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Degree of

Doctor of Philosophy

In Microbiology

"Studies on the occurrence of microbial infections as a consequence of frequent blood transfusions"

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CHAPTER 1

INTRODUCTION

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Blood has fascinated human beings from ancient times. Egyptians bathed in it, arestocrates drank it, authors and playwriters used it as themes and modern humanity transfused it. The road to an efficient, safe and uncomplicated transfusion technique has been rather difficult, but due to scientific developments, great progress has been made over the last decades.

Blood is a living tissue composed of cells and plasma. Cells which include red blood cells, white blood cells and platelets constitute about forty five percent of the blood volume; while plasma accounts for the remaining fifty five percent. About seven percent of a person's weight is blood and the volume of blood varies according to height and weight.

Once regarded as a miracle procedure, blood transfusion is now a routine life-saving process. In an age in which spectacular medical advances have become frequent occurances, field of transfusion medicine has somehow remained overshadowed. Attention is drawn to transfusion practices only and when untoward incidents come to light during blood transfusion. Yet, blood an essential human tissue is donated to and received by thousands of people each day to save lives. In India the study conducted by a group of experts on the directive of supreme court observed that for a population of over 100 crores hardly 4.5 million units of blood is available against a demand of 8 million units, thus leaving a gap of 3.5 million units. To meet such huge demand supply gap regorous blood donations and well established blood banks are the need of time. However, in India, as in many developing countries, officially recognized blood banks are rare and far apart. The strain on blood bank is not merely the volume of blood needed but also availability of 'safe' blood as and when needed.

The transfusion of blood began with the use of whole blood and continued for many years. Now-a-days, blood is separated into its cellular and protein components to be used in specific blood disorders and illnesses. However, unfortunately these blood products/components are associated with the risk of transmission of viral, parasitic

and bacterial diseases. The degree of problem caused by these microbial agents is directly related to their endemicity in a particular area. Handling and storage of blood, its components and products at room temperature as well as 4ºc may provide sufficient time and opportunity for microbial growth. There are two major hurdles in providing a very efficient and risk free transfusion therapy.

(1) Shortfall on the technical side; both in terms of trained technical personal as well as modern sophisticated instrumentation. This is specifically a very severe problem in remote regions of our country where majority of rural population reside.
(2) Dependence on same source of high risk professional blood sellers and only changed the title of professional blood sellers to relative donors or replacement donors.

Since the detection of Human Immuno Deficiency Virus (HIV) infection in Tamil nadu in 1986 there has been sudden awakening about the fact that a life saving regime for a patient in need of blood transfusion can lead to slow but painful death in form of Acquired Immunodeficiency Syndrome (AIDS). Blood transfusion is the most efficient way of transmission of HIV. Even a small transfusion of infected blood results in virtually 100% seroconversion.

Besides AIDS, a number of microbial infections; viral, bacterial as well as parasitic in nature are known to be transmitted via blood. The list dose not end here and new infections are making entry into it. Latest suspect is human form of Bovine spongiform encephalopathy (BSE) or "mad cow" disease – a prion disease of human, also known as 'varient crutzfeldt-Jacob disease' or vCJD. Prions are present in peripheral lymphatic tissues to a greater extend than classical CJD. (Ganem, 2004; Bergman, 1995)

Since the time when Dr. Bernard Fantus, Professor of Pharmacology, University of Illinois established the first blood bank in 1937, the objective has been to provide quality blood in required quantity. There are some genetically inherited diseases or some medical situations where a person can survive only if one is given 'multiple blood transfusions'. A multiple blood transfusion is prescribed under variety of situations namely; Thalassaemia, Hemophilia, Heamodialysis, Surgical

multitransfusion and Leukemia. Since the individuals are subjected to multiple blood transfusions, it is obvious that they fall into ‘high risk’ group likely to be more vulnerable to various microbial infections caused by blood transfusion therapy.

Present study is aimed at comparing prevalence of same of the microbial infections such as HIV, hepatitis, bacterial infections and parasitic infection mainly in different cohorts of multitransfused individuals with each other and also with the control set of blood donors who are proclaimed to be ‘healthy’.

The prevalence of viral markers such as HIV, HBsAg and HCV antigen along with Veneral Disease Laboratory Research (VDRL) test for detection of syphilis are ‘indices’ of safe blood as per the guideline of drug and cosmetic act of India. However, due to lapse in quality control as well as blood donation during ‘window period’ of infection result in transmission of these antigens to the recipient. In such context, patients with history of multiple blood transfusions can be considered at ‘high risk’ of receiving such contaminated blood.

For a comparative study of different cohorts of multitransfused patients for various microbial markers as well as biochemical parameters, various blood analysis methods were used. This included Enzyme Linked Immunosorbent assay (ELISA) for various antigens, Polymerase Chain Reaction (PCR) based analysis for detection of HBsAg and HCV antigens and related biochemical analysis for various cohorts.

OBJECTIVES

1. To study prevalence of microbial infections in different cohorts of multi-transfused patients namely thallasaemics, haemodialysis patients, hemophiliacs, surgical multitransfused and leukemia patients.
2. Comparative studies on prevalence of microbial infections in healthy donors and multitransfused patients.
3. To study correlation between various haematolological and biochemical parameters with presence of microbial infections in individual cohort.
4. To determine statistical significance of results obtained within different cohorts and in healthy donors.
5. To perform molecular analysis of some of the seropositive patients by advanced techniques like polymerase chain reaction (PCR).
6. To compare prevalence of microbial infections in various cohorts of multitransfused patients of Saurashtra region with that at global level from literature reviews.

CHAPTER 2

REVIEW OF LITERATURE

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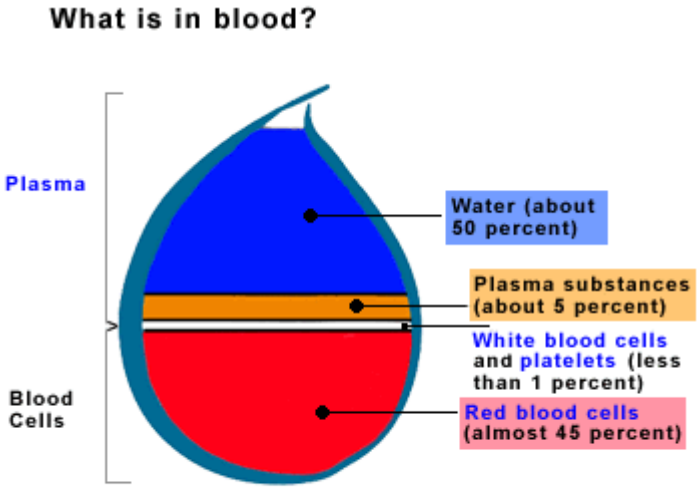
REVIEW OF LITERATURE

Blood is a fluid pumped by the heart that circulates throughout the body via the arteries, veins, and capillaries. An adult male of average size normally has about 6 quarts (5.6 liters) of blood. The blood carries oxygen and nutrients to the body tissues and removes carbon dioxide

COMPONENTS OF BLOOD



Blood is a mixture of cells and watery liquid, called plasma that the cells float in. It also contains other things like nutrients (such as sugar), hormones, clotting agents, and waste products to be flushed out of the body.

There are three kinds of cells in the blood: red blood cells, white blood cells, and platelets. Red blood cells carry oxygen from the lungs throughout the body, white blood cells help fight infection, and platelets help in clotting.

RED BLOOD CELLS

Red blood cells (also called erythrocytes) are the most numerous, making up 40-45 percent of one's blood, and they give blood its characteristic color. Red blood cells are shaped like tiny doughnuts, with an indentation in the center instead of a hole. They contain a special molecule called hemoglobin, which carries the oxygen. In the lungs,

where there is a lot of oxygen, the hemoglobin molecules loosely bind with oxygen. Each molecule of hemoglobin contains four iron atoms, and each iron atom can bind with one molecule of oxygen, allowing each hemoglobin molecule to carry four molecules of oxygen. In the capillaries, where there is little oxygen, the hemoglobin readily sheds the oxygen it is carrying and allows it to be absorbed by the body's cells. The iron in hemoglobin is what makes blood red.

WHITE BLOOD CELLS

White blood cells (leukocytes) are the body's mobile warriors in the battle against infection and invasion. There are three types of white blood cell: granulocytes, lymphocytes, and monocytes. There are, in turn, three kinds of granulocyte: neutrophils, eosinophils, and basophils. (Granulocytes are called so because they contain granules that hold digestive enzymes.) Neutrophils kill invading bacteria by ingesting and then digesting them. Eosinophils kill parasites, and are involved in allergic reactions. Basophils also function in allergic reactions, but are not well understood. Lymphocytes are key parts of the body's immune system. There are two kinds of lymphocyte: T cells and B-lymphocytes. T cells direct the activity of the immune system. B-lymphocytes produce antibodies, which destroy foreign bodies. Monocytes, the largest kind of white blood cells, enter the tissues of the body and turn into even larger cells called macrophages. They eat foreign bacteria and destroy damaged, old, and dead cells of the body itself.

PLATELETS

The blood cells called platelets (thrombocytes) help blood to clot, in several different ways. When bleeding occurs, platelets clump together to help form a clot. Also, when they are exposed to air (as they would be by a wound), platelets start breaking down and release a substance into the bloodstream. This substance starts a chain of chemical events that eventually causes a protein in the blood, fibrinogen, to turn into a different substance, fibrin, which forms long threads. These threads tangle up red blood cells to help from a clot, or scab, over the wound. In their "resting" state, platelets look like two plates stuck together (hence the name). When "activated" and helping to form a clot, they change shape and look like tiny roundish blobs with tentacles. At only two to three microns, they are the smallest kind of blood cell.

PLASMA

Plasma is a clear, straw-colored liquid that carries the blood cells and various hormones, nutrients, and so on through the body. It makes up a little Plasma is about 90 percent water. Much of the other ten percent comprises various kinds of protein molecules, including enzymes, clotting agents, immunoglobulin, and proteins that carry hormones, vitamins, cholesterol, and other things the body needs. Plasma also contains sugar (glucose) and electrolytes like sodium, potassium, and calcium, as well as other things like the aforementioned hormones, vitamins, and cholesterol.

Blood cells are produced in the bone marrow, a jellylike substance inside the bones that is composed of, among other things, fat, blood, and special cells that turn into the various kinds of blood cells. In children, the marrow of most of the bones produces blood. But in adults, only the marrow of certain bones -- the spine, ribs, pelvis, and some others -- continues to make blood. Bone marrow that actively produces blood cells is called red marrow, and bone marrow that no longer produces blood cells is called yellow marrow.

All blood cells come from the same kind of stem cell, which has the potential to turn into any kind of blood cell. These stem cells are called pluripotential hematopoietic stem cells.

Blood transfusions were not possible until Karl Land Steiner first identified the major human blood groups - namely O, A, B, and AB -- in a series of experiments in 1901 that earned him the Nobel Prize.(At the time, Land Steiner identified only groups A, B, and O; further analysis, two years, later revealed AB.)

There are many other antigens on the red cell surface. The most important is the Rh factor. A person is defined as either Rh positive or Rh negative depending on the presence of the primary Rh antigen on the red cell. In contrast to ABO antigens, however, a person only develops anti-Rh after exposure to Rh-positive red cells through transfusion or pregnancy. Modern blood-banking technology uses highly sensitive tests to properly identify and match blood between donor and recipient. (Greer, 2004).

What is a Blood Transfusion?

Blood transfusion is withdrawal of blood or its constituent parts from healthy donors and given to oligaemic patients.

Sometimes a person will have some of their own blood drawn & stored before having surgery & re injected after surgery this is called autologous blood transfusion

A blood transfusion is a safe, common procedure in which blood is given to you through an intravenous (IV) line in one of your blood vessels. Blood transfusions are done to replace blood lost during surgery or a serious injury. A transfusion also may be done if your body can't make blood properly because of an illness.

During a blood transfusion, a small needle is used to insert an IV line into one of your blood vessels. Through this line, you receive healthy blood. The procedure usually takes 1 to 4 hours, depending on how much blood you need.

Blood transfusions are very common.. Most blood transfusions go well. Mild complications can occur. Very rarely, serious problems develop.

Millions of lives are saved each year through blood transfusion. In many countries however people still die due to an inadequate supply of blood & blood products.

This has a particular impact on women (as a consequence of pregnancy related complications), children (malnutrition, malaria, severe life- threatening anemia, hemophilia, and thalassemia) and trauma victims especially poor & disadvantaged.

History of Blood Transfusion

From the earliest recorded, Blood has been a point of fascination and mystery. Early in time we see Blood represented as a source of passion in humans. We often hear "hot Blooded," "cold Blooded" and "bad Blood." These familiar expressions are found in every language, and are in common use the world over today.

As history records, Bloodletting was a common medical practice until the early **1800**'s. This combination of superstition and desire to do something meaningful for sick people, no doubt led to many deaths and the spread of disease. "Thinning the Blood," was also very popular, fruitlessly useless, and sometimes deadly.

Early on, 'Blood transfusion' meant direct donor-to-patient transfusion. This practice, however, was frequently disastrous because there was no quality knowledge of Blood types, and compatibility. At first, even animal- to- human transfusion was tried. (Farr, 1980).

In **1901**, Karl Land Steiner, an Austrian physician, whom we see as the most important individual in the field of human Blood, documented the first three human Blood groups (based upon substances on the Blood cells), A, B and O. Without this discovery and the subsequent research, there would be no Blood banking.

The practice of drawing and storing human Blood is a comparatively new science. Borne in these beginnings of human Blood transfusion, the need for storage of the rapidly perishable Blood became obvious.

In **1914**, long-term anticoagulants, among them sodium citrate, were developed and tried in Europe, promising longer term preservation of Blood.

In **1916**, Francis Rous and J. R. Turner introduced a citrate-glucose solution that permitted storage of Blood for several days after collection. Also, as in the 1915 Richard Lewisohn discoveries at Mt. Sinai Hospital in New York City, this allowed for Blood to be stored in containers for later transfusion, and aided in the transition from the vein-to-vein method to direct transfusion. This discovery also directly led to the establishment of the first Blood 'depot' by the British during World War I. Oswald Robertson was credited as the creator of the Blood depots, basically the forerunner Blood banking system that is in use today.

Further, during that same time period, R. Weil demonstrated the feasibility of refrigerated storage of anti-coagulated Blood.

Early in **1932**, the first facility, fully functioning as what would come to be known as a "Blood bank," was established in a Leningrad Russia hospital.

In **1936**, Bernard Fantus, director of therapeutics at the Cook County Hospital in Chicago, established the first hospital Blood bank in the United States In creating a hospital laboratory that can preserve and store donor Blood, Fantus originated the term 'Blood bank.' Within a few years, hospital and community Blood banks began to be established across the United States, some of the earliest documented were in Cincinnati, San Francisco, New York and Miami.

In **1940**, when he was at Columbia Presbyterian Hospital in New York, Dr. Charles Drew, a graduate of McGill University Medical School in Montreal, researched and documented a technique for long-term preservation of Blood plasma. By separating the liquid red Blood cells from the near solid plasma and freezing the two separately, he found that Blood could be preserved and reconstituted at a later date. In addition, as a prolific inventor, he was a major contributor to the advancement of Blood banking in the United States. In England, he helped supply thousands of units of plasma for World War II victims.

Later in **1940**, Edwin Cohn, a professor of biological chemistry at Harvard Medical School, developed a cold ethanol fractionation, the process of breaking down Blood plasma into components and products that were made available for clinical use. This was the birth of the Blood product manufacturing industry.

Blood banking began growing rapidly with the return from World War II of physicians who had seen the effectiveness of transfusion therapy and began to demand that Blood be made available for treatment of their patients.

In **1946**, the United States Public Health Service issued the first federal license permitting the 'manufacture' of whole Blood.

In **1947**, the American Association of Blood Banks (AABB) was formed to "promote common goals among Blood banking facilities and the American Blood donating public. Essential to this success story were the goals to support and encourage

continued Blood research, to facilitate the exchange of scientific information, and to develop standards of practice for Blood banks. This commitment to universal standards and communication changed the business of Blood banking forever.

By **1948** the American [National] Red Cross had began operating a comprehensive program to collect and distribute Blood.

By **1950**, the United States Blood collection system had grown to approximately 1,500 hospital Blood banks, 46 community Blood centers and 31 American Red Cross regional Blood centers, among others.

Also in **1950**, Carl Walter and W. P. Murphy, Jr. introduced the plastic bag for Blood collection and storage, replacing breakable glass bottles with rugged plastic bags. These plastic bags allowed, among other things, economical ultra low temperature freezing of Blood and Blood products.

Additionally in **1950**, an active year in Blood banking history, the use of glycerol cryoprotectant for freezing red Blood cells became widespread.

In **1951**, the AABB Clearinghouse (now the National Blood Exchange) was established, providing a centralized system in the United States for safely exchanging Blood among Blood banks.

By **1962** in the United States, there were 4400 hospital Blood banks, over 120 community Blood centers and more than 50 American Red Cross Blood centers, collecting a total of five to six million units of Blood per year.

1965 - Judith Graham Pool identifies the technique, now known as cryoprecipitation for concentrating factor VII from Blood plasma.

1972 - The FDA Bureau of Biologics begins regulating Blood resources. It assumed this responsibility from the National Institutes of Health, Division of Biologics Standards.

1973 - The FDA publishes more precise standards for Source Plasma and requires all operating plasmapheresis facilities to apply for and maintain licensure of Blood establishments and Blood products and submission to inspection standards.

1974 - The FDA declares Good Manufacturing Practices (GMP's) for the collection, processing, and storage of human Blood and Blood components.

1977 - The formal FDA compliance program is established. Annual facility inspections are transferred from the FDA Bureau of Biologics to FDA field investigators and program supervisors.

1989 - The FDA issues some light weight requirements for computerization of Blood related establishments, with higher quality requirements to come.

1992 - The International Plasma Products Industry Association, an influential trade group representing Blood manufacturers is formed (primarily of U. S.).

1994 - The European Association of the Plasma Products Industry, a trade group representing Blood manufacturers in Europe and the UK, is founded.

In **1996 and 1997** - The United States Government first issued reports suggesting problems with the Blood supply, along with suggestions of ways to improve Blood safety, including regulatory reform in the Blood processing and Blood banking industries. This has become a routine event.

An effort of many years in **1999** produced the implementation by the Blood manufacturing community of Nucleic Acid Amplification Testing (NAT) under the FDA's Investigational New Drug (IND) application process. NAT employs a testing technology that directly detects the genetic materials of viruses like HCV and HIV (Wildmann, 2000).

In the United States today, licensed Blood donation and banking establishments include centers that collect, process, store and distribute Blood and Blood products. These facilities are engaged in Interstate Commerce, and are therefore regulated under

federal law. Standardization, inspection and enforcement are bringing the science of Blood banking to an ever newer, more useful and trusted place.

Long term "cryo" storage of Blood and Blood products at hyper low temperatures is shaping the story of the future of Blood, Blood products and the safety of the public Blood supply. These processes also offer ever more economical autologous Blood donation and storage alternatives for those who need and/or can afford them.

Blood Donation

Blood donation is a process by which a blood donor voluntarily has blood drawn for storage in a blood bank, generally for subsequent use in a blood transfusion.

Process

The process of giving blood involves screening the donor, the actual donation, and a brief recovery period. This applies to both whole blood donations and plasmapheresis, or donating only.

Donated blood is tested by many methods, and a typical screening panel includes most of the tests below:

Blood Type

Blood is tested to determine the donor's ABO group (A, B, O, and AB) and Rh type (positive or negative). This is critical in selecting compatible blood for a patient in need of a transfusion.

Antibody Detection Test

All donors are tested to determine if their plasma contains unexpected antibodies to red blood cell antigens (genetic marker). These antibodies, if not identified, can cause problems in a blood recipient.

Tests for Infectious Diseases

Screening donated blood for infectious diseases that can be transmitted through blood transfusion is very important in ensuring safety. A positive screening test in any of the following tests for infectious disease is followed by a confirmatory test, since it is

possible to have false positive test results. A false positive occurs when the screening test is positive but it cannot be confirmed. This means a donor was not exposed to the infectious agent being tested for but the screening test was positive. The following tests to be performed on each unit of donated blood are required by the Food and Drug Administration (FDA):

Hepatitis B Surface Antigen (HBsAg)

The hepatitis B virus has an inner core and an outer envelope (the surface). The HBsAg test detects the outer envelope or surface of the virus.

Hepatitis C Virus Antibody (anti-HCV)

Two tests are done to detect hepatitis C infection. The anti-HCV test detects antibodies to the hepatitis C virus. A positive result suggests the donor has been exposed to the hepatitis C virus and may be infectious.

Syphilis Antibody

Syphilis tests detect the presence of an antibody to the organism *Treponema pallidum* that causes syphilis. This test has been performed on blood donors since shortly after World War II when the rate of infection was much higher. The risk of transmitting syphilis through a blood transfusion today is very small, since the rate of infection is low in blood donors and the organism causing syphilis is very fragile and unlikely to survive blood storage.

Human Immunodeficiency Virus 2 (HIV-1/2) Combo Antibody

HIV-1 and/or HIV-2 virus cause acquired immunodeficiency syndrome, or AIDS. HIV-1 is more common in the United States while HIV-2 is prevalent in Western Africa. One screens for antibodies to both HIV-1 and HIV-2 viruses.

Autologous donation

A person who anticipates the need for a blood transfusion at a later date (usually because of scheduled surgery) may make an autologous donation, in which their blood is stored and later transfused back into its original donor. Besides ensuring the availability of compatible blood (especially important for patients with rare blood types), this procedure also eliminates the risk of disease transmission from infected

donors. Autologous donation is sometimes done by the hospital instead of a community blood bank. Eligibility requirements are relaxed for autologous donors, as the blood is not used for anyone else. Generally, any patient who is eligible for elective surgery with some exceptions such as history of heart disease is eligible for autologous donation.

Blood Component Preparation and Therapy

INTRODUCTION

Modern blood transfusion envisages the optimal use of every blood donation by way of blood component therapy. The development of plastic blood collection bags with integral tubing, high speed refrigerated centrifuge, deep freezers and cell separator machines have made blood component preparation easier and practical. With the more advanced chemical techniques (plasma fractionation) various plasma derivatives or fractions are also now made available.

List of various blood components and plasma derivatives:

BLOOD COMPONENTS	PLASMA DERIVATIVES
• Red cell concentrate (packed red cells)	• Albumin
• Leukocyte poor red cell concentrate.	• Plasma protein fraction (PPF)
• Platelet rich plasma (PRP)	• Factor VIII concentrate
• Platelet concentrate	• Fibrinogen
• Granulocyte concentrate	• Immunoglobulin
• Fresh frozen plasma (FFP)	• Other coagulation factors
• Cryoprecipitate	
• Single donor plasma • Cryo – poor plasma	

Blood component therapy helps in two ways…

- Economy of blood as one unit of blood can be separated into different components and used in different patients according to their indication.
- Minimizes the hazards of whole blood transfusion.

Preparation of Blood Components

Various components of blood like red blood cells, platelets and plasma can be separated from one another by centrifugation because of their different specific gravities.

1. RED BLOOD CELLS

Red blood cells are prepared by removing approximately 200 to 250 ml of plasma from a unit of 450 ml of whole blood or 150 to 175 ml of plasma from a unit of 350 ml of whole blood. The red cells have approximately heamatocrit of 70-80% and have same oxygen carrying capacity as whole blood.

Preparation

Red blood cells are prepared by…

1. **Sedimentation**

   The blood after collection is kept in a refrigerator in a upright position, till the red cells settle down and the supernatant plasma is transferred into a transfer bag or satellite bag.

2. **Centrifugation**

   Blood is collected in double/triple CPDA blood collection bags and after balancing bags are centrifuged at 5000*g for 5 minutes at 4-6 degree temp.

INDICATIONS

1. Severe anemia to reduce chances of circulatory over load.
2. Hemolytic anemia especially in aplastic crisis.
3. Various hypo plastic anemias.
4. Less blood group antibodies in packed cell, so non-specific blood

i.e. O Negative blood can be given to patients with other groups.

5. Less plasma proteins with packed cells, so there are minimum aphylactic reactions.

Transfusion requirement of each patient should be based on clinical status, rather than hemoglobin value or heamatocrit. There are no set hemoglobin levels that indicate the need for transfusion. The level of 10 gm/dl has been used as transfusion trigger for many years for surgical and leukemia patients, which has now been lowered to 7.5 gm/dl.

2. LEUCOCYTE POOR RED BLOOD CELLS

Leukocyte poor red blood cells imply the removal of at least 70%of the leucocytes, with a loss of less than 20%of red blood cells.

Preparation

1. Centrifugation: Inverted centrifugation Double centrifugation
2. Filtration
3. Washing of red blood cells with saline.
4. Freezing and Deglycerolization.

Advantages

The use of leucodepleted blood products is gaining importance because of the scientific evidence that filtered blood products to remove leucocytes prior to transfusion prevent or reduce the incidence and severity of adverse transfusion effects like:-

- Febrile non-hemolytic transfusion reaction (FNHTR)
- Sensitization to blood products (HLA-allo-immunization)
- Transmission of certain transfusion associated diseases like CMV infection.

3. FRESH FROZEN PLASMA (F.F.P)

Plasma separated from whole blood, frozen within six hours of collection and stored at –20ºC and below.

Indications:

- Multiple coagulation factor deficiencies
- Reversal of coumarin drug effect
- Use in Antithrombin Deficiency

- Immunodeficiency syndromes
- In open heart surgery

4. CRYOPRECIPITATE

It is the insoluble portion of plasma remaining after the fresh frozen plasma has been thawed under controlled conditions.

Composition of cryoprecipitate:

CONSTITUENTS	AMOUNT
Factor VIII	80-100 Units/Concentrate
Fibrinogen	150-250 mg./concentrate
Von-Willebrand Factor	40-70% of original FFP

Indications:

Cryoprecipitate may be indicated for treatment of:

- Hemophilia A
- Von-Willebrands disease
- Congenital or acquired fibrinogen deficiency

5. PLATELET RICH PLASMA AND PLATELET CONCENTRATE

Preparation:

1. By using CDP-A/or ADSOL or SAG –M triple bags and refrigerated centrifuge.

2. By using aphaeresis machines (cell separators).

Indications:

1. A megakaryocytic thrombocytopenia: Leukemia (Kliman et al., 1964)

BLOOD TRANSFUSION SAFETY

Raktabiij – an asura described in our epics glorified the value of blood as he was blessed with the ability to have a clone generated automatically with every drop of his blood that touches the ground. If we translate this story to modern medicine, blood constitutes the lifeline of human body, and can generate life in a moribund soul. With nearly 13 million annual unscreened blood transfusions, 95 per cent of blood transfusions in India were deemed unsafe by researchers from Northwestern University. The WHO estimated that 80 per cent of global population living in developing world has access to only 20 per cent of safe blood. Therefore, safety of blood transfusion is key to delivery of quality of patient care.

The emergence of HIV in the 1980s highlighted the importance of ensuring the safety, as well as the adequacy, of national blood supplies. In many countries, even where blood is available, many recipients remain at risk of transfusion-transmissible infections (TTIs) as a result of poor blood donor recruitment and selection practices and the use of untested units of blood.

The WHO Blood Transfusion Safety (BTS) team supports the establishment of sustainable of national blood programmes that can ensure the provision of safe, high quality blood and blood products that are accessible to all patients requiring transfusion and their safe and appropriate use. In support of this mission, the WHO BTS team recommends the following integrated strategy to national health authorities:

- Establishment of a well-organized, nationally coordinated blood transfusion service that can provide adequate and timely supplies of safe blood for all patients in need;
- Collection of blood only from voluntary unpaid blood donors at low risk of acquiring transfusion-transmissible infections and stringent blood donor selection criteria;
- Testing of all donated blood for transfusion-transmissible infections, blood groups and compatibility;
- Production of blood components to maximize the use of donated blood and enables the provision of therapeutic support for patients with special transfusion requirements;

- Appropriate clinical use of blood and the use of alternatives, where possible, to minimize unnecessary transfusions;
- Safe transfusion practice at the bedside;

National blood transfusion services

The provision of safe and adequate blood is the responsibility of government. The formation of a nationally organized and managed blood programme should be an integral part of each country's national health care policy and health care infrastructure. The blood transfusion service (BTS) should be established in accordance with an agreed: National blood Policy and plan within a legislative framework. It should be responsible for establishing and maintaining a national quality system, including the development of guidelines and standards, staff training, a data/ information management system and a system for monitoring and evaluation of all the blood transfusion activities.

The BTS requires formal government commitment, support and recognition of the national health authority as a specific, identifiable programme with a budgeting and finance system that can ensure the BTS to fully achieve a stable and adequate blood supply. Safe, accessible supplies of blood and blood products cannot be achieved without cost. However, an unsafe or inadequate blood supply is ultimately even more costly - in both human and economic terms.

Voluntary blood donation

Safe blood donors are the cornerstone of a safe and adequate supply of blood and blood products. The safest blood donors are voluntary, non-remunerated blood donors from low-risk populations. Despite this, family/replacement and paid donors, which are associated with a significantly higher prevalence of transfusion-transmissible infections (TTIs) including HIV, hepatitis B, hepatitis C, syphilis and Chagas disease, still provide more than 50% of the blood collected in developing countries. WHO advocates and recommends to its member states to develop national blood transfusion services based on voluntary non-remunerated regular blood donation in accordance with World Health Assembly resolution 28.72, which was adopted in 1975.

The key to recruiting and retaining safe blood donors is good epidemiological data on the prevalence (and incidence, where possible) of infectious markers in the general population to identify low-risk donor populations coupled with an effective donor education, motivation and recruitment strategy to recruit new voluntary non-remunerated blood donors from these populations. A pleasant experience during blood donation, good donors care and effective communication between blood centre staff and blood donors are all important factors for the retention of safe blood donors.

Testing of donated blood

Testing of all donated blood for transfusion transmitted infections (TTIs) such as HIV, Hepatitis B, Hepatitis C and Syphilis is one of the strategies recommended WHO to ensure safe blood. Where appropriate and possible, donated blood should be tested for other infections such as Chagas Disease and Malaria. Blood is also tested to identify the blood group and for the presence of irregular red cell antibodies before transfusion. This is to make sure the patient who will receive compatible blood in order to avoid serious hemolytic transfusion reactions.

The inappropriate use of blood and blood products, coupled with the transfusion of unscreened or improperly screened units, particularly in countries with poor blood programmes, increases the risk of TTIs to recipients. It also widens the gaps between supply and demand and contributes to shortages of blood and blood products for patient requiring transfusion. Thus, it is necessary to reduce the unnecessary transfusions. This can be achieved through the appropriate clinical use of blood, avoiding the needs for transfusion and use of alternatives to transfusion. The transfusion is deemed appropriate when it is used to treat condition leading to significant morbidity and mortality that cannot be prevented or managed effectively by other means. The commitment of the health authorities, health care providers and clinicians are important in prevention, early diagnosis and treatment of diseases/ conditions that could lead to the need for blood transfusion.

Infectious Risks of Transfusion

Currently, the risk of transmission of infectious diseases through transfusion is minimal, because effective preventive strategies, including new laboratory tests, have been implemented. Nevertheless, many infectious agents, including viruses, bacteria,

and parasites, can be transmitted through blood transfusion. Well-recognized viruses include hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV), hepatitis G virus/GB-C virus (HGV/GBV-C), human immunodeficiency virus types 1 and 2 (HIV-1/2), human T-cell lymphotropic virus types I and II (HTLV-I/II), cytomegalovirus (CMV), Epstein-Barr virus (EBV), TT virus (TTV), human herpes virus type 6 (HHV-6), SEN virus (SEN-V), and human parvovirus (HPV-B19). Bacteria such as *Treponema pallidum* (the agent of syphilis), *Yersinia enterocolitica*, and *Staphylococcus* and *Streptococcus* species (common agents of bacterial contamination), and parasites such as *Plasmodium* species (the agent of malaria), *Trypanosoma cruzi* (agent of Chagas' disease), and *Babesia microti* (agent of babesiosis) have also been reported to be transmitted through blood transfusion. In addition, emerging blood-borne pathogens such as hepatitis E virus (HEV), human herpes virus type 8 (HHV-8), *Borrelia burgdorferi* (agent of Lyme disease), and the unknown agent of Creutzfeldt-Jakob disease (CJD) and variant CJD (vCJD) may pose a threat to the safety of blood.

Infectious agents may be classified into five categories based on transmissibility through transfusion, pathogenicity of the agent, availability of donor serologic test, and effectiveness of pathogen inactivation. The risk of transmission for the first group of agents is minimal because of donor screening and testing, and is normally associated with donations collected during the window period. For example, the risk of transmission of HIV and HCV in Canada through blood transfusion was estimated to be 1 in 752,000 and 1 in 225,000 donations respectively (Chiavetta et al., 2000). Infections caused by agents in the second and third group usually present clinical diseases in high-risk recipients, such as immunocompromised individuals; however, the transfusion transmission risk is also very small because of preventive strategies such as universal leukodepletion and solvent-detergent treatment. Transfusion transmitted bacterial infections resulting from bacterial contamination of blood components is the most common infectious adverse event. Approximately 1 in 2,000-3,300 units of platelets and 1 in 38,500 units of red cells are contaminated with bacteria; however, not every contaminated component causes reactions. The incidence of transfusion transmitted bacterial reaction is estimated to range from 1 in 500,000 units of red cells to 1 in 50,000 units of platelets (Blajchman et al., 1999).

Infectious agents in the fourth and fifth group may pose a potential risk following transfusion. Although agents in the fourth group have been proved to be transfusion transmissible, the pathogenicity of these agents is currently not established. Similarly, even though agents in the last group usually cause disease, their transfusion transmissibility has not been established.

Table 1

Categories of infectious agents according to transfusion transmissibility, pathogenicity, donor test and pathogen inactivation.

	First Group	**Second Group**	**Third Group**	**Fourth Group**	**Fifth Group**
Agents	HBV, HCV, HIV-1/-2, HTLV-I/-II, *Treponema pallidum*	CMV, EBV, *Yersinia enterocolitica*, *Staphylococcus* and *Streptococcus* species, *Trypanosoma cruzi*	HAV, HPV-B19, HHV-6, *Plasmodium* species, *Babesia microti*, *Rickettsia rickettsii*, *Leishmania donovani*, *Toxoplasma gondii*	HGV/GBV-C, TTV, SEN-V	HEV, HHV-8, *Borrelia burgdorferi*, *Ehrlichia phagocytophila*, prions
Transfusion transmissibility	Established	Established	Established	Established	Not established
Pathogenicity	Cause diseases	Cause diseases largely in high risk individuals	Cause diseases largely in high risk individuals	Not established	Cause diseases
Donor serologic test	Available	Not available	Not available	Not available	Not available
Effectiveness of pathogen inactivation	Largely inactivated	Largely inactivated	Largely not inactivated	Unknown	Unknown

Noninfectious Risks of Transfusion

Although Transfusion Transmitted infections have received the greatest attention, noninfectious adverse events remain the most common complications associated with transfusion. The majority of these noninfectious adverse events are immune mediated. Acute immune mediated reactions include acute hemolytic transfusion reaction (AHTR), transfusion-related acute lung injury (TRALI), febrile nonhemolytic transfusion reaction (FNHTR), urticarial reaction and anaphylaxis. Delayed immune mediated reactions consist of delayed hemolytic transfusion reaction (DHTR), transfusion associated graft-versus-host disease (TA-GVHD), and post transfusion purpura (PTP) (Gresens et al., 2001).

FNHTR and urticarial reaction are the most frequent non life-threatening acute transfusion reactions; however, AHTR is the most frequent severe reaction and the leading cause of death associated with transfusion. On the other hand, TRALI and TA-GVHD are rare and the most fatal transfusion reactions. The case fatality rate is 5% to 14% for TRALI and over 90% for TA-GVHD. TRALI has been recognized as the third leading cause of death associated with transfusion. (Kopko et al., 2001).

