بسم الله الرحمن الرحيم

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Serodetection of HBV, HCV, HIV and *Treponema pallidum* among Blood Donors in Almak Nimir University Hospital ,Shendi,Sudan

A thesis Submitted for the fulfillment of the requirement of M.Sc. in Medical Laboratory Sciences(Microbiology)

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الآية

بسم الله الرحمن الرحيم

قال تعالى:

﴿ يَا أَيُّهَا الَّذِينَ آمَنُوا إِذَا قِيلَ لَكُمْ تَفَسَّحُوا فِي الْمَجَالِسِ فَافْسَحُوا يَفْسَحِ اللَّهُ لَكُمْ وَإِذَا قِيلَ انشُزُوا فَانشُزُوا يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ ﴾

سورة المجادلة – الآية (11)

Dedication

Allah our creator and Master, Our greatest and most honored teacher

(May Allah bless and grant him)

To the fountain of patience and optimism and hope.

To each of the following in the presence of God and His Messenger our

(Mothers)

Whom he strives to bless comfort and welfare and never stints what he owns to push us in the way of success, to our dearest

(Brothers)

Our second magnificent home,

(All our teachers)

To all distinguished to the taste of the most beautiful moment our

(Friends)

Acknowledgement

First and foremost, we must acknowledge our limitless thanks to Allah the Ever-Magnificent; the Ever –Thankful, for his help and bless. We are totally sure that this work would have never become truth, without his guidance.

We owe a deep debt of graduate to our university for giving us this opportunity to complete this work.

We are grateful to some people who work hard with us from the beginning till the completion of present research particularly our supervisor **Dr: Ahmed Mohammed** who has been generous during all phases of research, and we highly appreciate the effort expended by **Dr: Khalid saeed** and my colleague **Nuha abdelmoniem**

And we would like to take this opportunity to say warm thanks to all of our beloved friends who have been so supportive along the way.

And also we would like to express wholehearted thanks to our families for their generous support through our entire life because of their unconditional love and prayers, without their support this study wouldn't have been possible.

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Abstract

Background: Screening for transfusion-transmissible infections (TTIs) to exclude blood donations at risk of transmitting infection from donors to recipients is a critical part of the process of ensuring that transfusion is as safe as possible. Effective screening for evidence of the presence of the most common and dangerous TTIs can reduce the risk of transmission to very low levels.

Aim: The aim of this study was to analyze the frequency of HIV, HBV, HCV, and syphilitic infections among blood donors in shendi during 3 months in 2018.

Methods:A total of 200 blood donors visited the blood bank center of Almak Nimir university in shendi during April - July were screened for antibodies against HIV, HCV, Treponema pallidum and HBsAg using ICTs rapid test and all reactive sera were retested by ELISA assay as confirmatory test.

Results: The study included 200 blood donors. The frequency of HIV , HBV , HCV , and syphilis was (0.5%) , (1.5%) , (0.5%) , (2.5%), respectively. Their were different between ELISA and screening test. This is different have statistical analysis show significant variation in result of p.value (0.000).

Conclusions: The Study concluded that the viruses Hb, HC were prevalent in the locality. Suspected case of HIV needed further investigation. Syphilis with higher prevalent rate than the above mention viruses encountered.

ELISA system is more sensitive and specific test than immunochromatogrphic test.

المستخلص

خلفية : فحص الامراض القابلة للنقل لاستبعاد المتبرعين الذين يعانون من هذه الامراض التي تنتقل من المتبرع الي المريض لانها جزء مهم من عملية النقل للتأكد من نقل الدم امن بقدر الامكان . فاعلية الفحص دليل علي وجود اهم و اخطر الامراض القابله للنقل تقلل من خطورة النقل الي اقل مستوي .

الهدف : الهدف من الدراسه تحليل معدل فيروس الكبد الوبائي بنوعيه و

, فيروس العوز المناعي و الزهري لكل متبرعين الدم في مدينة شندي في مدة ثلاثه اشهر في عام 2018

الطريقه : تم اخذ مجموع200 عينه في مستشفي المك نمر خلال ثلاثه اشهر و تم عمل الفحوصات بالطريقه التقليديه و التأكد منها بطريقه اكثر دقه .

النتائج : هذه الدراسه تضمنت اخذ200 عينه و كانت النتائج كالاتي معدل فيروس العوز المناعي و فيروس الكبد الوبائي (2.5%) , (0.5%) , (1.5%) , (0.5%) B,C والزهري علي التوالي.

الخلاصه :هذه الدراسه استنتجت أن فيروس الكبد الوبائي بنوعيه C،B سائد في هذه المنطقة اشتبهت أن حالة فيروس الكبد المناعي تحتاج لفحص آخر.

هذه الدراسة اظهرت زيادة معدل الزهري اكثر من معدل التهاب الكبد الوبائي بنوعيه و معدل فيروز العوز المناعي . والطريقة التأكيدية أثبتت أنها أكثر دقة من الطريقة التقليدية.

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Chapter one

Introduction, Rationale, objective

1. Introduction

It is the responsibility of governments to assure a safe and sufficient supply of blood and blood products for all patients requiring transfusion [1]. Each country should formulate a national blood policy and plan, as part of the national health policy, to define how safe blood and blood products will be made available and accessible to address the transfusion needs of its population, including how blood transfusion services will be organized and managed[2].

The provision of safe and efficacious blood and blood components for transfusion or manufacturing use involves a number of processes, from the selection of blood donors and the collection, processing and testing of blood donations to the testing of patient samples, the issue of compatible blood and its administration to the patient.

There is a risk of error in each process in this "transfusion chain" and a failure at any of these stages can have serious implications for the recipients of blood and blood products. Thus, while blood transfusion can be life-saving, there are associated risks, particularly the transmission of blood borne infections. [2]

Screening for transfusion-transmissible infections (TTIs) to exclude blood donations at risk of transmitting infection from donors to recipients is a critical part of the process of ensuring that transfusion is as safe as possible. Effective screening for evidence of the presence of the most common and dangerous TTIs can reduce the risk of transmission to very low levels [2]. Blood transfusion services Should therefore establish efficient systems to ensure that all donated blood is correctly screened for specific TTIs and that only non-reactive blood and blood components are released for clinical and manufacturing use.

