# A Review on Analysis Of Blood Grouping Discrepancies

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**Abstract**

**ABO and Rhesus blood group systems are clinically the most important and relevant for transfusion. A feature of the ABO system is the regular occurrence of anti A and anti B in the absence of the corresponding red cell antigen .A discrepancy exists when results of red cell tests do not complement that of serum tests. Blood Donors and patients must be correctly ABO and Rhgrouped because transfusing ABO in-compatible blood transfusion which may lead even to deathof the patient.**

**Keywords: ABO Grouping , ABO blood discrepancy, Rh grouping**

## 1. Introduction

At the beginning of the 20th century an Austrian scientist, Karl Landsteiner, noted that the RBCs of some individuals were agglutinated by the serum from other individuals. He made a note of thepatterns of agglutination and showed that blood could be divided into groups. This marked the discovery of the first blood group system, ABO, and earned Landsteiner a Nobel Prize.Landsteiner explained that the reactions between the RBCs and serum were related to the presenceof markers (antigens) on the RBCs and antibodies in the serum. Agglutination occurred when theRBC antigens were bound by the antibodies in the serum. He called the antigens A and B, and depending upon which antigen the RBC expressed, blood either belonged to blood group A or blood group B. A third blood group contained RBCs that reacted as if they lacked the properties of A and B, and this group was later called "O" after the German word "Ohne", which means "without". The following year the fourth blood group, AB, was added to the ABO blood group system. These RBCs expressed both A and B antigens. In 1910, scientists proved that the RBCs antigens were inherited, and that the A and B antigens were inherited codominantly over O. There was initially some confusion over how a person's bloodtype was determined, but the puzzle was solved in 1924 by Bernstein's "three allele model". (1)

**Importance Of Resolving Abo Discrepancies**

ABO and Rhesus blood group systems are clinically the most important. Blood Donors and patients must be correctly ABO and Rh grouped because transfusing ABO in-compatible blood may result in transfusion reaction which may even lead to death of the patient (2).A genomic study done on 324 clinical samples involved in ABO discrepancy showed that the number of definable alleles associated with ABO subgroups has increased from 14 to 29 than theirearlier study. (3)Another study on analysis of ABO discrepancies in 35 French hospitals suggests that incidence ofABO discrepancy was 1 per 3400.This figure was 10 times higher than incidence of ABO mismatched transfusion. (4).In reports from Dept of laboratory medicine in a National University, Korea chimerism and mosaicism are found to be important causes of ABO phenotype and genotype discrepancies by studying the STR (Short tandem repeat) loci by DNA-based techniques.(5) In an analysis of ABO discrepancy done by M.H.Kim (6) et al an 8 year study was done and foundan incidence of 82 cases out of 93,800 (0.08%) in Korean population .A study by Beenu Thakral, Karan Saluja, Meenu Bajpai, Ratti Ram Sharma, Neelam Marwaha on Importance of Weak ABO Subgroups in Indian population seventeen weak subgroup discrepancies were found in 86,687 donors units tested, making an overall incidence of 1:5,100 donors or 0.02% (7).In another Indian study by H.M.Bhatia and Malti.S.Sathe on Incidence of 'Bombay' (Oh) Phenotype and Weaker Variants of A and B Antigen in Bombay (India) it was found an incidence of weak A as 1:3,300,Weak B as 1:9,300 and Oh phenotype as 1:7,600 random persons (8).Most of the studies in Indian literature are on the donor population and on the Bombay group. Thisstudy was designed to find the incidence of ABO discrepancies (donors and patients) and to analyse the root causes in order to establish correct measures for resolving the discrepancy whichwill facilitate safe blood transfusion.

### 1.2 **Review of LiteraturesBLOOD GROUP ANTIGENS (9)**

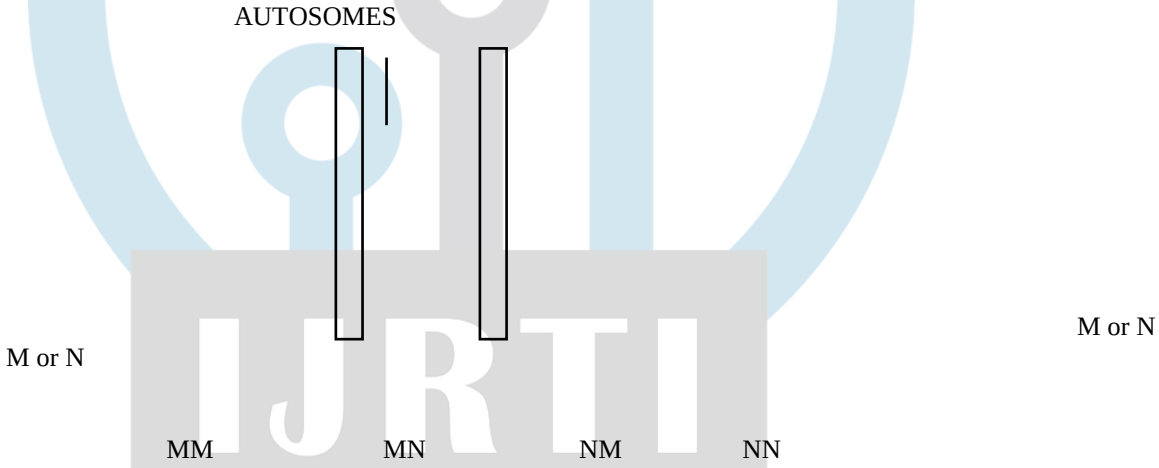
Approximately 280 red cell antigens have been recognized and authenticated by the international society of blood transfusion; of these 245 belong to one of the 29 blood group systems.

TABLE I: MAJOR BLOOD GROUP SYSTEMS

| No: | Name | Symbol | No: of antigens | Genes | Chromosome No: |
|---|---|---|---|---|---|
| 1 | ABO | ABO | 4 | ABO | 9 |
| 2 | MNS | MNS | 43 | GYPA,GYPB,GYPE | 4 |
| 3 | P | PI | 1 | PI | 22 |
| 4 | Rh | RH | 49 | RHD, RHCE | 1 |
| 5 | Lutheran | LU | 20 | LU | 19 |
| 6 | Kell | KEL | 25 | KEL | 7 |
| 7 | Lewis | LE | 6 | FUT3 | 19 |
| 8 | Duffy | FY | 6 | FY | 1 |
| 9 | Kidd | JK | 3 | SLC14A1 | 18 |
| 10 | Diego | DI | 21 | SLC4AE1(AE1) | 17 |
| 11 | Yt | YT | 2 | ACHE | 7 |
| 12 | Xg | XG | 2 | XG,MIC2 | X/Y |
| 13 | Scianna | SC | 5 | SC | 1 |
| 14 | Dombrock | DO | 5 | DO | 12 |
| 15 | Colton | CO | 3 | AQP1 | 7 |
| 16 | Landsteiner-Wiener | LW | 3 | LW | 19 |
| 17 | Chido-Rodgers | CH/RG | 9 | C4A,C4B | 6 |
| 18 | H | H | 1 | FUT1 | 19 |
| 19 | Kx | XK | 1 | XK | X |
| 20 | Gerdich | GE | 8 | GYPC | 2 |

| | | | | | |
|---|---|---|---|---|---|
| 21 | Cromer | CROM | 12 | DAF | 1 |
| 22 | Knops | KN | 8 | CR1 | 1 |
| 23 | Indian | IN | 2 | CD44 | 11 |
| 24 | Ok | OK | 1 | CD147 | 19 |
| 25 | Raph | RAPH | 1 | CD55 | 11 |
| 26 | John Milton Hagen | JMH | 1 | SEMA7A | 15 |
| 27 | I | I | 1 | GCNT2 | 6 |
| 28 | Globoside | GLOB | 1 | B3GALT3 | 3 |
| 29 | Gill | GIL | 1 | AQP3 | 9 |

Each system represents a single gene locus, or two or more very closely linked loci ofhomologous genes.
Most blood groups are inherited as Mendelian characters, although environmentalfactors may occasionally affect blood group expression.




| | | | | |
|---|---|---|---|---|
| Alternative genotype | + | + | + | _ |
| Results from tests with antiserum Anti M | _ | + | + | + |
| Result from tests with antiserum Anti N | _ | + | + | + |

**ABO BLOOD GROUP SYSTEM & Rh SYSTEM:(10)**

Karl Landsteiner in 1900 discovered the first human blood group system, the ABO system. The ABO system still remains the most important of the blood group in transfusion practice He classified human blood into A,B&O groups. A fourth blood group AB was discovered by Landsteiner's associates in 1902.A person's ABO blood group depends on A,B&O gene ( located on chromosome no: 9 ) inherited from each parent.