ABO incompatibility accounts for about 80% of AHTR-related deaths and occurs as a result of error. Well recognized errors include patient misidentification, sample error, wrong blood issued, transcription error, administration error, and technical error and storage error. Identification and prevention of these errors have become an increasingly important issue in transfusion safety.

In general, non immune mediated transfusion reactions result in minor clinical manifestations. These include hemoglobinuria, hyperkalemia, hypocalcemia, hypothermia, and iron overload. However, air embolus and circulatory overload may also be life threatening. Again, these reactions are mostly error related and can usually be prevented by proper preparation and storage of components, and better transfusion practices.

Despite the risks associated with transfusion, the Canadian blood supply is one of the safest blood systems in the world. Effective strategies and laboratory test procedures

are being implemented to further prevent and/or reduce transfusion-related adverse events.

Table 2

Classification of transfusion-related adverse events

Time of Occurrence	**Mechanism**	**Infectious Reaction**	**Noninfectious Reaction**
Acute	Immune mediated	--	Acute hemolytic transfusion reaction Transfusion-related acute lung injury Febrile nonhemolytic transfusion reaction Urticarial reaction Anaphylactic
	Non immune mediated	Bacterial contamination	Nonimmune hemolysis (e.g. hemoglobinuria) Circulatory overload Metabolic (e.g. hyperkalemia) Embolic
Delayed	Immune mediated	--	Delayed hemolytic transfusion reaction Transfusion associated graft-versus-host disease Post transfusion purpura
	Non - immune mediated	Transfusion Transmitted infections (viral, bacterial and parasitic)	Hemochromatosis (iron overload)

Acute adverse event is defined as any unfavorable event occurring in a patient during or within 24 hours after transfusion, but the definition has minor variations in the literature.

Delayed adverse event is defined as any unfavorable event occurring in a patient more

than 24 hours and up to 3 months after transfusion, but the definition has minor variations in the literature

Infectious complications in detail

There are many Blood borne, transfusion transmitted and related diseases. In the United States, many are openly and commonly written about in the press, and spoken of on the news.. Some are not. Following here is a partial and growing list of the most commonly known Blood transfusion transmitted diseases, but for which **no** routine cost-effective laboratory testing is available.

Hepatitis B

Despite the dramatic reduction in risk of viral transmission during the past three decades, viral hepatitis remains a serious complication of transfusion worldwide

The discovery in 1968 that the viremic phase of serum hepatitis could be recognized serologically (Blumberg et al., 1968) sparked the hope that all infectious donors could be identified. However, HBV remains a major human pathogen that causes acute and chronic hepatitis, cirrhosis and hepatocellular carcinoma (Ganem and Prince, 2004).

Hepatitis B surface antigen (HBsAg, formerly Australia antigen) is unassembled viral coat. Electron microscopy of concentrated serum containing HBsAg revels the presence of so-called Dane particles, diameter 42nm, which are known to be complete virus (Dane et al., 1970).The particles are made up of an inner protein core (HBc) containing partially double stranded DNA with a single stranded region of variable length, protein kinase and DNA polymerase surrounded by an outer coat of HBsAg. Antibodies to the surface antigen and the core antigen are known respectively as anti-**HBsAg and anti-HBc.**

Hepatitis B virus (HBV) is transmitted through parenteral and sexual exposure. Parenteral transmission is by transfusion of infected blood plasma or coagulation factor concentrate and by a variety of other percutanious exposures. Carriers with HBsAg below the detection level can transmit HBV by blood transfusion. Patients who were seronegative for all seronegative markers, but HBV –DNA by PCR, transmitted hepatitis B to two chimpanzees (Thiers et al.,1988). The mean incubation time is 90 days with a range of 30 to 180 days. Donor Blood is routinely tested for HBsAg and HBcAb. Nevertheless cases in which HBV is transmitted by HBsAg and

anti HBc negative donations still occur (Allain, 2004). Washing of red cells reduces the viral load and can render units seronegative. However, washing does not eliminate the risk of transmission of HBV. Red cells from blood that is only lightly contaminated with HBV and is washed, or frozen with glycerol and washed, although apparently HBsAg negative, can still transmit HBV to chimpanzees (Alter et al., 1978a) and humans (Rinker and Galambos 1981).

Screening for HBsAg in blood was initially done by immunodiffusion and counterimmunoelectrophoresis. These methods were soon replaced by more sophisticated immunoradiometric assay (RIA) and the ELISA, which detect less than 0.5 ng of HBsAg/ml of blood. However, taking the molecular weight of HBsAg as 3×10^6, 0.5 ng is equivalent to 1×10^7 particles. Thus with a negative result of these tests, as many as $4\text{-}10\times10^7$ antigen molecules may be present per milliliter. Complete prevention of post-transfusion hepatitis cannot be achieved by screening for HBsAg. Persons who have received a hepatitis B vaccination (recommended for all health care workers with patient contact) will have hepatitis B surface antibody present, but not HBsAg or HBcAb. Risk of transmission in the Unites States is said to be 1 in 66,000.

Hepatitis-D

Recipients of Blood products can also be infected with hepatitis delta virus (HDV), which is a defective RNA virus that needs an HBV super infection to replicate (Tiollais, 1988). The structure and replication of HDV are well described at molecular level (Taylor, 2003). Fewer than 10% of carriers of HBV are co-infected with HDV and the agent appears to be disappearing (Gaeta et al., 2003). HDV multiplies in the liver and is transmitted by blood and body fluids.

Superinfection of a carrier of HBsAg with HDV is associated with a chronic course of the delta infection in 70-90 % of cases with an increase in severity of the underlying chronic hepatitis (Monjardino and Saldanha, 1990). In HDV infection the delta antigen is present in liver and serum. Chronic HDV infection depends on the persistence of HBV.

Screening for HBsAg in blood donors minimizes but does not abolish the risk of PTH delta in HBsAg positive recipient (Rosina et al., 1985). The simultaneous presence of

HDV and HBeAg in the absence of detectable HBsAg has been reported (Shattok et al., 1985). HDV has been transmitted not only by blood and blood components, but also by coagulation factor concentrates (Purcell et al., 1985).

Hepatitis C

After the introduction of routine screening of blood donations for HBsAg, the great majority of cases of post transfusion hepatitis is due to hepatitis C virus, a flavivirus. (Alter et al., 1989a). Blood components and blood products including anti-D Ig transmit HCV. For intravenous use (Yap et al., 1993) and factor viii concentrate submitted to some of the virus inactivation procedures (Bergman, 1995). The hepatitis C virus (HCV) currently affects over four million people in the United States. This disease has reached epidemic proportions. It is the primary reason for liver transplantation in the United States hepatitis C virus (HCV) infection is still the most common transfusion transmitted infection. Persons at highest risk for the virus are those who received blood transfusions prior to 1991 or people with a history of IV drug abuse using shared needles. One attribute of this disease that contributes to the high rate of infection is that the time from infection with the virus until manifestation of liver disease can be decades. The hepatitis C virus subtype varies worldwide. In Europe, types 1, 2 and 3 are the predominant genotypes with types 1a and 3a in the northwestern countries and 1b in Hungary, Germany, Russia and Turkey. In North America, types 1a and 1b have been found. In Japan and Taiwan, types 1b, 2a and 2b predominate; type 6a in Hong Kong and Macau. Type 4 is found in Zaire, Central and North Africa; 5a in South Africa. The hepatitis C virus is taken into the body other than through the digestive tract. It must be inherited, transfused or injected in some manner. The sexual transmission rate is lower than once thought. At present, only testing for hepatitis C antibody is available. The antibody to the hepatitis C virus appears 54 to 192 days in a person's Blood after infection. If an infected person donates Blood prior to the appearance of this antibody the chance of that Blood being used in a transfusion is said to be one out of 103,000 donations, There are an estimated 15-million Blood transfusions each year. Risk of transmission in the Unites States is said to be 1 in 121,000.

Hepatitis E

Hepatitis E virus (HEV) is a non-enveloped RNA virus responsible for epidemic hepatitis in Asia, Africa, Latin America and the Middle East and sporadic hepatitis in developed countries. HEV is nearly always transmitted by faecal-oral route. However – like HAV transient viraemia develops after infection and transmission by blood transfusion does occur (Matsubayashi et al., 2004)

GB Virus (GBV / HGV)

Blind molecular cloning, similar to the strategy used to discover HCV, has resulted in the detection of several other transfusion- transmitted viruses. Using amplification techniques, cloning and sequencing, at first two viruses GBV-A and GBV-B found only in tamarins, and later a third virus, GBV-C, from a human serum were identified (Simons et al., 1995; Yoshiba et al., 1995). Of the three 'GB viruses GBV-C is the only human virus. GBV-C has been recovered from the blood of several patients with hepatitis, from multitranfused patients, hemophiliacs and intravenous drug users. The GB viruses are flavivirus – like agents similar to HCV, with a similar genomic organization. In relatively small studies, the prevalence of GBV-C has been found to be quite high; two percent in blood donors in the USA and the UK and twelve to fourteen percent in multiply transfused patients (Denis et al., 1996). GBV-C has been detected in three out of six patients with fulminant non-A, -B, -C or –E hepatitis but the real significance of the virus as a cause of hepatitis, PTH and chronic lever disease has still to be established (Yoshiba et al., 1995)

Human Immunodeficiency Virus (HIV)

Although retroviruses were among the first known viruses, for almost a century they were only found in animals, usually in association with neoplastic diseases. Until 1980, retroviruses had not been linked to human disease, let alone to transfusion-transmitted infection. However, the sensitive immunological, biochemical molecular approaches that became available in the 1970s led to the discovery of the first human retroviruses, isolated from lymphocytes of a patient with T-cell leukemia. And appropriately named human T- lymphotrophic virus type-1 (Poiesz et al., 1980). Knowledge of the existence of prototype retroviruses encouraged a search for a human retrovirus as the etiological agent of AIDS. The causative agent originally referred to as LAV or HTLV III and now called human Immunodeficiency virus (HIV) was discovered in 1983 (Barre-Sinoussi et al., 1983). They are RNA viruses,

which after entering host cells and losing their envelope, use 'reverse transcriptase' (RNA dependent DNA polymerase) together with cell derived RNA as a primer to transcribe a double stranded DNA copy of the single stranded viral RNA genome. All retroviruses contain three main structural genes; the *gag* gene codes for different molecular weight proteins that are integral to the nuclear core, the *pol* gene codes for reverse transcriptase; and the *ena* gene specifies for the envelope glycoprotein. The integrity of the envelope is essential for infectivity. The high lipid content of the retroviral envelope renders these agents very susceptible to disruption by detergents and organic solvents. All human retroviruses code for a small conservative major core protein, p24, and have similar modes of transmission, i.e. sexual, congenital and by blood or body fluids. (Wong-Staal and Gallow, 1985). Human retroviruses all share a tropism for lymphocytes inducing fusion and giant cell formation *in vitro* and impairing function *in vivo.* The high affinity receptor for HIV has been identified as CD4 on helper T-lymphocytes, macrophages and other cells.

HIV I and HIV II: In 1982 the first cases of AIDS transmitted from Blood or Blood components were reported, but little of the infection was known at that time, and even less was talked about publicly. The causative agent of AIDS was originally described as LAV (lymphadenopathy virus) (Vilmer et al., 1984). Gallow and his colleagues succeeded in culturing the same virus, which they called HTLV III in large quantities in continuously replicating T cells (Popovic et al., 1984). This virus, along with visna virus, belongs to the lentivirus subfamily of retroviruses. HIV was found to induce premature death of its host cells and to replicate rapidly (Gallow et al., 1984)

A second distinct retrovirus named HIV II causes a somewhat milder disease. Although this is also lymphotropic, cytotoxic and neurotropic, and shares epitomes of the core and *pol* proteins with HIV-I HIV-II nucleotide sequence identity with HIV I isolates is only 40-50%. The main difference between HIV II and I lie within the envelope nucleotides and proteins. The HIV I envelop glycoprotein gp120 binds specifically to CD4-bearing cells and interact with gp41 for virus-cell and cell-cell fusion events (Dalgleish et al., 1984). Two to four months after sexually transmitted infection, and 1-2 months after transfusion- transmitted infection, more than 95% of HIV-infected subjects exhibit a wide range of antibodies to the structural viral proteins. Disease develops when the CD4 helper cells have almost completely

disappeared, leading to impairment of the immune system and spread of HIV with signs of disease in multiple organs; anti-p24 declines and p24 antigen reappears at this stage.

Transmission of HIV infection and spread of AIDS by blood transfusion

Recognition of a possible relationship between blood transfusion and the acquired immune deficiency syndrome (AIDS) provided early clues that AIDS might have an infectious cause. In July 1982, three patients with severe classic hemophilia who developed Pneumocystis carini pneumonia were reported by the Centers for Disease Control in the morbidity and mortality Weekly Report (MMWR and CDC, 1982). All had been treated with large doses of commercially prepared factor VIII concentrate. Because HIV is both cell associated and present in plasma, all blood components are potentially infectious (Curran et al., 1985). The virus is well preserved in refrigerated and frozen blood; however, components that are washed, leucoreduced or cold stored for several weeks, reduce the likelihood of transfusion transmission (Donegan et al., 1990a).

In much of Asia and Africa the transmission of HIV by blood transfusion is still an important source of infection. Reasons for an alarmingly high rate of transmission, reported to be up to 10% of all cases, include

1. The demand for blood for patients with severe anemia and hemorrhage, mainly in obstetrics, gynecology and pediatrics; 2. The prevalence of HIV infection amongst the donor population. 3. The fact that HIV infection is not confined to a minority of the population who can be requested to refrain from blood donation; and 4. The inability of many laboratories to test for HIV or to perform and control the tests properly.

By 1983 radical changes began to occur in the donor criteria to exclude those at high risk for transmission of HIV. The testing of blood products for HIV started in 1985. It was a test to detect the presence of the antibody directed against HIV, rather than a direct test for HIV. Testing for HIV p24 antigen was mandated in 1996 in USA. Risk of transmission in the Unites States is said to be 1 in 563,000. Infections in persons Infected with HIV. However, in countries with a high percentage of infected subjects and where HIV is spread mainly by heterosexual intercourse, the risk of transmission of HIV by blood transfusion is still considerable.

Human T-lymphocytotrophic Virus (HTLV-1)

The human T-cell lymphocytotrophic virus Type-1 (HTLV-1), the first human retrovirus to be described, was isolated from cultured cells from a patient with an aggressive variant of mycosis fungoides and from a patient with Sezary syndrome (Poiesz et al., 1980). HTLV-1 is the causative agent of adult T-cell leukemia. The virus is also associated with lung infections, cancer of other organs, monoclonal gammopathy and renal failure and other opportunistic infections. These effects may be due to the immunodeficiency induced by HTLV-1 infection (Takatsuki, 1996).

HTLV-1 and –II belong to the oncovirus sub type of the retrovirus family and are able to induce polyclonal proliferation of T lymphocytes *in vitro and in vivo*.

This is a retrovirus that is endemic in Japan, with prevalence as high as 15% (Maeda et al., 1984) and in the Caribbean with 1-8% prevalence (Clark et al., 1985), in regions of Central and South America and in parts of sub-Saharan Africa (Gessain et al., 1986; Vrielink and Reesink, 2004).

HTLV-2 the second human retrovirus to be discovered, has a 65% nucleotide sequence identity with HTLV-1 and a significant serological cross reactivity (Hjelle., 1991).

HTLV-1 has been transmitted by cellular components, but not by cell-free plasma or plasma derivatives (Okochi, 1985). However, HTLV-RNA is detectable in plasma from infected subjects. Recipients of HTLV-1 infected concentrates may develop HAM (Araujo and Hall, 2004). ATL developed in two immuno-suppressed patients who had received multiple transfusions six and eleven year earlier (Chen et al., 1989).

HTLV-2 has also been transmitted by blood transfusion (Hjelle et al., 1990b).
Blood is routinely screened for antibodies to HTLV-1 utilizing this relatively inexpensive test. Risk of transmission in the United States at this time is said to be 1 in 641,000.

Transfusion Transmitted Virus

Transfusion Transmitted Virus is a relatively new virus becoming widely known in 1997 in patients with fulminant hepatitis and chronic liver disease of unknown etiology. TTV is an unenveloped, single stranded DNA virus. Two genetic groups have been identified, differing by 30% in nucleotide sequences. TTV DNA was detected in 47% of patients with fulminant non-A-G hepatitis and 46% of patients with chronic liver diseases of unknown etiology. The result suggests that TTV may be the cause of some cryptogenic liver diseases. In testing, the presence of TTV was found in approximately 10% of U. S. volunteer Blood donors, 13% of commercial Blood donors, and 17% of intravenous drug abusers. The rate of TTV infection among U. S. non-A, non-B, non-C, non-D, non-E hepatitis patients was only 2%.

Cytomegalovirus (CMV)

The prevalence of the CMV antibody ranges from 50% to 80% of the population. Blood contaminated with CMV can cause problems in neonates or immunocompromised patients. Potential problems in selected patient populations can be prevented by transfusing CMV negative Blood or frozen, deglycerolized RBC's. Donor Blood is not routinely tested for CMV.

Cytomegalovirus (CMV) is a large, enveloped, double stranded DNA, beta herpes virus that is cell associated, but may also be found free in plasma and other body fluids (Drew et al., 2003). CMV has a direct cytopathic effect on infected cells. The result may lead to neutropenia, some depression of cellular immunity and inversion of T-cell subset ratios, with a consequent increase in susceptibility to bacterial, fungal and protozoa infection in immunosuppressed patients (Grumet, 1984; Landolfo et al., 2003). CMV infection causes parenchymal damage, such as retinitis, pneumonitis, gastroenteritis, encephalitis, and can result in substantial morbidity and mortality.

The frequency of subjects with anti-CMV varies widely in different populations. Seroprevalence is lower (30-80%) in developed than in developing countries, where the figure may reach 100% (Krech, 1973; Preiksaitis, 1991). The prevalence of anti-CMV correlates with age and socioeconomic status (Lamberson, 1985; Tegtmeier, 1986).

The transmission of CMV by blood transfusion was first reported in the 1960s (Kaariainen et al., 1966; Klemola et al., 1969). CMV is now known as one of the infectious agents most frequently transmitted by transfusion. The pathogenesis of transfusion-transmitted CMV infection is not clearly understood. Primary infection rates depend on the number of transfusions, age of blood, and time of year and immunocompetence of the recipient (Tegtmeier, 1989; Preiksaitis et al., 1988; Preiksaitis, 2000). At present, no rapid, easy way to identify infectious subjects exists. Before an era of universal (or near universal) leucoreduction, 30% of anti-CMV-negative recipients undergoing cardiac surgery involving transfusion developed infection. Of patients who develop a primary or recurrent CMV infection following transfusion, fewer than 10% developed mononucleosis – like syndrome after three to six weeks of transfusion (Foster, 1966).

During the past decade, major advances have been achieved regarding the management of CMV (Meijer et al., 2003). Nevertheless, in immunosupressed patients, or in fetuses and premature infants CMV infections still cause severe disease that can be fatal.

Subjects infected with HIV and especially those with AIDS, if anti-CMV is negative, may acquire primary CMV infection by transfusion (Sayers et al., 1992).

Window period infections are the most likely source of antibody screening failures. Although the window periods for HIV-1, HCV and HBV have been reasonably well defined, the length of the CMV-seronegative window, estimated 6-8 weeks, is less well characterized (Zanghellini et al., 1999).

Epstein-Barr Virus

Infection with Epstein-Barr virus (EBV), a herpes virus – like CMV, is endemic throughout the world. EBV can cause primary symptomatic infection (infectious mononucleosis), but most commonly causes asymptomatic infection followed by latent infection (Henle, 1985).

Post transfusion infectious mononucleosis is seen only rarely in anti-EBV-negative immunocompetent patients and usually occurs only when a single unit of blood or

blood component, obtained from the donor during the incubation phase is given within 4 days of collection.

Other Herpes Viruses

Human herpes virus-6 (HHV-6) is a recently characterized virus, originally named HBLV (human B lymphotropic virus) for its ability to infect freshly isolated B cells. The virus was found in patients with various lymphoproliferative disorders. HHV-6 can infect monocytes, macrophages, T cells megalkaryocytes (Ablashi et al., 1987).The virus was found to be ubiquitous in blood donors when tested in London and the USA (Briggs et al., 1988). No transfusion-associated disease has been reported.

HHV-8, Kaposi's sarcoma virus is white cell associated and may be present in up to 30% of normal donors. Despite a relatively high HHV-8 seroprevalence in a Texas blood donor cohort (23%), HHV-8 DNA was not detected in any sample of donor whole blood using a highly sensitive PCR assay (Hudnall et al., 2003).

Human parvovirus B19

HPV B19 was discovered by an Austalian virologist in an assay for hepatitis B (Cossart et al., 1975). HPV B19 is a small single-stranded, non-enveloped, thermostable DNA member of the parvoviridae family (Young and Brown, 2004). The parvoviruses are dependent on help from host cells or other viruses to replicate. The red cell P antigen, a globoside present on a variety of cells in addition to erythrocytes, has been documented as the specific receptor for HPV (Brown et al., 1994).

Transmission of HPV by blood transfusion – HPV B19 has been transmitted by plasma fractionation products derived from large pool of plasma, particularly by factor VIII and factor IX concentrates (Blumel et al., 2002). Transmission of virus by single- donor components is unusual, but red cell unit has been associated with HPV B19 transmission and possible cardiac involvement in 22 years old women with thalassaemia major (Zanella et al., 1995).

West Nile Virus

West Nile Virus is a mosquito –borne flavivirus transmitted primarily to birds and some small mammals. Humans serve as an incidental host. Approximately 80%of human infections are assymptomatic, 20% result in a febrile illness known as West Nile Fever. About 1in 150 patients develop meningoencephalitis and residual neurological deficits have been reported. The period of infectivity has not been well defined. Of the 23 well-studied patients with transfusion transmitted infection, 14 were identified. The illness began between 2 and 21 days after the implicated transfusion. Red cells, platelets and fresh-frozen plasma (FFP) have all been implicated in transmissions. Six cases of WNV transmitted by transfusion occurred because of transfusion of components containing low levels of virus not detected by the testing of pooled specimens (Macedo et al., 2004). Other flaviviruses such as St Louis encephalitis virus, Japanese encephalitis virus and dengue virus are likely to be blood transmissible as well, although documentation is lacking.

Simian foamy virus

Simian foamy virus (SFV) (spumaretrovirus) is a highly prevalent retrovirus that has been shown to infect human cells. Tropisim is broad and includes B and T lymphocytes, macrophages, fibroblasts, endothelial cells and kidney cells. Persistent viremia has been detected in peripheral blood lymphocytes. (Boneva et al., 2002) Evidence of SFV infection included seropositivity, proviral DNA detection and isolation of the virus. There is no evidence as yet that SFV causes human disease but, recombination within the host, especially in immunocompromised hosts that may allow persistent infection, remains a concern.

Creutzfeldt-Jakob Disease (CJD)

A degenerative and fatal nervous system disorder. Affected individuals can remain asymptomatic for decades after infection and then progress rapidly to dementia, severe loss of coordination and death. The risk of CJD being transmitted through Blood products is ‘theoretical.’ However, all Blood banking organizations in Europe, and now in the United States, prohibit Blood donation by individuals who have symptoms or a family history of symptoms.

Unlike classic CJD, vCJD usually affects younger subjects (age 18-53 years) and is characterized by early psychiatric and sensory symptoms.

Prion protein has been identified on a wide range of circulating cells including platelets, myeloid cells, lymphocytes and red cells (Dodelet and Cashman, 1998; Holada and Vostal, 2000; Bessos et al., 2001; Li et al., 2001). Findings in experimental models show that blood not only contains infective agents of prion diseases, but that no barrier to transmission exists with intraspecies transmission and that the intravenous route of exposure to prions is fairly efficient (Casaccia et al., 1989; Cervenakova et al., 2003). In 2004, the first probable transfusion associated case of vCJD was described (Llewelyn et al., 2004).

Transmissible Spongiform Encephalopathy (TSE) agents are resistant to a range of physical and chemical means that have been used to inactivate viruses in plasma products. However, processes used to purify proteins, including factor concentrates can contribute significantly to removing both abnormal prions and infectivity (Lee et al., 2001).

Blood donors are carefully questioned about family history of CJD and surgeries that involved transplanted dura mater. If they answer affirmatively to any of these questions, they are permanently deferred as a donor. We see here again the potential danger in the 'honor system' of Blood donation in the United States. There is no possibility of contracting CJD by making a normal Blood donation.

Leishmaniasis

Cases of transfusion-associated Leishmaniasis are growing each year world wide. This increase is increasingly associated with patients who are positive for HIV. Transfusion-associated Leishmaniasis requires that the parasites be present in the peripheral Blood of the donor, survive processing and storage in the Blood bank, and infect the recipient. *L.tropica* has been demonstrated to survive in stored blood under blood bank conditions for 25 days (Grogl et al., 1993). In endemic areas where the population of potentially infected individuals may be much higher and the screening process for donors less rigorous, transfusion-associated Leishmaniasis is more common. Leishmaniasis is now found in over 90 countries. Again here we see the fact of world travel by a diverse population, and the 'honor system' Blood donor screening process, and too expensive testing, all contribute to the increase of transfusion transmitted Leishmaniasis.

Malaria

The popular statement, routinely given is that "malaria is rarely transmitted by Blood products." The number of transfusion associated cases of malaria, however, is at an all-time high. Malaria can be transmitted by the transfusion of any blood component likely to contain even small numbers of red blood cells; platelet and granulocyte concentrates, fresh plasma and cryoprecipitate have all been incriminated. An inoculum containing as few as 10 parasites can cause *Plasmodium vivex* malaria (Bruce-Chwatt, 1972).

Malaria parasites of all the species can remain viable in stored blood for at list 1 week (Hutton and Shute 1939) Malaria is particularly serious in pregnant women and in spleenactomised or immunosuppressed patients (Bruce-Chwart, 1985).

There are no practical laboratory tests available to test donor blood, so donors traveling to high risk malaria areas are often deferred from donating blood for six months.

Chagas Disease

Discovered a century ago by Brazilian doctor Carlos Chagas, this disease, properly named Chagas' Disease, is caused by a parasite that infects an estimated 18 million people worldwide, causing death from heart and digestive problems. Up to 20% of infected people never exhibit symptoms. Because of recent shifts in population, individuals from countries where this disease is common (South and Central America) are migrating in large numbers to the United States and other countries.

Babesiosis

An intraerythrocytic parasitic infection caused from the bite of the infected Ixodes tick. The disease closely resembles in some ways Lyme Disease, and in other ways, malaria. This significantly affects the hematological system, causing among other things, hemolytic anemia, thrombocytopenia, and atypical lymphocyte formation. The transmitted parasite only infects red blood cells by altering the cell membranes that causes decreased conformability and increased red cell adherence, which, in turn, can lead to development of acute respiratory distress syndrome (ARDS) among those severely affected. Babesia parasites invade and survive within erythrocytes. These blood-borne parasites remain viable under blood bank conditions. In Europe,

Babesiosis is a life-threatening disease and is a significant public health problem in regions of the northeastern United States. Of patients with Babesiosis, 84% are asplenic, and 53% become comatose and die. Those individuals with a history of the disease are to be permanently deferred from donating blood, if they know and admit before blood donation that they have carried the malady.

Toxoplasmosis

A systemic protozoan infection that causes symptoms similar to infectious mononucleosis. In immunocompromised individuals this infection can have serious neurological symptoms and can cause fetal death in pregnant women. Toxoplasmosis is also transmitted by common house cats.

Bacterial Contamination of Blood Products

This is another less often observed risk disorder directly associated with blood transfusion. It is increasingly rare but a very serious complication of blood transfusion. Most commonly associated with contamination during blood collection or during handling of blood products, such as preparation of platelet pools, and on occasion, associated with bacterial infection of the donor, it is sometimes recognizable by obvious changes in the appearance of the blood product. Studies indicate that the rate of contamination of blood products by bacterial pathogens may be significant. Since blood recipient death continues to occur, in 1997, the CDC entered into an agreement with national Blood collection and distribution agencies to determine the frequency of transfusion reactions associated with bacterial contamination of blood products. The new study will be a critical step in addressing this issue and will increase clinicians' awareness of bacterial contamination as a cause of transfusion reactions. Currently, the nation's blood supply is not screened for bacterial contamination. Amazingly, when this contamination issue is raised, blood donor deferral is merely recommended, and not mandatory.

Some common transfusion transmitted bacterial infections are as follows…

Treponema pallidum

Treponema pallidum is a motile spirochaete that spreads by sexual contacts, transfusion, percutaneous exposure and transmission from mother to infant. The incubation period from transfusion to clinical presentation varies from 4-weeks to 4.5

months,averiging 9-10 weeks. Donors at any stage of the disease,including late,latent syphilis, can transmit infection (Hartmann and Schone, 1942). Transfusion-transmitted syphilis was once considered a serious problem. Some 138cases had been reported by 1941 (De Schryver and Meheus, 1990). Since then, few cases have been reported in the developed world. The chief reasons for the decline of transfusion – transmitted syphilis seem to be almost universal practice of storing blood at 4degree C before transfusion, universal donor testing and the decline in the prevelance of syphilis in many countries. Spirochaetes are unlikely to survive in citrated blood stored for more than 72 hours at 4-6degree C (Bloch, 1941). Serological tests cannot prevent all cases of transfusion syphilis because most remain negative in early primary syphilis, when spirochaetaemia is most prominent (Spangler et al., 1964). The rational for continued syphilis testing relies upon the increasing demands for fresh blood components,specially platelets and fresh blood for exchange transfusion in new born infants. (Risseeuw-Appel and Kothe, 1983) and its questionable value as a surrogate test to exclude donors who are in high-risk groups for HIV and HBV infection.

Brucella abortus

This organism can survive for months in stored blood and ther are several reports of blood transfusion-transmitted symptomatic infection, mainly in children and splenectomizwd patients (Wood, 1955; Tabor, 1982). After an incubation period ranging from 6 days to 4 months, recipients of infected blood may develop undulant fever, headache, chills, exessive sweating, muscle pains and fatigue. Hepatosplenomegaly, lymphadenopathy, leucopenia and arthritis occur and vary rarely, complications such as purpura, encephalitis or endocarditis develop (Tabor, 1982).

Lyme Disease

The organism responsible for this disease is *Borrelia purgdorferi, a spirochaete.* Lyme disease is associated with the bite of the eastern deer tick, and can cause an illness that affects many systems within the body.

Although no cases of transmission of *B. burgdorferi* by blood transfusion has been reported so far, transmission is theoretically possible. The spirocheate has been

isolated from blood as old as 14 days. Donors with a history of Lyme disease can not donate Blood unless they no longer have symptoms whatever, have undergone a full course of antibiotic treatment, and are cleared by a physician. Public health and Blood agencies are closely monitoring this disease.

Exogenous and various endogenous bacteria and bacterial products contaminating stored blood or blood components

1. Bacteria may contaminate solutions or equipments to be used for transfusions but which have not yet been sterilized. After sterilization the solutions or equipments may remain contaminated with heat-stable bacterial products (pyrogenes). In contaminated solutions or equipments such as hydroxyethyl starch used for leucapheresis, bacteria may survive 'sterilization' or may contaminate solutions that have previously been sterilized, for example when a glass container is cracked during shipment (Wang et al., 2000).

2. Bacteria originating from skin flora, such as *Staphylococcus epidermis*, *Micrococcus* species, *Sarcina* species and diptheroids, may gain entrance during venesection. (Anderson et al., 1986).

3. Bacteria in the environment (*Pseudomonas* species, *Flavobacterium* species and *Bacillus* species) may gain entrance to blood components through minute lesions in the packs, during collection or processing in open systems (Szewzyk et al., 1993).

4. Bacteria circulating in the blood of an apparently healthy donor suffering from asymptomatic bacteremia may proliferate in red cell components stored at 4ºC or in platelet concentrates stored at room temperature.

CHAPTER 3

MATERIAL & METHODS

CHAPTER 3

MATERIALS AND METHODS

Study Group

We selected four groups of patients undergoing multiple blood transfusions. Groups are as follows.

1. A group of 130 thalassemic patients at K.T. Children Hospital, Rajkot initially included in the study in the year of 2003. At the end of the study we had registered 218 patients. These patients had been receiving blood transfusions regularly at K.T. Children Hospital since 2003 till the end of the study period. Patients who had received at least 2 previous blood transfusions were included for serological follow up for 5 successive years (2003, 2004, 2005, 2006 and 2007). Transfusion and clinical records of all patients were maintained. About 3 ml blood sample was collected and samples were preserved.

   Analysis of 121 patients was done at the end of the study and the others were excluded due to various reasons (05 patients moved, sample was inadequate in 02, one patient died and one was lost to follow up).
2. At Hemophilia Society, Rajkot a group of 156 hemophilic patients were registered initially in the study, at the end of the study in 2008 we have registered 232 hemophilic patients. Transfusion and clinical records of all patients were maintained throughout the study.
3. A group of 115 CRF patients at B. T. Savani Kidney Hospital, Rajkot undergoing hemodialysis were registered in 2003; at the end of the study in 2008 we registered 220 patients. We have recorded all the details of the patients like age, sex, prior H/O transfusion, frequency of dialysis etc.
4. We selected a group of patients from a multi speciality hospital who went for surgical procedure or any procedure that led them to multiple blood transfusions. Number of patients registered in 2003 was 58 and at the end of the study in 2008 the count was 61. We have recorded all the clinical history and transfusion records of all patients.

5. We selected a small group of Leukemic patients who went on multiple blood transfusions. We registered 10 patients for our study and we have recorded all clinical and transfusion history of all patients.

Serological study

Frozen samples were tested after the study period for various viral markers in the same laboratory by one person using the same batches of reagents and kits. Tests were carried out by commercially available, third generation, enzyme linked immunosorbent assay (ELISA) for the following TTD markers: (i) HBsAg (Microscreen HbsAg ELISA Test Kit by Span Diagnostics) (ii) antibodies to HCV (SP- NANBASE C-96 3.0 test kit by General Biological Corp.); (iv) anti HIV I/II (Enzaids HIV 1+2 ELISA test kit by Span Diagnostics); and (v) antibodies to CMV (IgM) (Equipar diagnostic kit.). Tests could not be repeated due to lack of serum samples and in some cases because of high reagent cost. We have done hematological studies of all samples like blood indices as well as bacteriological studies like culture of samples.

Screening for HIV

Introduction

Test was done by Enzaids HIV 1+2 ELISA test kit by Span Diagnostics.

AIDS is caused by Human Immunodeficiency Viruses viz. HIV Type-1 and HIV Type-2, as the etiological agents (Schupbach et al., 1984; Gallow et al., 1984) The serological analysis of the viruses, collectively referred to as HIV 1+2, laid the foundation of an ELISA based assay system for the qualitative detection of antibodies, in the human serum or plasma, indicating an exposure to the virus.

Conventionally used viral lysate proteins or their constituent polypeptides are now substituted by similar synthetic and/or recombinant (non-infectious) antigens (Schneider et al., 1987). Enzaids, with the use of these antigens, ensures highest level of specificity and sensitivity of the detection system.

Principle

The antigenic peptides/ proteins are adsorbed onto the wells of a microplate. These antigens are selected from immunodominant regions of HIV-1 and HIV-2, so as to provide maximum level of sensitivity. Special sample diluents is developed using proprietary formulation to minimize the non-specific binding. Test serum and controls, along with sample diluents are added to the respective wells and incubated. The conjugate binds to HIV antibodies that are already bound to immobilized antigens. A wash step removes the unbound conjugate. The substrate for the enzyme peroxidase and the chromogen 3,3',5,5' Tetramethylbenzidine (TMB) are added to all the wells and incubated further, resulting into a blue colored complex. The reaction is terminated by the addition of a stopping solution yielding a stable yellow colored end point. The intensity of color is proportional to the concentration of anti HIV-1 and/or HIV-2 present in the test specimen or control.

