Frequency of Human Immunodeficiency Virus, HBsAg, HCV and Syphilis Infections
In Blood Donors In Khartoum state

A thesis submitted as partial fulfillment for requirement of master in hematology

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(2011)

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2014
بسم الله الرحمن الرحيم
قال تعالى:
(وفي أنفسكم إفلا تبصرون)
صدق الله العظيم
الذريات الاتية (21)
DEDICATION

This work dedicated to:

My beloved mother

Spirit of my father

And my brother and my sister ’to my

faithful friend and colleagues

And special dedication to Professor. Sana El Itahir

I hope to receive your joy

Acknowledgments
First of all thanks my god for helping and supporting

My research and lighting the way for me.

Greatest thanks for Professor. Sana Eltahir
My greatness and thanks to my friends, who helping me to

complete my research in good way.

My faithfully and thanks to my

family, friends, and teachers.
<table>
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<td>ATR</td>
<td>Delayed Transfusion Error.</td>
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<td>CMV</td>
<td>Cyto-Megalo-Virus.</td>
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<td>ELISA</td>
<td>Enzyme linked Immune Sorbent Assay.</td>
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<td>HAV</td>
<td>Hepatitis A Virus.</td>
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<tr>
<td>HBV</td>
<td>Hepatitis B Virus.</td>
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<tr>
<td>HCV</td>
<td>Hepatitis C Virus.</td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus.</td>
</tr>
<tr>
<td>HRP</td>
<td>Horse Radish Peroxidase.</td>
</tr>
<tr>
<td>IBCT</td>
<td>Incorrect Blood Component Transfusion.</td>
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<tr>
<td>NAT</td>
<td>Nucleic Acid Testing.</td>
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<tr>
<td>PTP</td>
<td>Post-Transfusion Purpura.</td>
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<tr>
<td>TA-GVHD</td>
<td>Transfusion Associated Graft-Versus Host Disease.</td>
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<td>TRALI</td>
<td>Transfusion Related Acute Lung Injury.</td>
</tr>
<tr>
<td>TTI</td>
<td>Transfusion-Transmitted-Infection.</td>
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<tr>
<td>WBCS</td>
<td>White Blood Cells.</td>
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Abstract

Blood transfusion is essential and vital in the successful treatment of many malignant and nonmalignant hematological disorders. Blood transfusion is an important mode of transmission of infections to recipients. Although risk may be reduced by the vigorous screening of donors. The aim of this study to determine the frequency of human immunodeficiency virus, hepatitis B and C viruses and syphilis infections among blood donors in National Directorate of blood transfusion service at Khartoum state. This is a retrospective cross sectional study was conducted in the period between March 2014 to May 2014. Donors were selected by the standard criteria for donor fitness and screened for HIV, HBs, HCV and syphilis by ELISA. A total of 5100 donors were tested and analyzed by SPSS version 16. Out of 4014 (78.7%) were family donors and 1085 (21.3%) were voluntary donors. The frequency of HIV was 0.3% in the donors, and in HBV, HCV and syphilis was 5.9%, 0.5% and 3.8% respectively in total donors.

Conclusion: The positivity of hepatitis and syphilis was more in family donors as compared to voluntary donors. No voluntary donor was found to be positive for HIV. The present study concluded that motivating voluntary blood donors is the most effective way of ensuring adequate supplies of safe blood on a continuing basis.
المستخلص

نقل الدم أساسي وحيوي في المعالجة الناجحة لعدة امراض دموية خبيثة أو غير خبيثة. نقل الدم وسيلة لنقل العدوى لمستقبل الدم. يتم تقليل الخطر بالفحص للمتبرعين.

الهدف من هذه الدراسة، تحديد معدلات فيروس نقص المناعة البشرى، التهاب الكبد الوبائي، وعوامل الزهرى بين المتبرعين بالدم في الإدارة القومية لخدمات نقل الدم في ولاية الخرطوم.

هـذه الدراسة المتعلقـه بـثاث رجعـي اجريـت بين مـارس 2014 وحتى مايو 2014. تم اختبار المتبرعين بـمـعايير قياسية لل/~ياقـة البـدـنـيـه للمتبرع والفحص لـفيروس نـقص المناعة البشرى والتهاب الكبد الـوبائـي وـسي وزهرى بالـفحص المـتـرـبتـب بالـانـزـيمـات. تم اختبار 1500 متبرع اختبرـوا وحلـوا بالنـظام التحليـلـي SPSS نسـخـه 16. عدد 1400 (78.7%) من المتبرعين عـائليين و1085 (21.3%) من المتبرعين طوـعـيين.

معدلات فيروس نقص المناعة البشرى 0.3% بين المتبرعين والتهاب الكبد الوبائي وسـي وزهرى هـى 0.9%. 0.5% تتابـعـا بـين المتبرعين.

الخلاصة: المصابين بالتهاب الكبد الوبائي والزهرى علـى بين المتبرعين. الاستـروـيين مـقارـبـه بـالمتبرعين الطـوـعـيين. ولا يوجد متبرع طوـعـي يحمل فيروس نقص المناعة البشري.

خلصت هذه الدراسة التي تُحفيز التبرع الطوـعـي بالدم وـهو الطريـقة الأكتر فعالية لتحقيق مصادر كافية ومستمرة وسليـمة للدم.
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Chapter one

1. Introduction and literature review

1-1 Introduction

Blood transfusions today are an indispensable part of many medical and surgical therapies. The use of blood and its components temporarily replaces what may be lost or not produced before, during, or after a transmission of infectious diseases through donated blood is of concern to blood safety as transfusion forms an integral part of medical and surgical therapy. Blood transfusion carries the risk of transfusion-transmissible infections, including HIV, hepatitis, syphilis, malaria and infrequently toxoplasmosis, brucellosis more details and some viral infections like CMV, EBV and herpes. With every unit of blood, there is 1% chance of transfusion-associated problems including transfusion-transmitted diseases (Widmann, 1985).

The first case of transfusion-associated AIDS was described in an infant given transfusion for erythroblastosis foetalis (Hollan et al, 1990). Thereafter, many cases were reported all over the world in which transfusion of blood and its products was the only risk factor (Pita and Torres, 1997).

Despite implementation of various screening assays for detection of TTIs, occasional cases of post-transfusion infections are common. Majority of these problems are due to prevalence of asymptomatic carriers in the society as well as due to blood donations during the window period of infections. The hazards of transfusion were minimized by proper selection of donors and screening for infectious diseases by a high sensitivity screening assay. World health organization (WHO) recommends an integrated strategy to improve blood transfusion safety.
by establishment of well organized blood transfusion services, screening of donated blood for at least four major TTIs with quality assured system, rational use of blood and implementation of effective quality control systems (WHO, Geneva, 2002).
1-2 literature review

1-2-1 Blood transfusion

Is essential and vital in the successful treatment of many malignant and nonmalignant hematological disorders. Children with thalassemia, adults with myelodysplastic syndromes, and patients with autoimmune hemolytic anemias, leukemias, or a plastic anemias become chronically dependent on blood transfusions. Modern treatment procedures such as high dose chemotherapy and progenitor cell transplantation require intensive support with blood components and products (Reinhold et al., 2007).