In late 1930s Landsteiner & Wiener reported an antibody made by guinea pigs and rabbits when transfused with Rhesus on key red cells. These antigens on Rhesus monkeys were named as "Rh". **(2)**

**INHERITANCE OF ABO BLOOD GROUP:**

The theory for inheritance of ABO blood groups was first described by Bernstein in 1924.He demonstrated that each individual inherits one ABO gene from each parent and these two genes determine which ABO antigen is present on the red cell membrane. The ABO blood group antigens are encoded by one genetic locus, the ABO locus, whichhas three alternative (allelic) forms—A, B, and O. A child receives one of the three alleles from each parent, giving rise to six possible genotypes and four possible blood types (phenotypes).

FIG: 1 INHERITANCE PATTERN OF ABO GENE

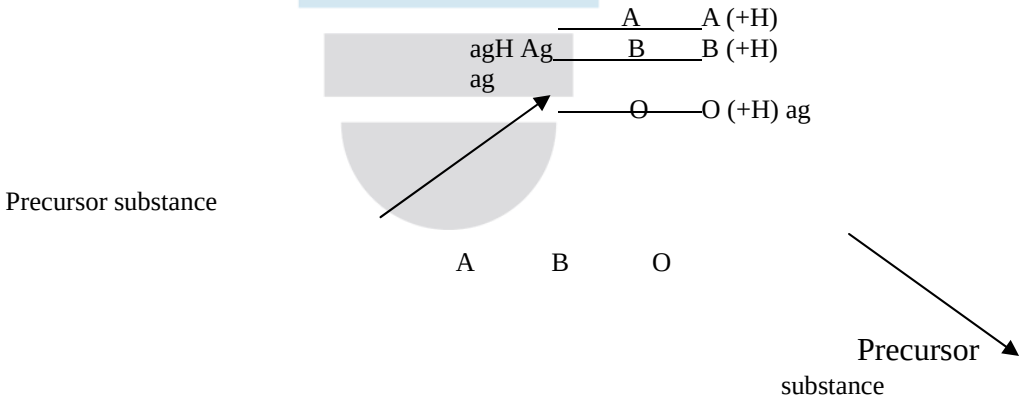
| ABO genotype in the offspring | | ABO alleles inherited from the mother | | |
|---|---|---|---|---|
| | | A | B | O |
| ABO alleles inherited from the father | A | A | AB | A |
| | B | AB | B | B |
| | O | A | B | O |

In ABO blood group system it seems at least two different genes contribute to the final antigen onred cells. **(2)**

→ 1st gene H gene controls changes of a precursor substance of H antigen

→ 2nd group of genes ABO genes control the further conversion of H antigenIn this way A & B antigens with a little "H" antigen can be produced.

"O" gene being an amorph leads to no change in H antigen.




Genes control the formation of specific enzymes responsible, which help to add up a carbohydrate structure on precursor substances with regard to basic ABO system rules can be summarized as follows.

**Rule 1**: factor A or B cannot be present in the blood of a child unless present in the blood of oneor both parents

**Rule 2**: a parent of group AB must transmit to the child either A or B and cannot be a parent of achild of group O.
**Rule 3**: a person of group O cannot be the parent of a child of group AB.
**Rule 4**: the agglutination A1 or A2 cannot appear in the child unless it is present in at least one ofthe parents.

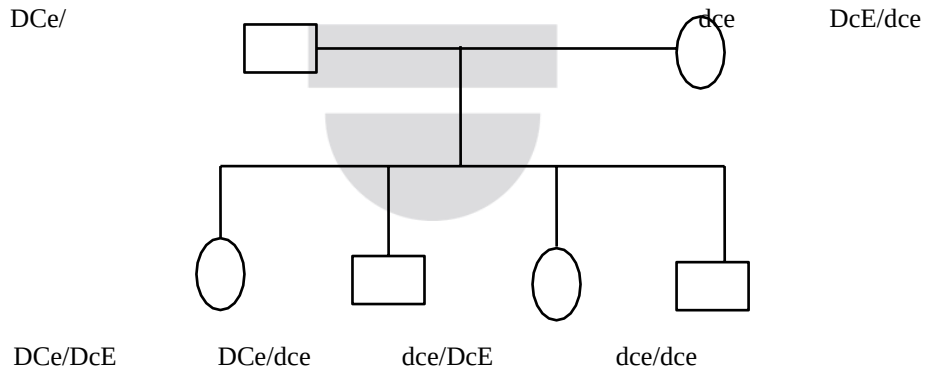
**TABLE II: Inheritance pattern of basic ABO blood group(11)**

| If parents are phenotype | phenotype possiblein children | phenotype not possiblein children |
|---|---|---|
| O&O | O | A,B,&AB |
| O&A | A,O | B,AB |
| O&B | B,O | A,AB |
| A&A | A,O | B,AB |
| A&B | B,O | None |
| B&B | AB,O,A,B | A&AB |
| O & AB | B ,O | O , AB |
| A & AB | A ,B | O |
| B & AB | A ,AB, A | O |
| AB & AB | A,B,AB | O |

INHERITANCE OF Rh BLOOD GROUP SYSTEM:

The phenotype of a given red cell is defined by the presence or absence of D, C, c, E, e, Fisher &race proposed that Rh locus contain 3 distant genes that control production of respective antigen.It is currently accepted that these are only two closely linked genes that control the expression ofRh.1 $^{st}$ gene codes for D and2 $^{nd}$ gene codes for CcEeLocus 1 contains one of the two alleles D or d. d may result from absence of D allele or may be anallele that is in an inactive gene.Loci 2 contains one of four alleles Ce, cE.ce or CE.It has been demonstrated through linkage studies that Rh locus is located on chromosomal Rh antigens are linked as codominant alleles offspring inherit one Rh halotype from each parent

FIG:2 INHERITANCE PATTERN OF RH GENE




***c. THE ABO LOCUS***

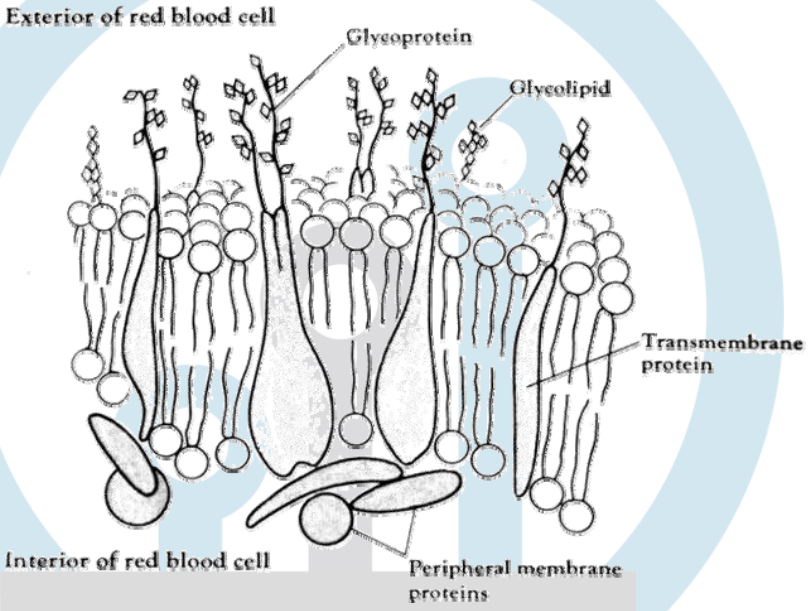
The ABOEntrez GeneMapViewer locus is located on chromosome 9 at 9q34.1-q34.2. It contains 7exons that span more than 18 kb of genomic DNA. Exon 7 is the largest and contains most of the coding sequence. Exon 6 contains the deletion that is found in most O alleles and results in a loss of enzymatic activity.The A and B alleles differ from each other by seven nucleotide

substitutions, four of which translate into different amino acids in the gene product (R176G, G235S, L266M, and G268A).The residues at positions 266 and 268 determine the A or B specificity of the glycosyl transferase they encode. The O allele differs from the A allele by deletion of guanine at position 261. The deletion causes a frame shift and results in translation of an almost entirely different protein that lacks enzymaticactivity(12).There are many variant ABO alleles that encode a number of variant ABO phenotypes, but theydo not encode specific antigens other than the A and B antigens. For example, weak A subgroups, such as $A_3$, $A_x$, and $A_{el}$, express the A antigen, and weak B subgroups, such as $B_3$ and $B_x$, express the B antigen.(13)