Contents of the Kit

Reagent: 1

Sample Diluent: Tris buffer, proteins and preservatives.

Reagent: 2

Conjugate: Peroxidase labeled anti-human immunoglobulin containing protein stabilizers and 0.01% Thimerosal as preservative.

Reagent: 3

Washing Buffer: Concentrated buffer containing Tween-20 and 0.01% Thimerosal as a preservative. Before use, dilute by adding one volume of concentrate to 9 volumes of distilled water.

Reagent: 4

Negative Control: Anti-HIV negative human serum, negative for Anti HIV-1 and Anti HIV-2 and containing 0.01% Thimerosal as preservative.

Reagent: 5

Positive Control: Inactivated serum containing anti-HIV antibodies and 0.01% Thimerosal as preservative.

Reagent: 6

Color reagent: Buffer containing hydrogen peroxidase and 3, 3', 5, 5' Tetramethyl benzadine (TMB) in solution.

Reagent: 7

Stopping Solution: Mineral acid.

Reagent: 8

Microwell strips: Coated with HIV 1+2 Recombinant and synthetic peptides.

Assay Procedure

- All the reagents were brought to room temperature before use, except color reagent. Required number of Microwells/ strips was removed from the packet. The wells were labelled appropriately.
- The reaction Blank well was left empty.
- 200 µl Sample Diluent (Reagent 1) was dispensed to rest of the required wells. Three Negative (Reagent 4) and one Positive (Reagent 5) controls in each run were used. 10 µl of Negative, Positive and test serum samples to the respective wells was added. The strips were mixed properly and covered with adhesive strip cover & Incubated for 30 minutes at room temperature (20°C-30°C).
- The adhesive strip cover was removed and discarded. The contents of the well were decanted into a waste container. The wells were filled with diluted Washing Buffer & allowed soak time of 30 seconds and then decanted in a waste container. The procedure was repeated for 4 more times. The wells were drained on a disposable absorbent pad or towel firmly to remove excess of fluid. Care was taken not to scratch the inner surface of well with pipette tips or tissue paper.
- 50 µl of Conjugate (Reagent 2) was added to each well, except the one used for the Reaction Blank Control.
- The contents of the Microwells were mixed by agitating the strips gently for 5-10 seconds.
- The strip was covered with fresh adhesive strip cover and incubated for 30 minutes at room temperature (20°C-30°C).
- Remove and discard the adhesive strip cover. Wash strip as in Step 4, five times with diluted Washing Buffer.
- 100 µl of Color Reagent (Reagent 6) was added into each well including Reaction Blank Control.
- It was incubated at room temperature for 15 minutes in dark.

- The reaction was stopped by adding 100 µl of Stopping Solution (Reagent 7) in all the wells.
- The contents of Microwells were mixed by agitating the strips gently for 50-10 seconds.
- Using bichromatic (450+630 nm) mode, absorbance readings were taken after blanking with A1 (Reagent blank) well.

Calculation for Determining Cutoff Value

Negative Control Mean (NCx)

Determine the Mean of Negative Control as shown below.

Example

Sample no.	Absorbance
1	0.064
2	0.055
3	0.076
Total:	0.195

NCx = 0.195/3 = 0.065

Positive Control

To consider the assay run to be valid, the absorbance value obtained for the Positive Control should be at least 1.00. If not, the assay must be repeated making sure that the procedure is being properly followed.

Blank

The absorbance value (O.D.) of Reagent blank should fall between 0.000 and 0.100 in bichromatic mode.

Cutoff Value

The cutoff value is the mean O.D. of the Negative Control plus 0.225.

Example

NCx = 0.065

Cutoff Value = 0.065+0.225= 0.290

Interpretation of Results

All the samples with the absorbance less than cutoff value were considered Negative for anti-HIV and the absorbance equal to or more than cutoff value were considered Positive for anti-HIV.

Non reactive results in a test for detecting antibodies against HIV-1 and/or HIV-2 do not exclude HIV infection with absolute certainty, if the patient happens to be in the window period (Cordes et al., 1995).

Limitation of the Test

- A non reactive result with this test does not preclude the possibility of HIV infection.
- Demonstration of the presence of HIV antibodies in test serum has extensive serious social implications. Hence, confirmation of a reactive sample by an acceptable technique such as a Western Blot test is of vital importance.
- Test procedure must be followed meticulously.

Screening for HBsAg

Hepatitis B virus causes Acute Viral Hepatitis, a common yet serious infection. HBsAg appears early during the course of infection and normally serves as a marker for the primary diagnosis (Blumberg et al., 1965).

Principle of Test

Microscreen HBsAg is a direct, non-competitive solid phase enzyme immunoassay for detection of various subtypes (ad & ay) of HBsAg in Serum or Plasma.

1. Wells are coated with immobilized HBsAg antibodies in which Test Serum or Plasma is added. If HBsAg is presnt in Test Serum or Plasma it will combine with HBsAg antibodies coated on wells. This complex further combines with peroxidase conjugate of anti-HBsAg antibody.
2. On additon of Substrate, chromogenic reaction with the enzyme produces a coloured end product.
3. This reaction is then stopped by addition of Mineral Acid and the absorbance is measured.

Kit components

Reagent 1

Sample Diluent: Tris Buffer, Proteins, Preservatives and Detergent.

Reagent 2

Conjugate: Monoclonal anti HBsAg antibodies conjugated with Peroxidase containing Protein Stabilisers and Preservatives.

Reagent 3

Washing Buffer: Concentrated Tris Buffer, containing Tween 20 & Thiomersol(0.01%) as Preservative. Before use, dilute by adding one volume of concentrate to 9 volumes distilled or Reagent Grade Water (Span product no.23668A or equivalent).

Reagent 4

Negative Control: HBsAg Negative Human serum, containing Preservative.

Reagent 5

Positive Control: HBsAg Positive Human Serum, containing Preservative.

Reagent 6

Color Reagent: Citrate Acetate Buffer, containing Peroxide & 3, 3', 5, 5' Tetramethylbenzidine (TMB) solution.

Reagent 7

Stopping solution: Mineral Acid

Reagent 8

Microwell Strips: Coated with anti-HBs Antibody.

Assay Procedure

Required number of Microwell strips to perform the test was taken out and the wells were labeled appropriately.

Remaining strips were kept in pouch provided, along with absorbent.

- 100 µL of Sample Diluent (Reagent 1) was added to Test and Control wells of the Microwell Strips (Reagent 8) except Blank well i.e. 1A. (as in Fig.). The Blank well was left empty.
- 100 µl of Negative Control (Reagent 4) was added to 1B, 1C & 1D and 100 µL of Positive Control (Reagent 5) to 1E and 1F. To rest of the wells, 100 µL of samples were added to the rest of the wells.

- Contents were properly mixed and the Microwell was covered with Adhesive Strip Covers provided and allowed to stand at Room Temperature for 60 minutes.
- The Adhesive Strip Covers were removed and discarded. The content of the wells were decanted into a waste container. The wells were filled with approximately 350 µL of diluted Washing Buffer (Reagent 3) and allowed soak time of 30 seconds per well (the Auto washer was programmed for soak time of 30 seconds) and then decanted in the waste container. The procedure was repeated for 4 more times. The wells were drained on a disposable absorbent pad or towel and tapped firmly to remove excess of fluid. Care was taken not to scratch the inner surface of the well with pipette tips or tissue paper.
- 50 µl of Conjugate Stabilizer (Reagent 2A) was added first, followed by 100 µL of Conjugate (Reagent 2) to each well except 1A i.e. Blank well. The contents of Microwells were mixed and the strips were covered with fresh Adhesive strip covers provided.
- It was incubated for 30 minutes at Room Temperature and then the Adhesive Strip Covers were removed and discarded.
- Each Microwell was washed five times.
- Microwells were tapped on a fresh disposable absorbent pad or towel and tapped firmly to remove all moisture present in the wells. Care was taken not to scratch the inner surface of well with pipette tips or tissue paper.
- 100 µl of Colour Reagent (Reagent 6) was added in all the Microwells including the Blank well. It was covered with fresh Adhesive Strip Covers.
- Incubated at Room Temperature far 30 minutes.
- Adhesive Strip Covers were removed and discarded and 100 µL of Stopping solution was added (Reagent 7) to all the Microwells to stop the reaction.
- The contents of the Microwells were mixed well by agitating the strips gently for 5-10 seconds.
- The results were read in Bichromatic (450+630 nm) mode. Absorbance readings were taken after blanking with A1 well (Reagent Blank). Readings were taken within 30 minutes of addition of Stop Solution (Van Weemen et al., 1971; Voller et al., 1978 and Fields et al., 1983).
    - Absorbance of Negative Control Should be <0.2

- Absorbance of Positive Control Should be >1.00
- Absorbance of Reagent Blank should be <0.100, when read in Bichromatic mode.

Cut Off Calculation

The Cut-off value was calculated based on the mean absorbance of 3 Negative Controls and addition of a factor (0.10) i.e.

Cut off = NCX + 0.10

Interpretation of Results

1. All samples with absorbance less than Cut off value were considered Non-Reactive for HBsAg.
2. Samples with absorbance more than Cut off value were considered Reactive and rested to confirm the positivity.

Screening for the detection of Hepatitis B by PCR

Hepatitis B Virus DNA detector Test was done at Ranbaxy Laboratories, Mumbai.

Detection of HBV by PCR would be desirable for a number of reasons…

a) Direct viral detection should contribute significantly in clarifying the status of individuals with intermediate serologies.
b) Variant strains of HBV cannot produce HbeAg in serum when an active infection is present. Therefore HbeAg to monitor disease progression may be of limited utility (Hoofnagle, 1990).
c) Also individuals under treatment have undetectable amounts of HbeAg but actively replicating HBV.

Method

For the quantitative assay, viral DNA was isolated from serum (Ansubel et al., 1990). For PCR amplification primers complimentary to the pol gene of the virus was used as it is highly conserved (Baginski et al., 1990). Detection of amplified sequence was done by 2% agarose gel electrophoresis (Sambrook, 1989).

Interpretation:

Each sample was run with a positive and a negative control visualizing a band of 244 bp in the test sample and positive control compared against a low mass DNA ladder confirmed HBV positivity. The negative control should not have any bands. Because of the exquisite sensitivity of PCR, special care are being taken to avoid cross

contamination of samples beginning from the collection till end of testing to avoid any false positive results.

Screening for HCV

Test was done by third generation Enzyme Immunoassay Kit for the detection of antibody to Hepatitis C Virus (Anti- HCV) in human serum or plasma by HCV MICROLISA.

Introduction

Hepatitis C is a disease caused by viral infection, which is primarily a result of blood transfusions or improper needle punctures (Dienstag, 1983). Since Hepatitis C is a significant problem for public health management, screening for Hepatitis C, therefore is urgently needed (Alter 1988; Tabor et al., 1980).

Principle of the Test

The kit adopts the second antibody "sandwich principle" as the basis for the assay to detect antibodies to Hepatitis C Virus.

The SP-NANBASE C-96 3.0 is an enzyme immunoassay kit, which employs synthetic HCV peptides and recombinant HCV antigens for the detection of antibodies to HCV in human serum or plasma. These antigens, which are reactive with the predominant antibodies of HCV, constitute the solid phase antigenic absorbent. When human serum or plasma added to the well, the HCV antigens and Anti-HCV will form complexes on the wells if Anti-HCV is present in the specimen. The wells are washed to remove the unbound materials. The Conc. Anti-Human IgG-HRPO Conjugate is added to the well results in the formation of (HCV)·(Anti-HCV)·(Anti-human-IgG-HRPO) complex. After washing out the unbound conjugate, TMB substrate solution is added for the color development. The intensity of color development is proportional to the amount of antibodies present in the specimen.

Contents of the kit

1. HCV Antigens Plate: Microtiter plate coated with HCV antigens.
2. Concentrated Anti-Human-IgG·HRPO Conjugate: Contained anti human IgG, Peroxidase in buffer with Bovine serum. Preservatives: 0.01% Thimerosal and 0.003% Gentamycin.
3. Anti-HCV Positive Control: Inactivated human plasma positive for antibody to HCV. Preservative: 0.1% Sodium azide.

4. HC Negative Control: Normal Human Plasma non-reactive for antibodies to HCV. Preservative: 0.1% Sodium azide.
5. Specimen diluents C: Tris-buffer with bovine serum and Tween-20. Preservative: 0.1% Sodium azide.
6. Conjugate diluents: Tris-buffer with bovine serum and Tween-20. Preservatives: 0.01% Thimerosal and 0.03%Gentamycin.
7. TMB Substrate solution A: 0.6 mg/ml of 3, 3', 5, 5'- Tetramethyl-benzidine solution.
8. TMB Substrate solution B: Citric acid buffer with H_2O_2.
9. Washing Solution D Concentrate: Phosphate buffer with Tween-20.
10. 2 N Sulfuric Acid.

Test Procedure

1. All reagents and specimens were brought to room temperature (20-30°C) before beginning the assay. The reagents were gently mixed well before use.
2. Two wells were kept reserved for blanks. No specimen or specimen diluents were added in that.
3. Needed number of wells for Blanks was prepared, two wells for Negative control, three wells for Positive control, and one well for each specimen.
4. Sampling: 1:21 dilution of each control and specimen with specimen diluents were made.
5. 10 µl of each control or specimen into the well of pre-dilution plate was dispensed.
6. 200 µl of specimen diluents was added to each well and mixed well.
7. 100 µl of each diluted control or specimen was transferred to the corresponding wells in HCV Antigens Plate.
8. The plate was sealed with an adhesive slip & incubated in a 37 °C water bath or circuited incubator for 60 minutes.
9. At the end of the incubation period, the adhesive slip was removed and discarded and the plates were washed by following the plate washing procedure.
10. 100µl of the diluted Conjugate was added in each well except two blanks. The plate was sealed with an adhesive slip, and incubated as in step 8 for 30 minutes.
11. Step 9 was repeated.

12. Equal volume of TMB Substrate Solution A and Substrate Solution B were mixed in a clean container immediately prior to use. 100 µl of mixture was added to each well including two blank wells.
13. The plate was covered with Black Cover and incubated at RT for 30 minutes.
14. The reaction was stopped by adding 100 µl of 2 N Sulfuric Acid to each well including two blanks.
15. Absorbance of Controls and Test Specimens were determined within 30 minutes at 450 nm with a dual filter instrument.

Calculation and Determination

1. Calculation of NC_x

   Example:

NC	Absorbance
1	0.045
2	0.060

   NCx = (0.045 + 0.060) ÷ 2 = 0.053

2. Calculation of PC_x

   Example:

PC	Absorbance
1	1.510
2	1.826
3	1.305

   PCx = (1.510 + 1.826 + 1.305) ÷ 3 = 1.547

3. Calculation of P-N Value

   P-N = PCx – NCx

   Example: 1.547-0.053 = 1.494

   P-N value must be ≥ otherwise the test is invalid.

4. Determination of Cutoff Value and Cutoff Index

   Cutoff value = NCx + 0.25 X PCx

   Example:

   Cut off Value = 0.053 + 0.25 X 1.547

   = 0.053 + 0.387

   = 0.440

   Cutoff Index = Sample OD Value/ Cutoff Value

Example:

Sample Value is 0.596

Cutoff index = 0.596/0.440 = 1.355

Interpretation of result

- Specimens with absorbance values LESS than the CUTOFF VALUE were considered NON-REACTIVE by the criteria of GBC's SP-NANBASE C-96 3.0
- Specimens with absorbance values GREATER than or EQUAL to the CUTOFF VALUE were considered initially REACTIVE. They were retested in duplicate. If both CUTOFF INDEXES of the duplicate were GREATER than 1.5, the specimen was considered to be repeatedly REACTIVE for antibodies to HCV by the criteria of GBC's SP-NANBASE C-96 3.0
- Initially reactive specimens, of which both CUTOFF INDEXES of the duplicate retest are LESS than 1.0, was considered NON-REACTIVE for antibodies to HCV.

Screening of CMV IgM

Introduction

Cytomegalovirus (CMV) infections are widespread and approximately half of the adult population has antibodies to CMV. CMV infections are frequent in individuals with deficient cellular immunity such as cancer patients or person with AIDS, or those receiving immunosuppressive agents. About 2% of pregnant women have either a primary or a reactivated CMV infection during pregnancy and it is estimated that 10-20% of congenitally infected newborns will show evidence of disease (Marx 1985, Kalmin 1981) (Stern and Tucker 1973). The detection of antibodies against CMV may be value as an aid in the diagnosis and in determining the immune status of the patient (Ahlfors, 1981). The test is done by Equipar diagnostic kit.

Principle

CMV antigens are fixed to the interior surface of microwells. Patient's serum is added and antibody present to cytomegalovirus will bind to these antigens. The microwells are washed to remove unbound serum proteins. Antibodies conjugated with Horseradish Peroxidase enzyme and directed against human IgG are added and will in turn bind to any human IgG present. The microwells are washed to remove any unbound conjugate and then chromogen/substrate is added. In the presence of

peroxidase enzyme the colorless substrate is hydrolyzed to a colored end-product. The color intensity is proportional to the amount of antibodies present in the patient's serum.

Components:

1. Microwell Plate
2. Negative control
3. Low positive control
4. High positive control
5. Sample diluents
6. Wash solution
7. Enzyme conjugate
8. TMB- Substrate
9. Stop solution

Assay procedure

- Required numbers of microwells were placed in the microwell holder. One end of each strip was marked for orientation.
- The samples were diluted 1:100 with serum diluents. As calibrators were ready for use they were not diluted.
- 100 µl of negative control, low positive control and high positive controls were pipetted into subsequent wells.
- The microwells were incubated at room temperature for 15 minutes.
- Microwells were washed by inverting and flicking them into a sink. Wash was repeated 3 times with wash buffer.
- 100 µl of Enzyme conjugate was pipetted into each well.
- The microwells were incubated at room temperature for 15 minutes.
- Microwells were washed.
- 100 µl of TMB-Substrate was pipetted into each well.
- The microwells were incubated at room temperature for 5 minutes.
- 100 µl of Stop solution was pipetted into each well using the same pipetting sequence.
- Color intensity of the solution in each well was measured by using a microwell reader with a 450 nm filter.
- Standard curve was prepared and results were calculated.

Quality control

Each time the assay is run the Low Positive and the High Positive Standard should be run in duplicate. The mean OD values for the standards should fall within the following ranges:

Control	OD Range
Negative control	<0.250
Low positive control	>0.300
High positive control	>0.700

The results are reported as Activity Index Values.

Reporting results: The activity index values of the controls and the serum samples were read from the standard curve prepared for each run.

Syphilis screening test

Principle

The RPR Syphilis screening test is a macroscopic non-treponemal flocculation card test for detection and to quantify the regain, an antibody like substrate present in serum or plasma and spinal fluid from syphilitic persons.

Qualitative test procedure

- All reagents and samples were brought to room temperature.
- Using the disposable sample dropper, one drop of serum was dispensed onto a separate circle on the test card. Fresh disposable sample dropper for each sample was used. The positive and negative control sera were kept on respective circles on test card.
- Using the disposable stirring rod, spread the sample was spread over the entire area of the test circle.
- The carbon antigen was mixed well and one drop of "free fall" Antigen suspension was placed onto each specimen using 20G dispensing needle.
- The card was placed on a rotator and rotated for 8 minutes at 100 rpm. Immediately after 8 minutes rotation, the results were read macroscopically in good light. (McGrew et al., 1968; Hunter et al., 1964 and Larsen et al., 1981)

Qualitative test results

Reactive: The presence of large aggregates in the center or the periphery of the test circle.

Weakly Reactive: The presence of small or fine aggregates.

Non- Reactive: Smooth grey appearance with no aggregates visible.

Culturing of blood

Bacteraemia

The presence of the bacteria in the blood is called bacteraemia. It is usually pathological although transitory asymptomatic bacteraemia can occur during the course of many infections and following surgical procedures.

Septicemia

This is a clinical term used to describe severe life threatening bacteraemia in which multiplying release toxins into the blood stream and trigger the production of cytokines causing fever, chills, toxicity, tissue anoxia, reduced blood pressure, and collapse. Septic shock is usually complication of septicemia with Gram negative bacilli, and less frequently, Gram positive organisms.

Procedure

- 10-12 ml blood was inoculated into Columbia agar diphasic medium (Hi-Media Make) aseptically.
- The medium was incubated up to 7 days.
- Simultaneously, buffy coat smear was prepared from EDTA blood.
- Smear was stained with Gram's stain & Giemsa stain.
- The smear was examined microscopically and reported accordingly.
- After an overnight incubation the diphasic culture medium was examined for growth and turbidity.
- Subculture was done on Blood Agar & MacConkey agar from 2^{nd} day onwards.
- Diphasic culture media were re-incubated.
- Blood agar & MacConkey agar were examined for bacterial growth (Cheesebrough, 2000).

Examination of blood for malaria parasites

Malaria is a major public health problem and cause of suffering premature death in tropical and subtropical countries. In many endemic areas, it is becoming increasingly difficult to control because of the resistance of the parasite to anti-malarial drugs and the failure of vector control measures.

Detecting and identifying malaria parasite in blood films

Blood was collected, followed by capillary blood method for Malaria blood films preparation. EDTA venous blood also can be used.

Capillary blood method (Thin and thick blood films on the same slide)

- Using a completely clean grease- free microscope slide a small drop of blood was added to the centre of the slide and a larger drop about 15 mm to the right.
- The thin film using a smooth edged slide spreader was spread immediately. Blood from anemic patients needed spreading more quickly with spreader held at a steeper angle.
- Without delay, the large drop of blood was spread to make the thick smear. The slides were labeled with the date and the patient's name and number.
- The blood films were allowed to air dry with the slide in a horizontal position and placed in a safe place.
- Absolute methanol and ethanol were used to fix thin blood films.
- Staining of malaria parasite was done by Field's stain - one of Romanowsky stain

Reporting

When the slide is completely dry after staining procedure, a drop of immersion oil was applied to an area of the film which appeared mauve colored.

Malarial parasites were examined in microscope using the 40X and 100X objectives.

The presence of parasites (trophozoites, schizonts, gametocytes) was reported and also whether malaria pigment is present in white cells.

Screening of Hematological parameters

All the samples were screened by poCFH-100i Automated Hematology Analyzer by Sysmax, Transasia.

It shows parameters like WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT, LYM#, LYM%, MXD#, MXD%, NEUT#, NEUT%, RDW-SD, RDW-CV, PWD*, MPV, P-LCR.It shows histogram of WBC, RBC and PLT.

Detection principles

WBC : Electric impedance method.

RBC/ PLT : Hydrodynamic focusing DC detection method.

HGB : Non-Cynaide hemoglobin analysis method.

Test procedure: Serum samples were run on poCH – 100i which is an auto analyzer instrument.

Screening of blood groups (ABO)

Monoclonal agglutinating sera were used for the determination of human blood groups (ABO) by Span Diagnostic kit.

Introduction

Discovery of ABO blood grouping in 1900 by Karl Landsteiner was a milestone in the history of Immunohematology. He reported the presence of two antigens viz. A and B, on the surface of human red blood cells (RBCs). Based on this discovery, he divided RBCs into three groups viz. A, B and O. Very soon, in 1902, a fourth group viz. AB was discovered by Decastello and Sturli. ABO blood group system is the most significant blood group system used for the transfusion and organ transplantation purposes. According to this system, the blood of each individual carries an antibody directed against the antigen which is absent from the person's RBCs.

Principle

Test is based on hemagglutination reaction. Human RBCs possessing A and/or B antigen will agglutinate with the corresponding antibody. Agglutination of RBCs with SPANCLONE ANTI-A Monoclonal, SPANCLONE ANTI-B Monoclonal and/or SPANCLONE ANTI –A B Monoclonal is a positive result and indicates the presence of the corresponding antigen. Absence of agglutination of RBCs with SPANCLONE ANTI-A Monoclonal, SPANCLONE ANTI-B Monoclonal and/or SPANCLONE ANTI-AB Monoclonal is a negative test result and indicates the absence of the corresponding antigen. SPANCLONE ANTI-AB is especially useful in detecting the

weak variants of A and B which are occasionally missed by the routinely used Monoclonal ANTI-A and ANTI-B sera. (Dacie and Lewis, 2001)

Reagents

SPANCLONE ANTI-A Monoclonal

SPANCLONE ANTI-B Monoclonal

SPANCLONE ANTI-AB Monoclonal

Assay Procedure

- 10% suspension of RBCs in normal saline was prepared. Alternatively, oxalated whole blood was used.
- Three circles were marked on a glass slide and labeled them as A, B and AB.
- One drop of the appropriate blood grouping reagent was placed at the above marked area.
- One drop of 10% cell suspension or oxalated whole blood was placed to each of the marked area.
- With the separate applicator sticks the contents were mixed well and spread over an area of approximately 2cm in diameter. The slide was tilted back and forth for 2 minutes.
- The evidence of agglutination was observed macroscopically (Mollison, 1997).

Interpretation of result

Positive: Agglutination indicates positive reaction.

Negative: No agglutination indicates negative reaction.

Possible reactions with SPANCLONE ANTI-A Monoclonal, SPANCLONE ANTI-B Monoclonal and SPANCLONE ANTI-AB Monoclonal and their interpretations are shown in the following table.

Screening of ANTI-D (Rho)

The test was done by using kits of Span diagnostics

Introduction

About 95% of the Indian population and 85% of the Caucasian origin possess D (Rho) antigen on their erythrocytes. Human red blood cells are classified as “Rh-Positive” or “Rh-Negative” depending upon the presence or absence of this antigen on their

surface. SPANCLONE ANTI-D (Rho) Monoclonal is used for the detection of the presence of Rho antigen on the red blood cells.

Principle

Human red blood cells possessing D antigen are agglutinated by the antibody, directed against D (Rho) antigen.

Assay Procedure

- Whole blood or 40% suspension of red blood cells in saline was used as specimen.
- One drop each of SPANCLONE Anti-D (Rho) Monoclonal and whole blood 40% suspension was placed on a clean glass slide.
- The contents were mixed well with an applicator stick, and spread over an area of approximately 2 cm in diameter. The slide was tilted back and forth for 2 minutes.
- The evidence of agglutination was observed macroscopically (Thomson et al., 1986; Goossens et al., 1987).

Interpretation of result

Presence of agglutination indicates D (Rho) Positive, cell type.

Absence of agglutination generally indicates D (Rho) Negative, cell type.

Screening for S.G.P.T. detection specially in Thalassemic patients

All the samples were screened by S.G.P.T. IFCC method, Kinetic by ERBA S.G.P.T. Test kit.

Principle

L-Alanine + 2-Oxoglutarate $\xrightarrow{ALT}$ Pyruvate + L- Glutamate

Pyruvate + NADH $\xrightarrow{LDH}$ L- Lactate + NAD

ALT: Alanine aminotransferase

LDH: Lactate dehydrogenase

Sample

Unhemolysed serum or heparinised plasma, Anticoagulants such as heparin or EDTA are suitable. ALT is stable for 3 days at 2-8ºC.

Assay procedure

- 100 µl test serum was added to 1000 µl of working reagent and aspirated to chemical analyzer.
- The reading was noted (Bradly et al., 1972; Wolf et al., 1972; Wroblewski et al., 1956).

Quality Control

To ensure adequate quality control each run should include assayed Normal and Abnormal controls.

Normal Values

Females: Up to 22 IU/L at 30°C or 0-31 IU/L at 37°C

Males: Up to 29 IU/L at 30°C or 0-40 IU/L at 37°C

Screening of Iron & TIBC Level by Crest Biosystems

Test is based on Ferrozine Method for the determination of Iron and Total Iron Binding Capacity in serum.

Summary

Iron found in blood mainly present in the hemoglobin of the RBCs. Its role in the body is mainly in the transport of oxygen and cellular oxidation. Iron is absorbed in the small intestine, and bound to a globulin in the plasma, called transferrin, and transported to the bone marrow for the formation of hemoglobin. Increased serum iron levels are found in hemolytic anemias, hepatitis, lead and iron poisoning. Decreased serum levels are found in anemias caused by iron deficiency due to insufficient intake or absorption of iron, chronic blood loss, late pregnancy and cancer. Increase in TIBC is found in Iron deficient anemias and pregnancy. Decrease in TIBC is found in hypoproteinemia, hemolytic/ pernicious/ sickle cell anemias, inflammatory diseases and cirrhosis.

Principle

Iron, bound to Transferrin, is released in an acidic medium and the Ferric ions are reduced to Ferrous ions. The Fe (II) ions react with Ferrozine to form a violet colored complex. Intensity of the complex formed is directly proportional to the amount of

iron present in the sample. For TIBC, the serum is treated with excess Fe (II) to saturate the iron binding sites on transferring. The excess Fe (II) is absorbed and precipitated and the Iron content in the supernatant is measured to give the TIBC.

Fe (III) in acidic medium →Fe (II)
Fe (II) + Ferrozine →Violet Colored Complex

Normal Reference Values

Serum Iron (Males) : 60 – 160 µg/dl
(Females) : 35- 145 µg/dl
(Neonates) : 150-220 µg/dl

TIBC : 250- 400 µg/dl
UBIC : 160- 360 µg/dl

Procedure

Wavelength/ Filter : 570 nm (Hg 578 nm) / Yellow
Temperature : Room temperature
Light Path : 1 cm

Iron Assay

The reagents and serum samples were pipette into clean dry test tubes labeled as Blank (B), Standard (S), Sample Blank (SB) and Test (T):

Addition Sequence	B (ml)	S (ml)	SB (ml)	T (ml)
Iron Buffer Reagent (L1)	1.0	1.0	1.05	1.0
Distilled water	0.2	-	-	-
Iron Standard (S)	-	0.2	-	-
Sample	-	-	0.2	0.2
Iron Color Reagent (L2)	0.05	0.05	-	0.05

The reagents were mixed well and incubated at R.T. for 5 min. The absorbance was measured of the blank (Abs. B), Standard (Abs. S), Sample blank (Abs. SB) and Test Sample (Abs. T) against D.W.

TIBC assay

The reagents and samples were pipette into a clean dry test tube

Serum	0.5 ml
TIBC Saturating Reagent (L1)	1.0 ml

The contents were mixed well and allowed to stand at RT for 10 min and added to that reagent 2 (L2)

TIBC Precipitating Reagent (L2)	Approx. 50 mg

The contents were mixed well and allowed to stand at RT for 10 min and centrifuged at 2500–3000 rpm for 10 min to obtain a clear supernatant. The iron content was determined in the supernatant as above mentioned iron assay (Siedel et al., 1984).

Calculations

Iron (µg/dl) = Abs.T – (Abs. SB + Abs. B)/ Abs.S – Abs.B ×100

TIBC (µg/dl) = Abs.T – (Abs. SB + Abs. B)/ Abs.S – Abs.B ×300

UIBC (µg/dl) = TIBC µg/dl - Iron in µg/dl

Prothrombin Time (PT)

Principle

This test reflects the overall efficiency of the extrinsic system. It is sensitive to changes in factor V, VII and X, and less so to factor II (prothrombin). It is also unsuitable for detecting minor changes in fibrinogen level, but may be abnormal if the fibrinogen level is very low or if there is an inhibitor present. The sensitivity of the test is influenced by the reagent and technique used and it is important to establish a reference range locally (Biggs and McFerlane 1962; Hirsh et al., 1995).

The pathway measured by the prothrombin time is shown in Figure 11.1 (Colman and Hrish 1994). The PT reagent, often termed thromboplastin, contains tissue factor and phospholipids.

Equipment

• Test tubes

• Stopwatch

• Pipettes

• Waterbath

• Coagulometer

Reagents

Many suitable reagents are commercially available. Notes on reagent selection are included in Section 8.

• Thromboplastin (this may contain calcium chloride)

• 25 mM Calcium chloride (only required, if thromboplastin reagent does not contain calcium.)

Manual Method

- To the first two tubes:
- ml normal plasma was placed and warmed to 37°C for 2 minutes.
- ml pre-warmed (to 37°C) thromboplastin reagent (if calcium is present in the reagent), were added, stopwatch started, gently mixed by shaking the tube and clotting times were recorded.
- Repeated for each test sample.
- Patient's clotting time were reported in seconds.

Results/ Interpretation

Duplicate clotting times should not differ by more than 10%.

Notes:

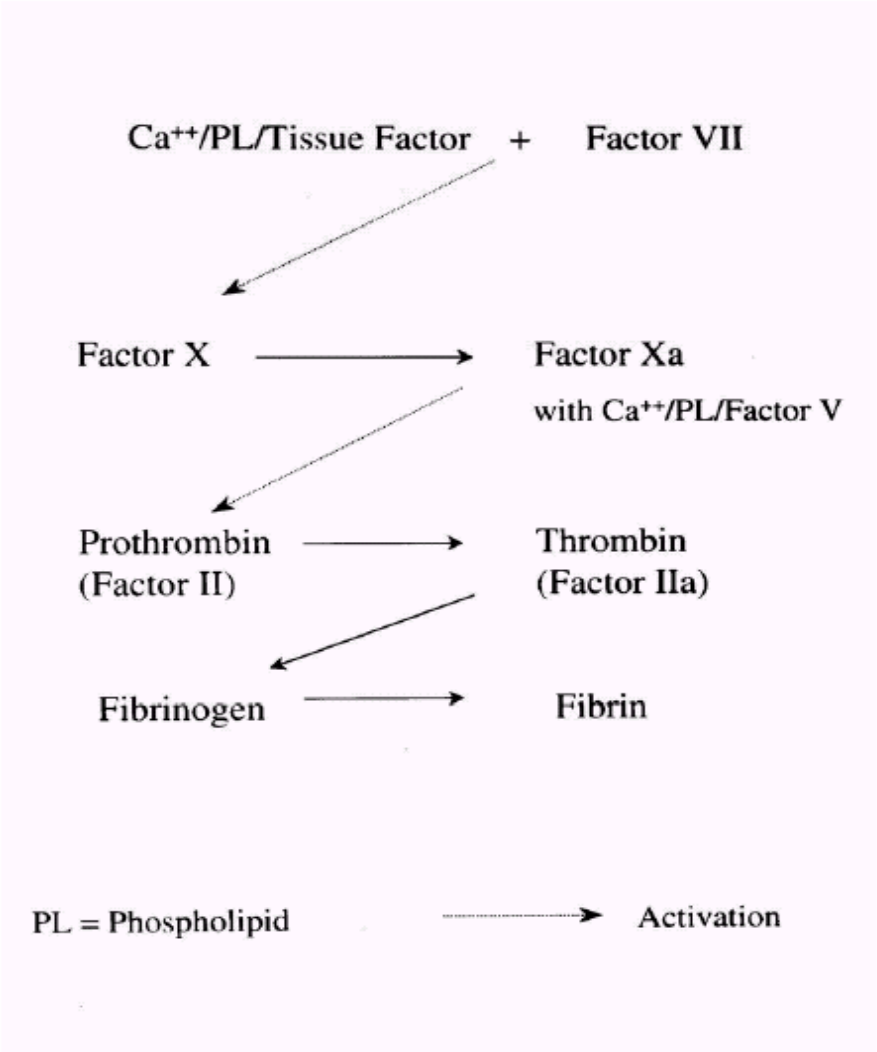
If thromboplastin reagent does not contain calcium the test procedure is 0.1 ml plasma, 0.1 ml thromboplastin and clot with 0.1 ml pre-warmed 25mM calcium chloride.

Activation of factor IX by tissue factor – factor VII occurs *in vivo*. Under the conditions of most PT tests factor X is so strongly activated that the assay is insensitive to deficiency of factor IX or VIII.

Thromboplastin/calcium chloride should be pre-warmed for 5-30 minutes prior to use. Clotting times are normally influenced by the use of different coagulometers depending on how and when the end point is detected. This underlines the importance of establishing normal ranges for the method currently in use in the laboratory.