1-2-2 History of blood transfusion

An early development leading to the establishment of blood banks occurred in 1915, when Richard Lewison of Mount Sinai Hospital in New York City initiated the use of sodium citrate as an anticoagulant. This discovery transformed the blood transfusion procedure from direct (vein-to-vein) to indirect. In the same year, Richard Weil demonstrated the feasibility of refrigerated storage of anticoagulated blood. The introduction of a citrateglucose solution by Francis Peyton Rous and JR Turner two years later permitted storage of blood in containers for several days, thus opening the way for the first "blood depot" established in Britain during i. Oswald Hope Robertson, a medical researcher and U.S. Army officer is often regarded as the creator of the first bank of stored blood in 1918. He stored blood for up to 21 days to treat haemorrhagic shock suffered in battlefield injuries. Although he recognised the advantages of adding glucose to blood, it was 20 years before this observation was fully appreciated in the development of large-scale blood
storage during the Spanish Civil War between 1937 and 1939. In Russia Sergei Yudin pioneered the transfusion of cadaveric blood and performed this successfully for the first time on 23 March 1930. Also in 1930 Yudin organized the world's first blood bank at the Nikolay Sklifosovskiy Institute, which set an example for the establishment of further blood banks in different regions of the Soviet Union and in other countries. By the mid-1930s the Soviet Union had set up a system of at least 65 large blood centers and more than 500 subsidiary ones, all storing "canned" blood and shipping it to all corners of the country. News of the Soviet experience traveled to the United States, where in 1937 Bernard Fantus, director of therapeutics at the Cook County Hospital in Chicago, established the first hospital blood bank in the United States (Morris et al., 1976).

In creating a hospital laboratory that preserved and stored donor blood, Fantus originated the term "blood bank." Within a few years, hospital and community blood banks were established across the United States. Willem Johan Kolff organised the first blood bank in Europe (in 1940). In 1939 Charles R. Drew researched in the field of blood transfusions, developing improved techniques for blood storage, and applied his expert knowledge in developing large-scale blood banks early in World War II. The University of Louisville is also credited for the blood bank. An important breakthrough came in 1939/40 when Karl Landsteiner, Alex Wiener, Philip Levine, and R.E. Stetson discovered the Rh blood group system, which was found to be the cause of the majority of transfusion reactions up to that time. Three years later, the introduction by J.F. Loutit and Patrick L. Mollison of acid-citrate-dextrose (ACD) solution, which reduces the volume of anticoagulant, permitted transfusions of greater volumes of blood and allowed longer term storage. Carl Walter and W.P. Murphy, Jr., introduced the plastic bag for blood collection in 1950.
Replacing breakable glass bottles with durable plastic bags allowed for the evolution of a collection system capable of safe and easy preparation of multiple blood components from a single unit of whole blood. It decreased wastage from expiration and facilitated resource sharing among blood banks. Newer solutions contain adenine (Morris et al., 1976).

1-2-3 Blood transfusions services

Blood transfusions today are an indispensable part of many medical and surgical therapies. The use of blood and its components temporarily replaces what may be lost or not produced before, during, or after a disease process and/or its treatment. The benefits of transfusion today far outweigh their minute (yet real) risks with all the current safeguards to select donors, test blood, and ensure that compatible blood is transfused to the correct patient. Transfusion medicine has come a long way due to multiple pathfinders, adventurous physicians, and courageous donors and patients, especially in the last half century. Until the human immunodeficiency virus (HIV) pandemic in the 1980s, transfusion services in many economically restricted countries remained poorly developed, primarily due to economic constraints. With the institution of HIV testing in 1985 in the United States and in other developed countries, economically restricted countries were encouraged to place a stronger focus on transfusion safety practices. Although transfusion safety has improved somewhat over the past few decades, many economically restricted countries continue to struggle with inadequate resources and infrastructure that hinder establishment of a safer blood supply. According to the world health organization (WHO), only 20% of the worldwide supply of safe and screened blood is available to people living in economically restricted countries, where approximately 80% of the world’s population resides (Seev et al., 2004).
Commitment of governments to a well developed and organized blood transfusion service is the first key to sustainable and successful blood transfusion systems. Many economically restricted countries are faced with a high disease burden but lack the infrastructure to address the problem. Various organizational structures of blood services are described in this chapter, as is the commitment of the WHO to worldwide blood safety. A second key to optimizing blood transfusion services includes selection of safe blood donors by recruitment of non remunerated (nonpaid or voluntary) individuals. In the developed world, donor screening focuses on excluding individuals who carry a higher “risk” of infectious disease than the general population such individuals include intravenous drug users, men who have sex with men, and commercial sex workers. In economically restricted countries, such as Africa, where heterosexual transmission has accounted for as much as 80% of all HIV infections, such focused donor screening is less effective. A strategy employed by economically restricted countries to select safe blood donors involves predonation laboratory testing of blood with rapid diagnostic tests (Chin and Mann, 1990).

1-2-4 Governmental organization of blood services

Development and organization of blood transfusion services in economically restricted countries has not only lagged behind blood services in developed countries but has also lagged behind other parts of the health care system as well (Chin and Mann, 1990).

The sustainability of high quality transfusion systems in economically restricted countries is best achieved with national oversight and a national commitment to safe blood transfusion as part of the health care system. Blood services in economically restricted countries, just as in more developed countries, should be defined by a legal framework that states
the national blood policy and describes the processes governing the collection, processing, and transfusion of blood. A variety of organizations collect and process the blood in economically restricted countries, typically including the ministries of health, social security systems, the armed forces, private organizations, and nongovernmental organizations. Blood transfusion services, which oversee the delivery of blood products to patients, are usually operated by health care facilities, such as hospitals (Cruz, 2003).

1-2-5 Effects of blood transfusions

ABO incompatible red cell transfusion remains one of the most important serious hazards of transfusion and is a much greater risk than that of human immunodeficiency virus (HIV) or hepatitis C virus (HCV) transmission by blood. Incorrect blood transfusion remains the most common transfusion error. ATR, acute transfusion reaction, DTR, delayed transfusion reaction, IBCT, incorrect blood component transfused, PTP, post-transfusion purpura, TA-GVHD, transfusion-associated graft-versus-host disease, TRALI, transfusion-related acute lung injury; and TTI, transfusion-transmitted infection (Marcela, 2009).