The adoption of screening strategies appropriate to the needs, infrastructure and resources of each country can contribute significantly to improvements in blood safety. In countries where effective blood screening programmes have been implemented, the risk of transmission of TTIs has been reduced dramatically Over the last 20 years [3] [4].

Nevertheless, a significant proportion of donated blood remains unsafe as it is either not screened for all the major TTIs or is not screened within a quality system.

Data on blood safety indicators provided in 2007 by ministries of health to the WHO Global Database on Blood Safety (GDBS) indicate that, of the 155 countries that reported performing 100% screening for HIV, only 71 screens in a quality-assured manner [5].

Concerted efforts are still required by a substantial number of countries to achieve 100% screening of donated blood for TTIs within quality systems.

1.1 Rationale:

It is the responsibility of governments to assure a safe and sufficient supply of blood and blood products for all patients requiring transfusion.

Immunochromatographic test is the main test used if blood transfusion Centers in my locality, this study aim to know the accuracy of using ICT in blood banking centers and compare it with ELISA as confirmation method.

1.2 Objectives:

1.2.1 General objective:

Serodetection of HBsAg , HIV , HCV ,and Syphilis among blood donors .

1.2.2 Specific objective:

- To find out prevalence of HBV, HCV, HIV, and Syphilis among blood bank donors.
- To assess the result of screening test for HBV, HCV , HIV and syphilis with reference to ELISA .
- To find out the association between prevalence of HBV , HCV , HIV , and T.pallidum and the risk factor such as age , education , marital status .

Chapter Two

Literature Review

2, Literature review

Human immunodeficiency virus:

The human immunodeficiency virus (HIV) is a retrovirus, an enveloped RNA virus, which is transmissible by the parenteral route. It is found in blood and other body fluids.

Once in the bloodstream, the virus primarily infects and replicates in lymphocytes [(6)].

The viral nucleic acid persists by integrating into the host cell DNA.

A number of different groups and subtypes (clades) have been identified

Withsome significant antigenic differences; HIV-1 and HIV-2 are the two

Major distinctvirus types and there is significant cross-reactivity between them.

HIV-1 is now endemic in many parts of the world, although its incidence and prevalence islow in some regions. HIV-1 group M is Responsible for more than 99% of the infections worldwide, whereas the Prevalence of Additionally, a few infections with HIV groupO and group N have been observed in Africa. The appearance of antibody marks the onset and persistence of infection, but not immunity [(6)].

Transmissibility

As HIV can be present in the bloodstream in high concentrations and is stable at the temperatures at which blood and individual blood components

Are stored, the virus may be present in any donated blood from an HIV-infected individual.

Infectivity estimates for the transfusion of infected blood products are much higher (around 95%) than for other modes of HIV transmission owing to the much larger viral dose per exposure than for other routes [(6)].

Screening

The methods used to identify the presence of HIV employ the following screening Targets:

Serological markers:

— anti-HIV-1, including group O, + anti-HIV-2

— HIV p24 antigen (p24 Ag)

Viral nucleic acid: HIV RNA.

The assay should be capable of detecting subtypes specific to the country or region.

Screening donations for both antibody and antigen will identify the vast majority of donations from infected donors [7].

Anti-HIV-1 + anti HIV-2 and p24 antigen

All screening strategies should employ, at minimum, the detection of antibody because the identification of specific antibody is still the most reliable screening method. They should preferably also employ the detection of antigen. Antibody may be detected approximately three weeks after infection and approximately six days after antigen is first detected [8]. HIV p24 antigen may appear from 3 to 10 days after viral RNA [9] and its detection can further reduce the serological window period by 3 to 7 days before antibody detection.

Screening for anti-HIV has been the basis for blood screening since the mid-1980s And HIV serology is therefore well understood. Although there is cross-reactivity between the main virus types (HIV-1 and HIV-2), it is not sufficient to rely on anHIV-1 specific assay to detect all cases of HIV-2. Since the early 1990s, anti-HIV assays have included specific antigens for both HIV-1 and HIV-2.

However, the use of antibody-only assays has been superseded by the use of combination HIV Antigen and antibody assays (combined HIV p24 Ag and anti-HIV-1 + anti-HIV-2),Wherever possible. These provide an enhanced level of sensitivity in early infection over antibody-only assays by reducing the serological window period [10].

HIV RNA

Viral RNA can be detected approximately 7 to 11 days after infection: i.e. When the results of HIV antigen-antibody assays are negative, but HIV RNA Detection is positive [8]. The detection of HIV RNA can reduce the risk of HIV Being transmitted through the transfusion of infected blood donated during the serological window period of antigen and antibody assays.

Hepatitis B virus

Hepatitis B virus (HBV) is a member of the hepadnasvirus group and is an enveloped DNA virus. HBV is transmissible by the parenteral route and may be found in blood and other body fluids. Once in the bloodstream, the virus travels to the liver where it replicates in hepatocytes.

HBV is endemic globally and hyper-endemic in parts of the world. It is difficult to determine the total number of cases of transfusion transmitted HBV globally [11] [12].

Transmissibility

While HBV is present in the bloodstream, the levels of the virus itself are variable.

In recently infected individuals, viral DNA is normally present, although not always at high levels. Chronically infected individuals may either be infectious (viral DNA present) or non-infectious (viral DNA absent) and viraemia would generally be expected to be very low or absent entirely.

Screening for hepatitis B surface antigen (HBsAg) indicates infection with HBV, but does not in itself distinguish between recent and chronic Infections [11] [12] .

