Detection of Epstein Barr Virus among Blood Donors using ELISA and PCR, Central Blood Bank, Wad Medani Teaching Hospital, Gezira State, Sudan (2017)

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Medical Microbiology

Department of Medical Microbiology
Faculty of Medical Laboratory Sciences
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Detection of Epstein Barr Virus among Blood Donors using ELISA and PCR, Central Blood Bank, Wad Medani Teaching Hospital, Gezira State, Sudan (2017)

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Date: 7/1/2018
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Declaration

The undersigned author hereby affirm that the dissertation entitled (Detection of Epstein Barr Virus Among Blood Donors using ELISA and PCR, Central Blood Bank, Wad Medani Teaching Hospital, Gezira State Sudan) which has been submitted for fulfillment of the requirements of M.Sc degree in Medical Microbiology at the University of Gezira, Sudan. The research has been conducted under the supervision of Prof. adam Dawoud Abakar Salim and Dr. Elbadawi Abdalbagi Talha, had been prepared by my own potential and it had not been copied from any other source, also it had not been presented by any other researcher for Scientific degree elsewhere.

NAME : REFGHA ALTAYEB AHMED EBRAHIM
Dedication

I dedicate this work to

My loving father
Who made me proud to be his daughter.

the source of unconditional love

My mother

To the piece of my heart

My sisters and my brothers

To everyone who give something to light my way
ACKNOWLEDGEMENTS

I would like first and most to thank almighty God for the blessings and power that made my project a reality.

I would like to express my gratitude to my supervisor Prof. Adam Dawoud Abakar Salim for the highly perceptive comments, constructive suggestions and encouragement.

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Finally it is impossible to convey, in a couple of sentences, my gratitude to many people for helping me to learn and whom cooperation made this work possible. Thanks everyone helps me if I forget to mention in these short lines. I appreciate all helps and supports had been given to me.

May Allah reward you generously.
Detection of Epstein Barr Virus among Blood Donors using ELISA and PCR, Central Blood Bank, Wad Medani Teaching Hospital, Gezira State, Sudan (2017)

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Abstract

Epstein-Barr virus (EBV) is a member of the ancient and highly successful herpes virus family. Wide range of population more than 90% were reported to be infected with the virus especially during the childhood and thereafter remains in the body for life and was reported to be spread by various means such as oral secretions, seminal fluid, sexual contact, blood transfusion, or eating utensils with an infected person, it can cause infectious mononucleosis and associated with a wide a variety of malignant and nonmalignant conditions. The aim of this cross sectional laboratory based study to detect EBV infection among blood donors attending Central blood bank in Wad Medani teaching hospital, study population consisted of 25 blood donors all of them were males, Their age range between (21-39), they were tested by ELISA and all of them are negative for EBV infection. Further, 10 of randomly selected blood donors were tested by PCR and one of them is positive for EBV infection (10%). It was concluded that PCR detection give 10% infection rate by EBV among study population. Despite sensitive antibody-based blood-donor screening, the nucleic-acid tests by PCR is more sensitive in detection of virus and provide data accurately to measure the risk. Virus DNA amplification using polymerase chain reaction especially among suspected EBV blood donors is strongly recommended.
الكشف عن فيروس إبشتاين بار بين المتبرعين بالدم باستخدام المقايسة الامتصاصية للإنزيم المرتبط وتفاعل البلمرة المتسلسل, بنك الدم المركزي, مستشفى ود مدني التعليمي, ولاية الجزيرة السودان (2017)