Table 1-1 Transfusion-transmissible agents

<table>
<thead>
<tr>
<th>Agents</th>
<th>Characteristics related to transfusion</th>
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<td>Viruses</td>
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<tr>
<td>Hepatotropic</td>
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<tr>
<td>HAV</td>
<td>Very rarely transfusion transmitted; no carrier state; faecal-oral transmission.</td>
</tr>
<tr>
<td>HBV</td>
<td>2- to 6-month incubation period; carrier state; readily transmissible by blood.</td>
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<tr>
<td>HCV</td>
<td>Majority of cases asymptomatic; carrier state; readily transmissible by blood.</td>
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<tr>
<td>Retroviruses</td>
<td>HIV1 and HIV2</td>
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<td>HTLV-I and</td>
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<td></td>
<td>EBV</td>
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<td>Others</td>
<td>Parvovirus B19</td>
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<td></td>
<td>West Nile virus</td>
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<tr>
<td>Bacteria</td>
<td>Treponema pallidum</td>
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<td>Endogenous</td>
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<td>Exogenous</td>
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<td>Parasites</td>
<td>Malaria</td>
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</tr>
<tr>
<td>Chagas’ disease</td>
<td></td>
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( Victor et al., 2005)

1-2-6 Blood donor recruitment, selection, and screening

Replacement donors are recruited from among the family or relatives of hospitalized patients who require blood transfusions. A number of studies have shown that voluntary donors often have a lower prevalence and incidence of infectious transfusion-transmitted diseases than do family/replacement donors and paid donors. In Kenya, a study comparing volunteer donors from mobile blood units with donors recruited by family members showed that HIV seroprevalence among voluntary donors was significantly lower than among family recruited donors. Information collected in the Global Database on Blood Safety has repeatedly shown the importance of recruiting voluntary blood donors (Beal, 1993).

Blood centers in sub-Saharan Africa routinely rely on family/replacement or paid donors for a significant percentage of their blood collection needs, often due to shortages in supply of donor blood in the blood banks. A lack of resources to train personnel and to provide transport for mobile blood campaigns contributes to blood shortages as well. From 50% to 70% of donations are collected from family/replacement and paid donors worldwide, often in countries with relatively high prevalence of HIV, hepatitis B virus (HBV) and hepatitis C virus (HCV) infection. A blood
bank in Armenia reported that 12% of the 1010 units of whole blood collected in 2004 were drawn from paid donors, and 88% were drawn from patient relatives. One of the blood banks in Armenia has, for the first time, organized blood drives and collected 172 units of blood from volunteer donors, including medical students. Some countries have reported that elimination of paid donors has paralleled a decline in the seroprevalence of HIV in individuals who present for blood donation. Although recruitment of voluntary blood donors is costly and difficult, marketing strategies aimed at increasing the proportion of repeat voluntary donors have met success in countries such as Venezuela, Nicaragua, and Cuba (Beal, 1993).

1-2-6-1 Donor Selection

Despite inherent difficulties in selecting donors in areas of high prevalence of infectious diseases, donor recruiters in economically restricted countries have attempted to identify effective risk factor deferral criteria using medical history, demographic information, and social history. In sub-Saharan Africa, age has been used as a surrogate marker for infectious risk. Although the age for peak seroprevalence of HIV varies in different regions in sub-Saharan Africa, peak seroprevalence of HIV is typically age 15 to 29 in women and age 25 to 39 in men. Therefore, recruitment of donors has shifted to secondary school students and older adults in such areas of the world. Another potential surrogate marker for donor infectious risk is demographic status. However, recruitment of rural donors can be difficult and is more expensive due to limited transportation infrastructure and potentially lower levels of nutritional health, which result in higher rates of anemia. One study reported that blood donor self deferral can reduce the risk of HIV transmission through blood transfusion. Self deferred donors demonstrated significantly higher rates of HIV, HBV, and HCV as
compared to general donors. For example, in Zimbabwe, attempts have been made to collect blood only from individuals with a recent prior blood donation history and to establish repeat donor groups. Because funding and resources for mobile donor recruitment are limited, blood centers are often forced to rely on family/replacement donors to fill the available blood deficit (Christopher et al., 2007).

1-2-6-2 Predonation Testing

Worldwide, prospective blood donors are screened for transfusion transmissible diseases. In addition, adequate hematocrit should also be assessed. The high prevalence of anemia often contributes to a dearth of eligible blood donors, such as at a transfusion center in Nigeria, where 75% of donors were rejected for anemia alone. In certain countries, blood services and hospitals test donors for HIV and other infectious disease markers by rapid test methods before donation. Proponents of predonation testing argue that the advantages of pretesting in areas of high prevalence of HIV, HBV, and HCV, such as in sub-Saharan Africa, outweigh the disadvantages. Advantages of pretesting include: 1. Savings on the cost of blood collection bags and waste disposal 2. Savings on the cost of the equipment and supplies needed for postdonation enzyme immunoassay testing 3. Fewer units of stored blood to include only screen-negative units 4. The ability to quickly inform deferred donors of their test results in regions where mail and telephone communication is poor and the likelihood of repeat donation is low (Clark et al., 2005).

Collection centers in populations with high infectious disease prevalence may find predonation testing strategies attractive from a cost and resource savings perspective. High prevalence of infectious diseases in donors not only compounds the risk to the recipient, but also increases the overall cost of the transfusion product. Because blood collection bags represent
as much as one third of the blood bank budget, money is wasted on bags thrown away due to infected donors. Rates of donor deferral after laboratory screening, based on a positive infectious disease result in the donor, may exceed 10%. Positive HBV surface antigen (HBsAg) tests on donor units accounted for more than half of the discarded units in one Ghanaian study. The decrease in cost associated with predonation screening may be significant when transfusion-transmitted infection prevalence among donors reaches 15% to 20%. Well-organized blood safety programs that depend mainly on voluntary, nonremunerated blood donors should expect discard rates of less than 10%, even in areas with high infectious disease prevalence. Even though prescreening donor units may be economical, it poses a challenge when used to prescreen for HIV or hepatitis, particularly in sub Saharan Africa. Most often performed in blood collection sites in small rural hospitals and clinics, predonation testing may be troublesome due to the lack of qualified staff, organized recruitment system for volunteer donors, or quality management system. In many countries, low skilled technicians may perform donor prescreening with rapid tests and may not have the skills to perform more complex tests for follow-up or to guarantee that the other basic operations and functions of a proper transfusion service are carried out (Clark et al., 2003).

Counseling and physician referral of positive donors may not routinely take place; when performed, counseling may be inadequate due to insufficiently trained staff. Caution must be exercised while practicing predonation testing, because blood transfusion centers have the potential to become test sites for persons who simply wish to know their HIV status (Christopher et al., 2007).
**1-2-6-3 Collection and processing**

In the U.S., certain standards are set for the collection and processing of each blood product. "Whole blood" (WB) is the proper name for one defined product, specifically unseparated venous blood with an approved preservative added. Most blood for transfusion is collected as whole blood. Autologous donations are sometimes transfused without further modification, however whole blood is typically separated (via centrifugation) into its components, with red blood cells (RBC) in solution being the most commonly used product. RBC units can also be frozen when buffered with glycerol, but this is an expensive and time consuming process, and is rarely done. Frozen red cells are given an expiration date of up to ten years and are stored at –85 °F (–65 °C) (Marik and Corwin, 2008).