The distinction between acute and chronic infection is not relevant to blood screening; all HBsAg positive donations should be considered to be at high risk of transmitting HBV and should not be released for transfusion. Additionally,Some studies indicate that even when HBsAg is negative, some individuals may have low levels of detectable viral DNA which will be transmitted by blood and may cause infection in the recipient [11] [12].

The use of unscreened HBV-infected blood and blood products will result in the transmission of HBV in the vast majority of cases. In general, the earlier in life that HBV is acquired, the more likely the individual is to develop chronic infection which then has a higher probability of progressing to cirrhosis and hepatocellular carcinoma.

Screening

The serology of HBV is complex. A number of different serological markers develop during the course of infection, including hepatitis B surface antigen (HBsAg) and hepatitis B core antibody (anti-HBc). In addition, HBV DNA can be detected in the majority of cases, although in HBsAg negative phases of infection the DNA levels are generally relatively low and the viraemia may be transient.

The methods used to identify the presence of HBV employ the following screening targets:

Serological markers:

— Hepatitis B surface antigen

— Hepatitis B core antibody, in some situations

Viral nucleic acid: HBV DNA.

Hepatitis B surface antigen

Hepatitis B surface antigen is the prime marker used in blood screening programmes. It normally appears within three weeks after the first appearance of HBV DNA and levels rise rapidly [11].

It can thus be detected easily by most of the highly sensitive HBsAg assays available. The presence of HBsAg may indicate current or chronic infection and thus potential infectivity. Most blood transfusion services screen donated blood for HBsAg using sensitive immunoassays. Particle agglutination assays are still available and used in some countries, although they are less sensitive than immunoassays or even simple/rapid assays.

Hepatitis B core antibody [13]

Antibody to hepatitis B core antigen is produced later in acute infection, after the appearance of HBsAg, and marks the start of the immune response to HBV infection. In general, anti-HBc persists for life, irrespective of whether the infection resolves or progresses to chronicity. In the vast majority of cases of hepatitis B, the detection of anti-HBc has limited value as HBsAg is already present. In some cases, however, during the resolution of the infection, HBsAg may decline to below detectable levels. Although anti-HBs usually then appears relatively rapidly, there may be a short period of time prior to its appearance when anti-HBc is the only detectable circulating serological marker of infection, even though the individual may still have low viraemia and would thus be potentially infectious [13] .

If anti-HBc screening is introduced for routine use, it would be necessary to distinguish between individuals who are anti-HBc reactive because of

Previous, resolved, natural HBV infection, and are thus non-infectious, from those who have unresolved HBV infection and are thus potentially infectious. In a population with a high prevalence of infection, the number of Blood donors with evidence of natural, resolved infection is likely to be Significant, resulting in the potentially unnecessary discard of many blood donations [13].

As the presence of anti-HBs is protective, anti-HBs testing of all anti-HBc reactive donations would therefore be required to distinguish between infectious and non-infectious individuals. In general, a level of anti-HBs at 100 mIU/mL is usually accepted as the minimum protective level in the context of blood screening; donations that are HBsAg negative, anti-HBc reactive with anti-HBs levels of 100 mIU/mL or more are generally considered to be safe and acceptable for release for clinical or manufacturing use.

Another important consideration is that anti-HBc assays often demonstrate a high level of non-specificity [13].

This, together with the problems associated With the confirmation of anti-HBc reactivity, often results in a situation where anti-HBc reactivity is identified in the absence of any other markers of HBV infection and where the majority of this reactivity is actually non-specific and does not reflect HBV infection.

Thus, although anti-HBc screening may have advantages in some situations, the problems associated with the performance of anti-HBc assays and the complexity of dealing with HBV immune individuals may outweigh any potential benefits.

Alanine aminotransferase

Testing for raised liver alanine aminotransferase (ALT) levels was originally introduced in some countries prior to the identification of hepatitis C and the introduction of HCV screening in an attempt to reduce the incidence of what Was then called post-transfusion non-A, non-B hepatitis (PTNANBH) [14].

ALT is an enzyme found predominantly in the liver. It circulates naturally at low levels in the bloodstream, but is released in high quantities as a result of liver damage; this is often, but not exclusively, due to viral infection.

ALT is a non-specific marker of infection. With the advent of HCV screening, screening for raised ALT levels provides no identifiable benefit in terms of improving blood safety [15].

Hepatitis B virus DNA

The detection of HBV DNA further reduces the risk of HBV transmission through the transfusion of infected blood donated during the acute window period: i.e. when the results of HBsAg assays are negative, but HBV DNA is positive [16].

Low levels of HBV DNA have also been detected in the blood of individuals after the resolution of acute HBV infection and the disappearance of HBsAg or in so called chronic occult HBV infection [11][12] .

Hepatitis C virus

Hepatitis C virus (HCV) is a member of the flavivirus group and is an enveloped RNA virus. It is transmissible by the parenteral route and may be found in blood and other body fluids. Once in the bloodstream, the virus travels to the liver where it replicates in hepatocytes, resulting in a similar picture to that seen with HBV infection. Seroreversion has been seen in

numbers of individuals who have resolved their infections. The loss of circulating antibody may leave no readily detectable evidence of previous infection [17].

HCV is endemic in many parts of the world, although in some regions its Incidence and prevalence may be low. Several genotypes are identified and Are associated with different geographical distributions and some differences in antigenicity and clinical features, including response to treatment with interferon alpha (IFN-α) [18].

Transmissibility

While HCV is present in the bloodstream, the levels of the virus itself are variable.

In recently infected individuals, virus is normally present. However, only around 70% of chronically infected individuals are viraemic and the length of time that viraemia persists is not fully understood. Nonetheless, it is expected that most HCV infected donations would contain virus and thus be infectious [18].

Screening for both HCV antigen and antibody does not in itself distinguish between recent and chronic infection. The distinction is, however, not relevant to the screening of blood for transfusion and all HCV antigen-antibody reactive donations should be considered to be at high risk of transmission of HCV and should not be used for clinical or manufacturing use.