Figure 11.1

The pathway measured by prothrombin time test



Activated Partial Thromboplastin Time (APTT)

Principle

This is a non-specific test of the intrinsic system. Taken together with a normal prothrombin time, it is the most useful screening test for detecting deficiencies of factors VIII, IX, XI and XII (Biggs 1972).

The APTT will also be prolonged in any deficiency involving the common pathways (Deficiencies of factors V, X, II and to a lesser extent fibrinogen) and in the presence of inhibitors. The presence of some therapeutic inhibitors of coagulation, such as heparin, will also prolong APTT. It is important to rule out these treatments as a cause of prolonged APTTs before continuing with other tests (Hoffmann and Neulendijk 1978).

The pathway measured by the APTT is shown in Figure 12.1.

Equipment

• Pipettes
• Stopwatches
• Water bath
• Test tubes

Reagents

• APTT reagent (There are many suitable commercial reagents which may differ in sensitivity.)
• 25mM Calcium Chloride

Method

- Tubes containing calcium chloride were placed at 37oC for 5 minutes prior to use.
- 0.1 ml of APTT reagent was pipette into each of 2 glass clotting tubes at 37oC.
- 0.1 ml control plasma was pipette into first tube. Master stopwatch started. Mixed.
- 0.1 ml control plasma was added to second tube. Mixed.

- After 5 minutes 0.1 ml calcium chloride was added to each tube in succession and started new stopwatch for each tube. Mixed.
- Time for clot formation was recorded.
- All tests were performed in duplicate (CRC, 1980).

Results/ Interpretation

A normal range should be established locally.

A long APTT with a normal PT indicates a possible deficiency of factor VIII, IX, XI, XII, high molecular weight kininogen, prekallikrein or the presence of an inhibitor. In cases with a long APTT, an equal mixture of normal and test plasma should be tested (i.e., a mixture of 1 part test and 1 part normal plasma, called a 50:50 mix, below). If the APTT corrects by more than 50% of the difference between the clotting times of the normal and test plasma, a factor deficiency is indicated. Poor correction suggests an inhibitor, possibly to one of the clotting factors in the system or of the non-specific type, such as lupus anticoagulant.

Example: APTT control : 35 secs
test : 60 secs

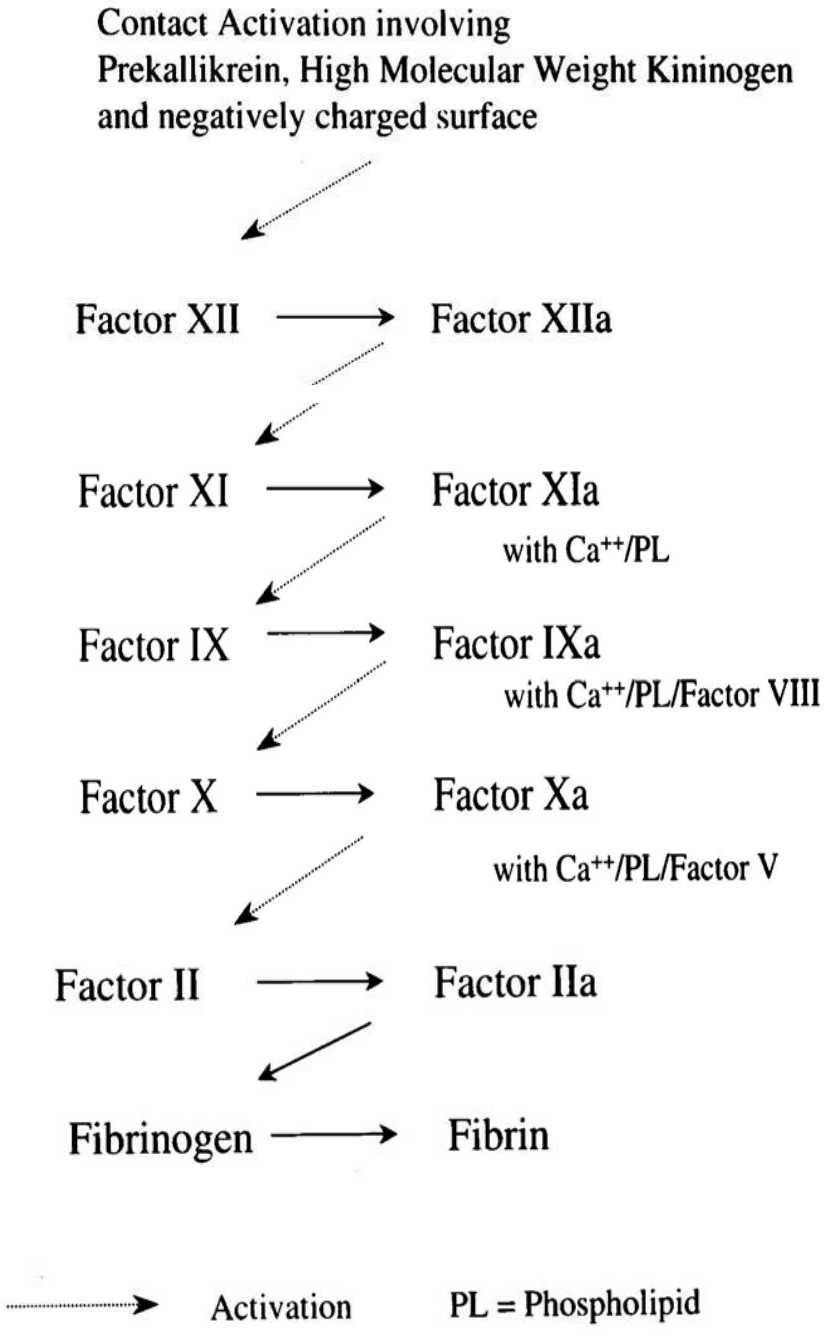
if 50:50 mix : 42 secs – this is a good correction, i.e. factor deficiency $60 - 42 = 18$, $60 - 35 = 25$, $25/2 = 12.5$, $18 > 12.5$

if 50:50 mix : 52 secs – this is a poor correction, i.e. probably an inhibitor present $60 - 52 = 8$, $8 < 12.5$

Note:

As for PT, clotting times can be influenced by the use of coagulometer. Within test plasmas, high levels of one clotting factor can compensate for lower levels of other factors. For example, a very high level of factor VIII during acute phase reaction can lead to a normal APTT in the presence of reductions of factor IX or XI which could be clinically important. If a patient has the appropriate personal or family history suggestive of a bleeding disorder, fuller investigation, including specific factor assays, may be justified even though the APTT is normal.

Pathway Measured by APTT



Mixing Tests for further Investigation of Abnormal PT and APTT

Principle

Plasma samples found to have abnormal screening tests, i.e. PT/APTT, can be further investigated to determine the cause of the abnormality. Information on the nature of the defect can usually be obtained by mixing experiments. The test plasma is mixed with normal plasma or plasma with a known coagulation defect. The test is repeated noting the degree of correction.

First of all it is important to demonstrate that the defect in a patient's plasma is corrected with normal plasma in order to eliminate the presence of an inhibitor. Correction of the abnormality by the addition of one of the reagents described below indicates that the added reagent must contain the substance deficient from the test sample.

Abnormal screening tests are repeated on equal volume mixtures (termed 50:50, below) of additive and test plasma (CRC 1980).

The agents which can be used for mixing tests are as follows:

- Normal plasma
- Aged plasma
- Adsorbed plasma
- Factor VIII deficient plasma
- Factor IX deficient plasma

The tables below show how the results from mixed tests should be interpreted.

Defect in test plasma	APTT	Aged or VIII deficient	Adsorbed or IX deficient	Normal plasma
VIII	abn	no corr	corr	corr
IX	abn	corr	no corr	corr
XI/XII	abn	corr	corr	corr
Inhibitor	abn	no corr	no corr	no corr

abn = abnormal; no corr = no correction; corr = correction

Defect in test plasma	PT	APTT	Aged plasma	Adsorbed plasma	Normal plasma
II	abn	abn	corr	no corr	corr
V	abn	abn	no corr	corr	corr
VII	abn	norm	corr	no corr	corr
X	abn	abn	corr	no corr	corr

Factor Assays based on APTT

(One stage Assay of FVIII: C, FIX, FXI or FXII)

Principle

The one-stage assay for factor VIII will be described here. The assay compares the ability of dilutions of standard and test plasmas to correct the APTT of a plasma known to be totally deficient in factor VIII but which contains all other factors required for normal clotting. For factors IX, XI and XII the assay is essentially the same and is performed by substituting the relevant deficient plasma for factor VIII deficient, and after selection of the appropriate reference plasma.

Equipment

• Plastic tubes

• Pipette

• 75 x 10-mm glass tube

• Ice bath

• Water bath

Reagents

Platelet poor citrated test and standard plasma:

The standard plasma used should be either a locally prepared plasma pool, kept at -70°C or lower or commercial standard plasma. In either case, this

reference plasma must be calibrated against an international standard for factor VIII. It is not acceptable to assume that a pooled normal plasma has 100 u/dl factor VIII:C since this is often not the case.

Factor VIII deficient plasma

This is available commercially or may be collected from a donor whose factor VIII level is less than 1 u/dl, who has no anti-VIII antibodies, has received no treatment for 2 weeks and has normal liver function tests. Abnormal liver function can lead to a decrease in other clotting factors. Such a decrease can lead to a non-specific assay. This plasma can be stored in aliquots at -35ºC. It is preferable to use factor VIII deficient plasma produced by immunodepletion of factor VIII from normal plasma using a monoclonal antibody. This type of material is available commercially. The commercial preparation has the advantage of viral safety compared to plasma from people with haemophilia who have been treated with plasma products. However, not all immunodepleted plasma is found to be < 1 u/dl and care should be taken to check this.

APTT reagent

Owren's buffered saline (OBS) or glyoxaline buffer

25mM $CaCl_2$

Method

- 1: 10 dilutions of standard and test plasma in buffered saline in plastic tubes were made.
- Using 0.2 ml volumes, doubling dilutions in OBS of standard and test plasma from 1/10 to 1/40 in plastic tubes were made. All dilutions were kept on ice as factor VIII is labile (easily destroyed). (This is only necessary if dilutions are not tested immediately after preparation or if room temperature exceeds 25°C).
- 0.1 ml of each standard dilution was pipetted into a 75 x 10-mm glass tube.
- 0.1 ml of factor VIII deficient plasma was added and transferred to 37oC water bath.
- 0.1 ml of APTT reagent was added and incubated for 5 minutes.
- At 5 minutes 0.1 ml CaCl2 was added and the clotting time was recorded.
- A "blank" should also be set up as follows:

0.1ml OBS

0.1ml FVIII deficient plasma

0.1ml APTT reagent

- Incubated for 5 min.
- 0.1ml $CaCl_2$ was added.
- The clotting time of the blank should be longer than the time of 1% FVIII activity of standard from the calibration graph. If the time is shorter this indicates that the plasma is not totally deficient in factor VIII and thus is not suitable for the test (Biggs, 1972).

Results/ Interpretation

The results were plotted on log/log or log/linear graph paper.

The 1: 10 dilution is arbitrarily assigned a value of 100%, the 1: 20 a value of 50%, and the 1: 40 a value of 25%. Straight lines, parallel to each other, should be obtained.

Read off concentration of test sample as shown on graph. In this example the test has a FVIII concentration which is 7% of that in the standard. If the standard has a concentration of 85 µ/dl, the test has a concentration of 85 µ/dl x 7% = 6 µ/dl.

If the lines are not parallel, the assay should be repeated.

Non-parallel lines may occur due to technical error. If technical error has been eliminated, nonparallel lines may be due to the presence of an inhibitor, which would show a converging pattern.

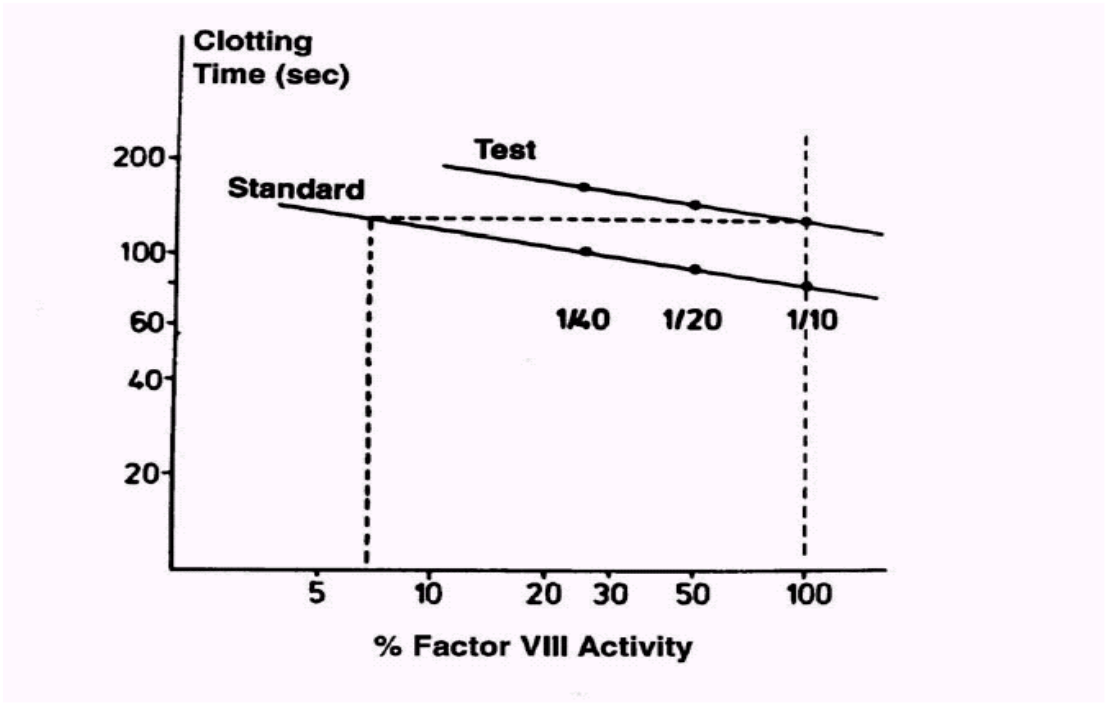
Note

If the test plasma factor VIII concentration is close to zero (i.e., the clotting times of all dilutions are similar to the blank) then non-parallel lines may occur.

Normal Range

The normal range should be established locally but often has a lower limit of 50-60 µ/dl.

Graph of factor VIII assay



CHAPTER 4

RESULTS

CHAPTER 4

RESULTS

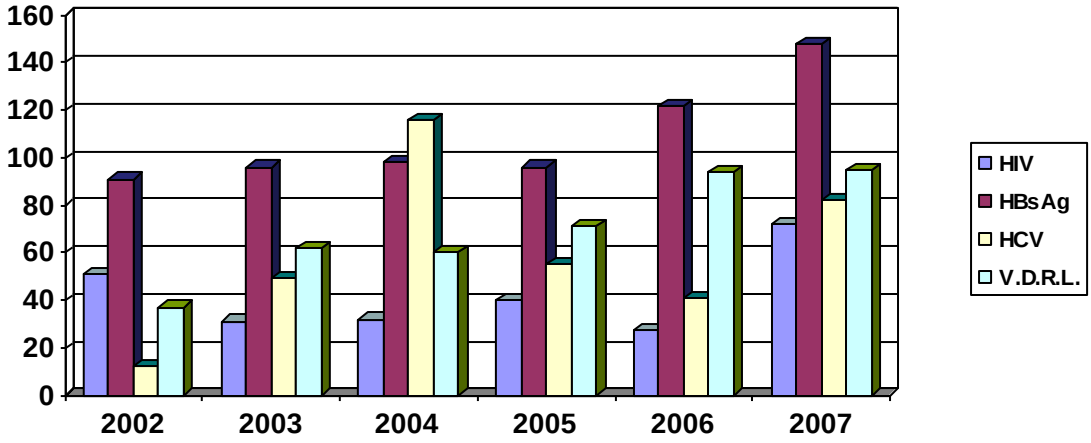
Risk of infectious complications due to blood transfusion is a major concern. Number and types of microbial infections depend on variety of factors as discussed. In developing countries like India where medical facilities are sporadic and not up to the best of the standards such incidences are obviously high. The risk is increased manifold if a person receives multiple blood transfusions.

Present study was conducted to observe incidence of microbial infection in patients who receive multiple blood transfusion in and around Rajkot city. Results obtained are divided in to five major categories:

(1) Incidence of Microbial infection and co-relative data in healthy donors
(2) Incidence of Microbial infection and co-relative data in Thalassaemic patients
(3) Incidence of Microbial infection and co-relative data in Hemodialysis patients
(4) Incidence of Microbial infection and co-relative data in Hemophilia patients
(5) Incidence of Microbial infection and co-relative data in surgical multi transfused and leukemia patients

Incidence of Microbial infection and co-relative data in healthy donors

Transfusion transmitted microbial infections such as HIV or Hepatitis or other infections are spread in community by apparently “healthy” blood donors. There are stringent measures to assure that the donor is adequately healthy while donating blood. Age of the donor should be between 18 and 55 years, weight should be above 45 kg. hemoglobin level should be in the normal range. Above all the donor should not be suffering from any major illness such as diabetes, hypertension, or even fever of unknown origin. However, infections are still transmitted through blood because of various reasons. Seropositivity of some of the markers which are indicative of quality of blood is shown in following result.



Prevalence of Microbial Infection among Donors

Figure 1

Year	Donor no.	HIV (%)	HBsAg (%)	HCV (%)	V.D.R.L.(%)
2002	5512	51 (0.92)	91 (1.65)	12 (0.21)	37 (0.67)
2003	5586	31 (0.55)	96 (1.71)	49 (0.87)	62 (1.10)
2004	5227	32 (0.61)	98 (1.87)	116 (2.21)	60 (1.14)
2005	6410	40 (0.62)	96 (1.49)	55 (0.85)	71 (1.09)
2006	6696	27 (0.40)	122 (1.82)	41 (0.61)	94 (1.40)
2007	7458	72 (0.96)	148 (1.98)	82 (1.09)	95 (1.27)

Figure 1 shows microbial infections among donors. In the year 2002, the rate of infections of HIV, HBsAg, HCV and V.D.R.L. were 51, 91, 12 and 37 respectively. In year 2003, the rate was 31, 96, 49 and 62 respectively. In year 2004, the rate was 32, 98,116 and 60 respectively. In 2005, the rate was 40, 96, 55 and 71 respectively. In 2006, the rate was 27, 122, 41 and 94 respectively. At the end of the study i.e. in the year of 2007, the rate was 72, 148, 82 and 95 respectively.

Percentage of donors found to be infected with HIV, Hepatitis B and C virus or *Treponema pallidum* is found varying over the years but there is a clear increase in all the four markers from 2002 to 2007. Most remarkable being rise in HCV seropositivity-0.21% in the year 2002 to 1.09% in 2007.

Statistical analysis of the data

Anova: Two factor analyses without replication were carried out to find out statistical significance of rate of infection over the years.

SUMMARY	Count	Sum	Average	Variance
2002	4	191	47.75	1091.583
2003	4	238	59.5	753.6667
2004	4	306	76.5	1425
2005	4	262	65.5	573.6667
2006	4	284	71	1988.667
2007	4	397	99.25	1144.917
HIV	6	253	42.16667	286.1667
HBsAg	6	651	108.5	494.3
HCV	6	355	59.16667	1285.367
VDRL	6	419	69.83333	490.9667

ANOVA

Source of Variation	SS	df	MS	F	P- value	F crit
Year	6097.333	5	1219.467	2.735593	2.35593	2.901295
Infection	14245.83	3	4748.611	10.65242	0.000527	3.287382
Error	6686.667	15	445.7778			
Total	27029.83	23				

1) Fcal= 2.735593 Ftab= 2.901295

Therefore Fcal < Ftab

Therefore there is no difference in donors blood samples year wise.

2) Fcal= 10.65242 Ftab= 3.287382

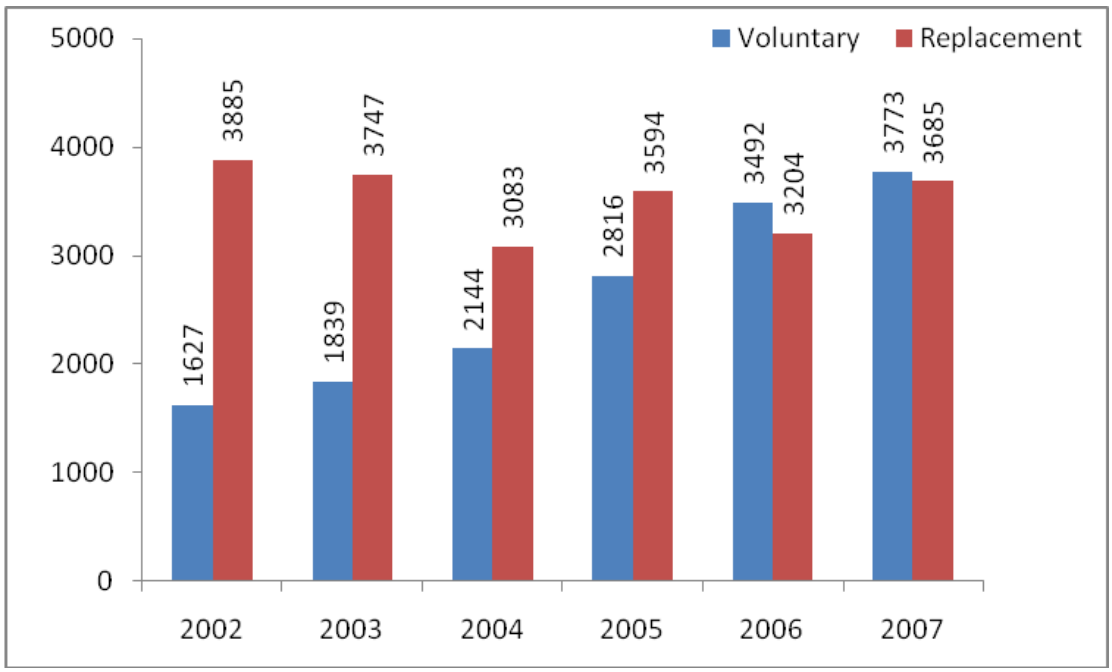
Therefore Fcal > Ftab

Therefore, there is difference in the rate of infection in donor’s blood samples.

In the beginning of our study replacement blood donation was much prevalent compare to voluntary blood donation. Change in the pattern of blood donation over a period of time was studied.

Number of Voluntary Vs Replacement Donors

Figure 2



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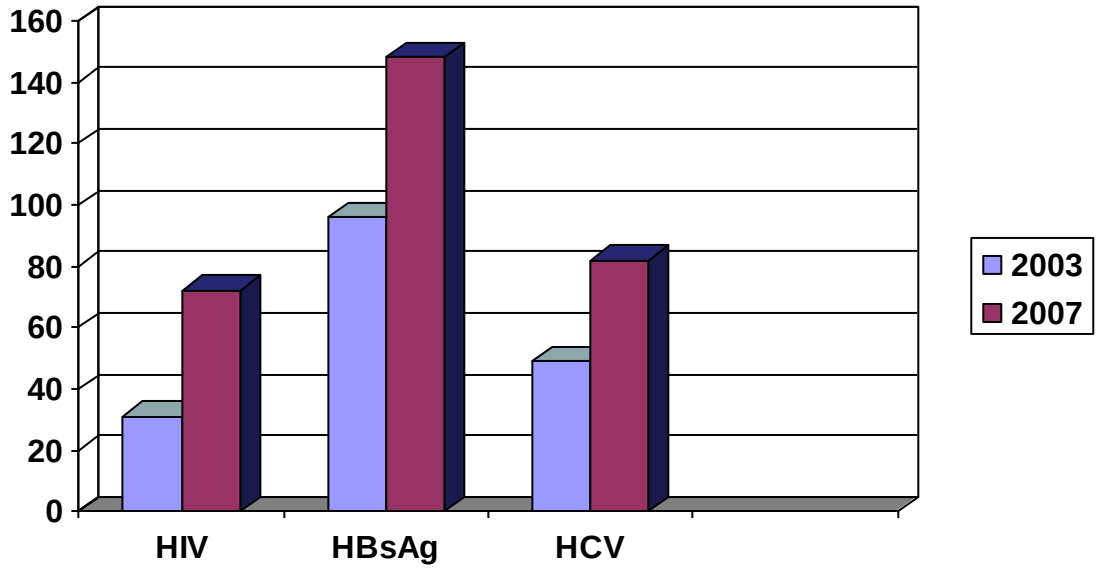
Year	Voluntary	%	Replacement	%	Total
2002	1627	29.51	3885	70.48	5512
2003	1839	32.92	3747	67.07	5386
2004	2144	41.01	3083	58.08	5227
2005	2816	43.93	3594	56.06	6410
2006	3492	52.15	3204	47.84	6696
2007	3773	50.58	3685	49.41	7458

Voluntary donation has increased from about 30% to more than 50% since 2002 to 2007.

This result shows increase in seropositivity of the three viral markers in donor population so that they can be compared with that of different cohort of multitransfused patients.

Donor's Status

Figure 3



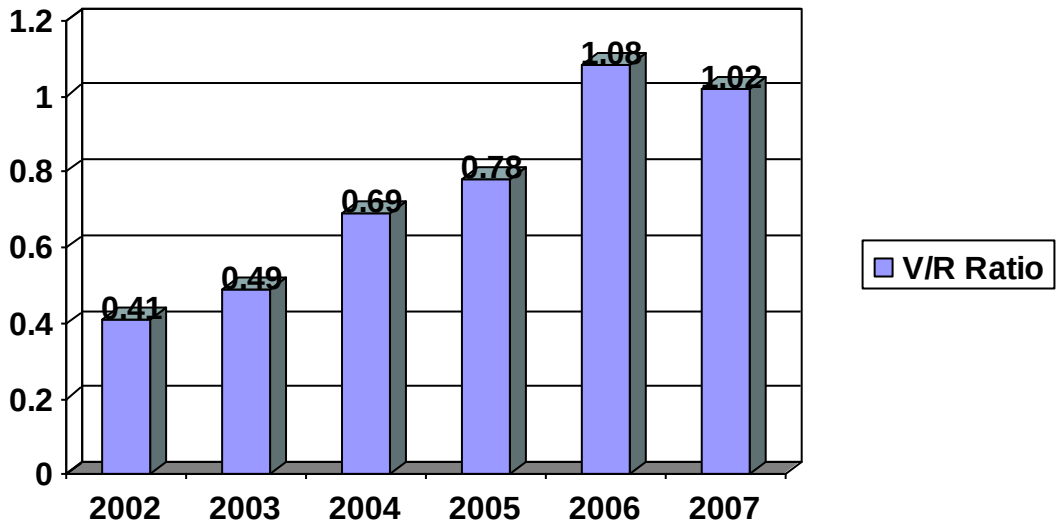
Year	Total	HIV +Ve	%	HBsAg +Ve	%	HCV +Ve	%
2003 (at the beginning of Study	5586	31	0.55%	96	1.71%	49	0.87%
2007 (at the end of Study)	7458	72	0.96%	148	1.98%	82	1.09%

Figure 3 shows donor's status in 2003 beginning of the study out of 5586 donors 31 were HIV, 96 were HBsAg and 49 were HCV Positive, while at the end of the study the rate was out of 7458 donors 72 were HIV, 148 were HBsAg and 82 were HCV Positive.

This figure shows a gradual shift from replacement (commercial?) blood donation to voluntary donation. In the year 2002 only 29.51% blood was obtained through voluntary donation which exceeded marginally (50.58% Vs 49.41%) in the year 2007.s

Voluntary/Replacement Donors Ratio

Figure 4



No.	Year	Voluntary %	Replacement %	Ratio V/R %
1	2002	29.51 %	70.48%	0.41%
2	2003	32.92%	67.07%	0.49%
3	2004	41.01%	58.08%	0.69%
4	2005	43.93%	56.06%	0.78%
5	2006	52.15%	47.84%	1.08%
6	2007	50.58%	49.41%	1.02%

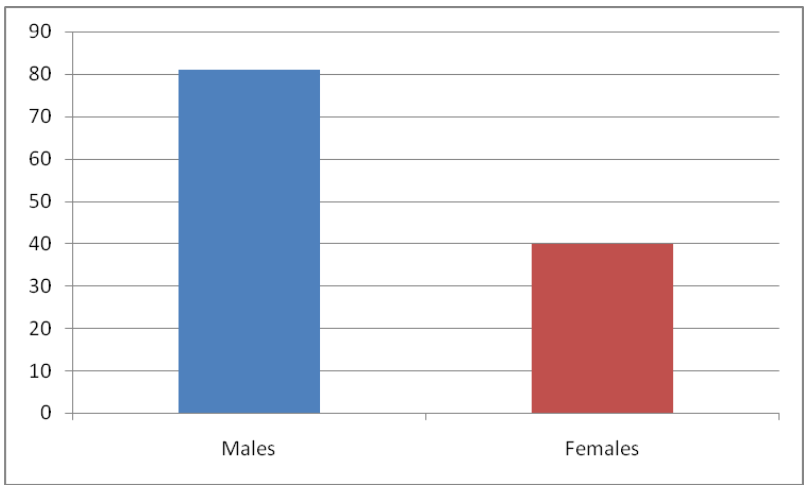
The above result shows a healthy trend of continuous increasing participation of voluntary blood donors from 2002 to 2007 but still almost 50% of the requirement of blood comes from replacement donors as shown by the ratio for the year 2007 (1.02).

Incidence of Microbial infection and co-relative data in Thalassaemic patients

Thalassemia is not a sex chromosome linked genetic disorder. However our study showed almost double the number of male patients compare to female patients as shown in the Graph.

Gender wise distribution of Thalassemia

Figure 5



Males	81	66.94%
Females	40	33.06%

Figure 5 shows Thalassemia major rules mainly Males than Females. At K.T. Children Hospital in our study we have registered 81 males and 40 female thalassemic patients.

From the literature review it was evident that Thalassemia is more prevalent in some casts compare to other. In order to check this in our present study castwise distribution of Thalassemic patients

Cast wise distribution of Thalassemic patients

Figure 6

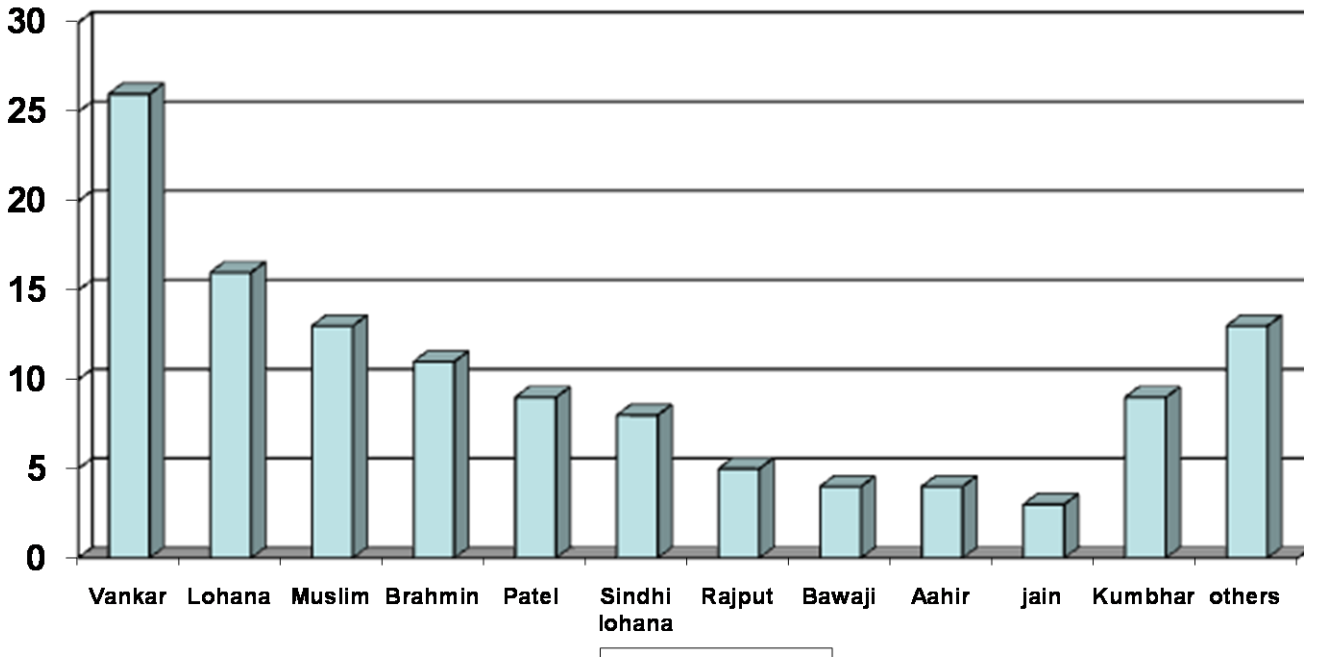


Figure 6 shows cast wise distribution in Thalassemic patients. Out of 121 patients screened, thalassemia mostly observed in Vankar cast that is about 26 (21.32 %), Lohana is second most common 16 (13.12%), Muslims 13 (10.66%), Brahmins 11 (9.02%), Patel 9 (7.38%), Kumbhar 9 (7.38%), Sindhi Lohana 8 (6.56%), Rajput 5 (4.1%), Bawaji & Aahir shares same numbers i.e. 4 (3.28%), Jain 3 (2.46%) and other casts shares approximately 13 (10.66%).

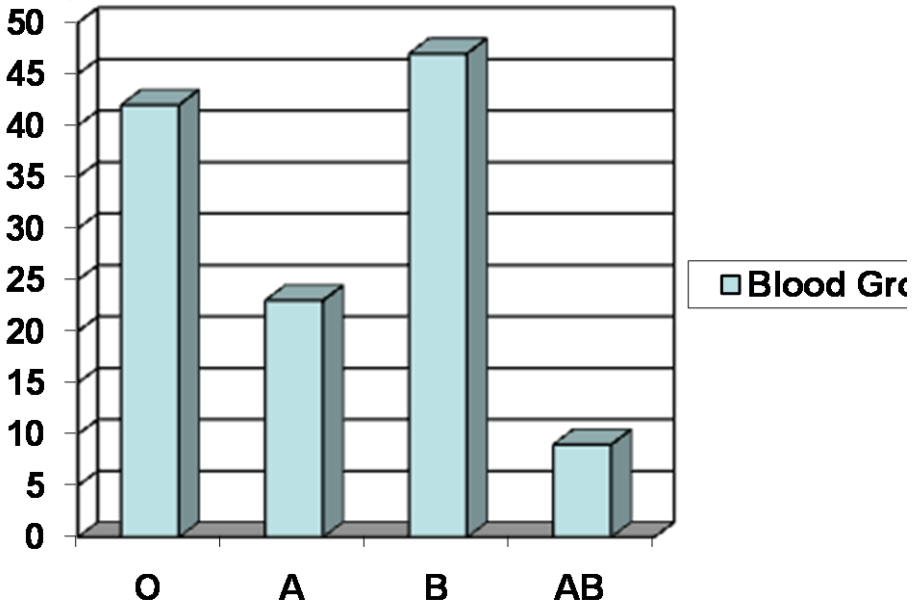
Caste	Number of Patients	%
Vankar	**26**	**21.32**
Lohana	**16**	**13.12**
Muslim	**13**	**10.66**
Brahmin	**11**	**9.02**
Patel	**9**	**7.38**
Kumbhar	**9**	**7.38**
Sindhi	**8**	**6.56**
Rajput	**5**	**4.1**
Bawaji	**4**	**3.28**
Aahir	**4**	**3.28**
Jain	**3**	**2.46**
Others	**13**	**10.66**
Total	**121**	**100**

Thalassemia is most prevalent in scheduled casts followed by Lohana, Muslim, Brahmin and other casts in decreasing order as shown in the table.