The less-dense blood plasma is made into a variety of frozen components, and is labeled differently based on when it was frozen and what the intended use of the product is. If the plasma is frozen promptly and is intended for transfusion, it is typically labeled as fresh frozen plasma. If it is intended to be made into other products, it is typically labeled as recovered plasma or plasma for fractionation. Cryoprecipitate can be made from other plasma components. These components must be stored at 0 °F (–18 °C) or colder, but are typically stored at –22 °F (–30 °C).

The layer between the red cells and the plasma is referred to as the buffy coat and is sometimes removed to make platelets for transfusion. Platelets are typically pooled before transfusion and have a shelf life of 5 to 7 days, or 3 days once the facility that collected them has completed their tests. Platelets are stored at room temperature (72 °F or 22 °C) and must be rocked/agitated. Since they are stored at room temperature in nutritive solutions, they are at relatively high risk for growing bacteria. Some blood banks also collect products by apheresis. The most common
component collected is plasma via plasmapheresis, but red blood cells and platelets can be collected by similar methods. These products generally have the same shelf life and storage conditions as their conventionally-produced counterparts. Donors are sometimes paid; in the U.S. and Europe, most blood for transfusion is collected from volunteers while plasma for other purposes may be from paid donors (Shander et al., 2007).

Most collection facilities as well as hospital blood banks also perform testing to determine the blood type of patients and to identify compatible blood products, along with a battery of tests (e.g. disease) and treatments (e.g. leukocyte filtration) to ensure or enhance quality. The increasingly recognized problem of inadequate efficacy of transfusion[1] is also raising the profile of RBC viability and quality. Notably, U.S. hospitals spend more on dealing with the consequences of transfusion-related complications than on the combined costs of buying, testing/treating, and transfusing their blood (Shander et al., 2007).

1-2-6-4 Serological testing

Serological tests are carried out on all donations to ascertain the blood group (A, B, AB or O) and for RhD typing; the results are checked against those previously obtained from that donor or by repeat typing with different batches of antibodies and test cells. Most UK centres also test for RhC, c, e, E and K antigens, and this information appears on the blood pack label. Blood units found negative for D antigen are labelled ‘RhD negative’. With the monoclonal typing antibodies in current use, most weak and variant forms of D antigen are detected on direct testing. Those below the limit of detection with monoclonal anti-D are labelled as RhD negative since they are not considered to be immunogenic to a D-negative recipient. Extended testing to detect, for example, weak D or Du in donors is not universally carried out. A proportion of the units is also
typed for Cw, Fya, Fyb, M, S, s, Jka and Jkb, thus making the phenotyped red cell stocks readily available for alloimmunized patients in need of transfusion. All donations are screened for clinically important red cell antibodies. Any donation found to have a high antibody titre should not be used for transfusion, although it may be a valuable source of red cell typing reagent. Low titres of antibodies should not automatically exclude a donation from therapeutic use as the antibody would be further diluted on direct transfusion. As well as this, about 90% of the plasma from most donations is removed and the cells are resuspended in an additive solution such as saline adenine glucose mannitol (SAG-M); most of the remaining red cells just have most of the plasma removed (Marcela, 2009).

1-2-7 Screening tests for blood donations

Screening tests are paradoxically usually directed at antibody to the agent rather than antigens from the agent, except in the case of hepatitis B virus. Antibody screening tests are markers for certain persistent or chronic infections and therefore indicate a potential for infectivity, especially when the inoculum is as large as a unit of blood or a blood component. Various agents may be transmitted by transfusion, but in the UK there are only five serological screening tests for blood donations that are currently mandatory. They are tests for: (i) hepatitis B surface antigen (HBsAg) for hepatitis B virus; (ii) antibody to HIV-1 and -2, in combination with HIV antigen; (iii) antibody to hepatitis C virus (HCV); (iv) antibody to Treponema pallidum (syphilis). Tests for several other agents are available, but it has not yet been considered necessary to extend the present range. The range of techniques for screening, to which process control can be applied, includes enzyme-linked immunosorbent assay (ELISA), haemagglutination and gelatin particle agglutination. For a test to be suitable for screening blood for transfusion, several conflicting
demands have to be met. Quality control of microbiological screening of blood donations for transfusion in the UK is essential because the occurrence of donations positive for hepatitis B virus, HIV, HCV or syphilis is rare. In contrast to blood grouping, in which every sample produces a ‘positive’ result of some sort, in microbiological screening tests most donor serum samples are negative. Great vigilance is therefore required in carrying out the routine screening tests. In low prevalence populations even an apparently low rate of false positive results from a screening test implies that a positive reaction has little predictive value. If, for example, an agent has an incidence of one in 100 000 donations, then a test with a specificity of 99.9% will produce a false positive reaction once in every 1000 donations, or 100 false positive reactions for every true positive. It is therefore imperative that any donor samples that give a positive reaction in any of the mandatory screening tests should be sent to a reference laboratory for confirmation before the donor is informed of the results. Blood centres in the UK and in other developed countries, use assays that have low false positive rates, and they all have access to centralized reference laboratories that carry out a battery of confirmatory tests, which virtually eliminates the possibility of mislabelling uninfected donors. In the UK these reference laboratories regularly coordinate their confirmation protocols. Specificity of microbiological assays is vital if the confidence of donors is to be maintained, and it must not be forgotten in the search for increased sensitivity. Fortunately recombinant and synthetic antigens and modern molecular biological methods have produced remarkable improvements in the sensitivity and specificity of assays, such as those for detecting HIV infection (Marcela, 2009).
1-2-7-1 Hepatitis B virus

The hepatitis B virus (HBV) is 42 nm in diameter and contains DNA. The virus is plasma borne and easily transmitted by all blood components and most blood products (for example factor VIII). It is not transmitted by pasteurized albumin. The chance of transmission is enhanced when plasma is pooled for the manufacture of blood products. However, the risk is removed with current viral inactivation procedures of fractionated blood components. The incubation period ranges from 2 to 6 months but is usually about 4 months. Although it is extremely infectious parenterally and is resistant to both chemical and heat inactivation, the number of transfusion-transmitted cases has been drastically reduced by screening blood donations. The few cases that do occur are due to seronegative donors undergoing acute infection or to carriers with subliminal levels of hepatitis B surface antigen (HBsAg) in blood donations. Screening for HBsAg is mandatory. Assays for antibody to hepatitis B core, HBc (total antibody and immunoglobulin M, IgM) are available for diagnosing acute hepatitis B infection. Assay for hepatitis B core (HBc) antibody should not replace that for HBsAg screening of donors; however in some countries both tests are done routinely on all blood units. In the UK only donors with a history of hepatitis or body piercing are tested for anti-HBc. Screening for the delta agent is unnecessary as delta depends on HBV to provide its surface antigen. Screening for antibody to HBsAg can be used to identify donors whose plasma is suitable for the preparation of hepatitis B immunoglobulin. In the UK, HBV is detected in approximately 1 in 25 000 donations overall, a lower rate than in the general population because individuals at high
risk of having human immunodeficiency virus (HIV) and, concomitantly, HBV are now excluding themselves from donation. Vaccine is available for protecting HBV-negative recipients of the products of pooled plasma (e.g. immunodeficient patients on permanent IgG therapy) and for patients who need regular transfusions (e.g. those with thalassaemia). Vaccine escape mutants of HBV have been reported. This necessitates careful validation of HBsAg assays (Marcela, 2009).

