will be performed under strict aseptic precautions and 3 mL of venous blood will be collected in EDTA test tubes. Antibody screening via Direct Coomb's Test will be performed in all collected samples.

PATIENT DETAILS

Name: Age:

Sex: IP no.:

Brief clinical history:

Age of onset of symptoms:

Age at first transfusion:

Blood group:

Type of blood received: [A] Whole blood

[B] Packed Red Blood Cells (PRBC)

[C] Leucodepleted blood

Frequency of transfusion:

Number of transfusions till date:

Hemoglobin (%): [A] Before transfusion -

[B] After transfusion -

Splenectomy status:

Direct Coomb's Test:

ANNEXURE IV

DIRECT COOMB'S TEST

Direct Coombs Test [DCT] / Direct Antiglobulin Test [DAT]

The direct Coombs test is used to detect antibodies (IgG or C3) that are stuck to the surface of red blood cells that sometimes destroy red blood cells and cause anemia.

To identify if a patient's RBCs are coated with immunoglobulin, complement or both, AHG with reactivity to human immunoglobulin and/or compliment is added to the patient's RBCs. If cross linking and subsequent agglutination is present, direct Coomb's test is positive.

Specimen: EDTA blood

Materials :

1. Test tubes
2. AHG reagent
3. Positive control cells (IgG coated)
4. Centrifuge and microscope

Procedure :

1. Make 5% cell suspension of patient blood by washing 3 times with normal saline.
2. After last washing decant the supernatant completely.
3. Take 1 drop of patient's cell suspension in a test tube.
4. Add 2 drops of A.H.G reagent with the patient's cell suspension in the test tube.
5. Mix well and centrifuge the mixture for at 1500 RPM for 1 minute.

6. Gently shake the tube and examine with naked eye and under microscope to see the agglutination.

7. If the test result is negative, add a drop of control cells.

8. Mix well and centrifuge the mixture for at 1500 RPM for 1 minute and look for agglutination. If no agglutination is seen, the result is invalid.

Result:

Presence of agglutination means a positive DAT indicating the presence of human immune globulin or complement bound to RBCs.

Absence of agglutination means a negative DAT.

Uses of DAT:

1. Presence of autoantibodies against RBC as in the case of warm autoimmune hemolytic anemia (AHA).

2. To detect hemolytic transfusion reactions when incompatible blood is transfused, the donor cells get coated with recipient's antibodies and the DAT is positive.

ANNEXURE V

KEY TO MASTER CHART

SN	-	Serial Number
YRS	-	Years
BG	-	Blood Group
PC	-	Parental Consanguinity
SWT	-	Sibling with Thalassemia
PWH	-	Parents with Hemoglobinopathy
Dx	-	Diagnosis
AD / AFT	-	Age at Diagnosis / Age at First Transfusion
TR	-	Transfusion Regimen
n	-	Number of Transfusions till date
S	-	Splenectomy
TBT	-	Type of Blood Transfused
VBT	-	Volume of Blood Transfused
mL	-	Milli-litre
iHb	-	Initial Hemoglobin
fHb	-	Final Hemoglobin
g/Dl	-	Gram per Decilitre
RGHb/wk	-	Rate of fall of hemoglobin per week
DCT	-	Direct Coombs Test
ALLO	-	Alloimmunization
M	-	Male
F	-	Female
Mo	-	Mother

Fo	-	Father
+	-	Positive
-	-	Negative
Y	-	Yes
N	-	No
TM	-	Thalassemia Major
TI	-	Thalassemia Intermedia
AT	-	Alpha Thalassemia
HT	-	HBE Thalassemia
Tt	-	Thalassemia Trait
St	-	Sickle Trait
HyrTr	-	Hyper-transfusion Regimen
HTr	-	Hypo-transfusion Regimen
STr	-	Super-Transfusion Regimen
D	-	Done
ND	-	Not Done
PRBC	-	Packed Red Blood Cells
TSW	-	Triple Saline Washed
O-TB	-	O Type Blood