***BIOCHEMISTRY OF ABO ANTIGENS***

The ABO genes do not actually code for the production of ABO antigen. It produces specific glycosyl ransferases that add sugar to a basic precursor substance.The action of H gene is intimately related to the formation of ABO antigen .The inheritance of H gene is independent of inheritance of ABO genes, but A,B and H antigens are all formed from the same basic precursor material, which is itself a genetic product.

FIG:3 RED CELL MEMBRANE

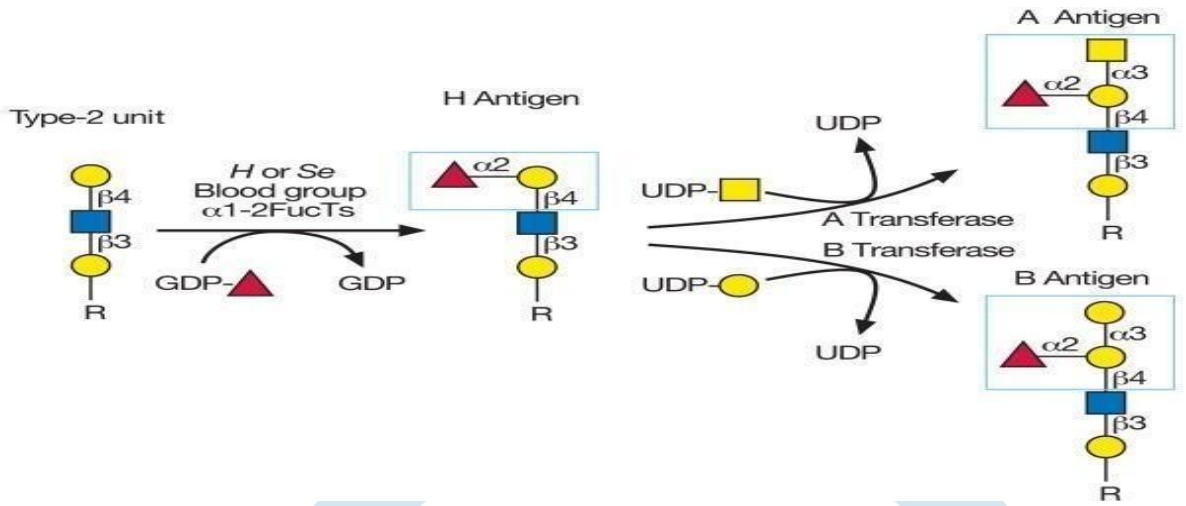



The basic material has a glycoprotein or glycolipid backbone (depending on whether an ABH red cell antigen or soluble substance is being produced) to which sugars are attached in response to specific enzyme transferases elicited by an inherited gene. The ABH glycolipid antigens are built on a common carbohydrate residue which represents a paragloboside.

INTERACTION OF Hh AND ABO GENES

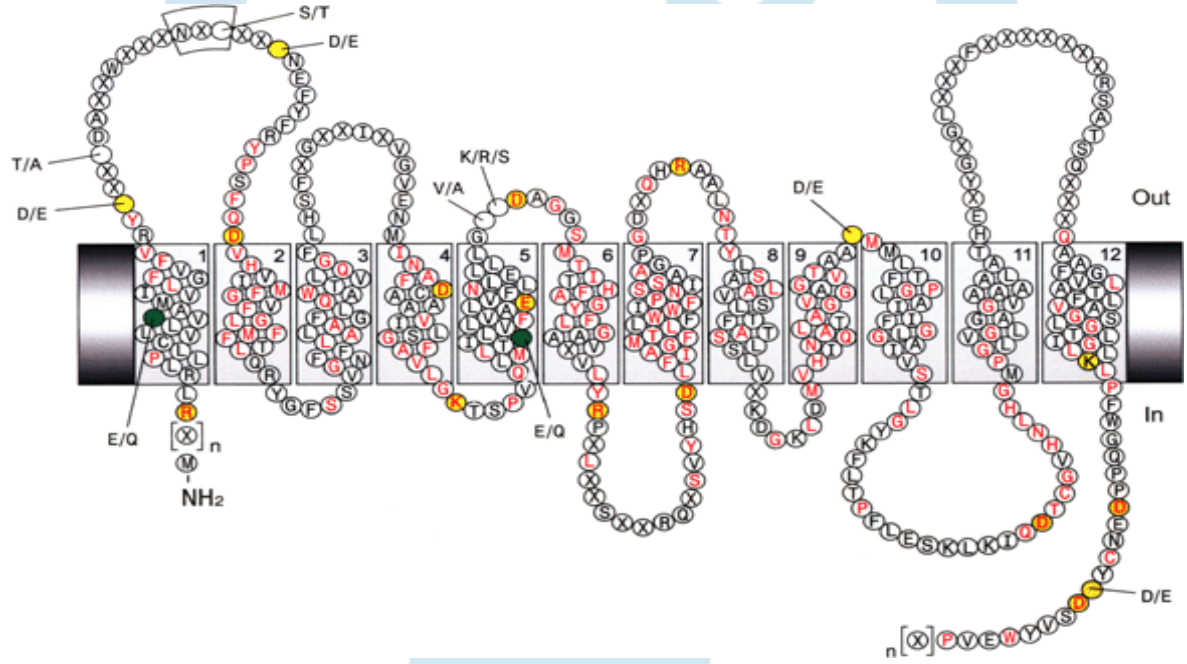
Inheritance of H gene elicit an enzyme L fucosyltransferase ,which transfers sugar L fucose from guanosine –diphosphate –L-fucose (gdp-fuc)donor nucleotide to terminal galactose of the precursorchain.The H gene is very common in random populations, with greater than 99.99%inheriting the H gene.The allele of H, h is quite rare and genotype hh is extremely rare. This hh genotype is called ***Bombayphenotype*** and lacks normal expression of ABO genes. Therefore H substance is formed first and then only other sugars attach to this depending on ABO genes inherited. The sugars that occupy the terminal position of this precursor chain and confer blood group specificity are called immunodominant sugars.The A gene codes for the production of N-acetyl galactos amyl transferase which transfers an N- acetyl galactosamine (Gal NAc) sugar from uridine – diphosphate –N-acetyl – D-galactose (UDP GaLNAc) donor nucleotide to H substance. This sugar is responsible for A specificity. A specific immunodominant sugar is linked to a type 2 chain glycolipid precursor that now contains H substance through action of H gene.The B gene codes for production of D galactosyltransferase which transfers a D galactose (Gal)sugar from uridine diphosphate galactose (UDP-Gal)donor nucleotide to H substance. This sugar is responsible for B specificity.When A and B genes are both inherited the B enzyme (D galactosyl transferase) seems to compete more efficiently for H structure. Therefore the average number of A antigen on AB adult cells is less compared to B antigen sites.The O gene is an amorph that does not elicit a transferase and therefore adds no additional sugar to the H structure. As a result the O blood group has the highest concentration of H antigen.