1-2-7-2 Hepatitis C virus

Hepatitis C virus (HCV) is a flavivirus causing most (in some countries, all) non-A, non-B hepatitis. Antibody to this agent can be detected by an enzyme-linked immunosorbent assay (ELISA). This uses antigen cloned from plasma known to transmit non-A, non-B hepatitis together with synthetic HCV antigens in some systems. There may be at least two different viruses that transmit non-A, non-B hepatitis. Assays have been developed in which cloned antigens or synthetic peptides can react with antibody to HCV. The virus is plasma borne and has some routes of transmission in common with HBV. The incubation period for hepatitis C is commonly from 6 to 12 weeks. Some countries require the screening of blood donors for antibody to hepatitis B core (anti-HBc) and the measurement of alanine aminotransferase (ALT) activity as surrogate markers for non-A, non-B hepatitis. Most donations exhibiting only one of these abnormal markers, however, do not transmit hepatitis C, so ‘surrogate’ screening leads to unnecessary wastage of blood donations. The main causes of increased ALT activity in British blood donors are obesity and alcohol consumption. Assays for hepatitis C antibody are used routinely to screen blood donations. Improved screening assays based on recombinant or synthetic antigens including viral core protein have been developed. Very occasionally HCV may be transmitted by
blood from donors in the early stages of infection. Direct testing for HCV nucleic acid using the nucleic acid amplification technique (NAT) can detect infection during most of the ‘window period’ before seroconversion. In the UK, this is usually performed on pools of samples. HCV antigen detection is almost as sensitive as pooled NAT and is cheaper and more convenient. In England and Wales, where ELISA for anti-HCV as well as NAT for HCV genome are used, the current residual risk per donation of HCV infection is one in 30 million. In the USA, before screening for HCV antibody was introduced, about 10% of transfusions caused significant increases in transaminase activity in recipients, and there were occasional cases of symptomatic hepatitis; this figure is now less than one in a million. Acute infection is usually mild, but a proportion of patients do develop chronic liver disease. Confirmed rates of positivity for anti-HCV (and thus carrier rates) in UK new donors are approximately one in 2000. Modern methods of viral inactivation of plasma-derived factors VIII and IX will prevent transmission. Haemophiliacs who have received effectively inactivated factor VIII have proved negative for antibody to HCV, in contrast to those who received uninactivated concentrate, with a worldwide anti-HCV prevalence greater than 70%, a prevalence similar to that in intravenous drug users. In the UK, haemophiliacs are no longer treated with plasma-derived products; they all receive recombinant coagulation factors. Another flavivirus distantly related to HCV has been cloned and named hepatitis G virus. However, it is not hepatotropic and the alternative name ‘GB virus C’ or GBV-C is more appropriate. Viraemia is present in 2% of blood donors and it has been shown to be transmissible by transfusion. It is considered to be non-pathogenic and is not causatively or predictively associated with elevated ALT levels in infected individuals. TTV and SEN-V are other cloned viruses initially thought to cause post-transfusion hepatitis.
These circoviruses are considered nonpathogenic. Hepatitis A and hepatitis E viruses only cause post-transfusion hepatitis very rarely because a carrier state does not occur (Marcela, 2009).

**1-2-7-3 Human immunodeficiency virus**

HIV-1 was transmitted by transfusion before screening for anti-HIV was introduced and before donors at high risk started excluding themselves from giving blood. HIV-2 occurs mainly in West Africa. Both are retroviruses, 100 nm in diameter, that carry their own RNA-dependent DNA polymerase (reverse transcriptase). Before screening was introduced, HIV had been transmitted by whole blood, red cell components, platelet concentrates and FFP. It can contaminate factor VIII and factor IX concentrates, but it can readily be inactivated chemically or by heat and modern concentrates do not transmit it. It has not been transmitted by albumin, immunoglobulins or antithrombin III. With current anti-HIV assays, the seroconversion period is rarely longer than 1 month, and a primary illness similar to glandular fever may occur during this time. The incubation period for the acquired immune deficiency syndrome (AIDS) is variable, with a likely median time of at least 7 years in adults (although the period is shorter for infants). Screening for HIV antibody is by an ‘antiglobulin’ or ‘sandwich’ ELISA, capture ELISA or, in some countries, gelatin particle or ‘rapid’ (e.g. dipstick) assays. ‘Competitive’ assays specifically for anti-HIV-1 have been superseded by assays that can detect both anti-HIV-1 and -2. In recent years ‘combi’ ELISAs that detect anti-HIV-1 and -2 together with HIV antigen in a single assay have reduced the window period of HIV infection to just a few days. Although some countries such as the USA and the UK employ NAT for HIV detection in addition to serological assays, the residual risk per donation for HIV in England and Wales, before NAT for HIV was introduced, was only one in 5 million because of the sensitivity of
serological testing and the low incidence of HIV infection in donors. This low incidence of ‘window period’ infections is not the case in countries with high rates of HIV infection, such as Thailand and South Africa, where donation screening for South Africa, HIV antigen has detected several positive donors who had not yet developed anti-HIV. Transmission of HIV by transfusion has been extremely rare since the introduction of screening. HIV antibody is found in one in 100 000 donations overall in the UK. The rate is significantly higher in new donors (one in 13 000) than in known donors (one in 176 000). ‘Seroconverting’ donations (those negative for HIV antibody but infectious because of recent infection) are therefore extremely rare. On only three occasions has a donation from a seronegative donor been known to have transmitted HIV infection to 5 recipients in the UK since screening started in 1985. The virus can be inactivated in fractionated blood products by treatment with heat or chemicals, but components such as red cells or platelets cannot be heat treated. Methods for inactivating such cellular components are, however, being assessed, although so far they are logistically and financially demanding (Marcela, 2009).