Screening

The methods used to identify the presence of HCV employ the following

Screening Targets:

Serological markers:

— HCV antibody

— HCV antigen

viral nucleic acid: HCV RNA.

HCV antibody and antigen

HCV antibody becomes detectable approximately 30 to 60 days after infection.

Viral antigen normally appears between 0 and 20 days after viral RNA first Appears. Antibody is generated and can be detected between 10 and 40 days After antigen is first detected [18].

The serology of HCV is still not fully understood. Serological screening has been highly effective in significantly reducing the transmission of HCV through the route of transfusion. Until recently, anti-HCV has been the prime serological marker for blood screening programmes. However, HCV antigen can be detected in the peripheral blood earlier than antibody in the course of early infection. HCV antigen assays, both antigen only and combined antigen-antibody, have been commercially available for a number of years. These have been introduced in some countries to improve the overall effectiveness of serological HCV screening [*18*].

Hepatitis C virus RNA

Viral RNA is normally detectable within a few weeks of infection and persists for 6–8 weeks prior to antibody seroconversion [*8*]. The detection of HCV RNA may further reduce the risk of HCV transmission through the transfusion of infected blood donated during the window period of antigen

and antibody assays: i.e. when the results of HCV antigen-antibody assays are negative, but HCV RNA is positive [8]. However, any benefit is dependent upon HCV incidence and the actual number of donations that may be collected in the window period [18].

Syphilis

Syphilis is caused by the bacterium *Treponema palladium palladium*. It is transmissible by the parenteral route and may be found in blood and other body fluids. Once in the bloodstream, the bacteria spread throughout the body. A primary lesion, chancre, usually occurs about three weeks after exposure, although the duration may be shorter in cases of transfusion-transmitted infection where the organism enters the bloodstream directly. Syphilis is endemic in many parts of the world.

Transmissibility

While *T. pallidum* may be found in the blood stream, levels are variable, even in acute primary syphilis, and the bacteraemia is often short-lived. In addition, the treponemes are relatively fragile, in particular being heat-sensitive; storage below +20°C for more than 72 hours results in irreparable Damage to the organism such that it is no longer infectious. Thus, although Clearly potentially infectious, the risk of transmission through the transfusion of blood and blood components stored below +20°C is very low. Blood components stored at higher temperatures (above +20°C), such as platelet concentrates, or those not stored at lower temperatures for any length of time, such as blood collected and used within 48 hours, present a significantly higher risk of transmitting syphilis. Thus, although the risk of transmission of syphilis from unscreened donations is variable, the screening test is nonetheless considered essential as most blood transfusion services

provide some blood components that are either stored above +20°C or are not stored below +20°C for sufficient time to kill any organisms present.

Screening

The methods used to identify the presence of syphilis employ the following Screening targets:

Non-specific, non-treponemal markers: antibody to lipoidal antigen (reagin)

Specific treponemal antibodies.

Treponemal serology is relatively complex with different profiles seen at different stages of infection and depending on whether treatment has been given. Infection with the four major types of pathogenic treponemes can not be distinguished by serological screening because the major immuno dominant epitopes are so similar that the antibodies produced are detected by any specific antibody assay for syphilis.

In general, syphilis assays can be divided into specific and non-specific assays; their use depends on whether the purpose of testing is screening or diagnostic testing.

Specific assays

Specific assays commonly used for blood screening are *Treponema pallidum* Haemagglutination assays (TPHA) and enzyme immunoassays (EIAs). These detect specific treponemal antibodies and thus identify donations from anyone who has ever been infected with syphilis, whether recently or long in the past, and whether treated or not.

Non-specific assays

Non-specific assays such as Venereal Diseases Research Laboratory (VDRL)

And rapid plasma reagin (RPR) tests identify those individuals who may have been more recently infected. They detect antibodies to cardiolipin or lipoidal antigen (reagin); the plasma levels of these antibodies rise Significantly in active infection due to the cellular damage. The use of non-specific assays is of most value in diagnostic testing where it can be used to identify recently infected individuals.

When the incidence and prevalence of syphilis in the blood donor population are high and cannot be reduced through donor selection strategies, it may be necessary to consider screening using a non-treponemal assay (e.g. VDRL or RPR) to identify only the highest-risk donors – those with evidence of recent infections For routine screening, however, this strategy carries a high risk of false negative results as the sensitivity of these assays is lower than specific assays and the test results may not always be positive, even when the infection is recent.

2.1 Previous study:

Seroprevalence rate of hepatitis B virus (HBV), hepatitis C virus (HCV)and human immunodeficiency virus (HIV) among blood donors in the north region of Jordan . Prevalence rates of HBV and HCV infections were 1.4% and 0.8%, and zero for HIV infections.The prevalence was not significantly higher in male than in female donors. Hepatitis B surface antigen (HBsAg) and anti-HCV positivity tend to increase with increase in age.

This study in Sierra Leone 2016 aimed to describe the overall prevalence of hepatitis B and C, HIV and syphilis among blood donors to Compare the differences between volunteer versus family replacement donors, as well as urban versus rural donors. There were 16807 blood samples analyzed, with 80% from males; 2285 (13.6%) tested positive for at least one of the four pathogens.

Overall prevalence was: 9.7% hepatitis B; 1.0% hepatitis C; 2.8% HIV; 0.8% syphilis. Prevalence was higher among samples from rural blood banks, the difference most marked for hepatitis C. The proportion of voluntary donors was 12%. Family replacement donors had a higher prevalence of hepatitis B, C and HIV than volunteers.

Prevalence of transfusion transmissible infections in blood donors of Pakistan. Of the 16,602 blood donors, 16,557 were males and 45 females (Mean age 28.6 ± 2). Nine hundred and Seventy three (5.8%) donations were reactive in any screening assay, with 58(0.35%) donations reacting in more than one assay. The prevalence of Hepatitis B, Hepatitis C, Human immunodeficiency viruses, Syphilis and Malaria was found to be 1.84, 1.7, 0.04, 2.1 and 0.07% respectively. Characteristics among the infections were evaluated and it was found that unmarried donors had a higher chance to be infected by Hepatitis B virus and Syphilis as compared to the other infections. On the other hand, construction workers and married donors were at more risk to be infected by Syphilis rather than the other infections. In case of co-infections, personnel with different occupations and marital status were infected by more than one pathogen.