SN	AGE (YRS)	SEX	BG	PC	SWT	PWH	Dx	AD / AFT (YRS)	TR	n	S	TBT	VBT (mL)	iHb (g/dL)	fHb (g/dL)	RFHb/wk (g/dL)	DCT / ALLO
1	8.1	M	A +	N	N	N	TM	7.7	HyrTr	101	ND	PRBC	5000	8.2	12	1.5	-
2	7.8	M	B -	N	N	N	TM	7.1	HyrTr	56	ND	PRBC	1000	7.5	13.4	0.5	-
3	1.8	F	A +	N	N	N	TM	1.1	HyrTr	11	ND	PRBC	100	9.7	13.5	0.7	-
4	9.1	M	A +	Y	N	N	TM	8.8	HyrTr	99	ND	PRBC	7500	8.6	12.6	0.6	-
5	18.5	M	B +	Y	N	N	TM	17.6	HTr	211	ND	PRBC	27500	6.7	9	0.1	-
6	4.6	F	B +	N	N	N	TM	3.1	HyrTr	32	ND	PRBC	200	8.4	12.9	1	-
7	10.8	F	A +	Y	N	N	TI	4.3	HTr	44	ND	PRBC	600	7.1	10.6	0.9	-
8	11.8	F	B +	N	N	N	TM	11.5	HyrTr	168	ND	PRBC	16750	7	12.1	1.6	-
9	21.1	M	B +	Y	N	N	TI	9.2	HyrTr	299	ND	PRBC	45000	6	11	2.2	+
10	18.1	M	B +	Y	N	N	TM	17.8	HyrTr	319	D	PRBC (TSW)	45500	8	10.7	1.8	+
11	5.5	M	AB +	N	N	N	TI	3.4	HyrTr	36	ND	PRBC	100	8.4	13.5	1	-
12	10	F	B +	Y	N	N	TI	3.8	HTr	36	ND	PRBC	200	7	10.2	0.8	-
13	1.6	F	B +	Y	N	N	TM	1	HyrTr	9	ND	PRBC	100	9.2	13.6	0.7	-
14	14.6	F	B +	Y	N	N	TI	8.1	HTr	97	ND	PRBC	800	4.9	10	1.8	-
15	19.3	M	O +	N	N	N	TI	15	HyrTr	238	ND	PRBC	-	7.8	9.9	0.6	-
16	16.8	F	A +	N	N	N	TM	15.3	HyrTr	207	ND	PRBC	24500	7.5	11.6	0.8	-
17	1.8	F	O +	Y	N	N	TM	2.4	HyrTr	15	ND	PRBC	600	8.4	12.9	1	-
18	15.7	M	O +	Y	N	N	TI	11.8	HyrTr	172	ND	PRBC	18000	9.6	13.2	1.2	-
19	11.9	F	O -	Y	N	N	TI	9	HTr	80	ND	PRBC	2400	6.6	11.4	0.8	-
20	6.3	F	O +	N	N	N	TM	6	HyrTr	65	ND	PRBC	600	9.7	14	0.9	-
21	11.2	F	A +	Y	N	N	TM	10.4	HyrTr	165	ND	PRBC	15000	7.1	11.4	1.8	-
22	11.9	F	O -	Y	N	N	TI	9.1	HTr	80	ND	PRBC	2400	6.6	11.4	0.8	-
23	6.4	F	B +	Y	N	N	TM	6.3	HyrTr	69	ND	PRBC	800	9.1	13.5	1	-
24	13.1	F	A +	Y	Y	N	TM	12.3	HyrTr	166	ND	PRBC	17250	9	13.2	1.1	-