FIG:4 FORMATION OF H ANTIGEN




***2.2..e. BIOCHEMISTRY OF RH ANTIGEN***

FIG:5 STRUCTURE OF Rh ANTIGEN




" " denote ,where the sequence of D diverges from C/c or E/eThe antigen specificity appears to depend on cofactors or specific conformation.

Requirements that are achieved only if the protein is embedded in the cell membrane bilayer in aspecific configuration .

The Rh antigen is a transmembrane polypeptide and is an integral part of the red cell membrane.Agre and carton have shown that D associated protein is linked to membrane skeleton.(14,15)

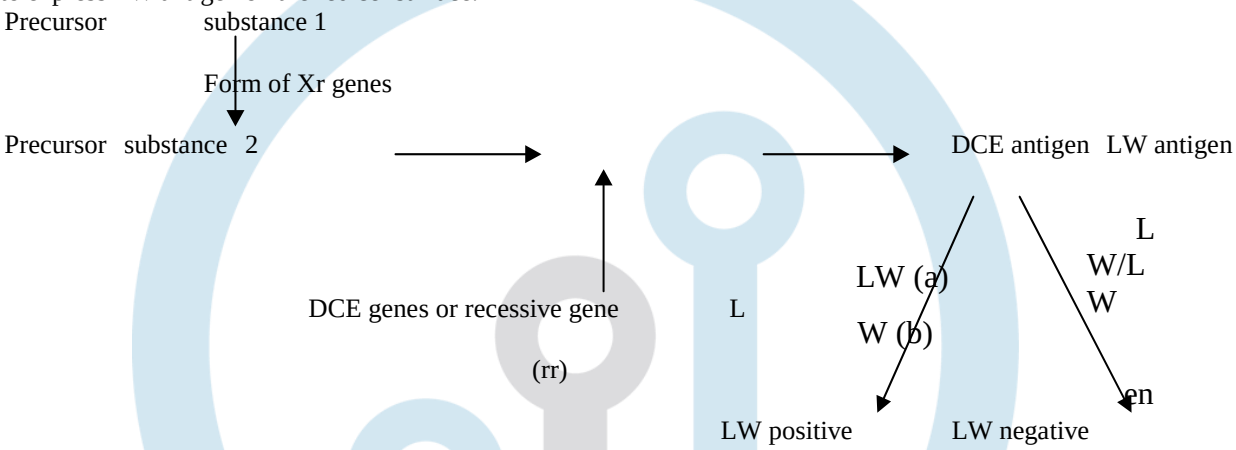
Many theories have been proposed to explain genetically the result of serologic and biochemicalstudies in the Rh system. Theories include: 6+

1. Wiener theory
2. Fisher and Race theory
3. Giblett's modification of Race's Rh genetic pathway

Gilblett's modification says precursor 1 is acted upon by one of several genes at Xr locus. Under normal circumstances precursor 1 is converted to precursor 2 in presence of X'r gene. In the presence of the DCE gene, precursor 2 is converted to the DCE antigen. The LW gene then acts toexpress the LW antigen. When Rh null suppressor genes ($X^0r$ $X^0r$) are present, the Rh and LW genes are not expressed on the red cell.

LW ANTIGEN

The antibody produced by injecting rhesus monkey red cells into guinea pigs and rabbits were identified as having the same specificity as the antibody Levine and Stetson described. This antibody is named anti LW.Phenotypically there is a similarity between the Rh and LW system. Anti LW reacts strongly with most D positive red cells, weakly with Rh negative red cells and never react with Rh null cells.There are 3 alleles at LW locus.$LW^a$, LWb and LW (a silent allele). Persons lacking LW antigen altogether are LW/LW and express no LW on red cells. LW $^a$ is very common and LW $^b$ less common .Under normal circumstances precursor substance is acted on by Rh genes to produce normal Rh antigens. The LW genes then exert their influence to express LW antigen on the red cellsurface.




When Rh null genes ($X^oR$ $X^oR$) are present, the Rh and LW genes are not expressed on red cells.

***2.2.f . SUBGROUPS OF A, B, O AND AB***

A and AB have been divided into subgroups $A_1$, $A_2$, $A_1B$ and $A_2B$ depending upon the reaction withanti A lectin, the extract from the seed of lectin dolichos biflorus or human anti $A_1$ serum. Anti A sera very seldom differentiate between $A_1$ and $A_2$ cells .Anti $A_1$ agglutinate $A_1$ and $A_1B$ but not $A_2$

A SUBGROUPS:(16)

Serum from group B individuals contain a mixture of two antibodies ,Anti A and Anti A1,which can be separated by absorption techniques using appropriate red cells. Group A red cells that react with anti-A only and not with anti-A1 are classified as A2 subgroups. Group A red cells that react with both anti-A and anti-A1 are classified as A1.

TABLE III: REACTIONS O "A" SUBGROUPS

| | REACTION OF PATIENT'S RED CELLS WITH | |
|---|---|---|
| BLOOD GROUP | Anti –A (from B sera) | Anti-A1 lectin |
| A1 | + | + |
| A2 | + | NEGATIVE |

B SUBGROUPS:

The subgroups of B are even more infrequent than the weaker subgroups of:

They are initially identified by variability of reaction with anti B and anti A, B. The subgroups $B_3$, Bx, Bm and $B_e$ are classified similarly to their counterparts in the classification of the A subgroups. **(10)**

TABLE IV: REACTIONS OF "B" SUBGROUPS

| | ANTI A | ANTIB | ANTI A,B | ANTI H |
|---|---|---|---|---|
| B | □ | ++++ | ++++ | ++ |
| BB3 | □ | ++ (mf) | ++ (mf) | +++ |
| BBX | □ | Weak/□ | Weak/+ | +++ |
| BBm | □ | □ | □ | +++ - ++++ |
| BBe1 | □ | □ | □ | +++ - ++++ |

BOMBAY GROUP:(16)

The H gene is supposed to be necessary for the formation of A and B antigens. The H gene is very common in random populations, with greater than 99.99%inheriting the H gene. The allele of H, his quite rare and genotype hh is extremely rare. This hh genotype is called Bombay phenotype and lacks normal expression of ABO genes. The Bombay phenotype was first reported by Blende in 1952 in Bombay, India. More than 130 Bombay phenotypes have now been reported in various parts of the world. General characters of Bombay $0_H$ ($H_{null}$) phenotypes:

- Absence of H, A and B antigens; no agglutination with anti $A_1$, anti B, antiA, B or anti H.
- Presence of anti A, anti B, anti A, B and a potent wide thermal range anti H in the serum.
- A, B, H nonsecretor.
- Absence of α-2-L-Fucosyltransferase (H enzyme) in serum and red cells.
- Presence of A or B enzymes in serum and red cells.
- Strong reactivity with anti I reagents(due to increase in no: of I receptors)
- recessive mode of inheritance.

**ANTIBODIES OF RED CELL ANTIGENS (17)**

Antibodies to red cell antigen vary widely in their characteristics and invivo significance .Red cell antibodies can be categorized based on their immunoglobulin class ; the antigen to which they are directed ,the method of stimulation, their optimal temperature of reaction invitro, whether they fixcomplement ,their action on red cells in vitro and their in vivo effects such as they cause hemolysis, transfusion reaction or HDN.

TABLE V: SOME MAJOR RED CELL ANTIGEN SYSTEM AND GENERAL CHARACTERISTICS OF THEIR CORRESPONDING ANTIBODIES.

| ANTIGEN SYSTEM | NO: OF ANTIGEN S | USUAL ANTIBODY | FIX COMPLEMENT | OPTIMAL REACTIVITY TEMPERATURE | TRANSFUSION REACTION | HDN | RED CELL IMMUNITY |
|---|---|---|---|---|---|---|---|
| ABH | 11 | IgM/IgG | YES | COLD | YES | YES | NO |
| P | 5 | IgM | NO | COLD | NO | NO | YES |
| Rh | 41 | IgG | NO | WARM | YES | YES | YES |
| LW | 3 | IgG | NO | WARM | YES | YES | YES |
| DUFFY | 5 | IgG | NO | WARM | YES | YES | YES |
| KELL | 21 | IgG | NO | WARM | YES | YES | YES |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| KIDD | 3 | IgG | NO | WARM | YES | YES | NO |
| MN | 46 | IgG/IgM | NO | COLD | NO | YES | YES |
| Ss | 46 | IgG/IgM | NO | WARM | YES | YES | YES |
| Lutheran | 7 | IgM | NO | WARM | YES | YES | YES |

ANTI A AND ANTI B

A feature of the ABO system is the regular occurrence of anti A and anti B in the absence of thecorresponding red cell antigen.