This study aimed to determine the Sero-prevalence of viral transfusion-transmissible Infectious diseases among blood donors, namely immunodeficiency virus, hepatitis B and C Virus among blood donors. The study was carried out in the blood bank at Kosti Teaching Hospital, White Nile State, Sudan. Screening of blood samples for hepatitis B surface Antigen (HBsAg), human immunodeficiency virus (HIV) and hepatitis C virus (HCV) Antibodies were done using immune chromatographic (ICT) strips. The study included 1204 Blood donors. All were males. The overall prevalence of viral transfusion transmissible Infections was 115 (9.6%).

The sero-prevalence for antibodies against HIV and hepatitis C virus was Positive in 8 (0.7%) and 41 (3.4%) donors respectively while HBsAg was Detected in 66 (5.5%) donors. This study showed that the sero-prevalence of hepatitis B and C viruses was high in the community local to Kosti hospital, a situation that reaffirms the Need for strict criteria for selection of blood donors and also methods of laboratory assays For detection of infectious agents must be improved. In addition; appropriate management must be ensured to prevent the further transmission of these infections.

Records covering the period between January 2010 and June 2010 at Kassala Teaching Hospital, eastern Sudan to determine the seroprevalence of HIV, HBV, HCV and syphilis infections among blood donors. A total of 810 Consecutive blood donors screened their age ranged between 19 – 58 year with mean (SD) 30.1(13).

the overall seroprevalence of HIV, HBV, HCV and syphilis was 3%, 4.3%, 3.1% and 2.7% respectively. Of all the subjects 106 had serological evidence with infection with at least one pathogen and 17 had multiple infections. HBV-HCV was the most common combination (35.3%) Followed by HBV - HIV (29.4%), HIV-syphilis (23.5%), HIV-HCV (5.9%) and HCV-syphilis (5.9%). The prevalence rate of HBV, syphilis and HCV among HIV positive donors was 20.8%, 16.7% and 4.2% respectively. This high seroprevalence of transfusion-transmissible infectious diseases call for Mandatory and routine screening of HIV, HBV, HCV and syphilis among blood donors in eastern Sudan, to ensure the safety of blood transfusion.

Chapter Three
Material and Method

3. Material and methods

3.1 Material

3.1.1 Study design:

This is prospective hospital base Descriptive study.

3.1.2 Study area:

The study was conducted in Almek nimir university hospital (shendi)

3.1.3 Study population:

All donors visited blood bank in Almek nimir university hospital from April – July.

3.1.4 Sample size:

The sample size used in the study was 200 blood donors who visited blood bank in Almak nimir university hospital from April – July 2018

3.1.5 Data analysis:

The data obtained from the questionnaires and the result of the laboratory analysis were entered into (SPSS), analyzed using calculator, and reduced to table.

3.1.6 Ethical consideration:

This study was approved by the Institutional Review Faculty of Postgraduate, Shendi. Approval was also obtained from the health authority of the Almak Nimir University hospital .The whole procedure was anonymous. Thus, coded serum samples received from the patients were used.

3.2 Method:

Sample collection and processing:

Five milliliters of venous blood samples were collected from each donors using standard technique in to plain tubes, the blood sample were allowed to stand at room temperature to allow for blood clotting then centrifuged at 2,500 rmp for 5 min and were separated and stored at -20c.

Screening tests:

A total of 200 blood donors were screened for HBV, HIV, HCV and syphilis by using immunochromatography test (ICT) . HBsAg (Ecotest china310030) for the qualitative presumptive detection of HBsAg in human whole blood serum or plasma, HIV-1/2(Alerechiba, 270-2214 Japan), forth detection of antibodies to HIV-1 and HIV-2 in human serum, plasma, or whole blood, HCV and Syphilis (Humasis-Republic of Korea)it is designed for qualitative determination of antibodies in human serum, plasma, or whole blood. All the reactive samples were retested by ELISA.

Sensitivity of HIV 100%, HBV > 99.9%, HCV, and Syphilis 100%

Specificity HIV 99.89%, HBV 99.9%, HCV, and syphilis 99.3%

Hepatitis B:

Principle:

HBsAg Rapid test device (whole blood, serum, plasma) detect HBsAg through visual interpretation of color development on the internal strip. Anti – HbsAg antibodies are immobilized on the test region of the membrane. During testing the specimen react with anti- HBsAg antibodies conjugated to colored particle and percoated on to the sample pad of the test. The mixture then migrated through the membrane by capillary action and interact with reagent on the membrane. If there is sufficient HBsAg in the specimen. A

colored band will form at the test region of the membrane. The presence of this colored band indicates a positive result. While its absence indicates a negative result. The appearance of a color band at the control region serves as a procedural control. Indicating that the proper volume of specimen has been added and membrane wicking has occurred.

Hepatitis C:

Principle:

Humasis HCV card is a chromatographic immunoassay, when sample is added to sample pad, it move through the conjugate pad and mobilizes gold protein A that is coated conjugated pad, the mixture moves along to the membrane by capillary action and reacts with recombinant HCV antigen that is coated on the region, if there are no anti-HCV antibodies in the sample, the region will remain color less, but the result is the formation of colored line in the test region if anti-HCV antibodies are present in the sample.

The mixed sample Continues to move to the control region and forms a colored line, indicating the test is working and its result is valid.

HIV:

Principle:

Alere determine HIV -1/2 is an immunochromatographic test for the qualitative detection of antibodies to HIV -1 and HIV-2.