25	8.8	M	B +	N	Y	N	TM	7.7	HyrTr	79	ND	PRBC	2400	8.7	12.4	1.7	-
26	10.3	M	B +	N	N	N	TM	9.7	HyrTr	133	ND	PRBC	10000	8.5	12.5	1.1	-
27	13	F	B +	N	N	N	TM	9.7	HyrTr	126	ND	PRBC	9600	9.1	12.5	1.1	-
28	5.9	F	A +	N	N	N	TM	5.3	HyrTr	22	ND	PRBC	3000	8.1	13.5	1.3	-
29	9	F	A +	Y	N	N	TM	8.2	HyrTr	100	ND	PRBC (TSW)	5500	8.2	12.2	1.4	-
30	11.7	M	B +	N	N	N	TM	10.2	HyrTr	157	ND	PRBC		7.5	11.1	1.3	-
31	2.9	F	AB +	N	N	N	TM	0.6	HyrTr	5	ND	PRBC	400	5.7	8.7		-
32	2.8	M	A +	Y	N	N	TM	2.2	HyrTr	25	ND	PRBC	230	8.6	13.1	1.1	-
33	9.7	F	AB +	Y	N	N	TM	8.3	HyrTr	108	ND	PRBC	6250	7.8	12.1	1.3	-
34	9.7	F	O +	Y	Y	N	TM	8.9	HyrTr	90	ND	PRBC	6300	7.9	11.2	1.1	-
35	17	M	B +	Y	N	N	TM	14.7	HyrTr	209	D	PRBC (TSW)		9.5	13.2	0.9	-
36	15.4	F	B +	Y	N	N	TM	14.8	HyrTr	212	ND	PRBC	212	7.7	10.2	0.7	-
37	6.5	M	O +	Y	N	N	TM	0.8	HyrTr	4	ND	PRBC	600	6.7	9.7		-
38	16.2	M	O +	N	N	N	TM	15.7	HyrTr	348	ND	PRBC (TSW)		8.5	12.7	1	-
39	4.4	M	B +	Y	N	N	TM	3.4	HyrTr	38	ND	PRBC	300	8.1	12.6	1.2	-
40	6.9	F	B +	Y	Y	N	TM	6.8	HyrTr	80	ND	PRBC	2750	8.7	13.5	1	-
41	21.3	M	O +	Y	N	N	TI	16.1	HTr	174	D	PRBC	24500	7.1	8.5	0.3	+
42	11.1	F	O +	Y	Y	N	TM	10	HyrTr	138	ND	PRBC	11750	9.1	13.5	1.6	-
43	13	M	A +	Y	N	N	TI	9.3	HyrTr	174	D	PRBC	11750	7.7	10.3	1.4	-
44	14.4	F	B +	Y	N	N	TM	13.9	HyrTr	195	ND	PRBC	24500	8.3	12	1.4	-
45	11.1	F	B +	N	N	N	TM	9	HyrTr	116	ND	PRBC	8250	9	13.4	1.1	-
46	15.3	F	B +	Y	Y	N	TM	14.8	HyrTr	160	D	PRBC	13200	9.3	11.4	0.8	-
47	8.7	M	A +	Y	N	N	TM	8	HyrTr	100	ND	PRBC	7000	4.9	7.9	0.3	-
48	17.4	M	O -	Y	N	N	TM	16.8	HyrTr	224	ND	PRBC	28800	7.1	9.2	0.8	-
49	11.8	M	A +	Y	N	N	TM	11.5	HyrTr	177	ND	PRBC	19000	8.6	10.8	1.4	-