Prevalence of blood group in Thalassemic patients was studied to check the preference of any particular blood group.

Blood group wise distribution of Thalassemic patients

Figure 7



Blood Group	Number of patients	%
O	**42**	**34.71**
A	**23**	**19.00**
B	**47**	**38.84**
AB	**9**	**07.45**

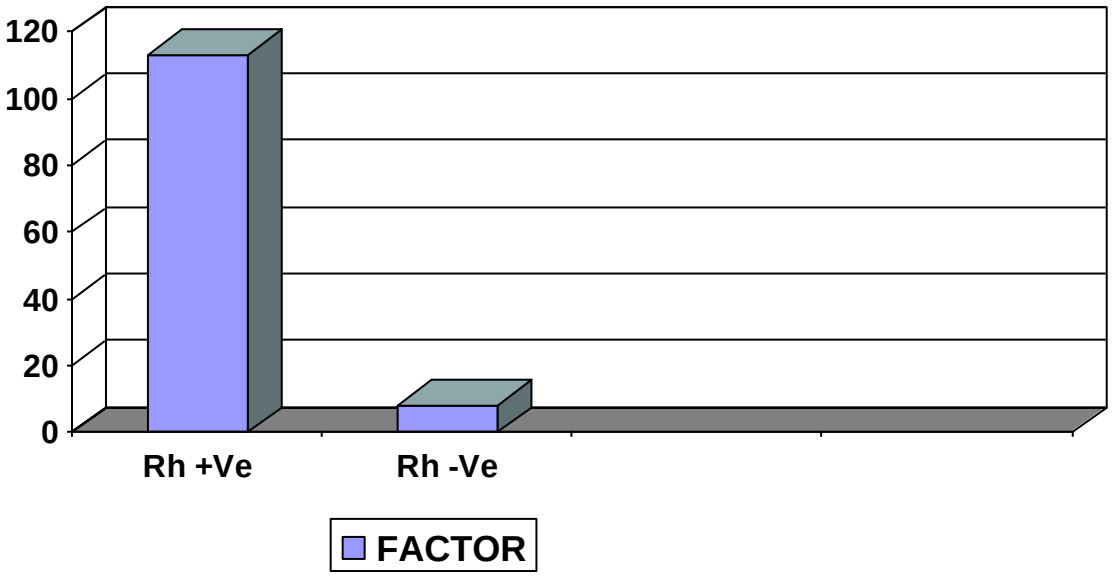
Figure 7 shows in our studies 42 patients out 121 possess Blood group O (34.71%), 23 out of 121 are of blood group A (19%), 47 out of 121 are of blood group B (38.84%) and 9 out of 121 having blood group AB (7.45%).

Results are similar to the donors data.

Rh Factor wise distribution was studied in our thalassemic subjects.

Prevalence of Rh Factor among Thalassemic Patients.

Figure 8



Rh Factor	No.	%
Rh + ve	113	93.38
Rh - ve	8	6.62

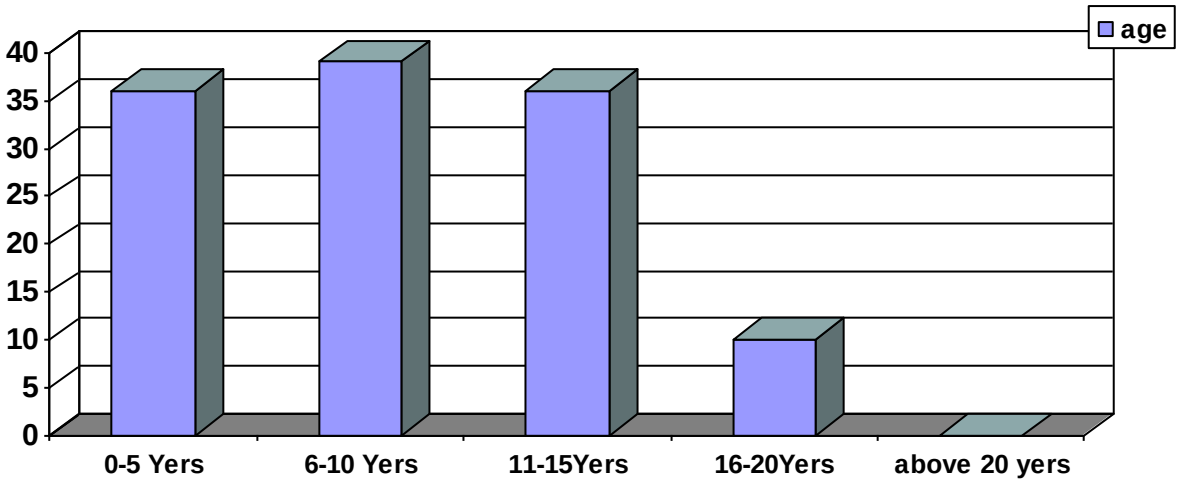
Above Figure shows Rh positivity in Thalassemic patients.

113 out of 121 patients are Rh Positive (93.38 %) and only 8 out of 121 patients are Rh Negative (6.62%).

Generally onset of Thalassemia is detected in a child is about 6 months of age. Only hope of survival of such a child is regular blood transfusion at specific time interval. Such a vigorous routine of blood transfusion from infancy result in many complications ranging from iron overload in vital organs to an array of microbial infections. Age-group wise distribution of Thalassemic children reflects this grave scenario.

Age wise distribution of Thalassemic patients

Figure 9



Age	No.	%
0-5 yrs	36	29.52
6-10 yrs	39	31.98
11-15 yrs	36	29.52
16-20 yrs	10	8.2
above 20 yrs	Nil	0

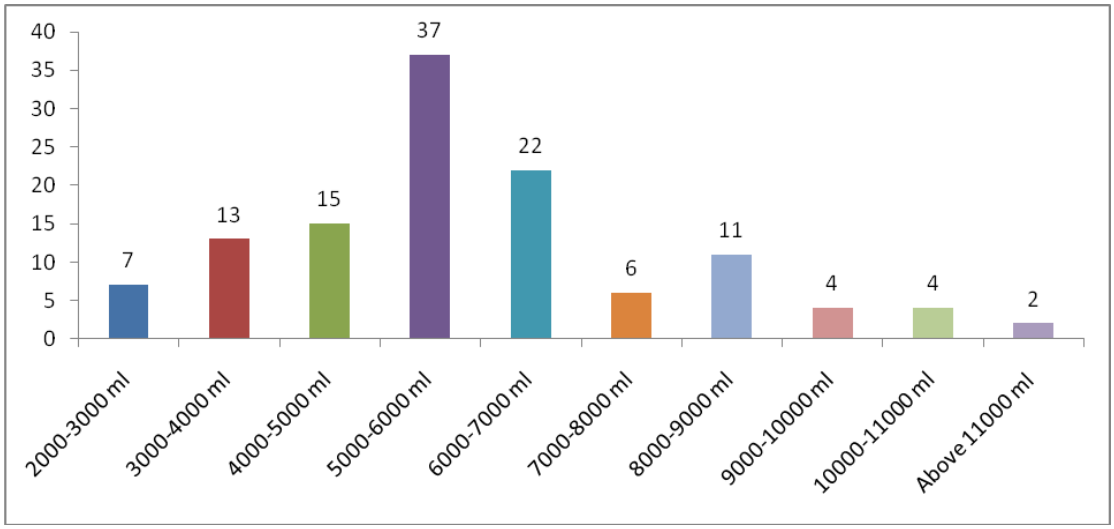
Figure 9 shows age-wise distribution of Thalassemic patients. In our study, there are 36 patients out of 121 fall in the group of 0-5 years of age (29.52%), 39 patients out of 121 are of 6-10 years of age (31.98%), 36 patients out of 121 are of 11-15 years of age (29.52%), 10 patients out of 121 are of 16-20 years of age (8.2%) and there is no patients above 20 years of age in our study.

Most children undergoing regular blood transfusion in K.T.Children hospital could not afford costly iron chelation therapy. As a result life expectancy of these children is very pathetic.

Blood transfusion requirement of Thalassemic patients depend on many factors such as age, spleen status etc.Requirement of blood transfusion per year was studied.

Requirement of Blood transfusion per year

Figure 10



ml/annum	No.	%
2000-3000 ml	**7**	**5.74**
3000-4000 ml	**13**	**10.66**
4000-5000 ml	**15**	**12.3**
5000-6000 ml	**37**	**30.34**
6000-7000 ml	**22**	**18.04**
7000-8000 ml	**6**	**4.92**
8000-9000 ml	**11**	**9.02**
9000-10000 ml	**4**	**3.28**
10000-11000 ml	**4**	**3.28**
Above 11000 ml	**2**	**1.64**

Figure 10 shows distribution according to blood transfusion per year.

Out of 121, 7 patients received 2000-3000 ml of blood/year (5.74%), 13 patients of received 3000-4000 ml of blood/year (10.66%), 15 patients received 4000-5000 ml of blood/year (12.3%), 37 patients received 5000-6000 ml of blood/year (30.34%), 22 patients received 6000-7000 ml of blood/year (18.04%), 6 patients received 7000-8000 ml of blood/year (4.92%) and 11 patients out of 121 receives 8000-9000 ml of blood/year.

Repeated blood transfusion results in Spleenomegaly in Thalassemic patients.

Spleen status in Thalassemic patients

Figure 11

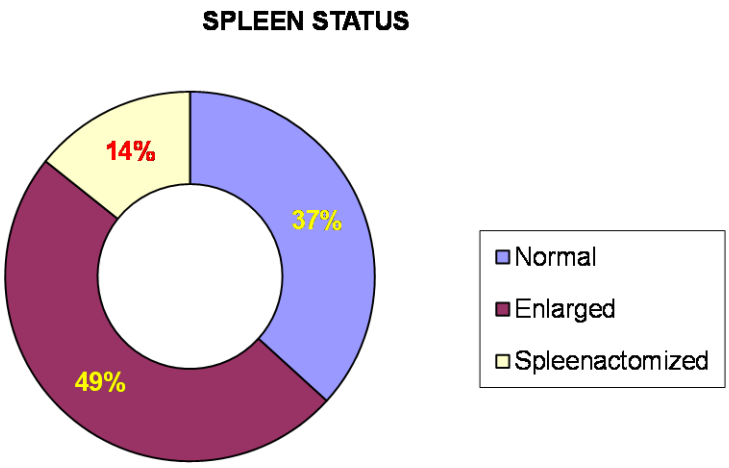
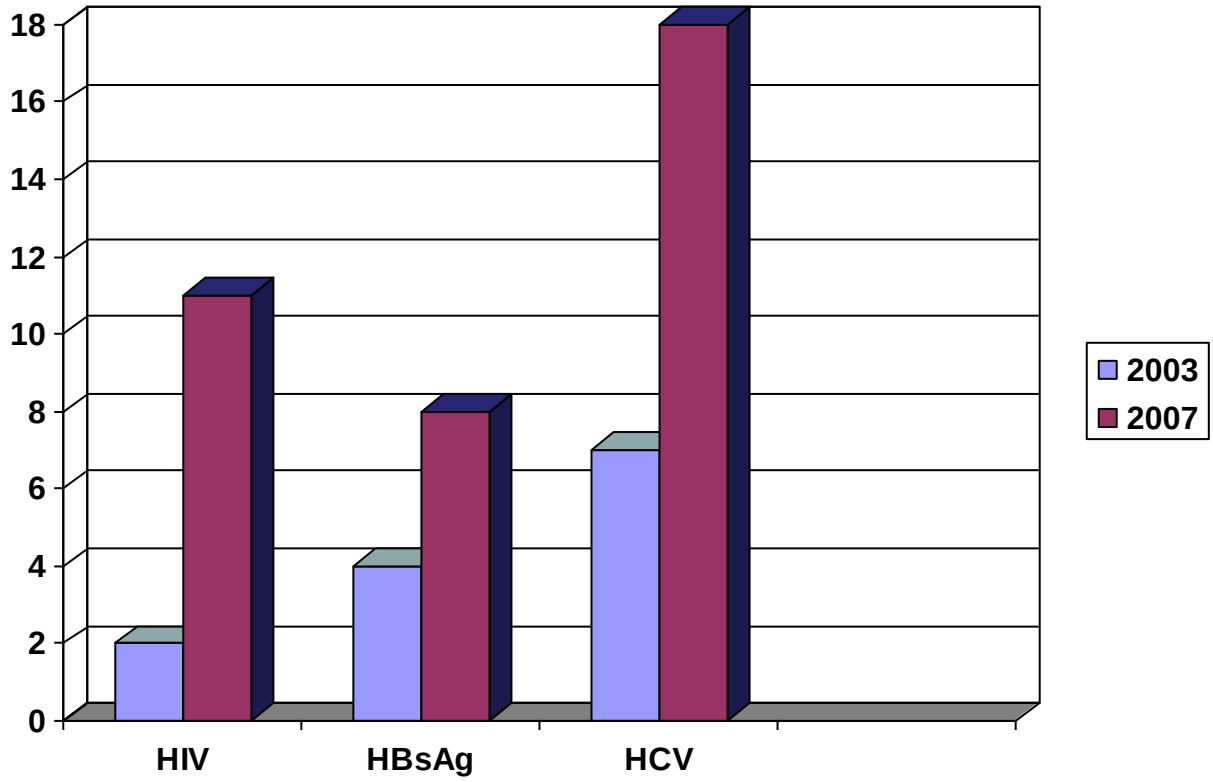


Figure 11 shows that 49% of the patients had spleenomegaly and 37% patients had normal size of the spleen. 14% of patients were spleenectomized which make them susceptible to microbial infection. However spleenectomy procedure is always followed by vaccination to the patients.

Vigorous transfusion regime makes thalassemic patients vulnerable to various microbial infections. In order to check prevalence of Transfusion Transmitted Viral infections following study was carried out.

Prevalence of viral infections in Thalassemic patients

Figure 12



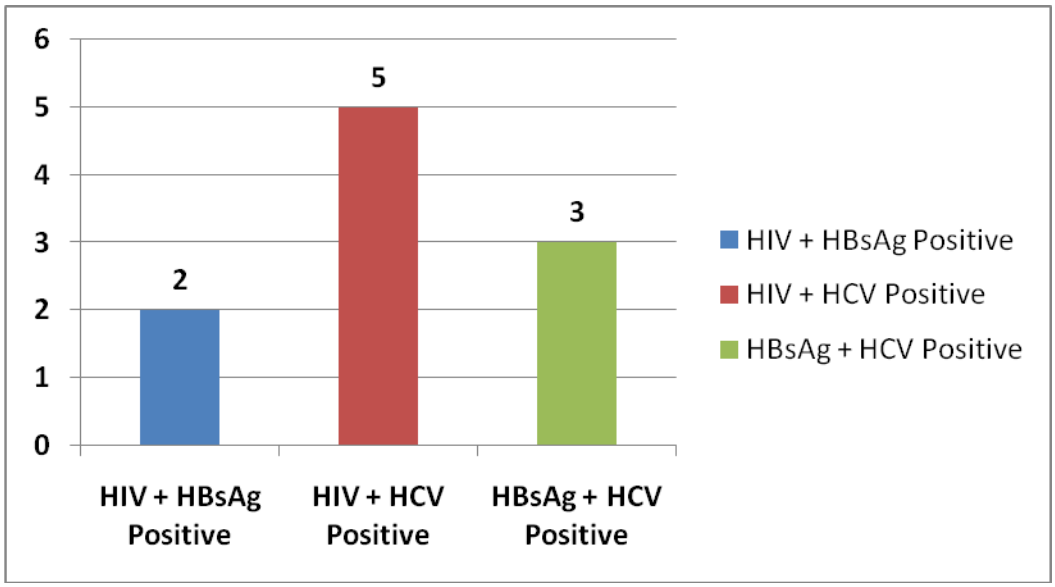
Year	Total	HIV +Ve	%	HBsAg +Ve	%	HCV +Ve	%
2003 (at the beginning of Study	121	02	1.65%	04	3.3%	07	5.78%
2007 (at the end of Study)	218	11	5.04%	08	3.66%	18	8.25%

Figure 12 shows prevalence of viral infections in thalassemic patients at the beginning of the study in 2003 the rate of infection was out of 121 only 2 were HIV Positive, 04 were HBsAg Positive and 07 were HCV Positive while 2007 the rate was out of 218, 11 patients were HIV positive, 8 were HBsAg positive and 18 were HCV Positive.

Multiple blood transfusions increase the possibility of patients being infected with more than one microbial infection. Following study was carried out to check infection with more than one virus.

Co-infection with more than one viral infection in Thalassemic Patients.

Figure 13



Total	HIV + HBsAg Positive	HIV + HCV Positive	HBsAg + HCV Positive
218	2	5	3

Figure 13 indicates higher number of co-infection with HIV+HCV compare to HBsAg+HCV and HIV+HBsAg.

The estimation of Hemoglobin always reveals the deviation of Hb level from the normal. Anything below 13.8 gm% in males and 12.0 gm% in females in considered as anemia.

Estimation of Hemoglobin in Thalassemics

Figure 14

		Hemoglobin %
1	Thalassemic Average	7.6%
2	Normal	15%

Above table shows patients with Thalassemia have lower Hemoglobin (Average 7.6%) than Normal (Average 15.0%).

Above Figure shows patients with thalassemia shows lower Hemoglobin % in our study, Thalassemic patients have average 7.6% of Hemoglobin as compare to normal patients have 15.0 % of Hemoglobin.

Blood requirement of viral infected thalasemic patients was studied.

Table 1

HIV	**BT ml/year**	**Average ml/year**
Patient 1	**12,500 ml/year**	**9250 ml/year**
Patient 2	**6000 ml/year**	

Table 2

HBsAg	**BT ml/year**	**Average ml/year**
Patient 1	**6000 ml/yr**	**6535 ml/yr**
Patient 2	**6750 ml/yr**	
Patient 3	**4000 ml/yr**	
Patient 4	**6000 ml/yr**	
Patient 5	**2500 ml/yr**	
Patient 6	**3750 ml/yr**	
Patient 7	**16750 ml/yr**	

Table 3

HCV	**BT ml/year**	**Average ml/year**
Patient 1	**12000 ml/yr**	**8150 ml/yr**
Patient 2	**11000 ml/yr**	
Patient 3	**6750 ml/yr**	
Patient 4	**4000 ml/yr**	
Patient 5	**7000 ml/yr**	

It can be seen that though there is individual variation in annual blood requirement of patients, average blood requirement is more compare to non infected thalasemic patients.

Table 4

Serological and Hematological Parameters in Thalassemic patients under the study

		S.G.P.T. Level
1	Thalassemic Average	55.1 IU/ml
2	Normal	6-21 IU/ml

Above table shows Thalassemic patients show higher level of S.G.P.T. enzyme (55.1IU/ml) than Normal people (6-21 IU/ml).

Table 5

Comparision of T.I.B.C. levels was carried out.

		T.I.B.C. Level
1	Thalassemic Average	506.49 mcg/dl
2	Normal	228-428 mcg/dl

Above table shows Thalassemic patients show higher level of T.I.B.C. levels (506.49 mcg/dl) than Normal people (228-428 mcg/dl).

Table 6

		S.Iron
1	Thalassemic Average	206.18 µg/100 ml
2	Normal (Male)	80-175 µg/100 ml
3	Normal(Female)	60-160 µg/100 ml

Above table shows Thalassemic patients show higher level of S. Iron. (206.18 µg/100 ml) than Normal people (Male:80-175 µg/100 ml and Female: 60-160 µg/100 ml)

Table 7

The hematocrite is about three times the hemoglobin value. It is also referred to as Packed Cell Volume (PCV). In anemic condition as in thalassemic expected hematocrit value is below normal. Our results are in support with this.

		H.C.T.
1	Thalassemic Average	21.6%
2	Normal	37-47%

Above table shows Thalassemic patients have lower levels of H.C.T. (21.6%) than Normal people (37-47%).

Table 8

		R.D.W.-CV
1	Thalassemic Average	21.44%
2	Normal	11.5-14.0%

Above table shows Thalassemic patients show higher level of R.D.W.–C.V. (21.44%) than Normal people (11.5-14.0%).

Table 9

Total count of leucocytes is always increased during thalassemia.

		W.B.C.
1	Thalassemic Average	12.5 X 10^3 cells/□ L
2	Normal	4.5-11.00 X 10^3 cells/□ L

Above table shows Thalassemic patients show high levels of W.B.C. (12.5 X 10^3 cells/□ L) than Normal people (4.5-11.00 X 10^3 cells/□ L)

Table 10

RBC count is low. Microcytic anemia is observed.

		R.B.C.
1	Thalassemic Average	2.82 X 10^6 cmm
2	Normal (Male)	4.5-6.5 X 10^6 cmm
3	Normal(Female)	3.9-5.6 X 10^6 cmm

Above table shows Thalassemic patients have lower levels of Red Blood Cells (2.82 X 10^6 cmm) than Normal people (Male: 4.5-6.5 X 10^6 cmm and Female: 3.9-5.6 X 10^6 cmm)

Table 11

Prevalence of Blood Groups in Viral infected Thalassemic patients.

1. For HIV infection

Total	Blood Group O	Blood Group A	Blood Group B	Blood Group AB
12	5	4	3	-
100%	41.66%	33.33%	25.00%	0.0%

2. For HBsAg infection

Total	Blood Group O	Blood Group A	Blood Group B	Blood Group AB
12	3	4	4	1
100%	25.00%	33.33%	33.33%	8.33%

3. For HCV infection

Total	Blood Group O	Blood Group A	Blood Group B	Blood Group AB
17	8	3	2	4
100%	47.05%	17.64%	11.76%	23.52%

There is no clear preference for any particular blood group in viral infected Thalassemic patients as number varies for each viral marker.

However much higher prevalence of HCV in patients with blood group O

Table 12

Prevalence of Syphilis among Thalassemic Patients.

Total	Reactive	Non-Reactive
218	00	218

No seropositivity for Syphilis was observed in thalassemic patients.

CORRELATION ANALYSIS

Correlation analysis of various parameters in hemogram as well as incidence of microbial infections with the amount of blood transfused was carried out.

	Hb	S.G.P.T.	S.Iron	RBC	HCT	MCV	RDW-CV	WBC	ml/year of blood	HIV	HBs Ag	HCV
Hb	1											
S.G.P.T.	0.034	1										
S.Iron	-0.228	0.001	1									
RBC	**0.769**	0.011	-0.183	1								
HCT	**0.774**	-0.019	-0.180	**0.959**	1							
MCV	0.134	-0.077	-0.033	0.118	**0.378**	1						
RDW-CV	-0.152	-0.226	0.083	-0.023	0.050	0.175	1					
WBC	0.094	0.137	-0.057	0.078	0.115	0.116	0.152	1				
ml/year of blood	-0.191	0.202	0.140	-0.066	-0.031	0.175	-0.057	-0.156	1			
HIV	0.023	0.065	-0.063	0.045	0.049	0.053	0.019	0.102	**0.812**	1		
HBsAg	0.004	**0.292**	0.125	0.058	0.023	0.047	0.033	0.123	**0.634**	0.239	1	
HCV	0.082	**0.342**	0.112	0.018	-0.062	0.038	0.013	0.149	**0.725**	**0.596**	**0.412**	1

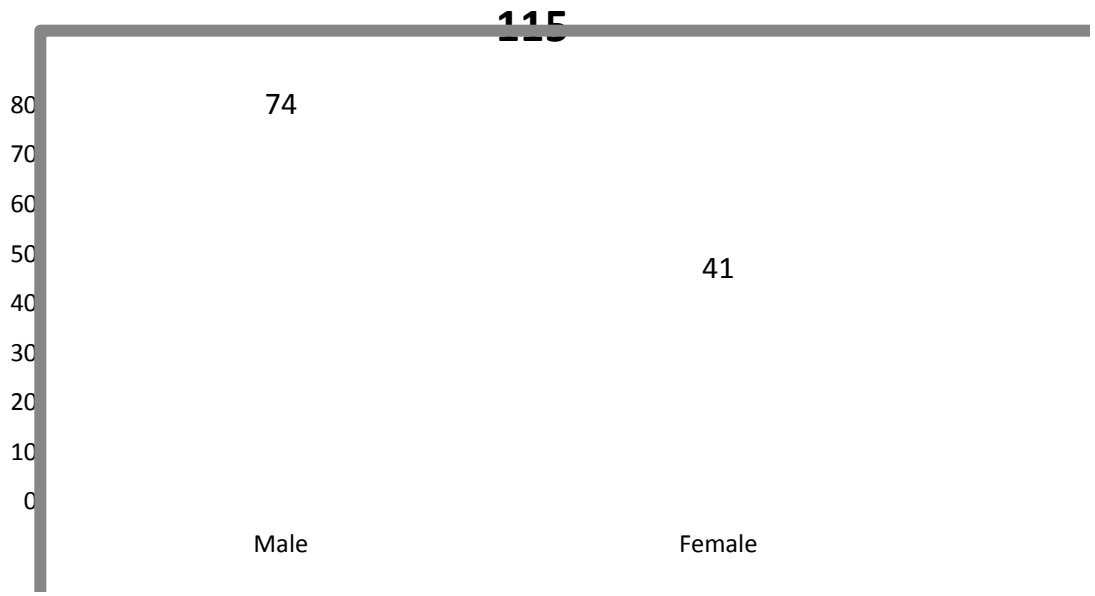
As shown in the table there is a significant correlation between hemoglobin value, RBC count and hematocrit value. There was also a significant correlation of moderate degree between hematocrit value and mean corpuscles value. Significance was also observed between the amounts of blood transfused per year with viral infections. Significance was more with HIV infection followed by HCV and HBV infection. Moderate degree of significance was observed in between HIV and HBV infection as well as HIV and HCV infection. There was a low to moderate correlation between S.G.P.T. levels and Hepatitis Viral markers namely HBV and HCV.

Incidence of Microbial infection and co-relative data in Hemodialysis patients

Male patients with autosomal dominant polycystic kidney disease (ADPKD) begin hemodialysis earlier than female patients. The rate of progression of many other renal diseases is also faster in men than women.

Figure 15

Gender wise distribution of patients on Hemodialysis



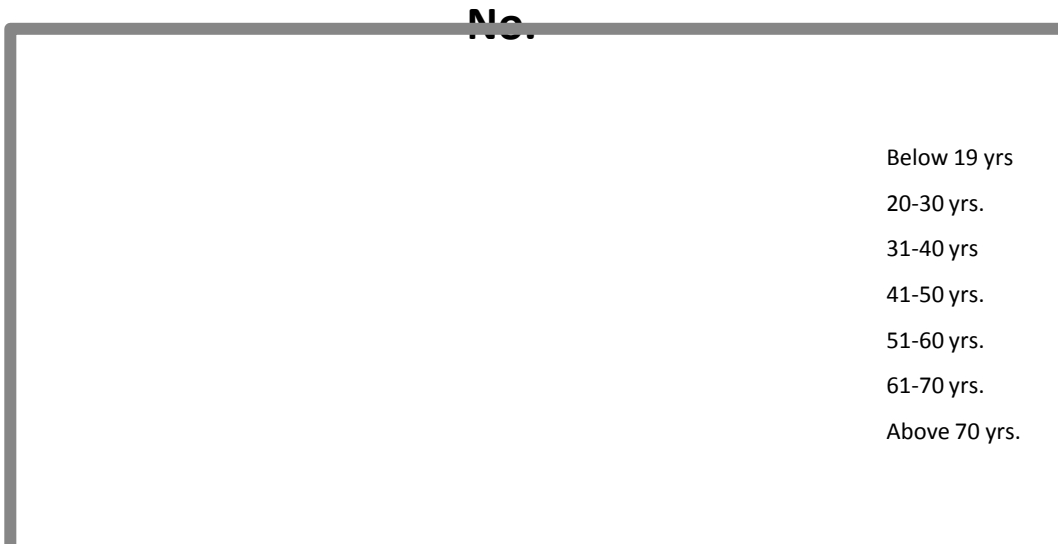
Total	115
Male	74
Female	41

Figure 15 shows Gender wise distribution of patients on Hemodialysis. We have registered 115 total patients out of them 74 patients were of male gender and 41 patients were females. Our results support literature data that males are more susceptible to kidney failure compare to females.

Chronic Renal Failure patients on maintenance hemodialysis were distributed according to their age to observe prevalence of CRF in any particular age group.

Figure 16

Age wise distribution of patients on Hemodialysis



Age	No.	%
Below 19 yrs	03	2.58%
20-30 yrs.	33	28.38%
31-40 yrs.	28	24.08%
41-50 yrs.	17	14.62%
51-60 yrs.	16	13.76%
61-70 yrs.	11	9.46%
Above 70 yrs.	07	6.02%

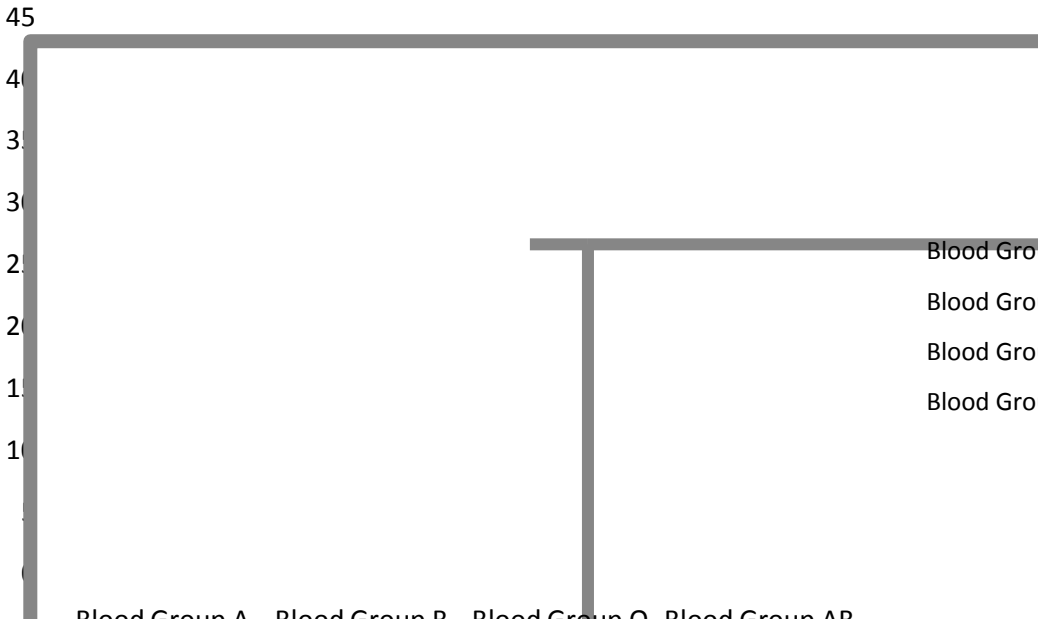
Figure 16 shows distribution of hemodialysis patients according to their age. 3 patients out of 115 were of below 19 yrs. 33 patients fall between 20-30 yrs. 28 patients are between 31-40 yrs. 17 patients were between 41- 50 yrs. 16 patients were between 51-60 yrs. 11 patients were between 61-70 yrs. And only 7 patients were above 70 yrs.

Almost 50% of the patients were in the age group of 20-40 years suggesting lower incidence of CRF in younger patients & relatively lower lifespan of these patients.

To observe the pattern of Blood Group distribution in Hemodialysis Patients the blood groups were determined.

Figure 17

Blood Group wise distribution of Patients on Hemodialysis



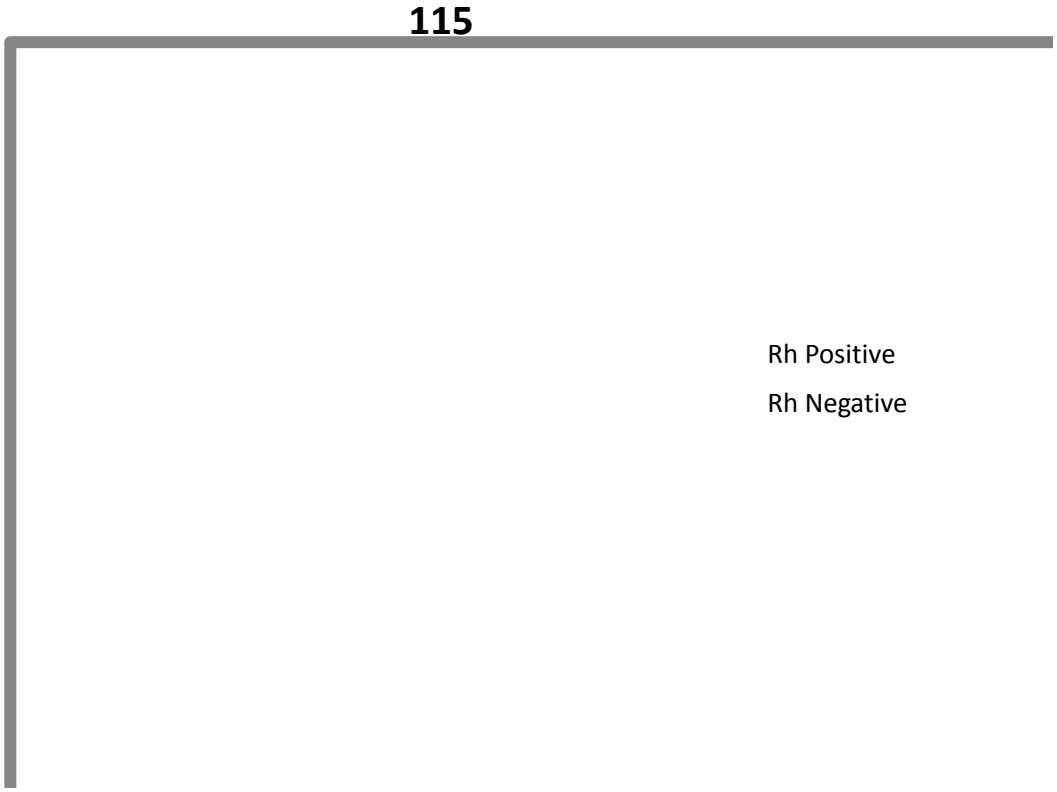
Total	115	%
Blood Group A	29	24.94%
Blood Group B	39	33.54%
Blood Group O	34	29.24%
Blood Group AB	13	11.18%

Figure 17 shows Prevalence of Blood Group in patients on Hemodialysis Out of 115 total patients 29(24.94%),39(33.54%),34(29.24%),13(11.18%) showed Blood Group A,B,O and AB respectively. Our result is in accordance with prevalence of blood group in general population suggesting no special linkage between blood group and CRF conditions.

Similarly Rh factor prevalence was determined in Hemodialysis patients.