Sample is added to the sample pad ,As the sample migrates through the conjugate .This mixture continues to migrate through the solid phase to the immobilized recombinant antigens and synthetic peptides at the window site , if antibodies bind to the antigen –selenium colloid and to the antigen at the patient window ,forming a red line at the patient window site .

If antibodies to HIV -1 and / or HIV-2 are absent, the antigen-selenium colloid flows past the patient window, and no red line is formed at the patient site.

To insure assay validity. A procedural control bar is incorporated in the assay device.

Syphilis:

Principle:

The Humasis syphilis card contain a membrane strip , which is precoated with recombinant treponena pallidum antigen on test band region . The recombinant treponena pallidum antigen - colloidal gold conjugate. Patient sample and sample diluents move along the membrane chromatographically to the test Region (T) and forms a visible line as the antigen – antibody – Antigen gold particle complex forms. Therefore, the formation of a visible line in the test region (T) indicate a positive result for the detection of

treponema pallidum specific antibodies (IgG ,IgM , and IgA) , when the treponema pallidum specific antibodies (IgG,IgM and IgA).

When the treponema pallidum specific antibodies are absent in the sample .

no visible color band in the test region (T).

Enzyme immunoassay (EIA):

HBsAg EIA test kits (Lot number BXE0742A , Fortress Diagnostics) , HCV (Lot number BXE0781A, Fortress Diagnostics) , HIV (Ag/Ab) (Lot number BXE0792A , Fortress Diagnostics) , Syphilis (Lot number

BXE0995A, Fortress Diagnostics) were used as confirmatory tests. Syphilis

tests are based on detection of anti-TP antibodies was achieved by antigen sandwich enzyme linked immunosurbend assay, Also HBsAg and HIV achieved by Sandwich method, except HCV tests achieved by indirect method. Excision of the test and interpretation of results were according to manufacture instructions. In all tests (HBV, HCV, HIV and T. pallidum) titers greater than >1 IU/ml were considered positive, 0.9 _ 1.00 IU/ml borderline, and <0.9 as negative. Optical densities were measured in microplate reader at 450 nm.

HBV, HCV, HIV, Syphilis sensitivity 99.79% and specificity 99.55%

Chapter Four

Results

4. Results

A total number of 200 blood donors in Elmak nimir university hospital were enrolled in this study during the period from April – July 2018 , the data obtained explained in tables (1,2,3,4,5,6), the result of the laboratory analysis was showed in Table (7,8,9,10) , P.value in Tables (11,12,13,14).

Table (1): Shows distribution the study population according to donation

Donation	Frequency	Percent
Voluntary	70	35
Relative	130	65
Total	200	100

Table (2): Shows distribution the study population according to Locality

Locality	Frequency	Percent
Urban	71	35.5
Rural	139	64.5
Total	200	100

Table (3): Shows distribution the study population according to occupation

Occupation	Frequency	Percent
Student	80	40
Medical	1	0.5
Farmer	50	25
Free work	40	20
Merchant	15	7.5
Teacher	5	2.5
Driver	4	2
Solider	5	2.5
Total	200	100

Table (4): Shows distribution the study population according to marital status

Marital status	Frequency	Percent
Married	50	25
Single	150	75
Total	200	100

Table (5): Shows distribution the study population according to education status

Education	Frequency	Percent
Illiterate	10	5
Primary	50	25
Secondary	80	40
University	60	30
Total	200	100

Table (6): Shows distribution the study population according to Age

Age	Frequency	Percent
<20	5	2.5
21-30	100	50
31-40	85	42.5
>40	10	5
Total	200	100

Table (7) Comparison between screening test and ELISA of HIV, HBV, HCV, and Syphilis among study group.

Serological marker	ICT		ELISA	
	Positive	Negative	Positive	Negative
HIV	5(2.5%)	195(97.5%)	1(0.5%)	199(99.5%)
HbsAg	3(1.5%)	197(98.5%)	3(1.5%)	197(98.5%)
HCV	2(1%)	198(99%)	1(0.5%)	199(99.5%)
Syphilis	10(5%)	190(95%)	5(2.5%)	195(97.5%)

Table (8) Show distribution of the HBV, HCV, HIV, syphilis according to education level.

Education Level	**Syphilis**	**HBV**	**HCV**	**HIV**
Illiterate	2(20%)	1(33.3%)	0(0%)	1(20%)
Primary	4(40%)	1(33.3%)	1(50%)	2(40%)
Secondary	2(20%)	1(33.3%)	0(0%)	0(0%)
University	2(20%)	0(0%)	1(50%)	2(40%)

Table (9) Show distribution of the HBV, HCV, HIV, syphilis according to Age.

Age	Syphilis	HBV	HCV	HIV
20 – 30	4(40%)	1(33.3%)	2(100%)	2(40%)
31 – 40	5(50%)	2(66.7%)	0(0%)	3(60%)
More than 40	1(10%)	0(0%)	0(0%)	0(0%)

Table (10) Show distribution of the HBV, HCV, HIV, syphilis according to Marital status.

Age	Syphilis	HBV	HCV	HIV
Single	3(30%)	1(33.7%)	1(50%)	2(40%)
Married	7(70%)	2(66.3%)	1(50%)	3(60%)

Table (11):Comparison between screening test and ELISA for HIV detection

			HIV ELISA		
			Positive	Negative	Total
HIV screening	Positive	Count	1	4	5
		% within HIV ELISA	100.0%	2.0%	2.5%
	Negative	Count	0	195	195
		% within HIV ELISA	.0%	98.0%	97.5%
Total		Count	1	199	200
		% within HIV ELISA	100.0%	100.0%	100.0%

P. value 0.000

Table (12): Comparison between screening test and ELISA for HBV detection

			HBV ELISA		
			Positive	Negative	Total
HBV screening	Positive	Count	3	0	3
		% within HBV ELISA	100.0%	.0%	1.5%
	Negative	Count	0	197	197
		% within HBV ELISA	.0%	100.0%	98.5%
Total		Count	3	197	200
		% within HBV ELISA	100.0%	100.0%	100.0%