50	16.8	M	O +	Y	Y	N	TM	16.5	HyrTr	172	D	PRBC	18000	9.7	12.6	1	+
51	5.1	M	O +	Y	Y	N	TM	4.2	HyrTr	45	ND	PRBC	50	10.02	13.5	0.8	-
52	8	M	O +	N	N	N	TM	7.7	HyrTr	98	ND	PRBC	10200	7.9	12.5	0.8	-
53	12.2	M	A +	N	N	N	TM	11.6	HyrTr	152	ND	PRBC		8.9	11.7	1.1	-
54	7.3	M	O +	N	N	N	TM	6.7	HyrTr	76	ND	PRBC	1500	8	12.3	1.2	-
55	26.5	M	B +	N	N	N	TM	20	HyrTr	372	ND	PRBC		9.6	13.5	0.8	-
56	7.5	M	AB +	N	N	N	TI	4.7	HTr	39	ND	PRBC	400	7.4	11	0.9	-
57	27.9	M	O +	N	N	N	TM	28.1	HyrTr	177	ND	PRBC	19250	9	11.7	1	-
58	9	M	A +	Y	N	N	AT	8.5	HTr	58	ND	PRBC	2250	7.5	10.1	0.5	-
59	15.1	M	O +	Y	Y	N	TI	10	HyrTr	148	ND	PRBC	13500	8.8	12.6	1.1	-
60	4.8	F	AB -	N	N	N	TM	4.5	HyrTr	46	ND	PRBC	60	8.1	12.6	0.9	-
61	12.9	M	A +	Y	N	N	TM	10.5	HyrTr	135	ND	PRBC	8750	7.8	11.9	1.1	-
62	1	F	O +	Y	N	N	TM	0.4	HyrTr	4	ND	PRBC	200	8.6	13.1		-
63	10.6	M	A +	Y	N	N	TI	7.7	HTr	115	D	PRBC (O-TB)	14400	7.3	10.4	0.7	-
64	8.3	M	A +	Y	N	N	TM	7.4	HyrTr	114	ND	PRBC	4000	8.2	11.7	1.2	-
65	6.7	M	O +	N	N	N	TM	5.2	HyrTr	70	ND	PRBC	3200	8.5	13.5	2.5	-
66	10.1	M	B +	Y	N	N	TM	9.4	HyrTr	128	ND	PRBC	10500	9.6	13.5	1.1	-
67	3.2	F	A +	Y	N	N	TM		HyrTr	29	ND	PRBC	100	8.1	12.6	1	-
68	3.7	F	A -	N	N	N	TM	2.7	HyrTr	27	ND	PRBC	240	5	8	1.1	-
69	8.3	M	B +	Y	N	N	TM	8	HyrTr	97	ND	PRBC	7500	9.5	13.5	0.8	-
70	8	M	A +	N	Y	N	TI	3.8	HTr	39	ND	PRBC	200	7.4	12.2	0.9	-
71	14.1	F	O +	Y	N	N	TI	10.8	HyrTr	134	ND	PRBC	9750	9.2	13.4	0.8	-
72	10.8	F	B +	Y	N	N	TM	9.9	HyrTr	134	ND	PRBC	12600	10.6	13.5	0.9	-
73	2.8	F	O +	Y	N	N	TM	2.3	HyrTr	15	ND	PRBC	800	7.5	12	1	-
74	9.5	F	O +	Y	N	N	TM	8.8	HyrTr	101	ND	PRBC	20000	8.9	13.3		-
75	13.2	M	O +	N	Y	N	TM	13	HyrTr	263	ND	PRBC	36000	8.4	11.5	1.4	+
76	9.5	F	O +	N	N	N	TM	8.9	HyrTr	110	ND	PRBC	6000	9	13.5	1	-
77	18.1	M	B +	N	N	ɔ - Tt, Mo -	HT	14.1	HTr	135	ND	PRBC	10250	7.8	10	0.8	-