TABLE VI: REACTIONS OF ANTI A AND B

| BLOOD GROUP | SUB GROUP | ANTIGEN ON RED CELL | ANTIBODY IN PLASMA |
|---|---|---|---|
| A | $A_1$ | $A+A_1$ | Anti B |
| | $A_2$ | A | Anti $A_1$* |
| B | – | B | Anti A , Anti $A_1$ |
| AB | $A_1B$ | $A+A_1+B$ | None |
| | $A_2B$ | A+B | Anti $A_1$* |
| O | – | H*** | Anti A,Anti $A_1$,Anti B,Anti $A_1B$** |

*Anti $A_1$ found in 1-2% of $A_2$ subjects and 25-30% of $A_2B$ subjects

** cross reactivity with both A and B cells.

**The amount of H antigen is influenced by the ABO group ,O cells contain mostH and $A_1B$ cells at least .Anti H may be found in occasional $A_1$ and $A_1B$ subjects.

The antibodies are a potential cause of dangerous hemolytic reaction if transfusion is given withoutregard to ABO compatibility .Anti A and Anti B are always ,to some extent ,naturally occurring and of IgM class.Although they react best at low temperatures, they are nevertheless potentially hemolytic at $37^0$c.Hyper immune Anti A and Anti B occur less frequently usually in response to transfusion or pregnancy ,early following injections of some toxoids and vaccines.They are predominantly of IgGclass and usually produced by group O and sometimes by group $A_2$ individuals .these antibodies react over a wide thermal range and are more effectively hemolyse than naturally occurring antibodies

ANTI $A_1$ AND ANTI H

An antibody reacting only with $A_1$ and $A_1$b cells called anti$A_1$ is occasionally found in the serum of group $A_2$subjects (1-2%) and not uncommonly in group $A_2B$ subjects (25-30%) .An antibody reacting most strongly with O and $A_2$ cells ,probably best referred to as Anti H ,is sometimes found in the serum of group $A_1$,$A_1B$ or B subjects.These two antibodies normally act as cold agglutinins and rarely react with appropriate red cell antigens at temperature over $30^0$c .They seldom cause hemolytic reaction invivo ,but may be a source of confusion in room temperature compatibility tests. A rare exception is anti H that occursin Bombay phenotype Oh, which is IgM antibody and causes lysis at $37^0$c.

ANTI Rh

Antibodies acting against all Rh antigens, except ‘d’ have been described namely Anti D, Anti C, Anti E, Anti e.Rh antigen are restricted to red cells and Rh antibodies are due to alloimmunization by previous transfusion or pregnancy ,except for some naturally occurring forms of anti E, they are usually IgG (sometimes with an IgM component) react best at $37^o$c,do not fix complement .Hemolysis ,when itoccurs ,is therefore extracellular and predominantly in the spleen.Anti D is the most important clinically ;it may cause hemolytic transfusion reaction and was a common cause of foetal death resulting from HDN before the introduction of anti D prophylaxis in 1970.The other Rh antibodies, although much less common ,may nevertheless cause

hemolytic transfusion reaction and HDN.

**POLYAGGLUTINABLE RED CELLS (18)**

Erythrocyte polyagglutination is the agglutination of red cells irrespective of blood group by manysera from normal adults. polyagglutinable red cells are not agglutinable by the patient's own serum.

There are 2 main categories of polyagglutination.

- Acquired
- Inherited

— Non microbial, due to somatic mutation There are 3 types of inherited polyagglutination

✓T activation- in hemolytic anemia, hemolytic transfusion reaction (*especially inchildren*), HUS, neonatal necrotizing enterocolitis.

✓Tk activation – associated with infections (*Clostridia, Candida albicans*)

✓ Tn activation – is a persistent abnormality by an abnormal clone of stem cells arising by somatic mutation. It is often associated with hematological abnormalities like chronic hemolytic anemia, leucopenia ,or thrombocytopenia., but may be present in healthy individuals.

## 2. Materials And Methods

### 2.1. MATERIALS

An analysis of ABO discrepancies was done on patients and donors samples during the period from January2019 to December 2019 at Indira Gandhi Technological And Medical Sciences University Paramedical Sciences college OPD & Gyati Taka General Hospital blood bank, Ziro. Forward and reverse grouping wereperformed on blood samples from inpatients, outpatients and donors. The root causes were analysed with clinical details to group the discrepancies and resolve them with suitable steps.

**METHODS**

INCLUSION CRITERIA :All the patient and donor samples, with EDTA or citrate anticoagulated blood for forward grouping and clotted blood samples for reverse grouping.

EXCLUSION CRITERIA

-Hemolyzed samples

-clotted samples of newborn upto 3 months of age for reverse grouping

PROFORMA:

IN DONORS:

Regulations of AABB and FDA require that two different tests be used to determine the ABO type of each donor unit .The detection of the antigens present on the red cells is commonly called a “forward or front type” and requires the use of anti A and anti B sera (the use of anti A, B sera is optional) for ABO typing and anti D for Rh Typing. The detection of the antibodies present in the plasma or serum is commonly called the “reverse or back” type .Reagent A1 cells, O cells and B cells are required, A2 cells are optional.

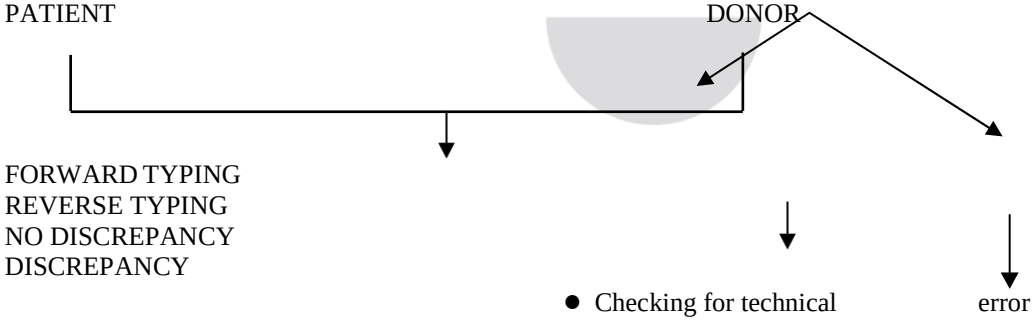
For donors the following procedures are done:

- Donor screening (selection of donors)
- Forward typing by slide method.
- Reverse typing from the pilot sample of the donor collected during bleeding.(serum)

Forward and reverse typing of donors are matched and if any discrepancy is found in forward typing, it isconfirmed with the tube method. If discrepancy is in reverse typing suitable steps are taken to resolve it.

IN PATIENTS:

Forward and reverse grouping of patients were done with an EDTA and clotted sample respectively. Ifforward shows a specific reaction with polyclonal antibodies anti A , anti B, anti D, reverse typing withpooled A,B and O cells should correlate with it.




**Resolving group 1 discrepancies:**

- Incubate the patient serum + reagent cells at room temperature for 15 minutes.
- Incubate the serum-cell mixtures at $4^0$c for 15 – 30 minutes
- An auto control and an o cell control for naturally occurring cold auto agglutinins.

**Resolving group 11 discrepancies:**

- Using lectins for subgroups of A and O.
- Acidifying the anti B typing reagent to pH 6.0 differentiate acquired B antigen. The acidified anti Bantisera agglutinate only true B antigen.
- Use Anti –B of different lot numbers for a low incidence antibody in the reagent antisera which reacts with a low incidence antigen on the patient's cells. The same can be used for a weak reacting Atyping.

**Resolving group 111 discrepancies:**

- Washing the red cells with saline.
- Wash the cord cells 6-8 times to remove viscous mucopolysaccharide that causes rouleaux.