رفقة الطيب أحمد إبراهيم

ملخص الدراسة

فيروس إبشتاين بار هو عضو من عائلة فيروسات الهربس القديمة. توضح التقاريران نسبة الإصابة 90% في المجتمع وخاصة خلال مرحلة الطفولة، ويتشر الباء في الجسم مدى الحياة وينتشر بعدة طرق منها إفرازات الفم، السائل المنوي، الاتصال الجنسي، نقل الدم أو استخدام أواني الطعام. مع شخص مصاب، يسبب الفيروس مرض كثرة الوعود العددية ويرتبط مع مجموعة واسعة من الأمراض الخبيثة وغير الخبيثة. هذه الدراسة المقطعية أظهرت أن فيروس الإبشتاين بار عبر المتبرعين بالدم في بنك الدم المركزي مستشفى ود مدني التعليمي. كان مجتمع الدراسة يتكون من 25 متبرع وكانت نسبة الإصابة عبر مجتمع الدراسة 10% وتم اختبارهم جميعاً بواضمة المقايسة الامتصاصية للإنزيم المرتبط وجميع النتائج سلبية. بالإضافة إلى ذلك تم اختيار 10 من المتبرعين عشوائياً واختبرهم بواسطة تفاعل البوليميراز المتسلسل وتم اختيار متبرع واحد فقط مصاب بالفيروس. تم الوصول إلى أن الكشف عن الفيروس بواسطة تفاعل البلمرة المتسلسل يعطي نسبة 10% من معدل الإصابة عبر مجتمع الدراسة، على الرغم من حساسية الاختبارات القائمة على الأجسام المضادة. اختبارات الحمض النووي عن طريق تفاعل البوليميراز المتسلسل هو أكثر حساسية في الكشف عن الفيروس ويتوفر بيانات دقيقة يتم بها قياس عوامل الخطر. ووصفي بالكشف عن الفيروس باستخدام تفاعل البوليميراز المتسلسل خاصة بين المتبرعين بالدم المشتبه اصابتهم بالفيروس.
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<td>ELISA</td>
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CHAPTER ONE
INTRODUCTION

1.2 General Introduction :-

Epstein-Barr virus (EBV) is a member of the ancient and highly successful herpes virus family. Wide range of population were reported to be infected with the virus especially during the childhood and thereafter remains in the body for life. EBV was first discovered in 1964 in B lymphocytes cultured from an African (endemic) Burkitt’s lymphoma (Carford et al, 2001). and the virus is now estimated to be present in around 96% of these tumors. The virus colonizes antibody producing (B) cells, which, as relatively long-lived resting cells are an ideal site for long term residence (Carford et al, 2001).

The virus was reported to be spread by various means such as oral secretions, cervix, seminal fluid, sexual contact, blood transfusion, food and drinks or eating utensils with an infected person (Silver et al, 2011).

Many people become infected with EBV in childhood. Infections in children usually do not cause symptoms or the symptoms are not distinguishable from other mild, brief childhood illnesses. People who get symptoms from EBV infection, usually teenagers or adults and get better in two to four weeks. However, some people may feel fatigued for several weeks or even months. After you get an EBV infection the virus becomes latent (inactive) in your body. In some cases the virus may reactivate. This does not always cause symptoms but people with compromised immune systems are more likely to develop symptoms if EBV reactivates (Crawford et al, 2016).

Blood transfusion is an essential part of modern medicine. While blood transfusion can be life-saving it is not without risk (Vamvakas, 2009). Specific pathologies associated with transfusion include circulatory overload, bacterial infections and hemolytic transfusion reactions are complications that can lead to deleterious outcomes (Tung et al, 2011). Additionally, there is a growing body of evidence indicating an association between transfusion, especially of aged/stored blood products, and increased levels of morbidity and mortality in patients (Bhaskar et al, 2012).

The primary goal of any blood transfusion is to provide the patient with donor red blood cells that optimally survive after transfusion and serve their function and to ensure that the patient actually benefits from the transfusion. To achieve this goal, donor red cells that are compatible with those of the patient’s blood are selected for transfusion. Blood safety is a major concern all over the world. One of the most important steps used to ensure blood safety is blood donor selection in patients. Blood
donor eligibility is determined by medical interview, based on national guidelines for donor selection (Katz et al, 2008).