78	4.2	M	B +	Y	Y	N	TM	3.9	HyrTr	53	ND	PRBC	90	7.4	11.2	1.3	-
79	7.6	M	A +	Y	N	N	TM	7.3	HyrTr	84	ND	PRBC	2750	9	13.5	1	-
80	6.7	F	B +	Y	Y	N	TM	6.1	HyrTr	88	ND	PRBC	500	7.1	11	1.5	-
81	4.3	F	A +	Y	N	N	TM	4	HyrTr	43	ND	PRBC	200	8.9	13.4	1	-
82	1.8	F	A +	Y	N	N	TM	1.2	HyrTr	12	ND	PRBC	150	8.8	13.3	0.7	-
83	20.9	M	B +	N	N	N	TM	20.4	HyrTr	412	ND	PRBC		8.5	10.2	1.1	-
84	22.3	M	O +	Y	N	N	TM	21.6	HyrTr	315	ND	PRBC		8.1	12.1	1.4	-
85	9.8	M	O +	Y	N	N	TI	6.7	HTr	9	ND	PRBC	800	9.8	13.2	1.1	-
86	14.1	F	B +	N	N	N	TM	13.5	HyrTr	217	ND	PRBC	19200	8.5	10	1	-
87	14.6	F	A +	N	Y	N	TI	3.7	HyrTr	88	ND	PRBC	221	6	8.8	1.2	-
88	2	M	A +	N	N	N	TM	1.3	HyrTr	12	ND	PRBC	900	8.3	12.8	0.9	-
89	4.1	M	A +	Y	N	N	TM	3.7	HyrTr	40	ND	PRBC	210	10.8	13.5	0.6	-
90	6.8	M	B +	Y	Y	N	TM	5.3	HyrTr	66	ND	PRBC	400	10.3	13.5	0.9	-
91	14.2	M	O +	N	N	N	TI	11.3	HyrTr	218	ND	PRBC	21250	8	13.3	1.2	-
92	9.8	M	B +	N	N	N	TM	9.5	HyrTr	139	ND	PRBC	12000	8.1	11.6	1.3	-
93	5.3	F	O +	N	N	N	TM	5	HyrTr	63	ND	PRBC	250	9.9	13.5	1.2	-
94	14.2	F	O +	N	Y	N	TM	14.1	HyrTr	225	ND	PRBC	28750	9.2	11.3	1.2	-
95	1.5	F	A +	Y	Y	N	TM	0.8	HyrTr	9	ND	PRBC	600	10.2	13.5	0.8	-
96	9.2	F	A -	Y	Y	N	TM	8.8	HyrTr	8.8	ND	PRBC	6250	8.3	11.7	1.3	-
97	21.4	M	A +	Y	N	N	TM	20.7	HyrTr	250	ND	PRBC	40000	9.7	11.5	1.1	-
98	11.7	F	O +	N	N	N	TM	11.3	HyrTr	158	ND	PRBC	19000	6.6	9.6	1.5	-
99	6.8	F	A +	N	N	N	TM	6.3	HyrTr	71	ND	PRBC	5300	8.5	12.6	1	-
100	12.3	M	AB +	Y	N	N	TM	11.6	HyrTr	140	ND	PRBC	11750	6.5	9.5	0.8	-
101	4.1	M	A +	N	N	N	TM	3.7	HyrTr	37	ND	PRBC	40	7.1	11.6	1	-
102	6.9	F	O +	Y	N	N	TM	6.6	HyrTr	91	ND	PRBC	1750	8.4	12.3	0.3	-
103	9.7	F	A +	N	Y	N	TI	4.8	HTr	35	ND	PRBC	200	7.2	11.2	0.8	-
104	7.3	M	O +	Y	N	N	TI	3.1	HTr	36	ND	PRBC	120	4.6	8.4	0.6	-
105	11	F	O +	Y	Y	N	TM	9.6	HTr	145	ND	PRBC	9600	6.5	9.8	0.9	-