**Resolving group IV discrepancies:**

Polyagglutination is suspected then lectin studies performed

The patient's cells were washed with 370c saline three times, and then retyped for cold autoantibody. Removing the bound immunoglobulin by treating the cells with chloroquine diphosphate (it removes thebound Ig )and cells can be retested.

**TECHNIQUE:**

ABO grouping was performed by 2 techniques.

1. Direct/ Forward/ Cell grouping.
2. Reverse / Serum Grouping.

Each of the methods can be done in 2 ways.

i) Slide Method

ii) The Tube Method was adopted for the present study.

**FORWARD GROUPING AND Rh GROUPING:**

Patients or donors RBC were tested for detecting Ag on them by known antisera.

**Reagents used:**

- Anti A – 1g M/1gG polyclonal Ab (ABO grouping)
- Anti B – 1g M/1gG polyclonal Ab (ABO grouping)
- Anti D – 1g M Monoclonal Ab (Rh grouping)

**Tube Method :**

1. Take 3 test tubes and mark A, B and O
2. Add 2 drops of patient's or donor's sera to each tube.
3. Add 2 drops of 2-5% suspension of pooled A, B and O cells to corresponding tubes.
4. Tubes are shaken and centrifuged at 1500 rpm for 1 minute.
5. Read the agglutination both macroscopically and microscopically.

**TABLE I: INTERPRETATION**

| Patient No | Forward Grouping | | | Reverse Grouping | | | Blood Group |
|---|---|---|---|---|---|---|---|
| | Anti A | Anti B | Anti D | A | B | O | |
| 1 | +++ | - | +++ | - | +++ | - | A positive |
| 2 | - | +++ | +++ | +++ | - | - | B Positive |
| 3 | +++ | +++ | +++ | - | - | - | AB Positive |
| 4 | - | - | +++ | +++ | +++ | - | O Positive |

## 3. Results

**GENERAL CONSIDERATION:**

In the present study on ABO discrepancies 32,435 (patients and donors) who satisfied the inclusion criteriawere included and ABO typed. There were 119 (0.36%) discrepancies observed.- 21 were donors and 98 were patients.

**IN DONORS:**

Forward and reverse typing of donors were done and those which show discrepancies were resolved bysuitable measures. Out of total donors of 13984 during January 1st to December 31st 21 (0.15%) discrepancies were noted (table II)

**Table II: Donor details**

| MONTH | NO: OF DONORS | DISCREPANCIES NOTED |
|---|---|---|
| JANUARY | 1292 | 1 |
| FEBRUARY | 1181 | 3 |
| MARCH | 1076 | 0 |
| APRIL | 1122 | 2 |
| MAY | 996 | 3 |
| JUNE | 1377 | 0 |
| JULY | 1101 | 2 |
| AUGUST | 1028 | 2 |
| SEPTEMBER | 1101 | 2 |
| OCTOBER | 1351 | 3 |
| NOVEMBER | 1318 | 1 |
| DECEMBER | 1039 | 2 |
| TOTAL | 13984 | 21 |

In donor population the incidence of discrepancies were found to be 21 out of 13984 (0.15%).Out of the 21discrepancies there were only males of which maximum number of donors were in an age group of 21-30 years (12 cases)There were no donors in an age group of >51 years with discrepancy . For an age group 31-40 there were 4 cases and between 15-20 years there were 2 cases. (fig.1)

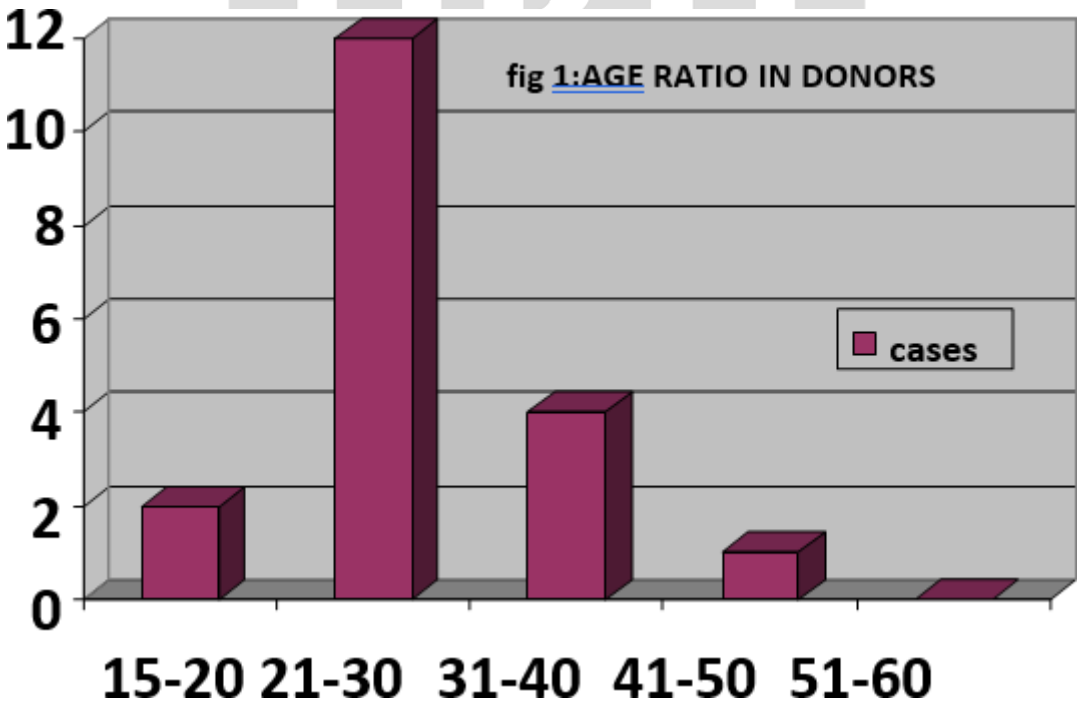
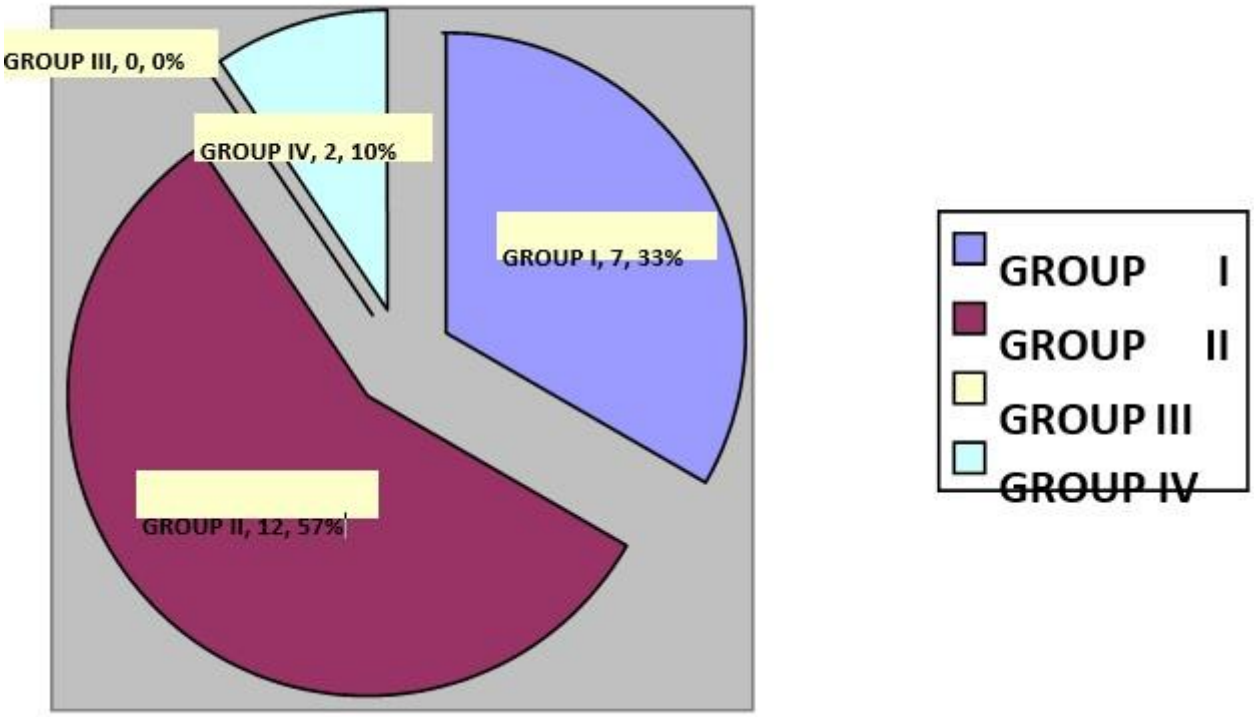