1-2-7-4 Other complications of transfusion

1-2-7-4-1 Treponema pallidum (syphilis)

Treponema pallidum can only be transmitted by fresh blood and platelet concentrates because it is readily inactivated by refrigeration for 72 hours. The incubation period varies from 4 weeks to 4.5 months, the average being 9–10 weeks. It is only rarely transmitted by transfusion, but when it is, it presents as a secondary eruption. It responds to treatment with antibiotics, usually a course of benzylpenicillin (2 megaunits). Screening for the antibody is mandatory, in the UK, by specific T.
pallidum haemagglutination or particle agglutination assays or ELISA. In early primary syphilis, at the height of infectivity, screening tests may be negative. The detection rate of infectious donors is low because most positive donors have had the infection and been treated. Donors with acute or latent infection, whilst still relatively rare are now being seen more frequently. Screening for syphilis may also identify donors who have contracted other sexually transmitted diseases (Marcela, 2009).

1-2-7-4-2 Malaria

Malarial parasites remain viable in blood stored at 4°C, and are readily transmissible by blood transfusion. In some endemic areas, all recipients are treated with antimalarial drugs. In non-endemic areas, there is a real risk of failure to recognize post-transfusion malaria owing to the rarity of the infection. This fact, combined with increasing travel to tropical areas, necessitates the careful vetting of blood donors by direct questioning and, in some centres, by tests for malarial antibodies. Visitors who have recently travelled to a tropical area are treated similarly for 12 months after the visit, unless malaria antibody testing is available. Such testing has recently become available in the UK (Victor et al., 2005).

1-2-7-4-3 Bacteria

Although rare in absolute terms (approximately three cases are reported and confirmed per year in the UK), bacterial transmissions by transfusion constitute two-thirds of all microbial transmissions by this route and often prove fatal. The vast majority of such cases are due to contaminated platelet preparations that are more than 3 days old, because bacteria (mostly skin commensals) will proliferate easily at room temperature. This risk is now far greater than viral risks because of the introduction of interventions such as NAT for HCV. UK blood services are in the
process of introducing enhanced methods of donor arm cleansing, ‘diversion’ of the first 20 mL of the donation to reduce the risk from skin contaminants and are considering routine bacterial screening of platelet preparations (Victor et al., 2005).
1-3 Rational

Blood transfusions today are an necessary part of many medical and surgical therapies. Blood transfusion is an important mode of transmission of infections to recipients: including HIV, hepatitis B and C, syphilis, malaria and infrequently toxoplasmosis, Brucellosis and some viral infections like CMV, EBV and herpes. Among all infections HIV and hepatitis are the most dreadful. Risk of transfusion transmission infection may be reduced by vigorous screening of donors and donated blood, the improved screening and testing of blood donors has significantly reduced transfusion-transmitted diseases in most developed countries.
1-4 Objectives

1-4-1 General objective

To determine the frequency of human immunodeficiency virus, hepatitis B and C viruses and syphilis infection amongst blood donors at National Directorate Of Blood Transfusion Services in Khartoum state.

1-4-2 Specific objective

1. To detect the human immunodeficiency virus (HIV), hepatitis C virus (HCV), hepatitis B surface antigen (HBsAg) and syphilis amongst blood donors test by using ELISA.

2. To determine the frequency of HIV, HBs, HCV and syphilis according to type of blood donors (voluntary and family).

3. To determine the frequency of transfusion transmitted infections according to sex and age of donors at national directorate of blood transfusion services in Khartoum state.
2. Materials and methods

2-1 Study design

This is a retrospective cross sectional study was carried out in National Directorate Of Blood Transfusion Service at Khartoum state to determine the frequency of human immunodeficiency virus, hepatitis B and C viruses and syphilis infections among blood donors in the period from March 2014 to May 2014.

2-2 Study population

A consecutive sample of apparently healthy adult voluntary (motivated blood donor, who donates at regular intervals) and family (usually one time blood donor only when a relative is in need of blood) blood donors agreed to participate after an informed consent.

2-3 Sample size

The sample included 5100 consecutive donors, 1085 voluntary and 4015 family in this groups 5008 males and 92 females, who donated blood between March to May 2014.

2-4 Inclusion criteria

Individuals who were included in the study were healthy men and non-pregnant non lactating women aged between 18 and 69 years, weighing \( \geq 50 \) kg and with hemoglobin levels above 12.5 g/dl for females and 13.5 g/dl for males.

2-5 Exclusion criteria

Donor below 18 years old or above 69 years old, weigh below 50kg, hemoglobin levels below 12.5 in males and 13.5 in females and current
history of medication and those with a history of operation, serious illness, jaundice, blood transfusion, radiotherapy or any form of cancer therapy were excluded from the study.

2-6 Ethical consideration

A written informed consent was obtained from all participants and prior to enrollment.

2-7 Data analysis

Data were coded, entered, cleaned, validated and analyzed using SPSS version 16.0 were done using Pearson Chi-Square, and presented in form of tables and graphs.

2-8 Sample collection

Blood samples were collected aseptically in 5 ml red top vacutainers. Sera specimens were separated after centrifugation, aliquot into 2 ml cryotubes tubes and stored at -20°C until the time for assay.

2-9 Methods

The screening for HIV, HBs, HCV and Syphilis were done by ELISA (Enzyme-linked immunosorbent assay) using kits (fortress diagnostic). Fortress kit is an enzyme-linked immunosorbent assay (ELISA) for qualitative determination of antigens or antibodies in human serum or plasma.

2-9-1 Materials and instruments required

1. Freshly distilled or deionized water.
2. Disposable gloves and timer.
3. Appropriate waste containers for potentially contaminated materials.
4. Disposable v-shaped troughs.
5. Dispensing system and pipette.
6. Absorbent tissue.
7. Dry incubator 37°C.
8. Microshaker for dissolving and mixing conjugate with sample.
9. Microwell plate reader, single wavelength 450nm or double wavelength 450nm and 630nm.
10. Microwell aspiration/wash system.

2-9-2 Reagents

Kit Contents:

1. Microwell plate 96 tests.
2. Negative control.
3. Positive control.
4. HRP-Conjugate Reagent.
5. Stock wash buffer.
6. Chromogen solution A.
7. Chromogen solution B.
8. Stop solution.
11. Package inserts.

2-9-3 HBs Ag

ELISA HBs Ag is an in vitro diagnostic kit for the detection of hepatitis B surface antigen (HBs Ag) in human serum or plasma.
2-9-3-1 Principle

The test is an enzyme-immunoassay based on a sandwich principle. Microtiter well have been coated with monoclonal anti-HBs(antibody to HBsAg). which constitute the solid-phase antibody. The test sample is incubated in such awell HBsAg, if present in the sample, will bind to solid phase antibody. Subsequently guinea-pig anti-HBs, which has been labelled with the enzyme horseradish peroxidase (HRP), is added. With a positive reaction this labelled antibody become bound to any solid-phase antibody HBsAg complex previously a blue colour in the test-well, which turns yellow when the reaction stopped with sulphuric acid. If the sample contains no HBsAg, the labelled antibody cannot be found specifically and only a low background colour develops.