106	14.6	F	AB +	Y	N	N	TM	13.6	STr	105	ND	PRBC (O-TB)	5750	7.8	12.3	2.8	-
107	15.8	F	O -	Y	N	N	TI	13.2	HyrTr	148	D	PRBC	17750	7.6	10.6	0.7	-
108	12.6	M	B +	N	Y	N	TI	8.3	HTr	108	D	PRBC	7000	8.8	10.6	0.2	-
109	23.8	M	B +	Y	Y	N	TI	23.2	HTr	185	D	PRBC	27500	7.7	13.5	0.5	+
110	16.1	M	O +	Y	N	N	TM	15.3	HTr	190	D	PRBC (TSW)	28500	6.8	8.2	0.8	+
111	8.1	M	B +	Y	N	N	TM	6.3	STr	79	ND	PRBC	1000	9.9	14.5	1.5	-
112	12.3	M	B +	Y	N	N	TM	10.8	HyrTr	133	ND	PRBC	10750	9.2	12.3	1.1	-
113	8.8	M	O +	Y	N	N	TI	5.7	HTr	76	ND	PRBC	200	7.5	10.4	0.9	-
114	5.8	F	A +	Y	N	N	TM	5.7	HyrTr	65	ND	PRBC	600	7.3	11.9	1.4	-
115	8.7	F	O +	Y	N	N	TM	8.3	HyrTr	99	ND	PRBC	5000	7.9	10.8	1.5	-
116	2.5	M	B +	N	N	N	TM	2.3	HyrTr	21	ND	PRBC	900	8.6	13.1	1.1	-
117	20.8	M	B +	Y	N	N	TM	18	HyrTr	246	ND	PRBC		7.9	9.7	1.1	-
118	13	M	AB +	Y	N	N	TM	10.2	HyrTr	100	ND	PRBC	6000	8	10.9	0.6	-
119	13.7	M	A +	Y	N	N	TM	13.1	HyrTr	175	ND	PRBC	21000	9.1	12.5	1	-
120	6.3	M	B +	N	N	N	TM	5.8	HyrTr	79	ND	PRBC	50	6.8	9.8	1.6	-
121	14.7	M	O +	Y	N	N	TM	14.5	HyrTr	238	ND	PRBC		9	13.5	1.2	-
122	14.3	F	O +	Y	N	N	TM	14.1	HyrTr	206	ND	PRBC	24000	9.2	13.5	1.2	-
123	4.8	M	B +	N	N	N	TM	4.6	HyrTr	24	ND	PRBC	1000	9.4	11.5	0.8	-
124	5	F	O +	N	N	N	TM	3.5	HyrTr	45	ND	PRBC	90	7.8	13.5	1.4	-
125	16.5	F	B +	N	Y	N	TM	15.5	HTr	205	D	PRBC	31500	9.4	11.1	0.3	+
126	12.4	F	B +	Y	N	N	TM	12.1	HyrTr	167	ND	PRBC (TSW)	16750	8.8	11.7	1.2	+
127	14	F	B +	Y	N	N	TM	13.7	HTr	173	ND	PRBC (O-TB)	23250	4.8	12.9	1.3	+
128	1.9	F	AB +	Y	N	N	TM	0.8	HyrTr	12	ND	PRBC	700	8.1	12.6	0.8	-
129	21.3	F	AB +	Y	N	N	TI	16.8	HyrTr	212	ND	PRBC	24500	9.5	12.1	0.9	-
130	8.4	F	O +	N	N	N	TM	8.2	HyrTr	111	ND	PRBC	7000	8.5	12	1.1	-
131	4.3	F	A +	Y	Y	N	TM	3.9	HyrTr	35	ND	PRBC	60	8.2	12.7	0.7	-
132	10.8	M	B -	Y	N	N	TM	10.8	HyrTr	171	ND	PRBC	13600	5.3	8.3	1.5	-
133	18.7	M	B +	Y	N	N	TI	18.1	HyrTr	90	ND	PRBC	500	7.4	10	1.5	-
134	8.3	M	O +	Y	N	N	TI	2	HyrTr	34	ND	PRBC	800	8.8	13.5	1.2	-
135	12.8	F	A +	Y	Y	N	TI	5.6	HTr	53	ND	PRBC	2400	6.9	9.8	0.3	-
136	1.6	M	O +	N	N	N	TM	1.1	HyrTr	11	ND	PRBC	60	9.7	13.5	0.8	-
137	9.7	F	O +	N	N	N	TM	8.3	HyrTr	141	ND	PRBC	12000	10.6	13.5	1.1	-
138	21	M	O +	N	N	N	TI	19.3	HTr	254	D	PRBC	43200	7.2	9.6	0.4	+
139	4.6	M	AB +	Y	N	N	TM	4.3	HyrTr	41	ND	PRBC	320	7.1	11.5	3.4	-
140	12.2	M	O +	Y	N	N	TM	11.4	HyrTr	176	ND	PRBC		8.2	11.9	1.5	-
141	14.6	M	B -	N	N	N	TM	13.2	HyrTr	163	D	PRBC	19200	8.7	11.6	0.6	-
142	20.3	M	B +	Y	Y	N	TM	14.9	HyrTr	241	ND	PRBC	27000	9.3	12.6	1.1	-
143	14.5	F	O +	Y	N	N	TM	12.9	HyrTr	183	ND	PRBC	21500	8.6	12.3	1	-
144	8.9	F	O +	Y	N	N	TM	8.6	HyrTr	120	ND	PRBC	9000	8.8	13.5	1.2	-
145	7.8	M	B +	N	N	N	TM	7.4	HyrTr	104	ND	PRBC	6250	9.2	13.5	0.6	-
146	6.4	M	O +	Y	N	N	TI	4.3	HTr	60	ND	PRBC	120	7.2	11.5	0.9	-
147	7.4	M	O -	N	N	N	TM	6.8	HyrTr	84	ND	PRBC	2750	7.5	11.9	1	-
148	16.9	M	A +	N	Y	N	TI	14.1	HTr	29	D	PRBC	2800	7.4	10.3	0.9	-
149	3.7	M	O +	N	N	N	TM	3.3	HyrTr	38	ND	PRBC	800	7.3	11.8	1.2	-
150	8.2	F	O +	Y	Y	N	TM	7.7	HyrTr	105	ND	PRBC	4200	9.2	12.1	1.2	-
151	14.7	F	B +	Y	Y	N	TI	13.1	HTr	83	ND	PRBC	2800	7.5	10.3	1.1	-