Donor screening criteria are established to protect both donors and recipients. To ensure blood safety, safe donors need to be recruited and high-risk donors should be discouraged from donation. The blood donors must be free from any blood transfusion diseases that can be determined by history and examination. (Hoffbrand et al, 2006).

1.3 Justification:-
Epstein Bar Virus has wide cosmopolitan distribution through most of countries with reported health complications.

Epstein Bar Virus has reported to have dangerous and easily transmitted in several ways, this threatens the healthy people, Workers in the health sector, autoimmunity and immune-compromised patients.

There is limited information or studies regarding the prevalence or incidence of Epstein Bar Virus among blood donors in Sudan.

Sensitive and specific laboratory diagnostic tools are needed for detection of the virus which will assist in protection healthy and under risk population by give them recommendations.

1.3 Objectives:

1.3.1 General objective:
To detect Epstein Bar Virus infection among blood donors attending Central blood bank in wad-Medani teaching hospital in Gezira state.

1.3.2 Specific Objectives:-
- To detect Epstein Bar Virus in blood bank donors using ELISA.
- To detect Epstein Bar Virus in blood bank donors using polymerase chain reaction.
- To validate molecular methods for detection of Epstein Bar Virus.
- To estimate the prevalence of Epstein Bar Virus among the blood donors in Central blood bank.
- To assess Risk Factors associated with Epstein Bar Virus infection among blood donors in Central blood bank.
CHAPTER TWO

LITERATURE REVIEW

2.1 History and definition of Epstein Barr Virus :-

Epstein-Barr virus (EBV) is one of herpes virus family also known as human herpes virus 4. Family: Herpesviridae; Subfamily: Gammaherpesvirina; Genus: Lymphocryptovirus (Hudnall et al, 2014). EBV is prevailing all over the world. Most people get infected with it at some point in their lives and can be spreads most commonly through bodily fluids, primarily saliva, can cause infectious mononucleosis, also called mono, and other illnesses (Crawford et al, 2016).

Epstein–Barr virus was discovered in 1964 from examining electron micrographs of cells cultured from Burkitt’s lymphoma (childhood tumors that is common in sub-Saharan Africa). Epstein Barr virus was found to be widespread in all human populations and to persist in the vast majority of individuals as a lifelong asymptomatic infection of the B-lymphocyte pool (Lawrence et al., 2017).

The virus naturally infects two major target cell types in vivo, pharyngeal epithelium and mature B lymphocytes. Pharyngeal epithelial cells appear to be naturally permissive with virus replication in differentiating squamous cells leading to the release of infectious virion into buccal fluid. In contrast, B lymphocytes harbor the virus as a nonproductive(latent) infection, and this interaction which appears central to the phenomenon of viral persistence. B lymphocyte pool is substantially reduced in size once the host cell mediated immune response develops, small numbers of infected B cells persist in the blood and lymphoid tissues of virus carriers (Steven et al., 1994).

2.2 Taxonomy :-

Two EBV subtypes, EBV-1 and EBV-2, differ in genetic sequence, geographic distribution, and biologic properties. The genetic sequences primarily differ in the latent genes EBER1, EBER2, EBNA2, EBNA3, and EBNA - LP. In most parts of the world the EBV-1 strain predominates (80–90 %), with EBV-2 more often isolated in equatorial Africa (Hudnall et al.., 2014).
2.3 STRUCTURE OF VIRUS :-

2.3.1 Virion morphology and size:-
Epstein Barr virus is Enveloped, icosadeltahedral nucleocapsid symmetry, spherical to pleomorphic particle, 120-220 nm in diameter. Between the capsid and the envelope is an amorphous layer of proteins termed the tegument. Nucleic acid: Linear, double-stranded DNA about 184 kbp in length.

2.3.2 Physicochemical properties:-
Nonionic detergents solubilize the envelope, virus inactivated by standard disinfectants, UV light, and gamma-irradiation, infectivity sensitive to acid pH and high temperatures, virus stable at low temperatures, especially at -60°C or below. Inactivated by heat (50-60°C for at least 30 min).
Epstein Barr virus survives at room temperature for a few days, 2-3 days at refrigeration temperature, and -70°C for many years (Chaganti et al, 2009).