Figure 18

Prevalence of Rh in patients on Hemodialysis



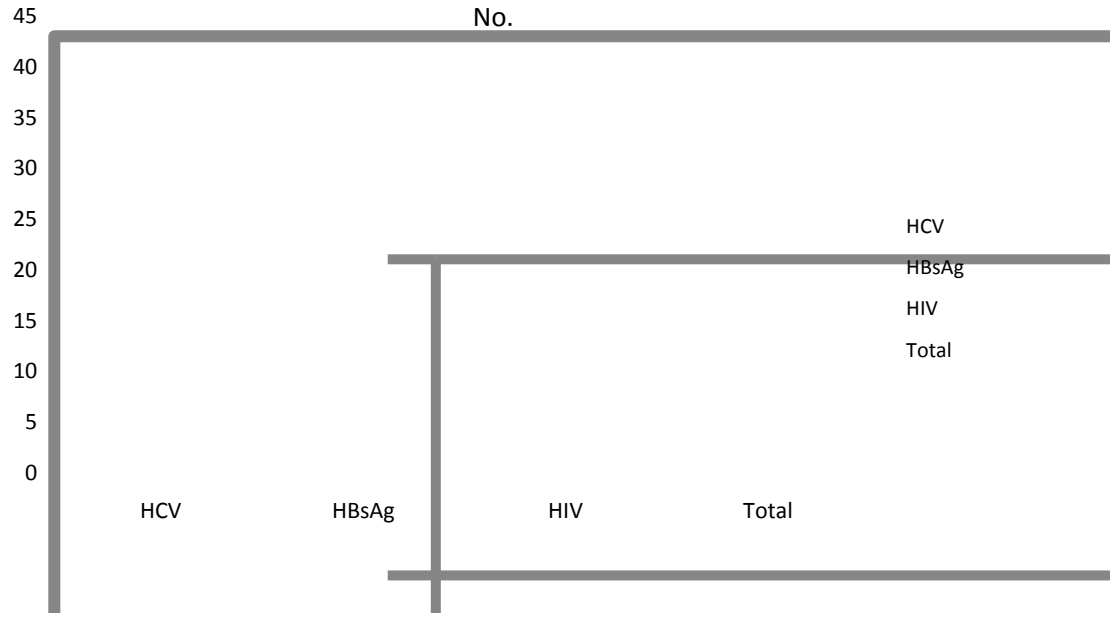
Total	115	100%
Rh Positive	106	91.16%
Rh Negative	09	7.74%

Figure 18 shows prevalence of Rh in patients on Hemodialysis. Out of 115 total patients 106 (91.16%) were Rh Positive and only 9 (7.74%) were Rh Negative.In accordance with the literature data Rh profile was observed in Hemodialysis patients.

Hemodialysis patients receive multiple blood transfusions over a long period of time. This makes them susceptible to various microbial infections much more than normal incidence. Frequency of incidence of viral markers was studied as follows.

Figure 19

Infectivity at the beginning of the study (2005)



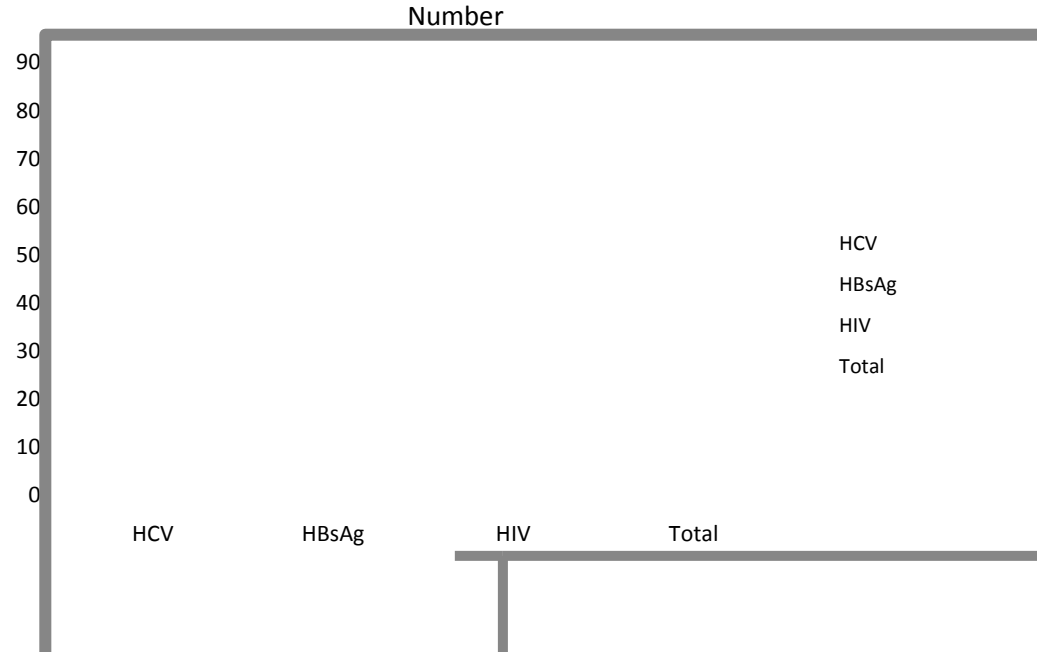
Microbial Infection	Number	%
HCV	35	30.43%
HBsAg	07	6.08%
HIV	00	0.00%
Total	42	36.52%

Figure 19 shows infectivity at the beginning of study. At the beginning we have registered 115 patients out of them 35(30.43%), 07(6.08%), 0(0.00%) patients found HCV, HBsAg and HIV positive respectively. Total 42(36.52%) patients out of 115 having microbial infections in 2005. An alarmingly high incidence of HCV was observed while HbsAg seropositivity was also observed comparatively high.

Infectivity in CRF patients at the end of the study was also studied.

Figure 20

Infectivity at the end of the study (2008)



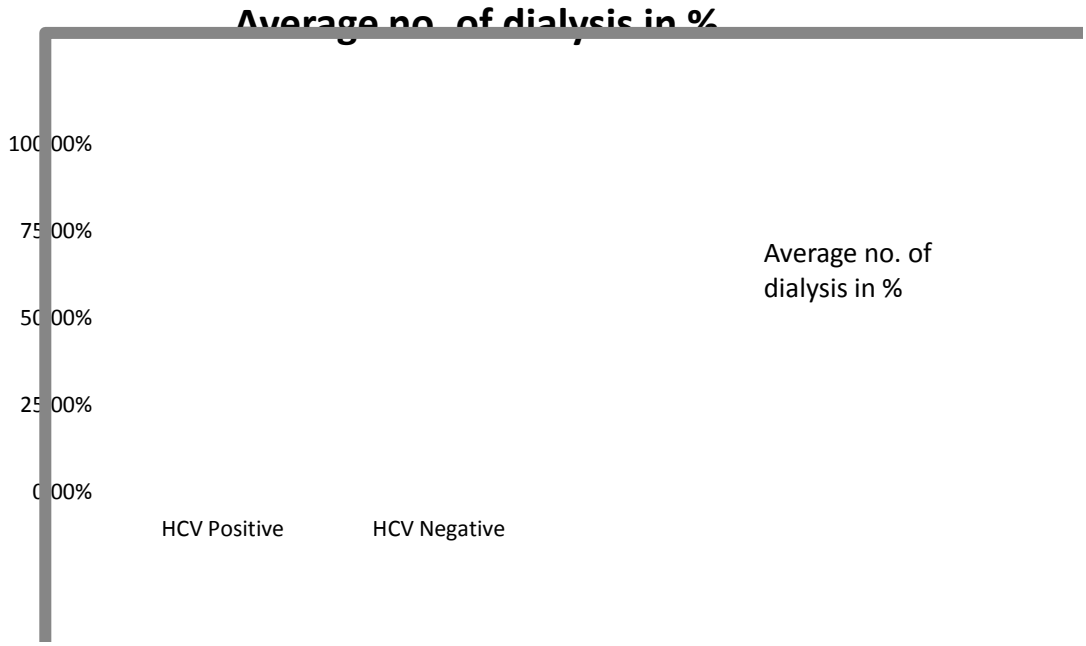
Microbial Infection	Number	%
HCV	68	40.00%
HBsAg	12	7.05%
HIV	00	0.00%
Total	80	47.05%

Figure 20 shows infectivity at the end of study. At the end we have registered 170 patients, out of them 68(40.00%), 12(7.05%) and 0(0.00%) patients were found HCV, HBsAg and HIV positive respectively. Total 80 (47.05%) patients out of 170 were having microbial infections in 2008. There was increased infectivity in HCV and HbsAg both viral markers.

Increased number of dialysis is suggestive of chronic illness of the patient. To observe the correlation between dialysis and infectivity following study was carried out.

Figure 21

Correlation between no. of Dialysis and HCV Positivity



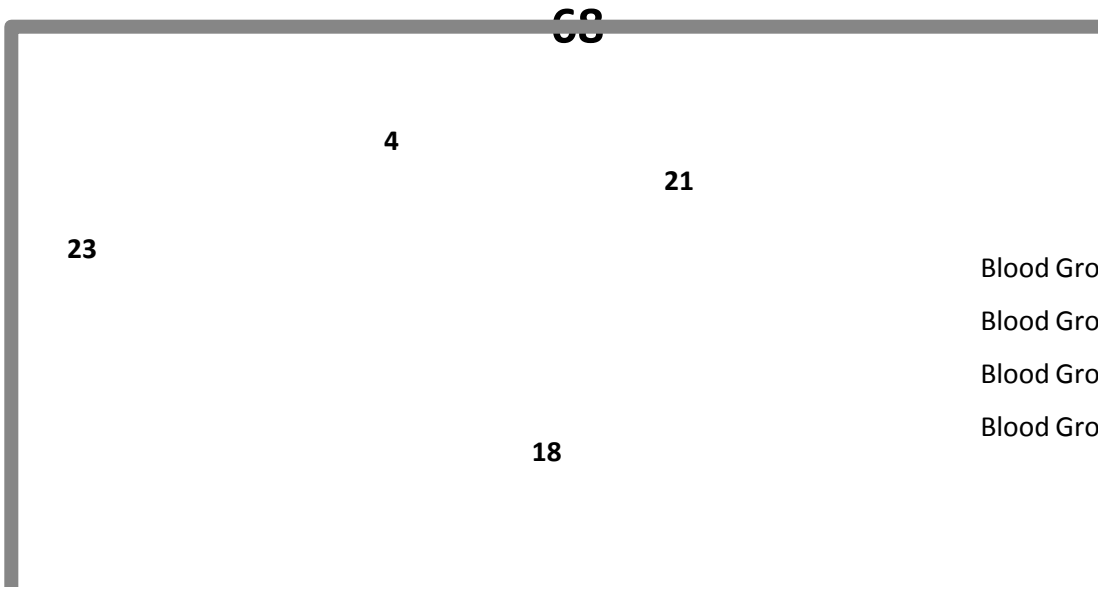
	Average no. of Dialysis (%)
HCV Positive	94.25%
HCV Negative	45.58%

Figure 21 clearly indicates that more the number of dialysis, (94.25% in HCV Positive patients and 45.58% in HCV Negative patients) more is the chance of infection by microbial agent.

There is a literature report that there may be correlation between Hepatitis infection and blood group. In our studies HCV infection was found in maximum number in Hemodialysis Patients.So prevalence of blood grouping in these patients was studied.

Figure 22

Prevalence of Blood Group in HCV infected patients on hemodialysis



Total	68	%
Blood Group A	21	30.88%
Blood Group B	18	26.47%
Blood Group O	23	33.82%
Blood Group AB	04	5.88%

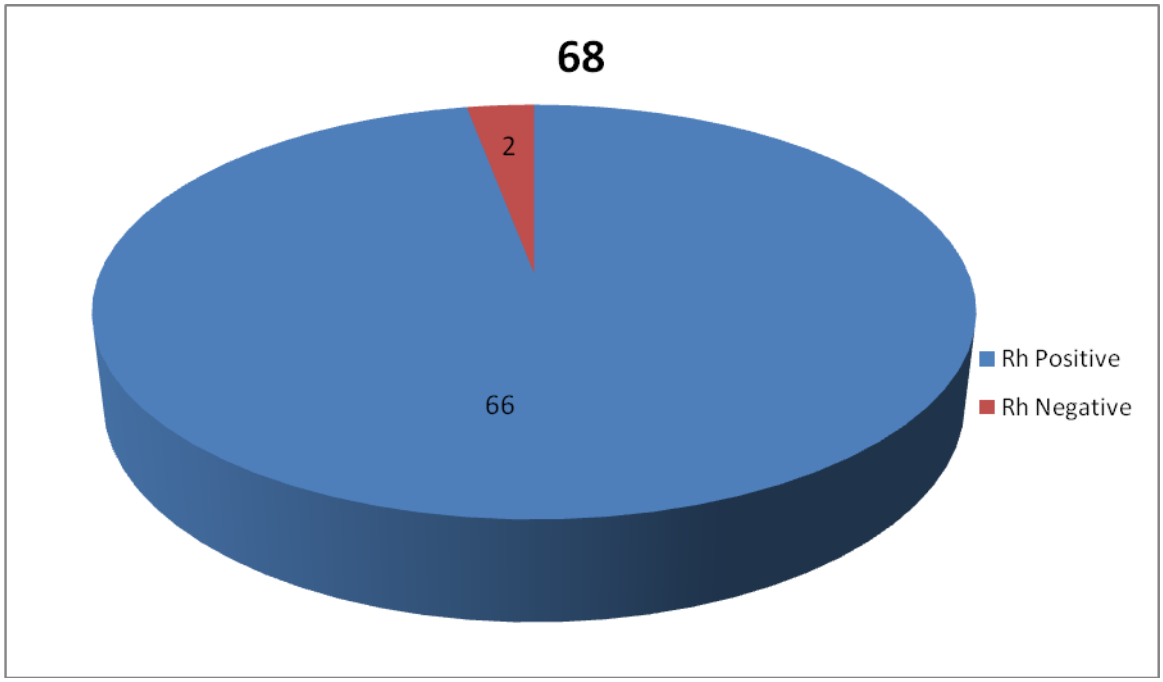
Above graph shows prevalence of blood group in HCV Positive patients out of 68 HCV infected patients 21,18,23 and 4 patients falls in blood group A,B,O and AB respectively.

In contrast to the normal profile of general population blood group O was prominent followed by blood group A , B and AB in HCV infected hemodialysis patients.

Similarly prevalence of Rh factor in HCV infected hemodialysis patients was studied.

Figure 23

Prevalence of Rh Factor in HCV infected patients on hemodialysis



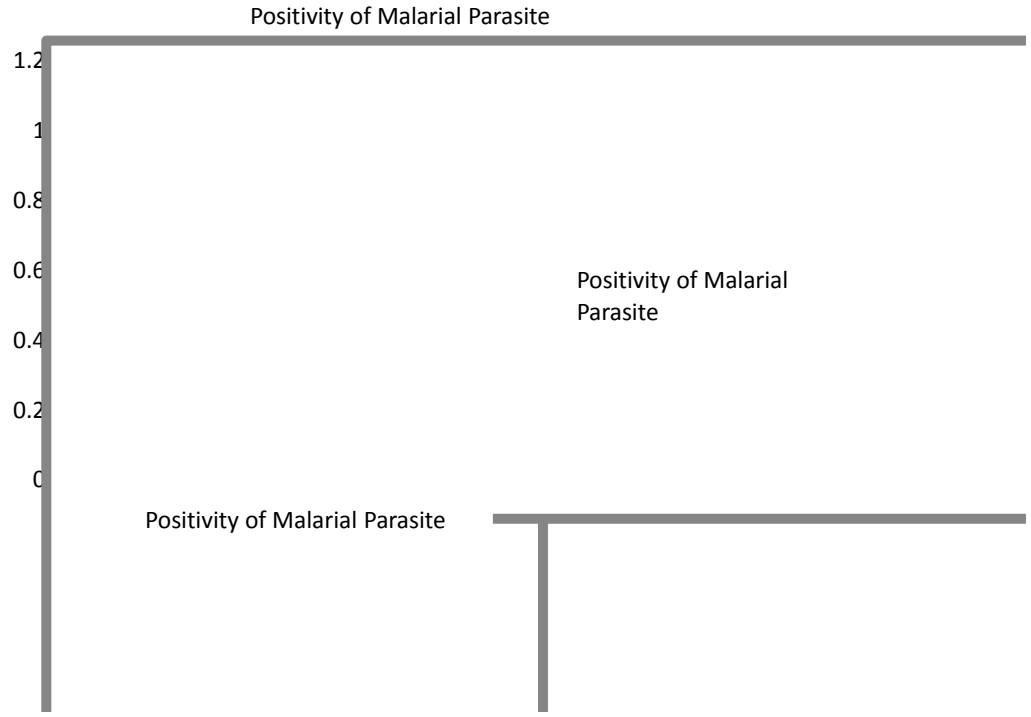
Total	68	100%
Rh Positive	66	97.05%
Rh Negative	02	2.94%

Figure 23 shows that out of 68 HCV positive patients 66(97.05%) patients are Rh Positive and only 2 (2.94%)of them are Rh Negative. Rh positive patients seem to be more prone to HCV infection compare to Rh negative patients. Since the number of patients is not sufficient for conclusively proving anything more patients need to be screened.

Malarial parasite may be transmitted through infective blood of the donor. However in rare cases patients receiving blood show positivity for malarial parasite.

Figure 24

Prevalence of Malarial Parasite among Hemodialysis Patients



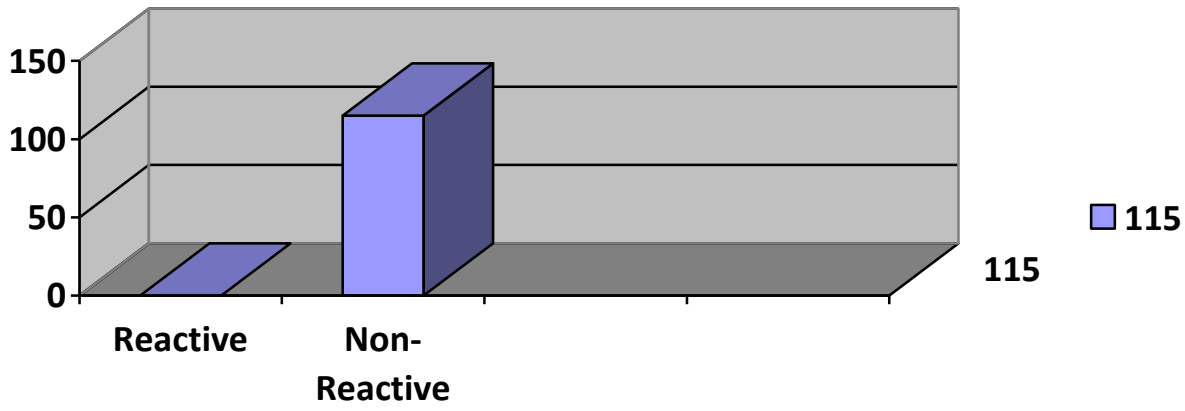
Total No. of Patients	115	%
Positivity of Malarial Parasite	01	0.86%

Figure 24 shows positivity of malarial parasite (0.86%) in Hemodialysis patients and patient was found symptomatically suffering from P.falciparum after blood transfusion and so was the donor on follow up.

V.D.R.L. positivity among sera of Hemodialysis patients was studied to check the prevalence of Syphilis among Hemodialysis patients.

Figure 25

Prevlence of Syphilis samong Hemodialysed Patients



Total	Reactive	Non-Reactive
115	00	115

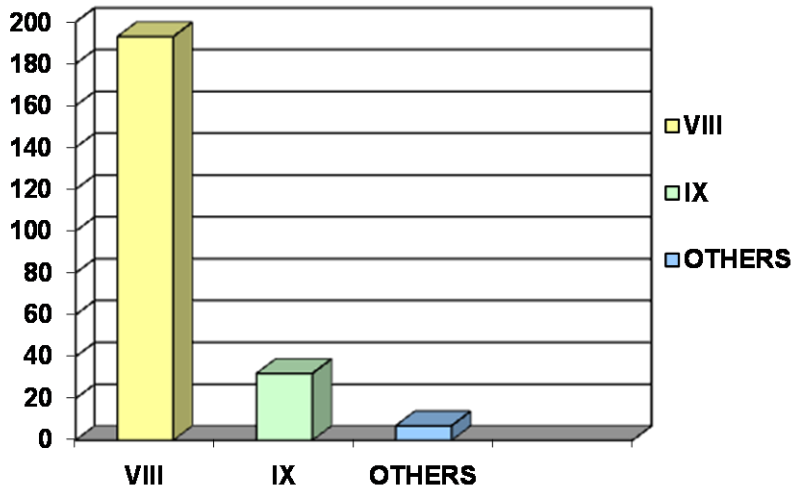
Figure 25 shows that no seropositivity for syphilis was observed. Spirochetes, the causative agent of Syphilis is a very fragile organism and cannot survive in stored blood for a longer period of time this could be possible reason for nonreactivity of the test.

Incidence of Microbial infection and co-relative data in Hemophilia patients

Hemophilia a hereditary disorder of Coagulation results in deficiency of Factor VIII (Hemophilia A) or Factor IX (Hemophilia B) or rarely due to deficiency of other clotting factors.

Figure 26

Comparison of Various Factors among Hemophilic Patients.



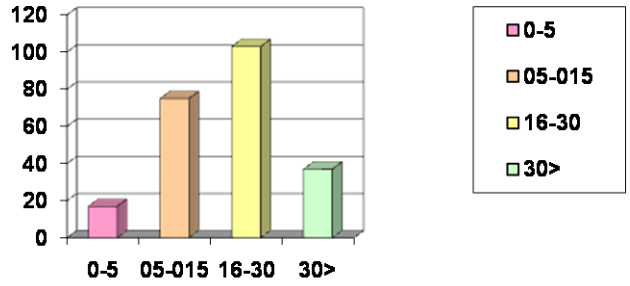
Sr.No.	Factor	MEMBERS NO.	%
1	VIII	193	83%
2	IX	32	14%
3	Others	07	3 %

Figure 26 explains Factor VIII deficiency was observed in 83% of patients while 14% patients were having deficiency of factor IX and 3% of patients were having deficiency of other factors such as factor V. Factor VIII deficiency is most commonly found among Hemophiliacs followed by deficiency of Factor IX and other factors.

Hemophilia is a chronic physical condition affecting the child from birth. Age group wise distribution was studied.

Figure 27

Age wise distribution of Hemophilic Patients



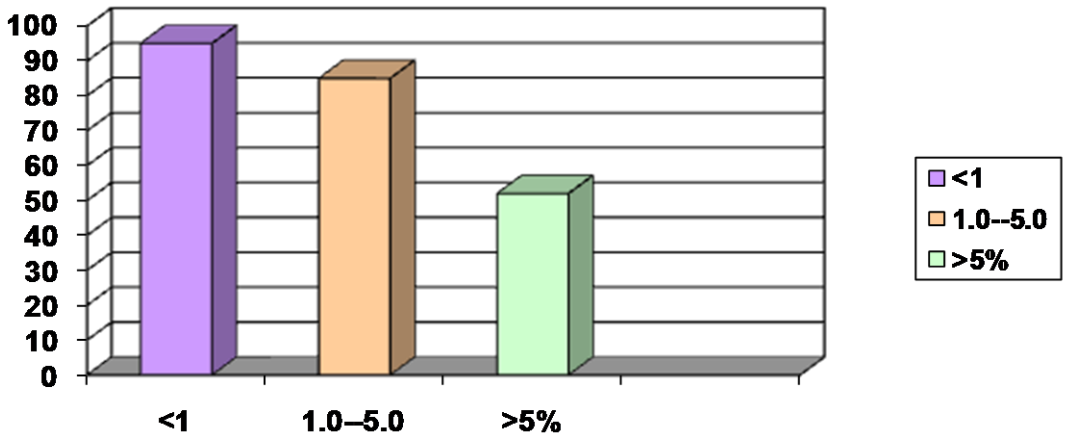
Sr.no.	Age group	Members No.	%
1	0-5 year	17	08
2	6-15 year	75	32
3	16-30 year	103	44
4	31 year	37	16

Figure 27 includes majority of the hemophilia patients under present study belonged to age group between 16 and 30 followed by 6-15 years, above 31 years of age and least number of patients were in 0-5 year age group. This data suggests good prenatal counseling and efficient management of the disease in patients.

Hemophilia can be mild, moderate, or severe, depending on how much clotting factor is in the blood. We carried out factor estimation in order to establish severity of the disease.

Figure 28

Distribution of Hemophilic Patients according to Factor Level



Sr. No.	Factor Level	Members No.	%
1	<1	95	40.94 %
2	1-5	85	36.64%
3	>5	52	22.42%

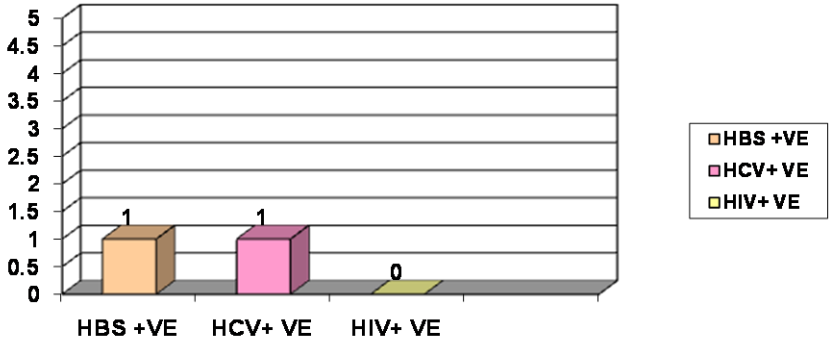
Figure 28 shows Factor level in most hemophilia patients was found to be either below 1(40.94%) or between 1 and 5 (36.64%). There were 52 (22.42%) patients with factor level of more than 5.

Majority of the patients under our study have severe hemophilia.

For the management of Hemophilia a patient needs to be transfused purified factor preparation or cryoprecipitated plasma. This increases the risk of transfusion transmitted infections.

Figure 29

Prevalence of Viral infections among Hemophilic Patients



Sr.no.	Positivity	No.	%
1	HBS Positive	1	0.43 %
2	HCV Positive	1	0.43 %
3	HIV Positive	00	00 %

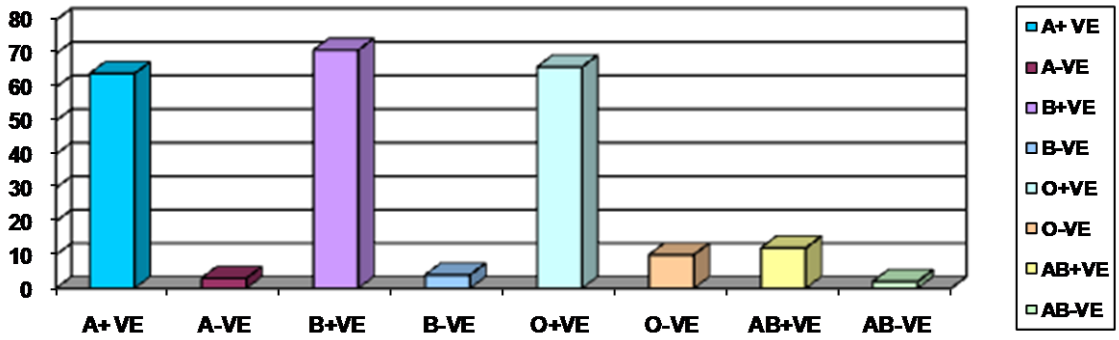
Figure 29 shows that out of 232 patients, only 1 patient was found to be HBsAg and 1 was HCV positive. All 115 patients were HIV negative.

Because of the advanced procedures of inactivation of viruses such infections are less likely to occur.

Determination of Blood Group in Hemophiliacs was carried out as follows.

Figure 30

Prevalence of Blood Group in Hemophilics



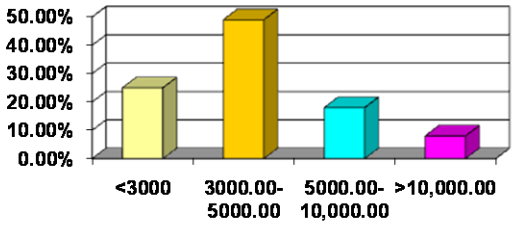
Sr.No.	Blood Group	Members No	
1	A Positive	64	27.58
2	A Negative	03	1.29
3	B Positive	71	30.61
4	B Negative	04	1.72
5	O Positive	66	28.45
6	O Negative	10	4.31
7	AB Positive	12	5.17
8	AB Negative	02	0.86

Figure 30 includes most prevalent blood group was B Positive followed by O Positive, It was closely followed by A Positive blood group and then it was AB Positive. As expected Rh negative blood groups were found comparatively less frequently.

Various socio-economic backgrounds of our patients were studied so that post-effective therapy can be suggested.

Figure 31

Distribution according to their Family Income



Sr No.	Income	Members No.	
1	<3000.00 Rs.	59	25
2	3000.00-5000.00 Rs.	114	49
3	5000.00-10000.00 Rs.	41	18
4	>10,000 Rs.	17	8

Figure 31 shows that 114 (49%) of the patients had an average family income of Rs. 3000-5000. 59 (25%) patients had less then 3000 Rs. family income while 41(18%) had family income between 5000 and 10,000.17(8%) had family income of more then 10,000 Rs. Since most of the patients come from lower middle income group factor replacement therapy could be a substantial burden compare to wet products.

Prevalence of CMV is common among general population

Figure 32

Prevalence of CMV among Multitransfused Hemophilic patients

Total	Positivity	%
232	03	1.29%

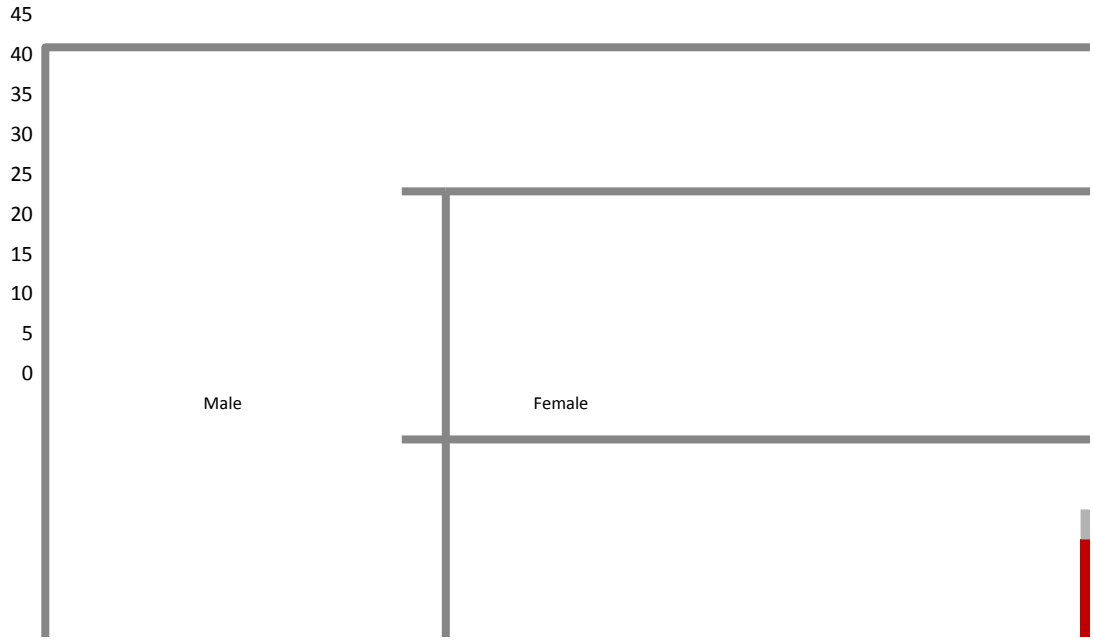
Figure 32 shows that out of 232 patients we have studied 3 patients (1.29%) were found to be positive for CMV.

Incidence of Microbial infection and co-relative data in surgical multi transfused and leukemia patients

Gender wise distribution of multitransfused surgical patients was studied.

Figure 33

Gender wise distribution of multitransfused surgical patients



Male	38	62.29%
Female	23	37.70%

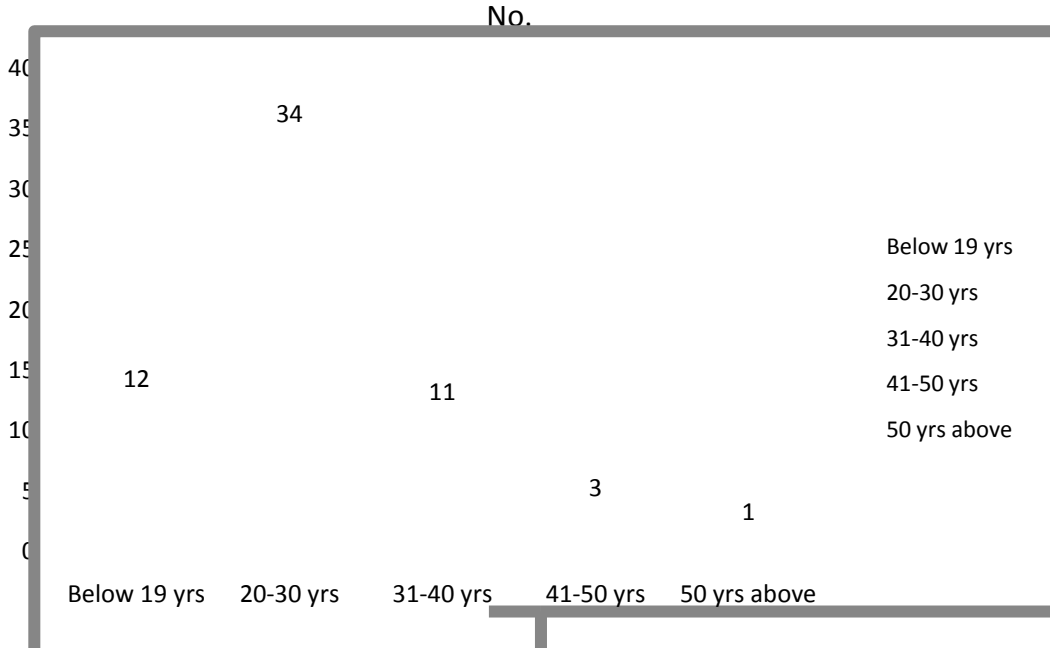
Figure 33 shows out of 61 patients 38 multitransfused surgical patients are males and 23 patients are females.

Random selection of patients suggests that because of more outdoor activities male are more prone to surgical transfusions than females.

Age distribution pattern in surgical multitransfused patients was studied.

Figure 34

Age wise distribution of multitransfused surgical patients



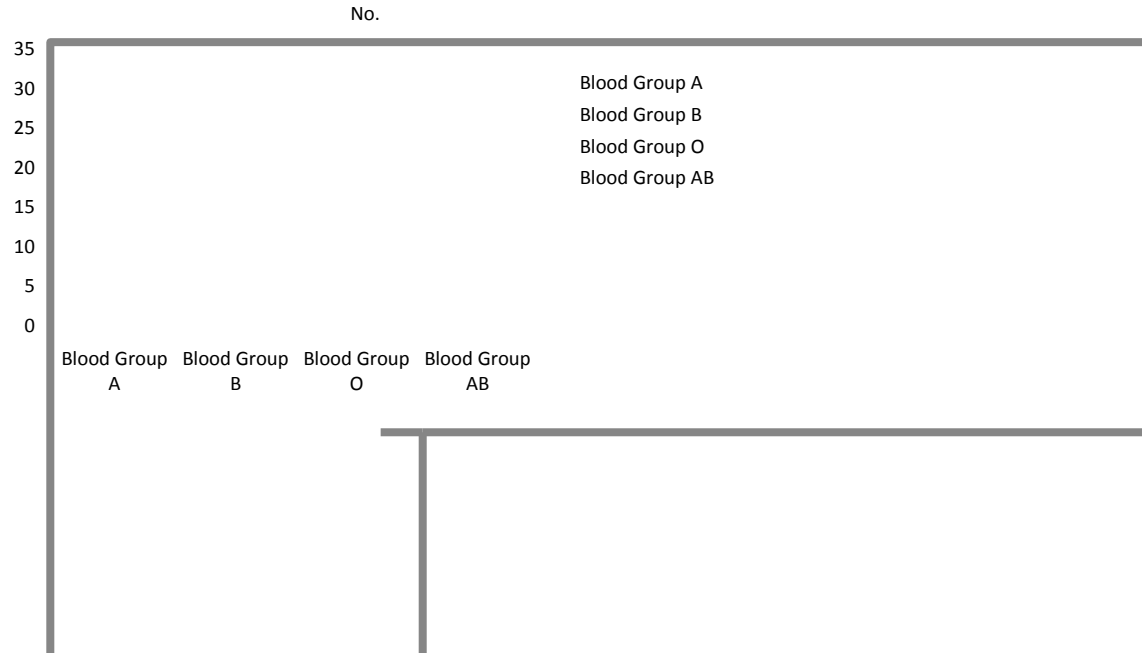
Age	No.	%
Below 19 yrs	12	19.67%
20-30 yrs.	34	55.73%
31-40 yrs.	11	18.03%
41-50 yrs.	03	4.91%
Above 50 yrs.	01	1.63%

Figure 34 shows age wise distribution of multitransfused surgical patients. There are total 61 patients out of them 12 patients are below 19 years, 34 patients are of between 20-30 yrs of age, 11 patients are of age between 31-40 yrs, 3 patients are of age between 41-50 yrs and only 1 patient is above 50 years and above.