Fig 1: AGE RATIO IN DONORS

In donor population the incidence of discrepancies were found to be 21 out of 13984 (0.15%).Out of the 21 discrepancies there were only males of which maximum number of donors were in an age group of 21-30 years (12 cases)There were no donors in an age group of >51 years with discrepancy . For an age group 31-40 there were 4 cases and between 15-20 years there were 2 cases. (fig.1)

FIG 2:DISCREPANCIES IN DONORS




Among the 21 cases, on grouping them based on the type of discrepancies 12 out of 21 cases were found to be the major category (57%) .Group 2 comprises 33% of total (7 out of 21) and group 4 only 10% (2 out of 21). No discrepancy came to group 3 category in
donors. (fig 2)

TABLE : III CAUSES OF DISCREPANCIES IN DONORS

| Sl no: | GROUPS | CAUSES | NO: OF DISCREPANCY |
|---|---|---|---|
| 1 | Group 1 | Weak expression of Rh Antigen | 6 |
| | | Weak expression of B Antigen | 1 |
| 2 | Group 2 | Bombay group | 3 |
| | | A2B | 1 |
| | | Weak expression of Antibody | 8 |
| 3 | Group 3 | | nil |
| 4 | Group 4 | ICT +ve | 2 |

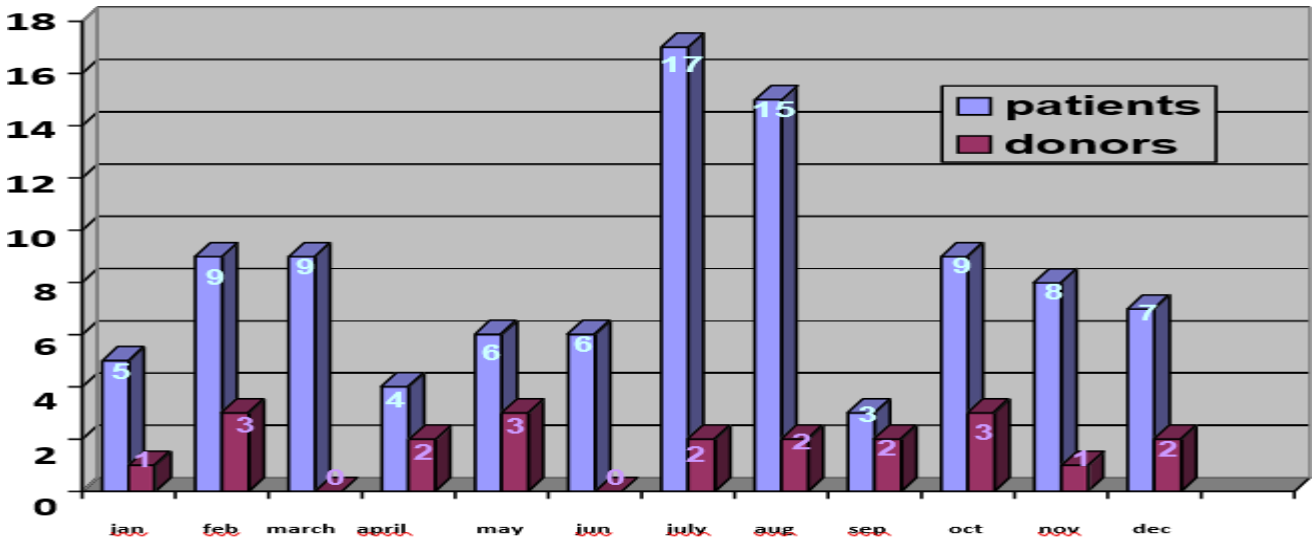



FIG 3: YEARLY ANALYSIS OF PATIENTS.

| | | | |
|---|---|---|---|
| | | TOTAL | 21 |

**IN PATIENTS:**

Forward and reverse typing of both in-patients and out-patients were done and those which showed discrepancies were resolved by suitable measures. Out of 18451 patients 98 ( 0.53%) patients showeddiscrepancy.(table IV)

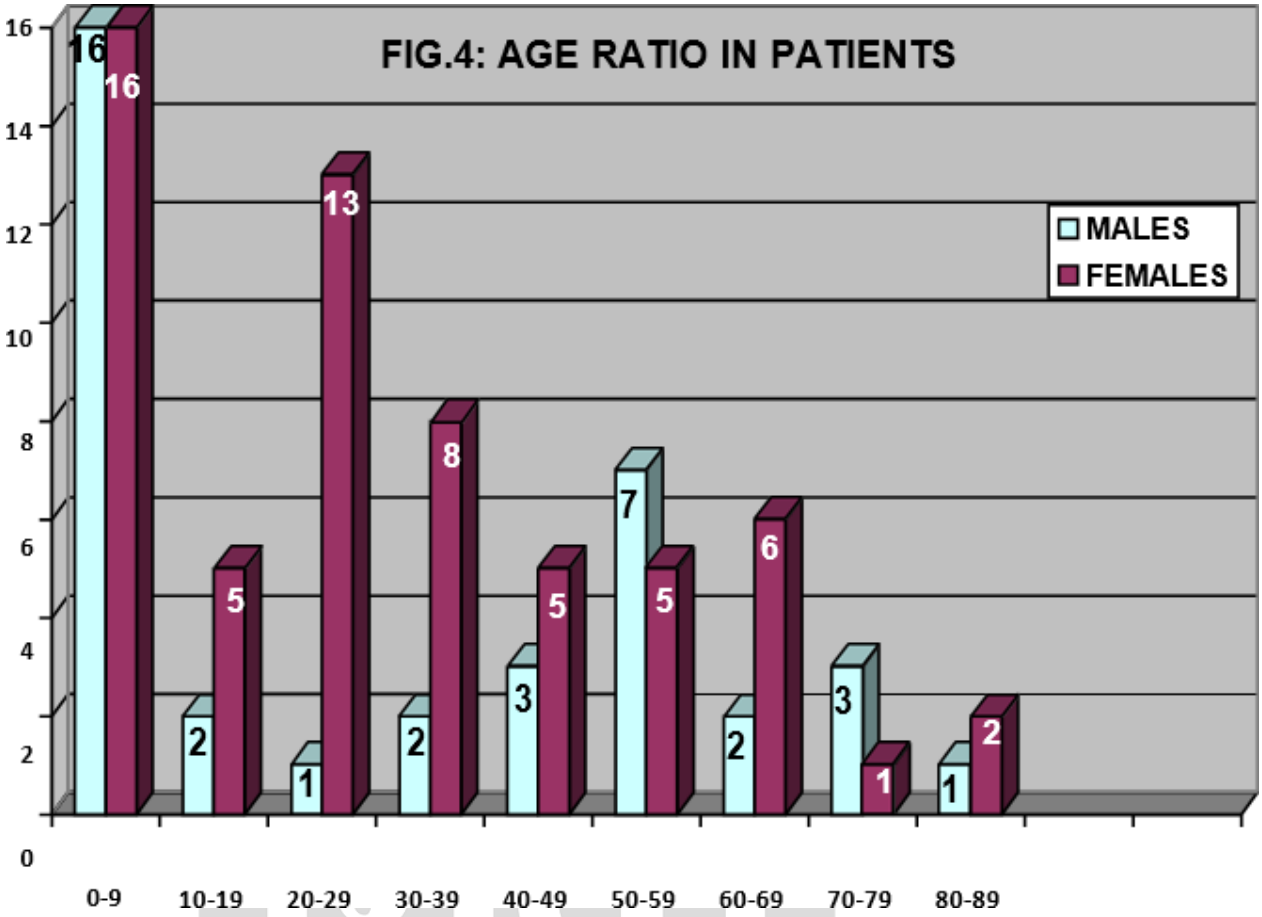
TABLE IV : PATIENT DETAILS

| MONTH | NO: OF PATIENTS | DISCREPANCIES NOTED |
|---|---|---|
| JANUARY | 1482 | 5 |
| FEBRUARY | 1364 | 9 |
| MARCH | 1543 | 9 |
| APRIL | 1446 | 4 |
| MAY | 1508 | 6 |
| JUNE | 1711 | 6 |
| JULY | 1688 | 17 |
| AUGUST | 1598 | 15 |
| SEPTEMBER | 1539 | 3 |
| OCTOBER | 1533 | 9 |
| NOVEMBER | 1593 | 8 |
| DECEMBER | 1446 | 7 |
| TOTAL | 18451 | 98 |