2-9-3-2 Procedure

- Reagents was prepared.

- The well was numbered.

- 20ml of sample diluents was added to each well.

- 100ml of sample, negative control and positive control was added.

- The plate was incubated 60 minutes at 37°C.

- 50ml HRP conjugate was added to each wells.

- The plate was incubated 30 minutes at 37°C.

- The sample was washed 5times.

- The sample was coloured by dispense 50ml of chromogen A and 50ml of chromogen B solution.

- Plate was incubated 15 minute at 37°C avoid light.

- 50ml stop solution was added to stopped reaction.

- The absorbance was measured at 450nm, 630nm.
2-9-3-3 Result

Cut-off value=\( *N_c \times 2.1 \)

\( *N_c \)=the mean absorbance value for three negative controls.

2-9-4 HIV(Ag/Ab)

Fortress kit is an enzyme-linked immunosorbent assay (ELISA) for qualitative determination of antigens or antibodies in HIV type 1 and/or type 2 in human serum or plasma.

2-9-4-1 Principle

This HIV(1+2)Ag/Ab ELISA kit is a two-step incubation “sandwich” enzyme immune assay, which uses polystyrene microwell strips precoated with recombinant HIV antigen (HIV-1gp4), gp120, and recombinant HIV-2 gp-36 and anti-HIV(p24) antibodies.

First steps: biotinylated anti-HIV(p24) antibodies with patient’s serum or plasma sample are added into the wells, during incubation, the specific HIV1/2 antibodies if present in sample will be capture inside the wells. Simultaneously, if HIV p24 antigen is present in sample, it will also be captured as a double antibody “sandwich” complex. The detection of the captured HIV p24 antigen-biotinylated antibody complex or HIV1/2 antibodies by adding the enzyme horseradish peroxidase (HRP) which has been conjugated to second HIV1+2 recombinant antigens and to avidin.

P24 detection: when p24 has been captured inside the wells, avidin will react with the biotin and attach HRP to the Ab-p24-Ab complex.

HIV1/2 antibody detection: When HIV1/2 antibodies have been capture inside the well the HRP-conjugated antigens will bind to the captured antibodies forming Ag-Ab-Ag(HRP) “sanduish” immunocomplex.
After washing and adding chromogens are hydrolyzed by the bound HRP to a blue coloured product in wells containing the Ag-Ab-Ag(HRP) and/or Ab-p24-Ab(HRP) ‘sandwich” immunocomplex. The blue colour turns yellow after stopping the reaction with sulfuric acid. The amount of colour intensity can be measured and is proportional to the amount of antibodies or p24 captured in the wells, and to the sample respectively. Wells containing sample negative for anti-HIV1/2 or p24 remain colourless.

**2-9-4-2 Procedure**

- Reagents was prepared.
- The wells was numberd.
- 20 ml of biotin-conjugated reagent was added into the each wells.
- 100ml of sample, control positive and control negative was added.
- The sample was incubated 60 minutes at 37°C.
- The sample was washed 5times.
- 100ml of HRP-conjugate was added into each well.
- The sample was incubated 30 minutes at 37°C.
- The sample was washed 5times.
- The sample was coloured by dispense 50ml of chromogen A and 50ml of chromogen B solution.
- Plate was incubated 15 minute at 37°C avoid light.
- 50ml stop solution was added to stopped reaction.
- The absorbance was measured at 450nm, 630nm.
2-9-4-3 Result

Calculation Cut-off value=*Nc +0.12
*Nc=the mean absorbance value for three negative controls.

2-9-5 HCV

ELISA assay for qualitative detection of antibodies to hepatitis C virus in human serum or plasma.

2-9-5-1 Principle

Indirect ELSA method for detection of antibodies to HCV in two-step incubation procedure. polystyrene microwell strips are pre-coated with recombinant antigens. During the first incubation steps, anti-HCV specific antibodies. If present ,will be bound to the solid phase pre-coated HCV antigens.

The well are washed to remove unbound serum proteins and rabbit anti-human IgG antibodies conjugated to horseradish peroxidase is added. During the second incubation steps, these HRP-conjugated antibodies will be bound to any antigen-antibody complexes previously formed and the unbound HRP-conjugated is then removed by the washing. Chromogen solutions containing tetramethylbenzidine(TMB)and urea peroxide are added to the wells and in presence of the antigen-antibody-ANTI-IgG(HRP) immunocomplex: the colourless chromogens are hydrolyzed by blue colour turns yellow after stopping the reaction with sulphuric acid. The amount of colour intensity can be measured and is proportional to the amount of antibody capture in the wells, and to the sample respectively. Wells containing samples negative for anti-HCV remain colourless.
2-9-5-2 Procedure

- Reagents was prepared.
- The well was numbered.
- 100ml of diluent was added into each well.
- 10ml of samples and control positive and negative was added.
- The sample was incubated 30 minutes at 37°C.
- The sample was washed 5 times.
- 100ml of HRP-conjugate was added into each well.
- The sample was incubated 30 minutes at 37°C.
- The sample was washed 5 times.
- The sample was coloured by dispense 50ml of chromogen A and 50ml of chromogen B solution.
- Plate was incubated 15 minute at 37°C avoid light.
- 50ml stop solution was added to stopped reaction.
- The absorbance was measured at 450nm, 630nm.

2-9-5-3 Result

Calculation cut-off value = *Nc + 0.12

*Nc = the mean absorbance value for three negative controls.

2-9-6 Syphilis

Fortress Syphilis Elisa is an in vitro diagnostic kit for the detection of antibodies to Treponnema pallidum in human serum or plasma.
2-9-6-1 Principle
Microwells are coated with recombinant treponema palidum antigen express in E.coli. The sample incubated with recombinant TP antigens conjugated to HRP. The pre-coated antigens express the same epitopes as the HRP conjugate antigens. In case of presence of anti-TP in sample, conjugated antigen will be bound to the two variable domains of the antibody and the specific antigen-antibody immunocomplex is captured on the solid phase. After washing chromogen solution containing TMP and urea peroxidase are added to the wells, in presence of antigen-antibody sandwich complex the colourless chromogen is hydrolysed by the bound HRP conjugate to blue coloured product, which turns to yellow upon the edition of the stop solution. This colour is read photometrically and directly proportion to the amount of the antibody in the sample. Well containing sample negative for anti-TP remain colourless.