2.3.3 Proteins: -
More than 20 structural polypeptides with molecular weights from 12,000 to 220,000.

2.3.4 Lipid:-
Exact percentage of total weight unknown, probably variable; located in virion envelope.

2.3.5 Carbohydrate:-
Exact percentage of total weight unknown, identified largely as covalently linked to envelope proteins.

2.3.6 Antigenic properties:-
Neutralizing antibody reacts with major viral glycoproteins located in the viral envelope. An Fc receptor for immunoglobulin G may be present in the virion envelope.

2.3.7 Effects of virus suspensions on cells:-
Fusion and agglutination occur rarely or only under very special conditions in the absence of replication.

2.3.8 Epstein–Barr virus gene :-
2.4 Virus replication:-

- **Entry**: The viral envelope adsorbs to receptors on the plasma membrane of the host cell, ultimately fuses with the membrane, and releases the capsid into the cytoplasm. A DNA-protein complex is then translocated into the nucleus.

- **Replication**: Viral DNA is transcribed in the nucleus. Messenger RNAs generated from the transcripts are translated in the cytoplasm. Viral DNA is replicated in the nucleus and is spoled into preformed, immature nucleocapsid.

- **Maturation and egress**: The ability to infect cells is acquired as capsid become enveloped by budding through the inner lamella of the nuclear membrane and, in some instances, through other membranes of the cell. Virus particles accumulate in the space between the inner and outer lamellae of the nuclear membrane and in cysternae of the endoplasmic reticulum. Virus particles are released by transport to the cell surface through the modified endoplasmic reticulum (Roziman et al, 1993).

2.5 Transmission:-

Epstein Barr virus is spread by saliva through: Kissing , Sharing drinks and food ,Using the same cups, eating utensils or toothbrushes and Having contact with toys that children have drooled on (Crawford et al, 2016)

The virus probably survives on an object such as a toothbrush or cup at least as long as the object remains moist. There is no evidence that disinfecting the objects will prevent EBV from spreading.

Epstein Barr virus can also spread through semen during sexual contact, blood transfusions and organ transplantations.

When person get infection for with EBV for the first time , he can spread the virus for weeks, even before he has symptoms. Once the virus is in his body, it stays there in a latent (inactive) state. If the virus reactivates, he can potentially spread to others no matter how much time has passed since the initial infection (Crawford et al, 2016).

Vector and Reservoir of the virus involved infected humans and persists life-long in B-lymphocytes as latent virus that can be reactivated. survival/Persistence in Blood Products the genomes are detectable in viable B-cells for duration of RBC storage .Transmission by Blood Transfusion Documented through seroconversion in sero negative recipients and in case reports using molecular methods (Chaganti et al,2009).
2.6 Epstein Barr virus infection :-

Persistently infected individual shed EBV either constantly or intermittently into saliva and there be spread the virus to the uninfected individual through close oral contact (Crawford et al., 2001).

Epstein–Barr virus (EBV) preferentially infects B lymphocytes through the binding of the major viral envelope Glycoprotein gp350 to the CD 21 receptor on the surface of B cells, and through the binding of a second Glycoprotein, gp42, to human leukocyte antigen (HLA)CLASS II MOLECULES as a coreceptor. Infection of other cell types (principally epithelial cells) is much less efficient and occurs through separate, as yet poorly defined, pathways (Young et al., 2004).

Following initial exposure to infectious saliva, EBV likely undergoes a brief period of lytic replication in oral and nasal epithelium. Subsequent infection of naïve B cells within subjacent tonsillar lymphoid tissues leads to a brief “pre-latent” period of lytic and latent gene expression prior to epigenetic repression of viral genes.