During the period of study maximum discrepancies were obtained in the month of July (17 out of 98).On the same month ,among donors there were 2 discrepancies ,so a total of 19 (15% of the total discrepancies ) wereobtained in July. (fig 3). Least number of discrepancies were in September (12% of 98).

Among patients age ratio was calculated and discrepancies were found to be more in females (52%) than inmales (48%).(fig 3)Among the 18451 patients, age group distribution of discrepancy was calculated and found to bemore in the age group of 0-9 years i.e. 32 out of 98 (32.6%).This is mainly due to absence/reduceddevelopment of antibodies in infants >3 months old. The interesting observation is that in the age group 0-9 there are equal numbers of both genders. When coming to the age group 21-30 there is a female predominance (13 out of 14 cases).The condition persists in most age groups except 50-59 yrs and 70-79 yrs in which there is a predominance of males.

Increase in the no: of discrepancies in females in an age group 21-39 is clinically significant. (FIG: 5)




On grouping the discrepancies based on their causes 98 cases were grouped and found that:

- Technical Error – 3 errors
- Group 1- 22 cases
- Group 2 -25 cases
- Group 3 -5 cases
- Group 4 -43 cases.

In this group 4 discrepancies were found to be most frequent which included AIHA, ICT , DCT + ves cases n which atypical antibodies may be the probable reason. Group 3 were found to be least (5 out of 98 cases).These occur mainly due to problems related to plasma proteins, like multiple myeloma. Among 5cases 1 was a case of multiple myeloma and rest were due to rouleaux formation due to plasma protein abnormalities. (FIG :5)

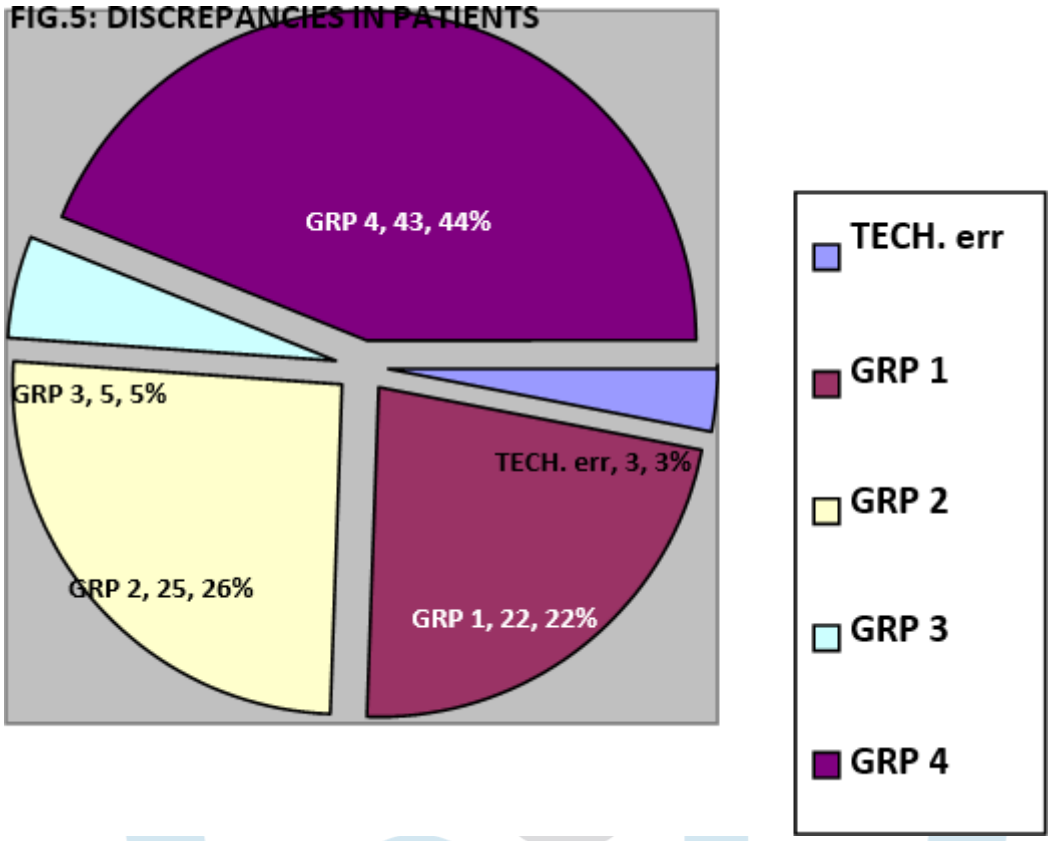



TABLE V : COMPARISON OF CAUSES OF DISCREPANCIES IN PATIENTS

| CASES | CAUSES | No: of patients with discrepancy | No: of donors with discrepancy |
|---|---|---|---|
| TECHNICAL ERROR | | 3 | |
| GROUP 1 | NEONATES | 20 | |
| | WEAK EXPRESSION OF ANTIGEN | 2 | 7 |
| GROUP 2 | SUBGROUPS | 10 | 4 |
| | WEAK EXPRESSION OF ANTIBODY | 1 | 8 |
| | INFECTION | 10 | |
| | MALIGNANCY | 4 | |

| | | | |
|---|---|---|---|
| GROUP 3 | MULTIPLE MYELOMA | 1 | |
| | ABNORMAL PLASMA PROTEINS | 4 | |
| GROUP 4 | AIHA | 28 | |
| | OTHER DCT,ICT + ves | 9 | 2 |
| | ITP | 2 | |
| | PREGNANCY | 4 | |
| | TOTAL | 98 | 21 |

## 4. Discussion

**INCIDENCE OF ABO DISCREPANCIES:**

This study was designed to determine the incidence and causes of all ABO discrepancies detected in IndiraGandhi echnological And Medical Sciences University Paramedical Sciences college OPD & Gyati Taka General Hospital blood bank, Ziro. Blood Donors and patients must be correctly ABO and Rh grouped because transfusing ABO in-compatible blood may result in transfusion reaction which may even lead to death of the patient [(1).] As a result of a study by Olsson et al, the number of definable alleles associated with weak ABO subgroups has increased from 14 to 29.[(2)] Linden and associates reported that incidence of ABO mismatched transfusion was 1 per 33,000 [(3)]. In the present study out of 32,435 (patients and donors) who satisfied the inclusion criteria there were 119 (0.36%) discrepancies. In the donor population the incidence is21 out of 13984 (0.15%). Out of 18451 patients 98 ( 0.53%) patients showed discrepancy.The higher incidence and the presence of clerical errors indicate the need for awareness studies and strictcompliance to standard guidelines.

## 5. Conclusions

The study on analysis of ABO discrepancies showed the incidence in the donor population as 21 out of 13984 (0.15%) and 98 out of 18451 (0.53%) in the patient population. So in general population incidence of ABO discrepancy was found to be 0.36%. The higher incidence in patients and the presence of clerical errorsindicate the need for awareness studies and strict compliance to standard guidelines. A problem solving strategy was formulated based on the existing guidelines which will help to reduce mismatched blood.

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