152	13.8	F	B +	N	N	N	TM	13.2	HyrTr	189	D	PRBC	24000	8.3	11.9	1	+
153	31.3	M	A +	Y	N	N	TI	25.5	HTr	179	D	PRBC	21750	7.2	8.9	0.2	+
154	9.5	M	O +	Y	N	N	TM	9.3	HyrTr	113	ND	PRBC	8750	8.7	13.1	1.2	-
155	22.3	M	B +	Y	Y	N	TM	6.3	HyrTr	163	ND	PRBC	7750	8.9	11.8	0.9	-
156	4.5	F	A +	Y	N	N	TM	3.9	HyrTr	46	ND	PRBC	40	9.8	13.5	0.9	-
157	16.3	M	A +	Y	N	N	TM	15.1	HTr	219	ND	PRBC	28000	7.2	9.2	1.1	+
158	19.9	F	O +	Y	N	N	TM	19.3	HyrTr	263	D	PRBC	37000	9.8	11.8	0.5	+
159	10.9	M	O +	N	N	N	TI	7.8	HyrTr	109	ND	PRBC	2750	9.1	11.9	1.4	-
160	3.9	M	O +	Y	N	N	TM	3.6	HyrTr	45	ND	PRBC	150	9.1	13.5	0.9	-
161	14.1	F	B +	Y	N	N	TM	14.1	HyrTr	168	D	PRBC	19200	8.6	11.9	0.7	-
162	3.8	M	O +	Y	N	N	TM	3.6	HyrTr	47	ND	PRBC	100	9.3	13.5	0.9	-
163	11.8	F	A +	Y	N	N	TI	8.8	HTr	76	ND	PRBC	3500	4.3	8.1	1.3	-
164	14.1	M	O +	Y	N	N	TM	13.7	HyrTr	157	ND	PRBC	17500	6.3	9.3	0.8	-
165	9.5	F	A +	Y	Y	N	TI	6.1	HyrTr	79	ND	PRBC	250	8.6	12.5	1	-
166	7.2	F	O +	Y	N	N	TM	6.9	HyrTr	81	ND	PRBC	3000	8.5	13.1	1.1	-
167	6.8	M	O +	Y	Y	N	TM	5.6	HyrTr	66	ND	PRBC	300	10	13.5	0.9	-
168	6.8	M	B +	Y	N	N	TM	6.3	HyrTr	76	ND	PRBC	800	7.1	11.8	1.4	-
169	8.3	F	O -	N	Y	N	TM	8.1	HTr	108	ND	PRBC	7500	5.7	9.4	1.3	-
170	9.8	F	O +	N	N	N	TM	8.8	HyrTr	83	ND	PRBC	4000	7.1	13.2	0.5	-
171	11.1	M	AB +	Y	N	N	TM	9.8	HyrTr	122	ND	PRBC	6600	8.8	13.1	1.1	-
172	20.3	M	O +	Y	N	N	TI	166	STr	166	ND	PRBC	13000	8.2	12.2	1.4	-
173	7.4	M	B +	N	N	N	TM	6.8	HTr	79	ND	PRBC (O-TB)	2400	7	11.9	1.1	-
174	19.2	F	B +	Y	N	N	TM	19.1	STr	149	ND	PRBC	16800	9.3	10.7	0.6	-
175	10.8	F	O +	Y	N	N	TM	10.2	HyrTr	154	ND	PRBC	13250	8.1	12.3	1	-
176	12.9	F	O +	Y	Y	N	TM	11.3	HyrTr	157	ND	PRBC (TSW)	15000	9.3	11.7	0.8	-
177	18.8	M	B +	N	N	N	TI	13.3	HTr	189	D	PRBC	31200	6.8	8.5	0.2	+
178	3.4	F	O -	Y	N	N	TM	3	HyrTr	31	ND	PRBC	50	8.9	13.4	0.7	-