Pattern of blood group prevalence was studied in our group of patients.

Figure 35

Blood Group wise distribution of multitransfused surgical patients



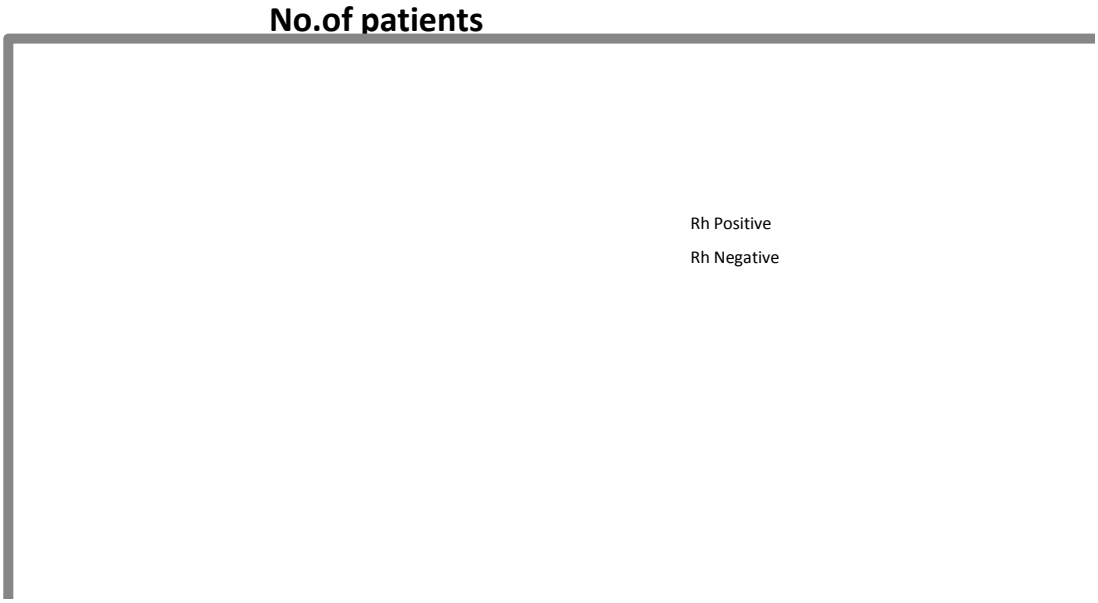
Total	61	%
Blood Group A	19	31.14%
Blood Group B	32	52.45%
Blood Group O	07	11.47%
Blood Group AB	03	4.91%

Figure 35 shows out of 61 patients 19 patients shows Blood Group A, 32 patients shows Blood Group B, 7 patients shows Blood Group O and only 3 patients shows blood group AB.

Following results show distribution of Rh factor among multitransfused surgical patients.

Figure 36

Prevalence of Rh factor among multitransfused surgical patients



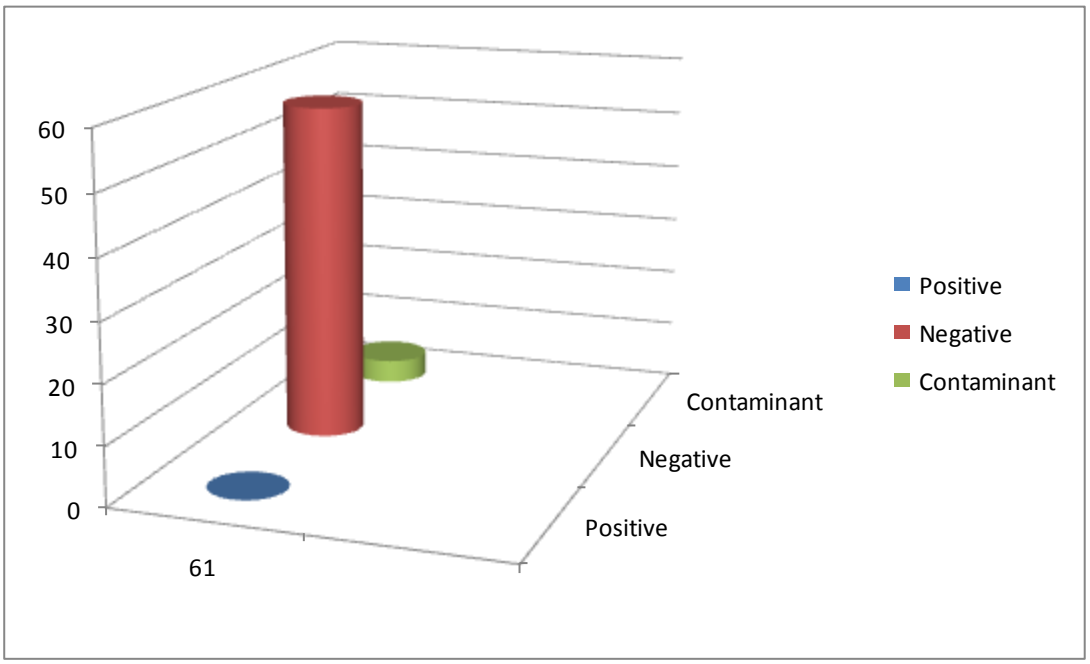
Total	61	100%
Rh Positive	52	85.24%
Rh Negative	09	14.75%

Figure 36 shows prevalence of Rh factor out of 61 patients 52 patients are Rh Positive and only 9 patients shows Rh Negative factor. Incidence of Rh negative patients is somewhat higher than literature data.

As a case study blood samples of surgical multitransfused patients were checked for bacterial growth to study incidence of bacteremia due to blood transfusion.

Figure 37

Blood Culture data of multitransfused surgical patients



Blood Culture Reports	Positive	Negative	Contaminant
61	00	57	04

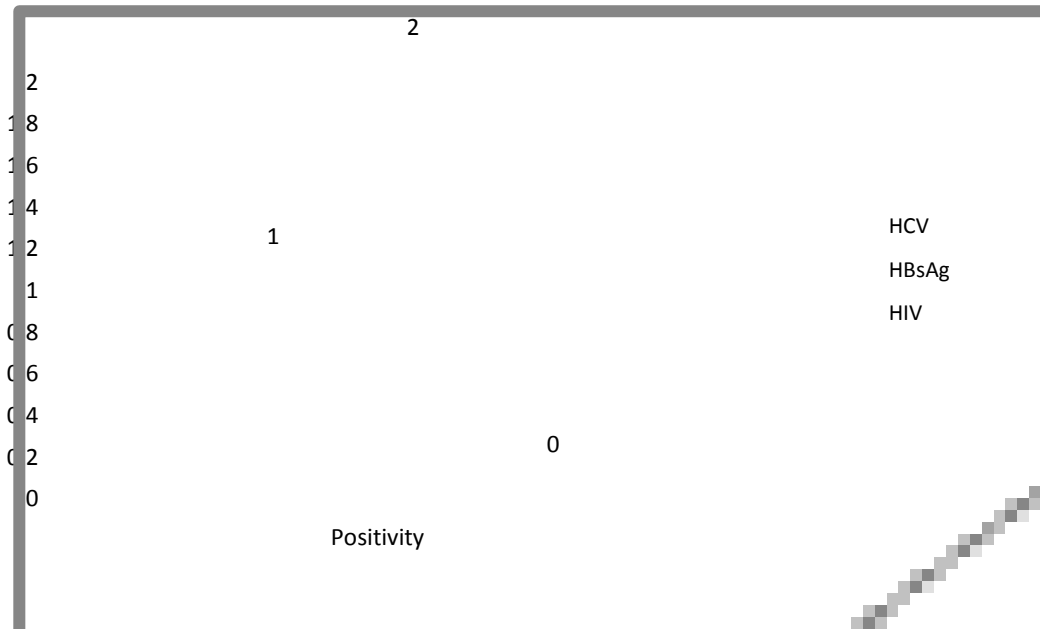
Figure 37 shows out of 61 blood cultures no positive blood cultures were reported, 4 contaminants were isolated form 61 samples.

Generally patients are given stored blood and surgical patients are on broad spectrum antibiotics. Due to these reasons bacteraemia is extremely rare.

Occurance of viral infection among our study group was checked.

Figure 38

Infectivity rate among multitransfused surgical patients



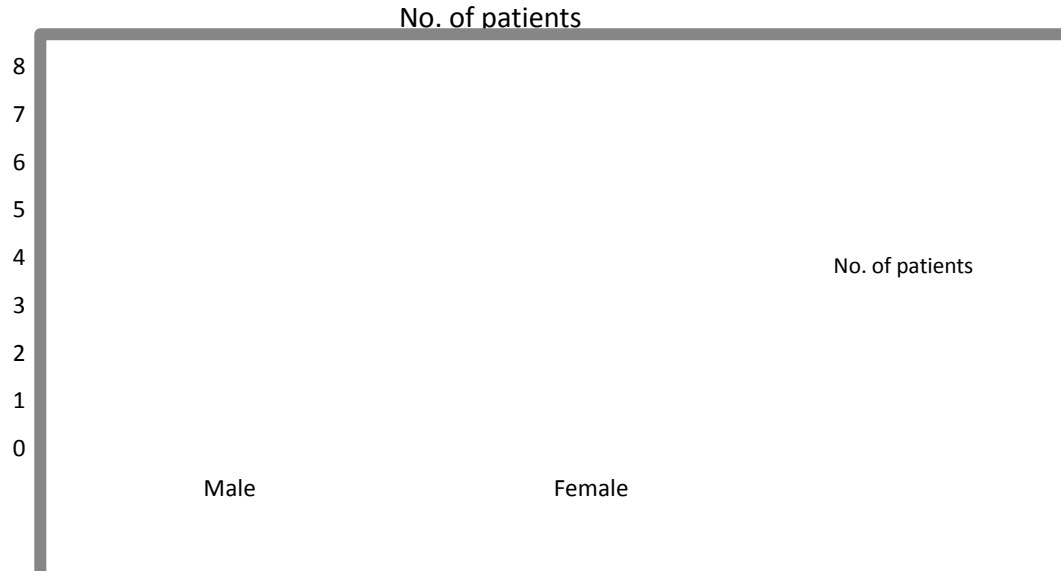
Viral infections	HCV	%	HBsAg	%	HIV	%
Posivity	1	1.64%	02	3.28%	00	00.00%

Figure 38 shows out of 61 patients 1 patient was HCV Positive, 2 patients were HBsAg Positive and none of them show HIV positive result. s

Gender wise sdistribution of multitransfused leukemic patients was studied to check its prevalence in any particular gender.

Figure 39

Gender wise distribution of multitransfused Leukemic patients



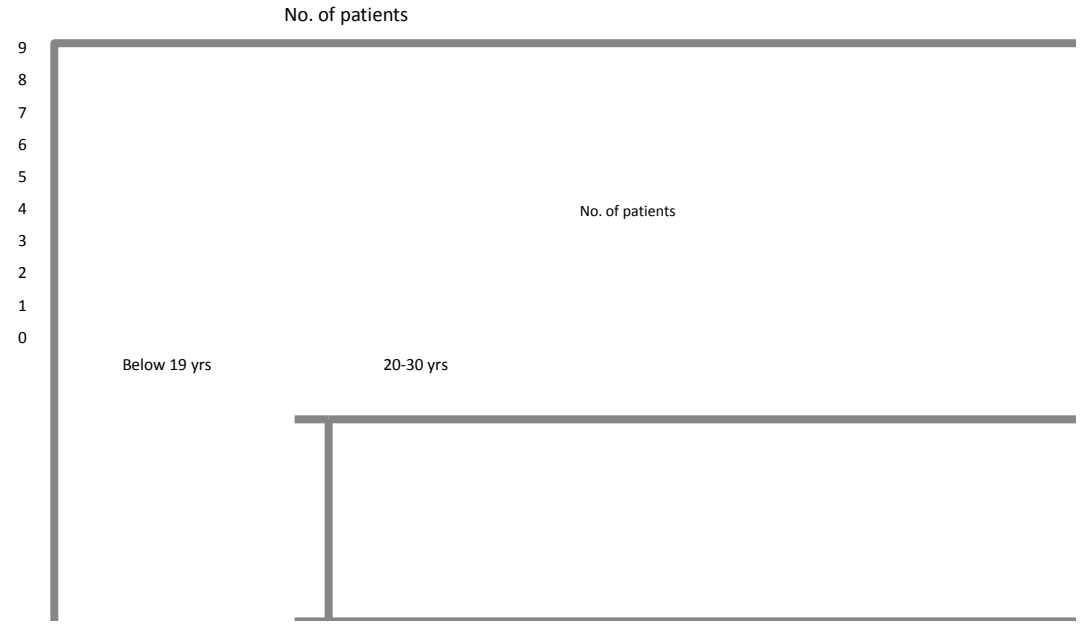
Total	10
Male	07
Female	03

Figure 39 shows gender wise distribution of leukemic patients. Total 10 patients we have registered out of them 7 patients were male and 3 patients were females.

Age wise distribution of Leukemic patients was studied.

Figure 40

Age wise distribution of Leukemic patients



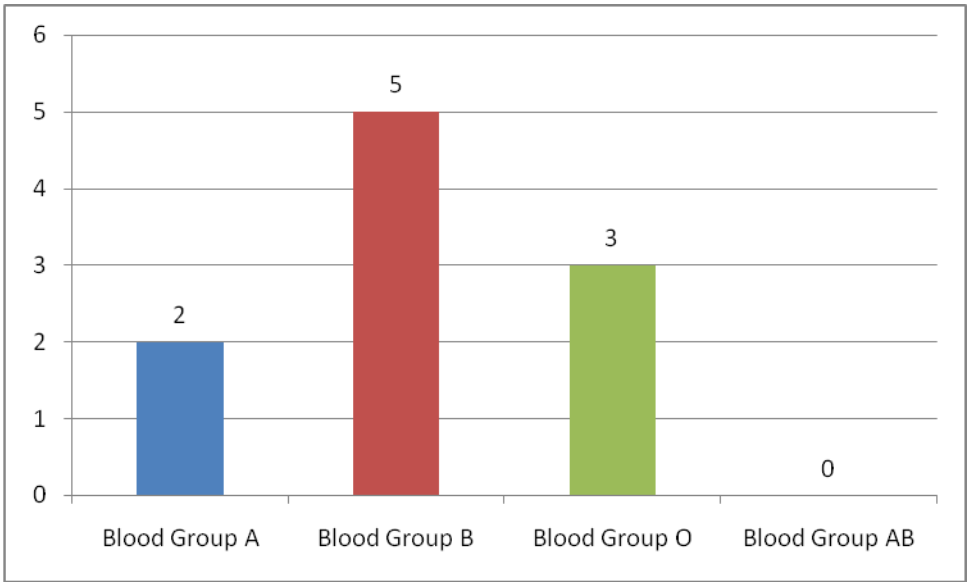
Age	No.	%
Below 19 yrs	08	80.00%
20-30 yrs.	02	20.00%

Figure 40 shows out of 10 leukemic patients, 8 patients fall between 0-19 yrs and 2 patients fall between 20-30 years.

Determination of Blood Group in Multitransfused Leukemics was carried out as follows.

Figure 41

Prevalence of Blood Group among multitransfused Leukemic patients



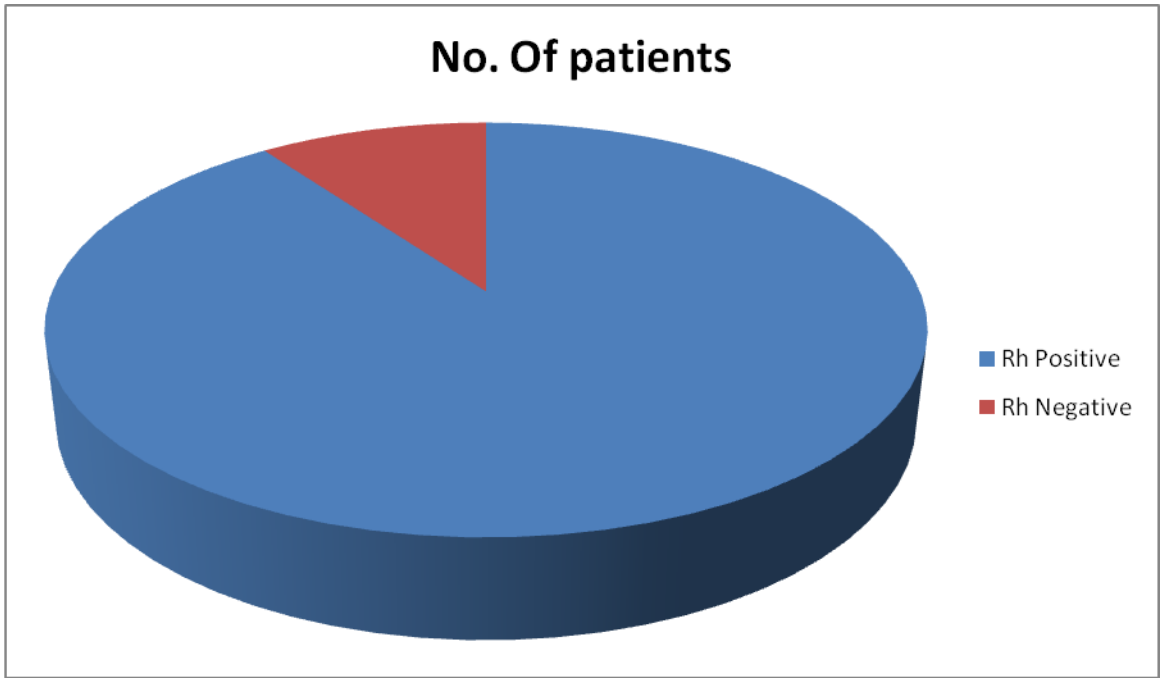
Total	No.	%
Blood Group A	02	20%
Blood Group B	05	50%
Blood Group O	03	30%
Blood Group AB	00	00%

Figure 41 shows prevalence of Blood Group in accordance with literature reports. Blood Group B was the most prominent(50%) followed by Blood Group O(30%), Blood Group A (20%).There is higher prevalence of Blood Group B as compared to the normal population. As the number of subjects under the study is less, no conclusive remark can be made.

Rh Factor distribution was observed as follows in our study.

Figure 42

Prevalence of Rh Factor among multitransfused Leukemic patients



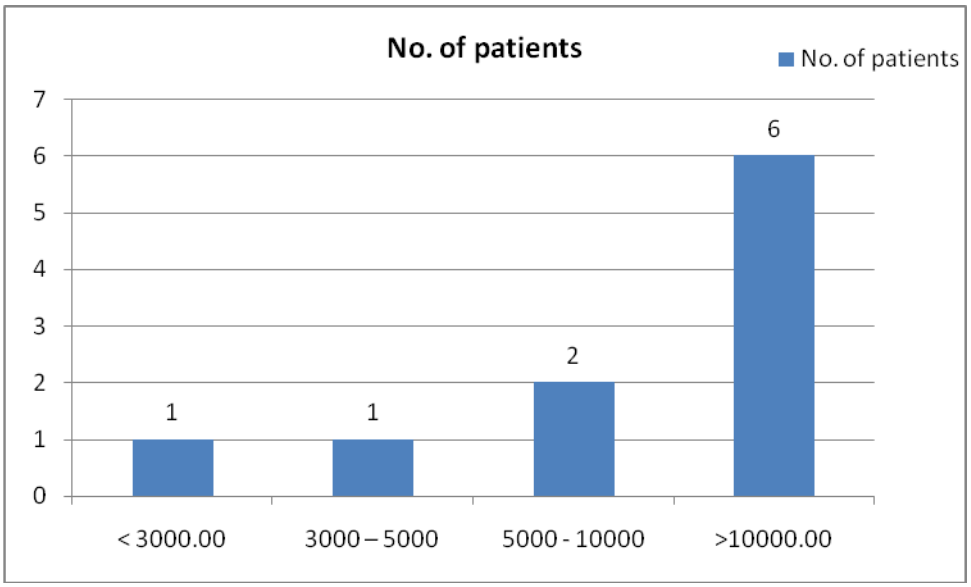
Total	10	100%
Rh Positive	09	90.00%
Rh Negative	01	10.00%

Figure 42 shows prevalence of Rh factor in leukemic patients out of 10 patients 9 patients shows Rh Positive factor and 1 patient shows Rh Negative factor.

Leukemia is a disease which requires intense and prolonged treatment which may be very expensive. In order to study socio-economic impact of the disease following study was carried out.

Figure 43

Distribution of Leukemic patients according to their family Income



Income per month	No. of patients	%
< 3000.00	01	10.00%
3000 – 5000	01	10.00%
5000 - 10000	02	20.00%
>10000.00	06	60.00%

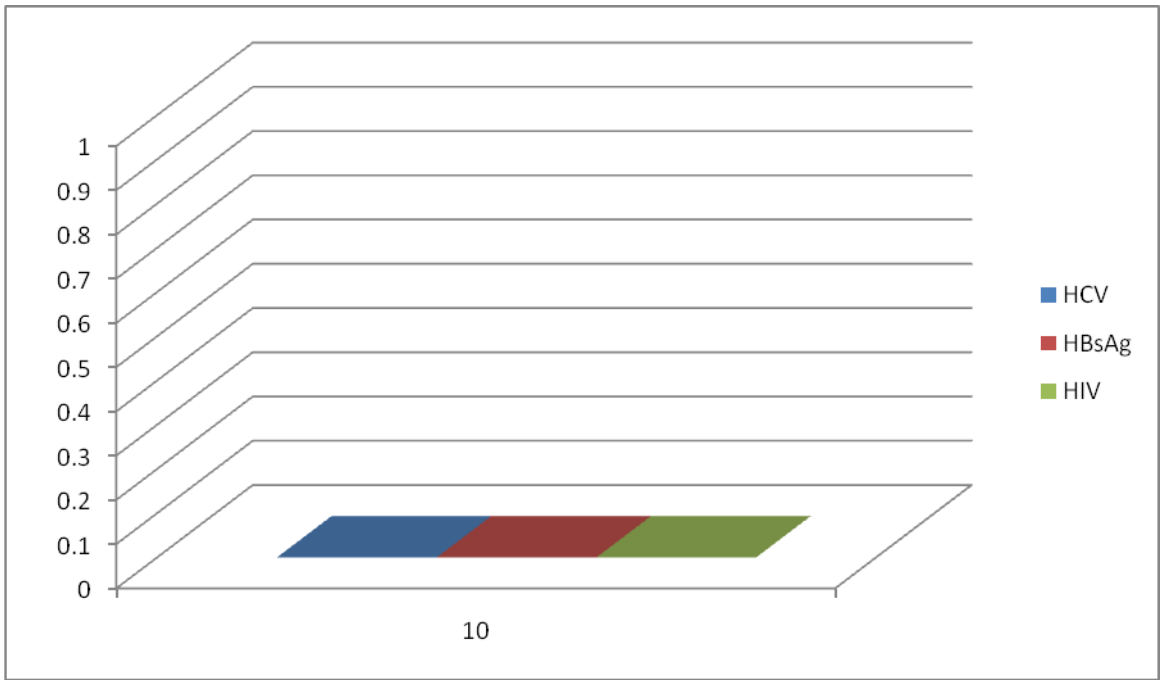
Figure 43 shows family income per month of multitransfused Leukemic patients. Out of 10 patients we have registered 1 patient shows income <3000.00 Rs. 1 patient shows income between 3000-5000 Rs. And 2 patients show family income between 5000-10000 Rs. And 6 patients show family income more than 10000 Rs.

Patients having monthly income of less than Rs. 10,000 (40%) have to bear burden of treatment of Leukemia with great difficulty as per our study.

Rate of infectivity of viral markers namely HCV, HbsAg and HIV were studied in our subjects.

Figure 44

Infectivity rate of multitransfused leukemic patients



Total no. of patients	HCV	HBsAg	HIV
10	00	00	00

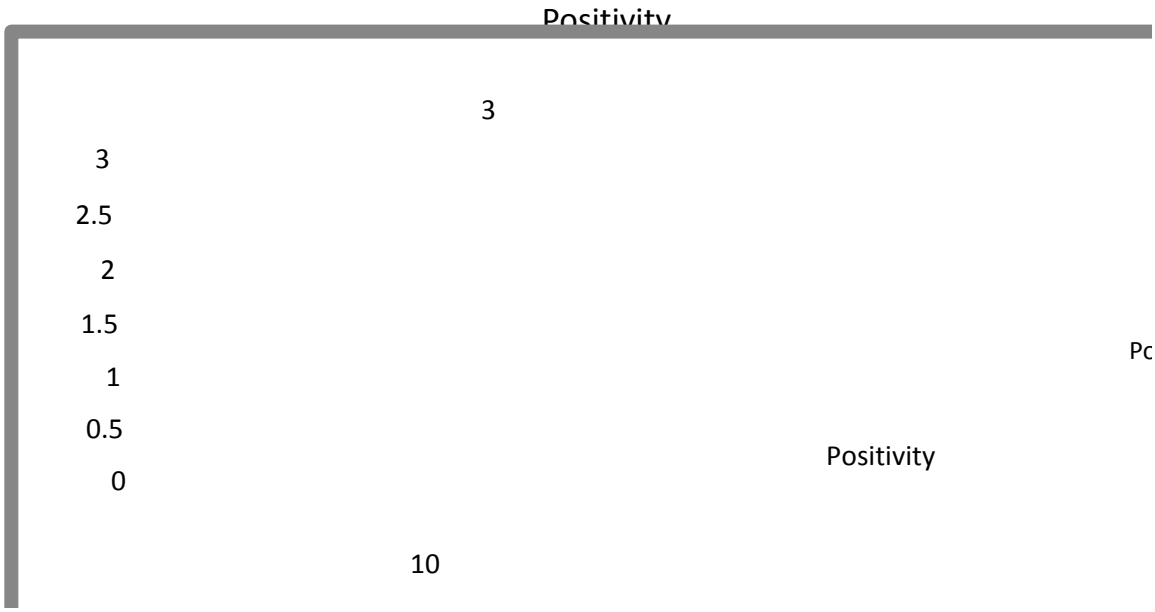
Figure 44 shows infectivity rate among Leukemic patients. All ten patients were HIV, HBsAg and HCV negative.

Fortunately none of our subjects had additional complication of life threatening viral infections due to multiple blood transfusions.

Cytomegalovirus infection is common among Leukemic patients. Seropositivity of Leukemic patients for CMV was studied in our subjects.

Figure 45

Prevalence of CMV among Multitransfused Leukemic patients

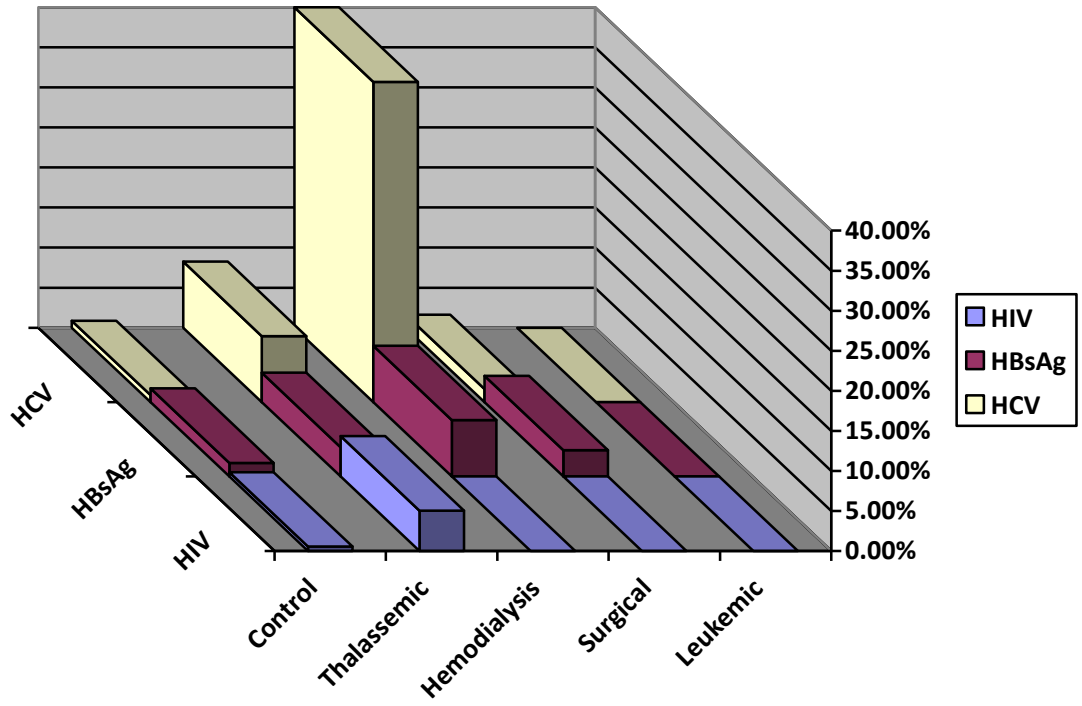


Total	Positivity	%
10	03	30.00%

Figure 45 shows infectivity rate of CMV among Leukemic patients. Out of ten patients three (30%) were CMV Positive. CMV infection is common among immunocompromised individuals. Chemotherapy treatments in Leukemia patients result in poor immune status of these patients. Our results indicate CMV infection may be due to immunocompromised status of our subjects.

Figure 45

Comparative Data



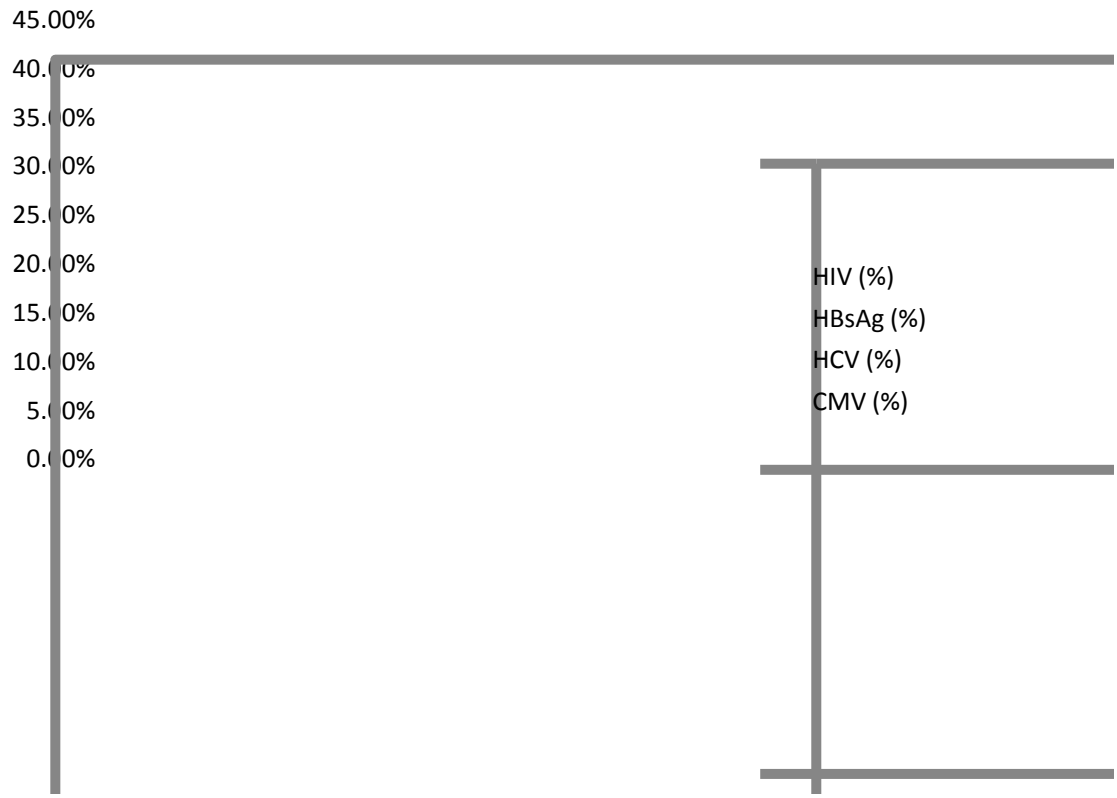
Viral Infections	Control (%)	Thalassemia (%)	Hemodialysis (%)	Hemophilia (%)	Surgical (%)	Leukemic (%)
HIV	0.55%	5.04%	-	-	0.00%	-
HBsAg	1.71%	3.66%	7.05%	0.43%	3.28%	-
HCV	0.87%	8.25%	40.00%	0.43%	1.64%	-

Figure 45 shows comparative incidence of viral markers in all five cohorts as compared to the control population of healthy donors.

Comparative aspect of viral infections in different cohorts of multitransfused patients is studied.

Figure 46

Comparative incidence of viral infections in various cohorts of multitransfused patients



Various Cohorts	HIV (%)	HBsAg (%)	HCV (%)	CMV (%)
Thalassemia	5.04%	3.66%	8.25%	-
Hemophilia	00.00%	0.43%	0.43%	1.29%
Hemodialysis	00.00%	7.05%	40.00%	-
Surgical Patients	00.00%	3.28%	01.64%	-
Leukemic Patients	00.00%	00.00%	00.00%	30.00%

Figure 46 shows overall comparison of viral infections in different cohorts. Prevalence of HIV among Thalassemic patients,Hemophilics,Hemodialysed patients, surgical patients and Leukemia patients is 5.04%,0.00%,0.00%,0.00% and 0.00% respectively. Prevalence of HBsAg among Thalassemic patients, Hemophilics,

Hemodialysed patients, surgical patients and Leukemia patients is 3.66%,0.43%,7.05%,3.28% and 0.00% respectively. Prevalence of HCV among Thalassemic patients,Hemophilics,Hemodialysed patients, surgical patients and Leukemia patients is 8.25%,0.43%,40.00%,1.64% and 0.00% respectively. . Prevalence of CMV among Thalassemia patients,Hemophilics,Hemodialysed patients, surgical patients and Leukemic patients is 0.00%,2.64%,0.00%,0.00% and 30.00% respectively.

CHAPTER 5

DISCUSSION

CHAPTER 5
DISCUSSION

Blood transfusion currently faces interesting challenges. While advances have been dramatic, both in terms of technology and organizational up gradation in developed countries, blood transfusion in developing countries still tend to stagnate with acute shortages, lack of component therapy and safety problems.

Many a times, these problems are perpetuated by financial limitations, political instability, endemic infections transmitted by transfusion and cultural taboos which inhibit blood donation. The net effect is that blood transfusion takes place in dangerous conditions, its lifesaving purpose subverted by lack of effective control. Microbial adaptation, climate and weather changes, war and famine, and the spectre of bioterrorism all raise the concern of emerging infectious threat to the blood supply. The situation has been further compounded and thus demands urgency with the emergence of AIDS and the growing understanding of the complexity of Hepatitis and its threat to transfusion recipient. Some infectious agents e.g. cytomegalovirus, human T-lymphotropic virus, *Treponema pallidum* are transfused more readily by relatively fresh blood components, whereas other agents (HBV, HIV) are stable in stored, and even in frozen red cells or plasma.

As far as transfusion transmitted infections are concerned in patients receiving multiple blood /blood components it depends on the demographic epidemiology of the etiological agent as well as socioeconomic factors associated with treatment.

Our present study is an attempt to highlight prevalence of some transfusion transmitted infections in and around Rajkot and compare it with available literature data in multiple blood transfusion individuals.

Source of transfusion transmitted microbial infection in various cohorts of multitransfused patients is ultimately donor's blood. In our studies incidence of microbial infections in donors were examined to get the idea of frequency as well as demographical variation of these microbial markers in the population.