P. value 0.000

Table (13):Comparison between screening test and ELISA for HCV detection

			HCV elisa		
			Positive	Negative	Total
HCV screening	Positive	Count	1	1	2
		% within HCV ELISA	100.0%	.5%	1.0%
	Negative	Count	0	198	198
		% within HCV ELISA	.0%	99.5%	99.0%
Total		Count	1	199	200
		% within HCV ELISA	100.0%	100.0%	100.0%

P. value 0.000

Table (14):Comparison between screening test and ELISA for Syphilis detection

			Syphilis ELISA		
			Positive	Negative	Total
Syphilis screening	Positive	Count	5	5	10
		% within Syphilis ELISA	100.0%	2.6%	5.0%
	Negative	Count	0	190	190
		% within Syphilis ELISA	.0%	97.4%	95.0%
Total		Count	5	195	200
		% within Syphilis ELISA	100.0%	100.0%	100.0%

P. value 0.000

Chapter Five

Discussion, Conclusion, Recommendation

5-0 Discussion

It is the responsibility of governments to assure a safe and sufficient supply of blood and blood products for all patients requiring transfusion [(1).]

Each country should formulate a national blood policy and plan, as part of the national health policy, to define how safe blood and blood products will be made available and accessible to address the transfusion needs of its population, including how blood transfusion services will be organized and managed.

Two hundred venous blood samples were collected from blood banking center in Almik Nimer hospital from April to July 2018 form different ages and tested by using immunochromatographic test (ICT) for HBsAg, HCV, HIV and syphilis .

The study found that among screening test for HBsAg, HCV, HIV and syphilis, the prevalence of HBsAg among blood donors are 1.5%, HCV is 1%,HIV 2.5% and syphilis 5% .

The screened blood samples were tested by Enzyme linked immunosorbent assay for HBsAg, HCV, HIV and syphilis, the prevalence of HBsAg among blood donors are 1.5%, HCV is 0.5%,HIV 0.5% and syphilis 2.5% .

The immunochromatographic test results were compared with ELISA method for HBsAg, HCV, HIV and syphilis, this was different in the result of two test this different have statistical analysis show significant variations in results of P-value (0.000).

Evolved in this study and found that the positive rates of HBsAg, anti-HCV, anti-HIV and anti-TP were 1.5% , 0.5% , 0.5% , and 2.5% respectively.While frequency of HBV in Jordan[(19)], sierraleono[(20)] ,

Pakistan[21] , Kosti[22] , and Kassala[23] was observed to be 1.4%, 9.7%, 1.84%, 5.5%,4.3% respectively .

Frequency of HCV Jordan, sierraleono, Pakistan, Kosti, and Kassalaalso showed 0.8 %, 1%, 1.7%, 3.4% and 3.1% respectively. Frequency of HIV in Jordan, sierraleono, Pakistan, Kosti, and Kassala,also showed Zero, 2.8%, 0.04%, 0.7%, and 3% respectively. fequency of T.pallidum also showed 2.1% in Pakistan, and 2.7% in Kassala .

In this study HBV was detected 3 (1.5%) Frequency of HBV in various studies were observed to be 1.4% , 1.84% was constant with this study , 9.7%, 5.5% and 4.3% was higher than this study.

Incidence of HCV in the study 1(0.5%) was low if compared with various studies 1%, 1.7%, 3.4%, 3.1% and 0.8% was constant with this study.

In this study showed the Frequency of HIV was (0.5%), the Frequency of HIV in various studies 0%, 0.4%, was lower than this study, 2.8%, 3% was higher than this study, 0.7% was constant with this study.

In this study showed the Frequency of Syphilis 5 (2.5%), the Frequency of syphilis in Pakistan 2.1% was lower than this study and in Kassala 2.7% was constant with this study.

The blood donors of primary education levels have high percentage of infection with syphilis about (40%) flowed by non-educated donor of Percentage about (20%) due decreased knowledge about the disease and it is mode of transmission.

In age from 31 – 40 years old the HBV and syphilis are of high rate of prevalence among donors (66.7%, 50%) respectevly, while the age group over 40 years old has high rate of syphilis infection only in percentage about (10%).

Syphilis infection, HBV and HIV are prevalent in married donors of percentage about (70%, 66.3% and 60%) respectevly.and HCV is more prevalent in singles about (50%).

5.1 Conclusions:

The Study concluded that the viruses HBV, HCV were prevalent in the locality. Suspected case of HIV needed further investigation. Syphilis with higher prevalent rate than the above mention viruses encountered.

ELISA system is more sensitive and specific test than immunochromatogrphic test.

5.2 Recommendation:

Screening for HBsAg, HCV, HIV and T.pallidum should include immunochromatography test and confirmed by ELISA.

The study revealed high rate T.pallidum seroprevalence,further studies should conducted in this aspect.

References, Appendix

6.0 References

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6.1 Appendix:

Hepatitis B:

Procedure:

Bring tests, specimen, and /or controls to room temperature (15-30) before use.

1. Remove the test from its sealed pouch, and place it on a clean, level surface label the device with patient or control identification. For best results, the assay should be performed within one hour.
2. Transfer 3 drops of whole blood /serum / plasma to the specimen well (s) of the device with the provided disposable pipette, and start the timer. or

   Allow 3 hanging drops of finger stick whole blood to fall in to the center of the specimen well (s) of the test device , and start the timer avoid trapping air bubbles in the specimen well (s) and do not added any solution to the result window .

   As the test begins to work, color will migrate a cross the membrane.
3. If the test fails to migrate a cross the membrane after one minute, added one drop of buffer to specimen well (s).
4. Wait for color band (s) to appear, the result should be read at 15 minutes. Do not interpret the result after 20 minutes.

Hepatitis C:

Procedure:

1-Bring all materials and specimens to room temperature before beginning the test and then open the foil pouch and place the device on a clean , dry and level surface .