179	9.5	M	O +	N	N	N	TM	9.1	HyrTr	116	ND	PRBC	7250	8.8	12.3	1.2	-
180	10.3	M	B -	Y	N	N	TI	7.2	HyrTr	102	D	PRBC	52250	8.9	12.1	0.9	+
181	7.6	M	O +	N	N	N	TM	7	HTr	83	ND	PRBC	3500	9.2	13.5	1	-
182	15.6	M	AB +	Y	N	N	TI	8.5	HTr	131	D	PRBC (O-TB)	7500	8	9.7	0.9	-
183	9.7	F	O +	Y	N	N	TM	8.3	HyrTr	113	ND	PRBC (TSW)	5000	8.4	11.2	1.3	-
184	9.9	M	A -	Y	N	N	TI	6.2	HTr	104	ND	PRBC	2000	7.5	11.54	0.7	-
185	9.5	F	O +	N	N	N	TM	8.6	HyrTr	122	ND	PRBC	8500	8.5	12.5	1.1	-
186	10.8	M	B +	N	Y	N	HT	8.1	HTr	65	ND	PRBC	400	7.8	11.4	1.1	-
187	12.9	M	AB +	Y	N	N	TM	12.7	HyrTr	186	ND	PRBC		8.2	13.5	1.4	-
188	16	F	B +	N	Y	N	HT	13	HyrTr	77	D	PRBC	600	9.1	11.7	1.1	-
189	20.5	M	A +	Y	N	N	TM	20.1	HyrTr	274	D	PRBC	44000	9.2	11.7	1.2	+
190	3.4	M	O +	N	N	N	TM	3.1	HyrTr	35	ND	PRBC	100	10.5	13.5	0.7	-
191	7.9	F	O +	N	N	N	TM	7	HyrTr	74	ND	PRBC	4800	9.8	13.5	0.9	-
192	16.3	M	A +	Y	N	N	TM	15.8	HyrTr	257	ND	PRBC	32000	7	9.9	0.6	+
193	8.1	M	O +	Y	N	N	TM	8	HyrTr	81	ND	PRBC	9000	7.7	12.1	1.6	-
194	10.1	M	O +	N	N	N	TI	7.4	HTr	111	ND	PRBC	4750	6.2	10	1.1	-
195	17.3	M	O +	N	N	N	TI	13.2	HyrTr	177	ND	PRBC	19200	10.8	13.2	0.5	-
196	22.3	M	O +	Y	N	N	TM	22.1	HyrTr	307	D	PRBC	48000	9.9	13.3	0.4	+
197	14.5	M	A +	N	N	N	TM	14	HyrTr	158	D	PRBC	19200	7.3	13	0.1	-
198	17.5	M	A +	N	Y	N	TI	13	HyrTr	194	ND	PRBC	17750	7.5	8.9	1.2	-
199	7.3	M	A -	N	N	N	TM	6.8	HyrTr	79	D	PRBC	10000	9	13.5	1.1	-
200	28.1	M	A +	Y	N	N	TM	27.8	HyrTr	172	ND	PRBC	18250	8.6	11	1.1	+
201	9.5	M	A +	Y	Y	N	TM	8.5	HyrTr	120	ND	PRBC	8000	7.2	10.2	0.4	-
202	5.8	M	O +	N	N	N	TM	5.3	HyrTr	56	ND	PRBC	200	9.1	13.5	1	-
203	8.6	F	O +	Y	N	N	TM	7.4	HyrTr	112	ND	PRBC	8250	7.7	12.7	1	-
204	7.1	M	O +	Y	Y	N	TM	5.4	HyrTr	63	ND	PRBC	400	7.4	12.1	1.3	-
205	5	F	O +	N	N	N	TM	4.8	HyrTr	55	ND	PRBC	500	8.7	13.5	0.9	-