HBsAg seropositivity in Donors

No.	Author	Year	Place	Positivity %
1	Talib et al	1983	Delhi	17.70%
2	Shanmugham et al	1978	Kerala	9.60%
3	Dutta & Mohammed	1972	Delhi	2.65%
4	Tejsingh Thakur	1990	Himachal Pradesh	2.56%
5	Pal et al	1973	Chandigadh	2.20%
6	Elvina et al	1989	Mumbai	2.02%
7	Vipul Khakhar	2002	Jamnagar	1.67%
8	Thaygarajan S.P.	1981	Himachal Pradesh	1.62%
9	Sama et al	1973	Delhi	1.60%
10	Graves & Biswas	1973	Calcutta	0.40%
11	Present Study	2007	Rajkot	1.98%

Hepatitis B virus infection is a major cause of morbidity and mortality in humans and it is endemic all over the world. About 350 million people of the world are infected with this virus (Lee, 1997)

Hepatitis B virus is major cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma especially in Asian countries. Due to crowded and unhygienic living conditions, horizontal transmission in early childhood is the major mode of infection of HBV in India (Tandon et al., 1996).

Hepatitis C virus first identified in 1989, was major cause of non-A, non-B hepatitis. Around 100 million people worldwide are estimated to be infected with HCV. Chronicity occurs in about 80% of infected patients. The health burden of chronic hepatitis C infection in the western world is gradually being realized. In most Asian countries, Hepatitis B virus is still the major cause of chronic liver disease and hepatocellular carcinoma. In Japan, the pattern is changing in the past decades and now HCV is the predominant cause of HCC. A comprehensive assessment of HCV infection in Asia is important. Any factor or activity that increases the risk of

transmission must be identified and built into strategy for infection control. This is particularly vital when effective HCV vaccine is not available.

HCV seropositivity in Donors

No.	Author	Year	Place	No. Tested	Positivity %
1	Khan M.	1993	Dhaka	163	2.4%
2	Panigrahi A.K.	1997	Delhi	15922	1.85%
3	Kakaepoto G.N.	1996	Karachi Pakistan	16704	1.80%
4	Jaiswal S.P.	1996	Indore	280	1.78%
5	Irshad M.	1995	Delhi	234	1.54%
6	DeSilva H.J.	1998	Sri Lanka	1748	0.74%
7	Choudhury N.	1995	Lucknow	313	0.30%
8	Jha J.	1995	Pune	NA	0.25%
9	Present Study	2008	Rajkot, India	7458	1.09.%

Hepatitis C Virus has been identified as an important etiological agent responsible for transfusion associated hepatitis and accounts for about fifty percent of the sporadic cases of non-A, non-B hepatitis (CDC, 1991).

The transmission of human immunodeficiency virus (HIV) through blood transfusion and the consequent emergence of transfusion associated acquired immune deficiency syndrome (AIDS) epidemic have arguably transformed the field of transfusion medicine over past several decades. HIV-1 and HIV-2 are the etiologic agents. The rate of confirmed positive infections detected amongst blood donors declined markedly due to notification and deferral of repeat donations from individuals, who had tested positive and with implementation of better strategies (donor informational documents and donor questionnaires) to exclude "at risk donors".

HIV seropositivity in Donors

No.	Author	Year	Place	No. Tested	Positivity %
1	Kwesigabo G.	2002	Tanzania	454	11.90%
2	Kiwanuk N.	2002	Uganda	6868	3.92%
3	Durosinmi M.A.	2002	Nigeria	16080	2.30%
4	Tallur S.	1997	Hubli	19705	1.74%
5	Rao P.	1994	Pune	44190	0.69%
6	Chandra M.	1991	Bombay	30632	0.60%
7	Sombasiva Rao R.	1995	Pondichary	19023	0.55%
8	Kulshreshtha R.	1999	Lucknow	39965	0.53%
9	Yan Y, Zhengz	1999	Fiji	569873	0.12%
10	Chievetta J.A.	2001	Canada	2000000	0.10%
11	Bhushan et al	1993	Vellore	79591	0.10%
12	Present Study	2008	Rajkot, India	7458	0.96%

Recognition of a possible relationship between blood transfusions and the AIDS provided early clues that AIDS might have an infectious cause. In much of Asia and Africa, the transmission of HIV by blood transfusion is still an important source of infection. Reasons for high rate of transmission, include 1) high demands for blood 2) prevalence of HIV infection amongst donor population and 3) The fact that HIV infection is not confined to a minority of the population who can be requested to refrain from blood donation and 4) the inability of many laboratories to test for HIV or to perform and control the tests properly. As can be seen from the table some of the African countries like Tanzania has a very high prevalence of HIV among donors whereas in India seropositivity is variable in different regions of the country depending upon socio-cultural activities.

Hepatitis B is an infection of major public health importance. More than one third of world's population has been infected with Hepatitis B virus (Sarin, 2002).

HBsAg seropositivity in Thalassemic patients

No.	Author	Year	Place	No. Tested	Positivity %
1	Hwang K.C.	1990	China	68	82.4%
2	Juneja M.	1998	New Delhi	64	31%
3	Moroni G.A.	1984	Italy	128	25%
4	Mollah A.H.	2003	Dhaka	152	13.8%
5	Cacopardo B.	1992	Italy	152	8%
6	Williams T.M.	1992	London	54	7.4%
7	Khakhar V.	2006	Surendranagar,Gujarat	90	6.6%
8	Singh H.	2003	Lucknow	70	5.7%
9	Chakravarti A.	2005	New Delhi	50	2.0%
10	Mirmomen S.	2006	Shiraz, Iran	732	1.5%
11	Karimi M.	2001	Shiraz, Iran	755	0.53%
12	Present Study	2008	Rajkot, India	218	3.66%

The frequency and natural history of Hepatitis B virus infection vary with geographic regions. A correlation study of literature data and our results suggested that Hepatitis B seropositivity in the Thalassemic subjects under this study was not alarmingly high. Though it was higher compare to data from Iran and Northen India, it is much less in comparison to China.

Hepatitis C virus has affected 150 million people worldwide and the number is increasing to term the disease as one of the most deadly disease of the century.

HCV seropositivity in Thalassemic patients

No.	Author	Year	Place	No. Tested	Positivity %
1	Silipoulou I.	1995	Greece	24	91.6%
2	Locasciulli A.	1993	Monza,Italy	25	76.0%
3	Angelucci E.	1994	Pesaro, Italy	256	60%
4	Chakravarti A.	2005	New Delhi	50	60%
5	Cocopardo B.	1992	Italy	152	47%
6	Kazemi M.	2008	Rafsanjan, Iran	60	45%
7	Nigro G.	1992	Rome, Italy	36	41%
8	Wonke B.	1990	London	73	23.3%
9	Juneja M.	1998	New Delhi	64	21%
10	Singh H.	2003	Lucknow	70	20.0%
11	Mirmomen S.	2006	Iran	732	19.3%
12	Agrawal M.B.	1993	Mumbai	72	16.7%
13	Karimi M.	2001	Shiraz, Iran	466	15.7%
14	Mollah A.H.	2003	Dhaka	152	12.5%
15	Williams T.M.	1992	London	54	11.1%
16	Samimi-Rad K.	2007	Tehran, Iran	98	5.1%
17	Present Study	2008	Rajkot, India	218	8.25%

Individuals with chronic hepatitis C are at much higher risk of developing hepatocellular carcinoma. The incidence of HCC is on an increasing trend in many parts of the world. The possible routes of transmission include the use of unscreened blood, blood products, tissue or organs, inadequately sterilized equipments etc. (Sarin, 2002b)

Seroprevalence of anti-HCV worldwide and in India showed substantial variation as can be seen from the comparative data.

HIV is a serious disorder of immune system makes the most vulnerable to a host of life threatening infections including unusual malignancies. A watchful WHO publishes data on spread of infection across the globe. According to them virtually every country is in the grip of this deadly disease and the epidemic is out of control in many places.

HIV seropositivity in Thalassemic patients

No.	Author	Year	No. Tested	Positivity %
1	Dubey A.P. et al.	1993	75	9.3%
2	Sen S. et al.	1993	203	8.9%
3	Vohra R.	1995	89	8.9%
4	Khan M.A.	1992	203	8.37%
5	Bichile S.K. et al.	1992	50	6.0%
6	Sudarshan et al.	1994	223	3.5%
7	Amrapurkar D.N. et al.	1992	40	2.5%
8	Hazami M.A.F.E.L. et al.	1989	212	2.35%
9	Sur D. Chakraborty AK.	1990	330	0.9%
10	Bhargava M. et al.	1991	185	0.54%
11	Singh S. et al.	1993	100	0.0%
12	Choudhary V.P. et al.	1993	91	0.0%
13	Chandra S. et al.	1993	22	0.0%
14	Choudhary N. et al.	1995	19	0.0%
15	Mirmomen S.	2006	732	0.0%
16	Present Study	2008	218	5.04%

Increasingly the spotlight is on the spread of HIV through the Asian continent, specially in South Asia and East Asia while rates remain low relative to some other regions, well over 7 million Asians are already infected and HIV is clearly beginning to spread in earnest through the vast populations of India and China (HIV testing manual).

Incidence of HIV in multitransfused Thalassemic patients in our study was higher than a few studies carried out by other workers. However, incidence as high as 9.3% has been reported by other group of workers. Our results show a moderate rate of HIV incidence.

Patients of Hemophilia receive various blood components upon episodes of bleeding. Depending upon the infrastructure and socio-economical background, the patient may receive purified factor preparation to fresh frozen plasma (FFP). Incidence of

microbial infection depends on the component given to the patient as well as viral inactivation procedure followed.

HBsAg Seropositivity in Hemophilic patients

No.	Author	Year	Place	No. Tested	Positivity %
1	Nebbia G.	1986	Milan, Italy	44	55%
2	Sengupta B.	1992	Calcutta	37	24.3%
3	Chow M.P.	1991	Taiwan	11	9%
4	Ghosh K.	2000	ICMR, Mumbai	400	6%
5	Sharifi-mood B.	2007	Iran	81	4.9%
6	Present Study	2008	Rajkot, India	232	0.43%

As shown in above table there is a great variation from different study group not only in the percentage positivity but also the size of the sample. In our present study Rajkot Chapter of National Hemophilia Society receives FFP from a selected pool of donors carefully screened and restricted only for this purpose. This explains low seropositivity of Hepatitis B marker in our study. The reason for low seropositivity can be attributed to vaccination against Hepatitis B to all our Hemophiliac subjects.

Prevalence of HCV among Hemophiliacs depends primarily upon the amount and type of product transfused. Almost universal exposure to HCV is observed in Hemophiliacs receiving untreated commercial clotting factor concentrates. However, Hemophiliacs receiving appropriately inactivated coagulation components from single donor cryoprecipitate generally remain Anti-HCV negative (Brettler, 1990).

HCV Seropositivity in Hemophilic patients

No.	Author	Year	Place	No. Tested	Positivity %
1	Chow M.P.	1991	Taiwan	11	100%
2	Ghany M.G.	1996	Louisiana, USA	100	79%
3	Samimi-Rad K.	2007	Tehran, Iran	76	43.4%
4	Sharifi-mood B.	2007	Iran	81	29.6%
5	Sengupta B.	1992	Calcutta	37	27%
6	Ghosh K.	2000	ICMR, Mumbai	400	23.9%
7	Present Study	2008	Rajkot, India	232	0.43%

HCV seropositivity in hemophilic patients found to be varying from 100% to 0.43% in various studies. As explained earlier policy of giving single donor cryoprecipitate to hemophiliacs is the reason of low seropositivity in our study.

Because of the enormous risk involved in transmission of HIV through blood, safety of blood and blood product is of paramount importance. Currently, the risk of transmission of HIV through transfusion is minimal, because effective preventive strategies, including new laboratory tests, have been implemented.

HIV Seropositivity in Hemophilic patients

No.	Author	Year	Place	No. Tested	Positivity %
1	Chow M.P.	1991	Taiwan	11	82%
2	Sultan Y.	1987	Paris, France	2049	48%
3	Ghany M.G.	1996	Louisiana, USA	100	42%
4	Sengupta B.	1992	Calcutta	37	24.3%
5	Ghosh K.	2000	ICMR, Mumbai	400	3.8%
6	Present Study	2008	Rajkot, India	232	00.00%

The virus that causes AIDS (HIV) can be carried in clotting factors as well as plasma. However, there has been no documented case of these viruses being transmitted during replacement therapy in our present study. Transmission of viruses has been prevented by careful screening of blood donors, testing of donated blood products, treating donated blood products with a detergent and heat to destroy viruses.

Infectious agents like Cytomegalovirus are transmitted more readily by relatively fresh blood components. The transmission of CMV by blood transfusion was first reported in the 1960s (Klemola et al., 1969). In most cases CMV appears to be transmitted only by cellular blood components, and the virus reactivates from donor leucocytes after transfusion. Host as well as donor factors are involved in CMV infection.

CMV Seropositivity in Hemophilic patients

No.	Author	Year	Place	No. Tested	Positivity %
1	Okubo S.	1990	Japan	49	85.70%
2	Rollang H.	1989	Norway	377	75.00%
3	Rabkin C.S.	1993	Rockville	393	49.00%
4	Chesseman S.H.	1984	NA	100	42.00%
5	Nogueira E.	2000	Brazil	100	25.00%
6	Present study	2008	Rajkot, India	232	01.29%

Generally, the line of treatment in Hemophiliac patients after the episodes of bleeding is either FFP or pure commercially available clotting factors. Leucodepletion in both these cases reduce the chances of acquiring CMV infection. However, improper preparation may account for low incidence as reported in our study.

The incidence of post-transfusion Hepatitis B throughout the region has reduced tremendously after introduction of HBsAg screening of donor blood in most countries.

HBsAg Seropositivity in Patients on Hemodialysis

No.	Author	Year	Place	No. Tested	Positivity %
1	Aghanishinikar P.N.	1992	Sultanate Of Oman	102	52%
2	Barton E.N.	1998	Jamaica	63	34.9%
3	Al-Mugeiren M.		Riyadh	20	15%
4	Hmida S.	1995	Tunisie	235	8%
5	Ballester J.M.	2005	Havana, Cuba	318	5.3%
6	Jaffers L.J. et al	1990	California	90	1.11%
7	Present Study	2008	Rajkot, India	115	7.05%

Viral liver diseases, especially due to hepatitis B virus (HBV) and hepatitis C virus (HCV), were observed with a high frequency in hemodialysis units throughout the world. Due to the deficient immune response of uremic subjects, which renders them unable to eliminate viruses, hemodialysis patients act as reservoirs of the viruses and transmit infection to other patients, to the dialysis unit staff and eventually to their own family, in the case of infection with HBV.

Hepatitis C virus (HCV) infection is a global health problem, common worldwide, leading to acute and chronic hepatitis and its consequences of hepatocirrhosis and hepatocellular carcinoma. Patients on hemodialysis belong to the high-risk group of HCV infection. The prevalence of HCV infection in dialysis patients ranges from 4% to more than 70% in some countries. The main reasons for such a high incidence of infections are a high prevalence of HCV infection in the general population, lack of standard infection precautions and effective vaccination, inadequate disinfection procedures of dialysis machines and other medical equipment, as well as spread of infection from patient to patient, especially in dialytic centers with a high percentage of infected patients. The diagnostic procedures useful in the evaluation of HCV infection are detection of anti-HCV antibodies.

HCV Seropositivity in Patients on Hemodialysis

No.	Author	Year	Place	No. Tested	Positivity %
1	Capsa D.	1991	Romania	133	91.7%
2	Gohar S.A.	1995	Ain-shams	46	87.5%
3	Ballester J.M.	2005	Havana, Cuba	318	51.6%
4	Hmida S.	1995	Tunisie	235	45.10%
5	Al-Mugeiren M.	1992	Riyadh	20	45%
6	Al-furayh O.	1992	Riyadh	52	40.4%
7	Wu J.S.	1991	Taiwan	63	36.5%
8	Jaffers L.J. et al	1990	California	90	12%
9	Barton E.N.	1998	Jamaica	63	7.9%
10	Present Study	2008	Rajkot,India	115	40.00%

As can be seen from the table patients undergoing maintenance hemodialysis constitute an especially important high risk group for HCV acquisition. Overall, Anti-HCV positivity among donors is not significantly high and therefore, blood transfusion alone does not seem to be responsible for the majority of HCV infections among the dialysis patients. Taking into consideration well documented evidence of role of poor infection control practices in HCV transmission among dialysis patients, dialysis units need to evolve stricter protocols for eliminating this mode of the spread of virus (Lamballerie, 1996).

In India, the main mode of transmission of HIV is through sexual contact. However, HIV infected blood/blood products can also transmit this infection. This is the most efficient way of transmission of HIV. Even a small transfusion of infected blood results in virtually 100% seroconversion. Many social and economic factors play role in incidence of AIDS in different part of the world ranging from low literacy to unsafe sex to alcohol use (HIV testing manual b).

HIV Seropositivity in Patients on Hemodialysis

No.	Author	Year	Place	No. Tested	Positivity %
1	Barton E.N.	1998	Jamaica	63	9.5%
2	Aghanishinikar P.N.	1992	Sultanate Of Oman	102	2.0%
3	Assogba U.	1998	Paris	347	1.1%
4	Ballester J.M.	2005	Havana, Cuba	318	00.00%
5	Hmida S.	1995	Tunisie	235	00.00%
6	Al-Mugeiren M.		Riyadh	20	00.00%
7	Present Study	2008	Rajkot, India	115	00.00%

Although each donated unit of blood is tested for evidence of infection by specific agents, there are at least four potential reasons why transmission of these agents may still occur: a) the donor has negative laboratory test results during the early stages of infection, known as the window period, b) the existence of a chronic carrier state in which a clinically asymptomatic donor will persistently test negative on a screening assay, c) donors harboring a mutant or atypical variant and d) laboratory errors when performing the screening tests. Measures to assure the safety of blood and blood components include use of voluntary donors, donor selection and questioning, laboratory testing for serological markers of infections, maintenance of registries of disqualified donors.

HBsAg Seropositivity in Multitransfused Surgical Patients

No.	Author	Year	Place	No. Tested	Positivity %
1	Utkan A.	2006	Turkey	646	2.50%
2	Ganczak M.	2008	Poland	100	1.00%
3	Present study	2008	Rajkot, India	61	3.28%

High incidence of Hepatitis B infection in our study compare to the available literature data may be attributed to low sample size and general reliability on replacement donors in surgical cases.

HCV infections continue to be a source of clinical interest resource utilization for the near future. It is highly likely that a marked increase in the prevalence of hepatic cancer will be the next most common disease associated with chronic hepatitis C. Thus, research directed at preventing and treating HCV infection, therapies aimed at blunting the antigenic effect of HCV and treating HCV induced hepatic cancer should be initiated and pursued aggressively. As HCV may soon account for more deaths annually than are due to HIV infections, it is essential to identify mechanisms of HCV virulence, persistence and oncogenicity. Only with such information will it be possible to ease the disease burden due to this rather single and complex infectious agent.

HCV Seropositivity in Multitransfused Surgical Patients

No.	Author	Year	Place	No. Tested	Positivity %
1	Utkan A.	2006	Turkey	646	0.60%
2	Ganczak M.	2008	Polish	100	0.00%
3	Present study	2008	Rajkot, India	61	1.64%

Similar to the results obtained with HBV infections there was higher prevalence of HCV infection among our subjects compared to the literature study.

Because HIV is both cell associated and present in plasma, all blood components are potentially infectious. The virus is well preserved in refrigerated and frozen blood; however, components that are washed, leucoreduced or cold stored for several weeks, procedures that diminish the number of viable leucocytes or the amount of virus reduced the likelihood of transfusion transmission (Donegan et al.1990).

HIV Seropositivity in Multitransfused Surgical Patients

No.	Author	Year	Place	No. Tested	Positivity %
1	Utkan A.	2006	Turkey	646	0.00%
2	Ganczak M.	2008	Polish	100	0.00%
3	Present study	2008	Rajkot, India	61	0.00%

The reduction in risk of transfusion transmitted HIV over the past fifteen years has been dramatic and reassuring. Donor demographics have proved effective at identifying and excluding donors at high risk for infection and transmission of HIV.

Platelet rich plasma and platelet concentrates are indicated in Leukemia. Post transfusion hepatitis in leukemia patients is not very frequent since the patients are vaccinated against this virus.

HBsAg Seropositivity in Multitransfused Leukemic Patients

No.	Author	Year	Place	No. Tested	Positivity %
1	Tavil B.	2007	Turkey	160	15.00%
2	Yeo W.	2000	China	626	12.00%
3	Kebudi R.	2000	Istanbul	50	10.00%
4	Rossi D.	2009	Italy	173	10.00%
5	Monteleone P.M.	1994	Massachusetts	45	00.00%
6	Present Study	2008	Rajkot, India	10	00.00%

As the sample size in present study was not large enough, no concrete conclusion can be made about the incidence of HBsAg seropositivity in multitransfused Leukemia patients. However, literature studies as shown on in the table show the incidence up to 15%.

Hepatitis C infection is considered to be the most common transfusion transmitted infection in the developed world. At present, only testing for Hepatitis C antibody is routinely employed. The antibody to the hepatitis C virus appears 54-192 days in a person's blood after infection. If an infected person donates blood prior to the appearance to this antibody the chance of that blood being used in a transfusion is said to 1 out of 103,000 donations.

HCV Seropositivity in Multitransfused Leukemic Patients

No.	Author	Year	Place	No. Tested	Positivity %
1	Kolho E.	1993	Finland	49	24.50%
2	Kebudi R.	2000	Istambul	50	14.00%
3	Monteleone P.M.	1994	Massachusetts	45	8.90%
4	Gharagozloo S.	2001	Iran	23	4.30%
5	Tavil B.	2007	Turkey	160	1.90%
6	Brodine S.K.	1995	California	2875	0.20%
7	Locasciulli A.	1994	Italy	15	00.00%
8	Present Study	2008	Rajkot, India	10	00.00%

It appears that due to the implementation of new epitopes of viral antigens in third generation ELISA it has become safer to the patient to rule out HCV infection. Present study does not have any positive report for this marker.

Risk of transmission of HIV after p24 antigen screening in donor's blood in developed countries has decreased the risk of transmission very drastically. Even in developing countries like India awareness about HIV status has reduced incidence of transfusion transmission of HIV.

HIV Seropositivity in Multitransfused Leukemic Patients

No.	Author	Year	Place	No. Tested	Positivity %
1	Kebudi R.	2000	Istambul	50	00.00%
2	Monteleone P.M.	1994	Massachusetts	45	00.00%
3	Present Study	2008	Rajkot, India	10	00.00%

Present study matches with that of literature data of 0% seropositivity for HIV marker in multitransfused Leukemia patients.

Subjects at higher risk of severe primary CMV infection are those who are anti-CMV negative patients with impaired immunity. Unfortunately, most of the Leukemic patients fall into this category. Transfusion transmitted cytomegalovirus infection (TT-CMV) has been documented as a cause of significant morbidity and mortality in immunocompromised as well as immunocompetent recipients. Window period infections are the most likely source of antibody screening failures.

CMV Seropositivity in Multitransfused Leukemic Patients

No.	Author	Year	Place	No. Tested	Positivity %
1	Vinelli E.	NA	Honduras	100	43.00%
2	Present Study	2008	Rajkot, India	10	30.00%

During the past decade major advances have been achieved regarding the management of CMV infection through the development of new diagnostic techniques for the detection of the virus. Nevertheless, in immunosupressed patients, CMV infection still causes severe disease that could be fatal.

From the above comparative data it can be said that:

- There is increasing incidence of sero-positivity of viral markers in donors over the years. This suggested that donation in the window period of infection or technical and demographical limitation of a developing country such as India.
- Except for hepatitis B marker in thalassamic patients, there was significant rise in viral markers in thalassamic as well as hemo-dialysis patients at the end of the study.
- As compared with the initial sero-positivity for HCV marker in hemo dialysis patients, there was a sharp increase at the end of the study. Medical fraternity seriously needs to look at the entire process of treatment to prevent renal failure patients acquiring HCV infection.
- Incidence of CMV sero-positivity was observed in hemophilia and leukemia patients. Later being immunocompromised, it was expected that opportunistic pathogen like CMV may lead to secondary complications.

- Incidence of post transfusion parasitic infection by *Plasmodium falciparum* was noted in one hemodialysis patient.
- With reference to HBV and HCV infection in hemophilia patients policy of strictly adhering to selected donors for fresh frozen plasma and using high quality viral inactivated clotting factors seem to be the cause of low incidence of sero-positivity in these patients.
- Survey of any particular blood group having predilection for HCV infection in moderately sizable population of HCV sero-positive patients show that blood group O people were most affected followed by blood group A patients.
- Socio economic parameters reflected that the burden of costly treatment was mainly on lower middle income group of people.
- Children in the age group below 19 years maximally reflected higher incidence of leukemia as per the available literature data.

CHAPTER 6

SUMMARY

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Blood transfusion is a routine life saving regime for millions worldwide. It is like a double edged sword which if not used with discretion and technical expertise can lead to many undesirable after events. Sudden spurt of awakening to the issue of blood safety arise only and when there is mass morbidity or mortality.

Certain medical conditions require that blood be transfused to patients on a regular basis over a period of months or years. Due to repeated transfusion of blood such individuals are at higher risk of complications related to blood transfusion.

Variety of factors ranging from window period of infection in donors to negligence in part of blood handlers to lack of infrastructure, rate of microbial infection due to blood transfusion is higher in developing countries like India.

Present research was carried out to study incidence of microbial infections in various cohort of patients who receive multiple blood transfusion.

Data of blood donation pattern and prevalence of microbial infection in "supposedly" healthy donors was studied to obtain insight into the quality of blood received by these patients.

Patients receiving multiple blood transfusion or blood component on regular basis were divided into four categories:

(1) Thalassaemia patients
(2) Hemodialysis patients
(3) Hemophilia patients
(4) Surgical multi transfused and leukemia patients

Various parameters were studied in each group such as:

- Socio economic parameter in which patients were categorized on basis of their age, gender, caste and family income.

- Hematological parameters like hemoglobin level and haemogram which included WBC, RBC, HCT, MCV and RDW-CV.
- Serological parameters like detection of viral markers e.g. HIV, HBsAg, HCV and CMV by ELISA method and detection of *Treponema pallidum* infection by RPR test.
- Biochemical analysis like S.G.P.T. enzyme level, T.I.B.C. and Serum Iron.
- Bacteriological analysis like blood culture.
- Microscopic examinations for detection of malarial parasites
- Molecular analysis for detection of viral genetic material by PCR technique.
- Statistical analysis of some of the data obtained was also carried out using correlation analysis, two way ANOVA for analysis of some of the results.

Results from all the cohorts were studied and inter-cohort comparison as well as comparison with the trend of infection was carried out. Following trends were emerging out of our present study:

Donor Cohort

- Trend is changing from replacement to voluntary donors. The ratio at the beginning of the study was 0.41(voluntary/replacement), now it is almost 1 suggesting that now 50% of the blood comes from voluntary donation.
- Incidence of microbial markers is increasing even in donor population.
- Most frequently occurring blood group is blood group B followed by blood groups O, A &AB in decreasing order in donor population.

Thalassemic Cohort

- Thalassemic cohort has higher seropositivity for HIV, HBsAg & HCV compared to donors.
- At the end of the study increase in incidence of HIV & HCV has been observed. Seropositivity in terms of percentage for HBsAg is decreased suggesting efficiency of vaccination for this marker in thalasemic subjects.
- About 50% thalassemic patients had spleenomegaly and 14% were spleenectomized.

- Hematological & serological parameters show typical microcytic anemia. Elevated levels of SGPT was observed but was not always associated with seropositivity of hepatitis viral markers.

Hemodialysis Cohort

- In cohort of hemodialysis patients higher incidence of hepatitis viral markers was observed especially hepatitis C.
- Number of male patients on maintenance hemodialysis was more than female patients.
- There was an increase in the incidence of HBV and HCV infection at the end of the study observed.
- An alarmingly high rate of HCV infection in hemodialysis patients and remarkable increase at the end of the study was observed.
- Other microbial infection like parasitic infection by malarial parasite was found in one patient undergoing maintenance hemodialysis.
- Blood group O and A seem to have predilection for hepatitis C virus infection in haemodialysis patients.
- On an average, number of dialysis was more for HCV positive patients than HCV negative patients.
- Socio-economical parameters such as age & gender were studied. Where number of male patients were 64.34% while female patients were 35.65% suggesting faster progression of renal failure in males compare to females. The age group that was affected most was 20-40 years which comprised more than 50 %(52.48%) of the total CRF patients on maintenance hemodialysis.

Haemophilia Cohort

- Majority of the patients in Hemophilia cohort were suffering from severe factor VIII deficiency in the age group of 15 to 30.
- Lower incidence of HBsAg and HCV marker may be due to excellently screened donors for FFP and viral inactivation of factors.
- CMV infection was more prevalent in hemophilia patient compare to HBsAg and HCV seropositivity.

- Low socio-economic status of most of the families made the treatment and management of the disease even more difficult.

Surgical multitransfused and Leukemia Cohort

- Bacteriological analysis of surgical multitransfused patient did not reveal presence of any bacterial growth in laboratory set up.
- Incidence of HBsAg and HCV was observed higher from the control results.
- A small group of multitransfused leukemia patients was also studied with respect to socio-economic criteria and microbial infections.
- There was no seropositivity for HIV, HBsAg and HCV in our subject group.
- Immunosuppressive treatment makes leukemia patients vulnerable to opportunistic pathogens like CMV as shown in our study.

CHAPTER 7

CONCLUSION

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CONCLUSIONS

Donors

- There is an increased incidence of all the microbial markers among donor population suggesting rise in rate of infection in general population.
- Seropositivity of Hepatitis C virus among general population has increased alarmingly from 2002 to 2007. The increase is 5.19 times suggesting rapid spread of infection in general population.
- Trend of voluntary donation of blood has increased steadily from 2002 to 2007; it is a very welcome result in our study. Healthy voluntary non-remunerative donors will lead to safe blood supply to the patients.
- This trend is also reflected in ratio of voluntary to replacement blood donation. In 2002 it was only 0.41 which exceeded 1.02 in 2007 suggesting more then 50% of blood comes from voluntary donation.

Thalassemia

- Socio-economic studies reflect that number of male patients is more than double compared to female patients suggesting bias towards treatment of only male children.
- Thalassemia is most prevalent in scheduled caste followed by Lohana, Muslim and Brahmin in decreasing order. Inclusion of Brahmin caste in higher incidence category is the new finding of our result.
- Higher incidence of viral markers such as HIV, HBsAg and HCV compared to donor population is indicative of thalassemics being at higher risk of Transfusion Transmitted Infections.
- Incidence of all the three above said markers was compared in the beginning and at the end of the study. Rate of HIV and HCV infections was markedly high at the end of the study compare to the beginning. However, rate of HBsAg infection was not so high suggesting success of immunization program against hepatitis B infection.

- Haemogram of Thalassemic patients reflect typical picture of Microcytic anemia.
- Elevated levels of S.G.P.T. enzyme in majority of Thalassemic patients suggest hepatospleenomegaly due to multiple blood transfusions. However higher S.G.P.T. level not necessarily meant seopositivity for Hepatitis B or C virus.
- Because of higher turnover rate of RBCs serum iron levels were higher in these patients compare to normal. Total Iron Binding Capacity (T.I.B.C.) was also found to be higher in these patients.
- No seropositivity of transfusion transmitted Syphilis was observed in our study.

Hemodialysis

- Number of male patients with Chronic Renal Failure (CRF) was higher compare to the female patients. Our data is in confirmation with earlier findings that the rate of progression of renal disease is faster in male than female.
- Distribution of blood groups in Hemodialysis patients is same as normal population suggesting no particular relationship between blood group and CRF condition.
- Seropositivity with viral markers mainly Hepatitis B and Hepatitis C is higher in CRF patients compared to normal population.
- An alarmingly high rate of HCV infection in hemodialysis patients and remarkable increase at the end of the study i.e. from 30.45% to 40.00% suggests need to reexamine all the aspects of possible entry of infectious agent into hemodialysis patients right from donor's screening to spread of infection during the process of dialysis to immune status of the patient.
- Average number of dialysis is also significantly high in HCV positive CRF patients compared to HCV negative CRF patients.
- Other microbial infection like parasitic infection by malarial parasite was found in one patient undergoing maintenance hemodialysis.
- No reactivity was observed for Syphilis by RPR test.

- An interesting result was observed with regard to distribution of blood group in HCV infected hemodialysis patients in contrast to the normal distribution of blood groups in general population. Prevalence of Blood Group O (33.82%) was followed by Blood Group A (30.88%), B (26.47%) and AB (5.88%) in decreasing order. Higher rate of infection in Blood Group A patients is reported in our study.

Hemophilia

- Comparable with literature data Factor VIII deficiency was most prominent in our subjects followed by Factor IX and other factors.
- Maximum number of Hemophilia patients was in the age group of 15-30 years. Number of young patients was least, suggesting good prenatal counseling. Survival of 16% patients above the age of 31 years suggests good management of the disease.
- Most of the patients had a relatively high factor requirement as shown in our study. This suggests severe hemophilia is prevalent in our cohort of study.
- Blood group B and O were almost equivalently prevalent.
- Family income of most hemophilic patients was not enough to be able to afford costly factor replacement therapy so most of them are dependent on Fresh Frozen Plasma (FFP) instead of pure factor thereby increasing risk of transfusion transmitted infections.
- CMV infection was more prevalent in hemophilia patient compared to HBsAg and HCV seropositivity. Good donor selection for FFP explains lower seropositivity for HBsAg and HCV. Immunocompromised status of these patients explains higher CMV infections.

Surgical multitransfused and Leukemia

- Males are more prone to accidental injury leading to multiple blood transfusions. In female patients, gynecological and obstretic surgery is one of the major conditions requiring multiple blood transfusions.
- Young people in the age group of 20-30 years comprise of more than 50% of surgical multitransfused patients in our study.

- Bacteriological analysis of surgical multitransfused patient did not reveal presence of any bacterial growth in laboratory set up. This could be because of the usual prescription of broad-spectrum antibiotics to the patients and bactericidal property of the blood.
- Incidence of HBsAg and HCV was higher from the control results suggesting multiple blood transfusions as high risk activity. However, no seropositivity of HIV marker was observed in our study.
- A small group of multitransfused leukemic patients was also studied with respect to socio-economic criteria and microbial infections.
- Most of these patients belong to lower middle income group.
- There was no seropositivity for HIV,HBsAg and HCV in our subject group.
- Immunosuppressive treatment makes leukemia patients vulnerable to opportunistic pathogens like CMV as shown in our study.

General Conclusions

Comparative data of viral markers in various cohorts of multitransfused patients show variable results. This could be because of many reasons. Few of them are listed below.

- Different immune status.
- Variable number of transfusions.
- Type of blood component.
- Source of Blood.
- Socio-economic parameters.

Among different cohorts incidence of viral markers were as follows

- Higher rate of HIV seropositivity was found in Thallaesamic patients compared to control population.
- HBsAg seropositivity was highest in Hemodialysis patients followed by Thalassemics and Surgical multitransfused patients respectively, least being in Hemophiliacs.
- Alarmingly high rate of infection by HCV is in Hemodialysis patients, second but distinctively far is Thalassemic children followed by surgical multitransfused patients.

- CMV infections were an alarming 30% in Leukemia patients and 1.29% in Hemophiliacs.
- Presence of Malarial parasite *P. falciparum* was observed in one hemodialysis patient.
- As regard to examination of blood samples from these patients no reactivity with RPR test was observed.
- No blood culture was found conclusively positive suggesting low prevalence of bacteria in blood. This could be attributed to bactericidal activity of blood components in stored blood.

CHAPTER 8

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