2-9-6-2 Procedure
- Reagents was prepared.
- The well was numbered.
- 100ml of HRP-conjugate was added.
- 20ml of Sample negative and positive control was added.
- The sample was incubated 60 minutes at 37°C.
- The sample was washed 5times.
- The sample was coloured by dispense 50ml of chromogen A and 50ml of chromogen B solution.
- Plate was incubated 15 minute at 37°C avoid light.
- 50ml stop solution was added to stopped reaction.
- The absorbance was measured at 450nm, 630nm.
2-9-6-3 Result

Calculation Cut-off value = *Nc + 0.18

*Nc = the mean absorbance value for three negative controls.
Chapter three

3. Results

Out of 5100 blood donors 1085(21.3%) were voluntary donors and 4014(78.7) were family donors in Table(3.1). 91.50% of voluntary donors were male, and 8.50% were female. And all of the family donors were male are shown in Figure(3-1). 70.30% of family blood donors and 57.90% of voluntary blood donors were in age from 18 to 30 years, and 37.10% of family donors and 26.90% of voluntary donors were in age from 31 to 40 years, and frequency 18.70% of family donors and 10.70% of voluntary donors were in age from 41 to 50 years, 8.0% of family donors and 4.40% of voluntary donors were in age more than 50 years that shown in Figure(3-2).

Percentage of HBs positive in voluntary blood donors were 0.6%, and in family blood donors 7.3% , and percentage of HBs negative in voluntary blood donors were 99.4% , and 92.7% in family blood donors that present in Table(3.2).

No positive results for HIV among voluntary blood donors while in family blood donors 13 (0.3%) , and percentage of HIV negative 99.7% in family blood donors that present in Table(3.3).

Frequency and percentage of HCV positive in voluntary blood donors were 1(0.1%), and in family blood donors 24 (0.6) , and percentage of HCV negative in voluntary blood donors were 99.9% , and 78.3% in family blood donors that present in Table(3.4).

Percentage of VDRL positive in voluntary blood donors were 1.80%, and in family blood donors 4.40% , and percentage of HCV negative in voluntary blood donors were 98.20% , and 95.60% in family blood donors that present in Figure(3.3).
Table 3.1: Frequency and percentage of the donors according to type of donors:

<table>
<thead>
<tr>
<th>Donors</th>
<th>voluntary</th>
<th>family</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency</td>
<td>1086</td>
<td>4014</td>
<td>5100</td>
</tr>
<tr>
<td>Percentage</td>
<td>21.3%</td>
<td>78.7%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Figure 3-1: Distribution of blood donors according to sex
Figure (3-2): Distribution of age group according to type of blood donors.

Table (3.2): Frequency and percentage of HBs according to type of donors:

<table>
<thead>
<tr>
<th>HBs results</th>
<th>donors</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>voluntary</td>
<td>family</td>
</tr>
<tr>
<td>Positive</td>
<td>Frequency</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>Percentage</td>
<td>0.6%</td>
</tr>
<tr>
<td>Negative</td>
<td>Frequency</td>
<td>1078</td>
</tr>
<tr>
<td></td>
<td>Percentage</td>
<td>99.4%</td>
</tr>
</tbody>
</table>
Table (3.3): Percentage of HIV according to type of donors:

<table>
<thead>
<tr>
<th>HIV</th>
<th></th>
<th>donors</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>voluntary</td>
<td>family</td>
</tr>
<tr>
<td>Positive</td>
<td>Frequency</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Percentage</td>
<td>.0%</td>
<td>.3%</td>
</tr>
<tr>
<td>Negative</td>
<td>Frequency</td>
<td>1085</td>
<td>4001</td>
</tr>
<tr>
<td></td>
<td>Percentage</td>
<td>100.0%</td>
<td>99.7%</td>
</tr>
</tbody>
</table>

Table (3.4): Percentage of HCV according to type of donors:

<table>
<thead>
<tr>
<th>HCV</th>
<th></th>
<th>donors</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>voluntary</td>
<td>family</td>
</tr>
<tr>
<td>Negative</td>
<td>Count</td>
<td>1084</td>
<td>3990</td>
</tr>
<tr>
<td></td>
<td>Percentage</td>
<td>99.9%</td>
<td>99.4%</td>
</tr>
<tr>
<td>Positive</td>
<td>Count</td>
<td>1</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Percentage</td>
<td>.1%</td>
<td>.6%</td>
</tr>
</tbody>
</table>
Figure(3-3): Percentage of VDRL according to type of blood donors
Chapter four

4. Discussion, Conclusions & Recommendation

4.1 Discussion

In this study in 5100 units of blood were collected from donors (voluntary and family donors). The majority of the donors were males which is comparable to the study done by Arora et al.,(2010) was (96.2%). The majority of voluntary donors under 30 years old, and this age is increase in family donors. No voluntary donor was found to be positive for HIV, and positivity for HIV in family donors was increase. Result of HIV in this study was lower in family and voluntary donors than the results of the study done by Sentjens et al,(2002) 5.9%, AbdelSalam et al,(2008) 0.8% ,Tulika et al,(2009) 5% , Arora et al,(2010) 1.7%, Nilima et al,(2010). And results of HBsAg in this study was higher in family donors and lower in voluntary donors than the results done by Arora et al,(2010) 0.9%, Kulkarni et al,(2012), 1.7%,and in family donors lower than AbdelSalame et al,(2008) 10%. HCV result is lower in family and voluntary donors than the study done by Gilany and Fedawy(2006) 2.7%, Arora et al(2010) 3.2%, Kulkarni et al(2012) 1.0%, and higher than study of AbdelSalam et al,(2008) 0%. Syphilis result is higher in family and voluntary donors than results done by Arora et al (2010) 0.04%, kulkarni et al(2012) 1.0%, and lower than result of AbdelSalam et al(2008) 15%. Frequency of HBsAg is more than other infectious diseases because of asymptomatic carriers. Moreover, it should never be forgotten that blood donations collected in the latent period of infection may be infectious despite a negative antibody test. Adding nucleic acid testing (NAT) to routine blood screening protocol helps in detecting very low levels of viral RNA or DNA. Relative donors most of the times are
family members and during emergency they donate blood without giving proper history of exposure (Arora et al., 2010).

### 4.2 Conclusions

- Positivity of HBS, HCV, HIV and syphilis in this study was more in family donors as compared to voluntary donors.
- Majority of voluntary donors in small age (range 30 and less).
- Positivity of HIV was high in family and no voluntary donor was found to be positive for HIV.
- HBsAg and syphilis was high in comparison to other results, and HCV is low from other results.
4.3 **Recommendation:**

This study recommends;

- Educating people and creating awareness about voluntary blood donation is an important factor.
- Motivating and recruitment of voluntary blood donors by conducting voluntary blood donation camp is the most effective way of ensuring adequate supplies of safe blood.
- Introducing nucleic acid testing (NAT) for HIV, HBsAg and HCV is recommended to detect the infection during window period.
References


Christopher D, Hillyer, (2007), Blood Banking And Transfusion Medicine, second edition ,615;39.


Appendix
ELISA Plate
ELISA Kits
ELISA washer