2-Use disposable pipette to transfer of sample (serum, plasma: 25 ML, whole blood: 50 ML) in to the sample well. And then add 1 drop (approximately 40 ML) of assay diluents in the sample well immediately.

3-Wait for 10 minutes and then read the results. Do not interpret the test results after 15 minutes.

HIV:

Procedure:

1- Remove the protective foil cover from each test.
2- For serum or plasma samples :
    a- Apply 50 ML of sample (precision pipette) to the sample pad (marked by arrow symbol)
    b- Wait a minimum of 15 minutes (up to 60 minutes) and read result.
3- For whole blood (venipuncture) samples :
    a- Apply 50 ML of sample (precision pipette) to the sample pad (marked by arrow symbol)
    b- Wait 1 minute, and then apply on drop of chase buffer to the sample pad.
    c- Wait a minimum of 15 minutes (up to 60 minutes) and read result.

4- For whole blood (finger stick) sample :

A-Apply 50 ML of sample (by EDTA capillary tube) to the sample pad (marked by arrow symbol)

B-Wait until blood is absorbed in to the sample pad, then apply one drop of chase buffer to the sample pad c-Wait a minimum of 15 minutes (up to 60 minutes) and read result.

Syphilis:

Procedure:

1. Allow the test device , specimen , buffer and / or controls to reach room temperature prior to testing
2. Remove the test device from the sealed foil pouch and use it as soon as possible.

Best result will be obtained if the assay is performed with in 1 hour

3. Place the device on a clean and level surface.

Serum or plasma specimen:

- Hold the dropper vertically and transfer 50 ML of serum or plasma (approximately 2-3 drops) in to the sample well (s) of the test device, then added 1 drop of buffer (approximately 40 ML) and start the timer.

Venipuncture whole blood specimens:

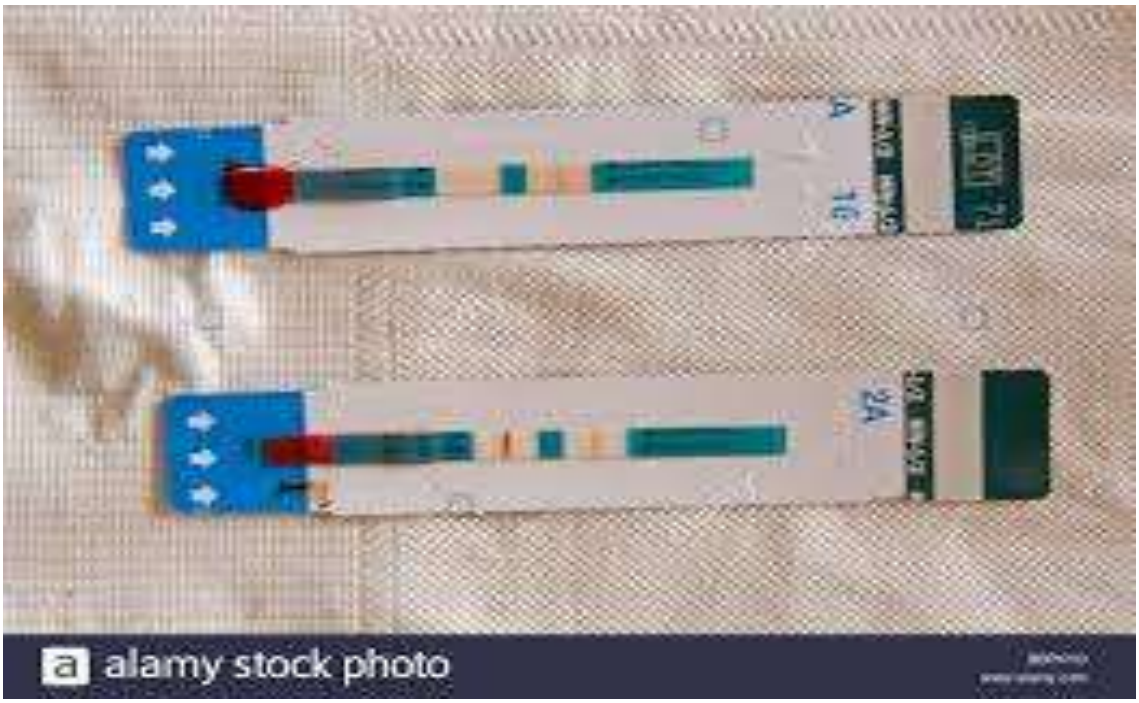
- Hold the dropper vertically and transfer 2 drops of whole blood (approximately 50 ML) to the sample well (s) of

    - The test device then added 1 drop of buffer (approximately 40 ML) and start the timer.

4-Wait for the colored line to appear, read result at 10 minutes. Do not read result after 30 minutes.


Anti HBs
HEPATITIS VIRUS
HBsAg
Anti-HBs
HBeAg
Anti-HBe
Anti-HBc (Total)
Anti-HBc (IgM)
Anti-HAV (Total)
TORCH Agent
VDRL
DRUG
Acetami
Amikin


HCV
HCV :
HORMONES


alamy stock photo


Syphilis
ID
C
T
S

Questionnaire

Date: …….

No: ……

Reason for donation Voluntary: ……..

Relatives (directed): …….

Nationality: Sudanese ……. Other ……

Specify……

Age: ……

Resident:

Locality: Urban (Shendi) ……..…

Rural (areas around Shendi)……..……

Occupation: student……. Medical field …….

Farmer …….. Others …… specify……...

Marital status: …….

Education status:

Illiterate …….. Primary …… Secondary …….

University and > ……

Results of the rapid test:

HBsAg: Positive ☐ Negative ☐

HCV: Positive ☐ Negative ☐

HIV: Positive ☐ Negative ☐

Syphilis: Positive ☐ Negative ☐

Result of ELISA:

HBsAg: ……..…….

HCV: ……..……..

HIV: …….

Syphilis: …….