This brief pre-latent period is marked by limited expression of a small set of lytic genes with regulatory function, excluding lytic genes essential for DNA replication and virion assembly. It is likely that by promoting cell growth and inhibiting apoptosis, pre-latent lytic gene products, including BART miRNA, viral BCL-2 homologs, and BZLF1, contribute to the early survival of EBV-infected B cells. Following epigenetic repression of the full complement of lytic genes and a subset of latent gene promoters, rapid growth of latent-infected B cells is induced by expression of the full growth-promoting complement of latency genes, i.e., the latency III program.

Expression of the full complement of lytic and latent antigens by infected epithelial cells and B cells triggers a vigorous humoral and cellular immune response that leads to suppression of viral replication. Latent-infected B cells persist by switching from the highly immunogenic latency III program to the less immunogenic latency II program, with virus gene expression restricted to three proteins, EBNA-1, LMP-1, and LMP-2A. EBNA-1 maintains the viral genome, while LMP-1 and LMP-2A maintain cell growth and preventing apoptosis.

The absence of EBNA-2-mediated transactivation allows for latency II B cells to adopt a germinal center B-cell phenotype and, survive germinal center and/or extrafollicular proliferation and maturation into EBV-infected memory B cells.

Epstein Barr virus infected memory B cells persist by switching from the latency II program to the latency 0 program, with near-complete absence of viral gene expression, with only intermittent LMP-2a expression. The EBV-positive resting memory B cells circulate in the blood, seeding lymphoid tissues throughout the body. Plasma cytotoxic differentiation of EBV-positive memory B cells leads to
end-stage viral replication. Intermittent virus replication in oral and nasal tissues also leads to low-level shedding of virus in saliva and lifelong persistence of IgG anti-VCA antibody and EBV specific cytotoxic T cells (CTL), more frequently directed against lytic antigens than latent antigens (Hudnall et al., 2014).

2.7 Signs and Symptoms :-

Many people become infected with EBV in childhood and usually do not cause symptoms, or the symptoms are not distinguishable from other childhood illnesses. People who get symptoms are usually teenagers or adults and get better in two to four weeks. However, some people may feel fatigued for several weeks or even months (Crawford et al, 2016).

Primary infection:-
- Childhood asymptomatic
- Adolescence 35-60% Infectious mononucleosis (glandular fever) (Joseph et al., 2000).

Glandular fever:-
- Fever
- Sore throat
- Swollen spleen
- Swollen lymph
- nodes
- Rash
- Fatigue (Joseph et al., 2000).

2.8 Tests and Diagnosis :-

Diagnosing EBV infection can be challenging, since symptoms are similar to other illnesses. Epstein Barr virus infection can be confirmed with a blood test that detects antibodies. About 90 percent of adults have antibodies that show that they have a current or past EBV infection. The molecular analysis of the virus continues to illuminate the mechanisms of action of the viral proteins. Now, the challenge is to exploit these mechanistic insights both to gain a better understanding of the biology of EBV infection in vivo and to develop novel therapies for treating virus-associated disease (Crawford et al, 2016).

2.9 History of Blood Transfusion :-

History of blood transfusion is part of the fabric of the history of humankind, including religion and superstition as well as science; ranging from circulating humors to modern medicine. Few, if any, other substances cause the same emotions, have the same associations, lead to the same fears, or have found as many ways into our common parlance and lexicon. Indeed, blood transfusion and blood-letting (now called therapeutic phlebotomy and apheresis) are some of the oldest and most common medical practices (AuBuchon and Whitaker et al 2007).
2.10 Early Transfusions:-

The record of man’s attempt to treat suffering and disease by blood transfusion extends back at least to 1667, when Jean Denis published in the Philosophical Transactions his experience in Paris with transfusing lamb blood (because of its presumed soothing qualities) to an agitated man (resulting in hemolytic transfusion reaction). In 1818, Dr. James Blundell was the first to successfully transfuse human blood into a patient with post-partum hemorrhage. Blundell recognized that he was replacing lost blood volume, not providing a ‘vital force’. Advances, some of which are described below, have allowed the development of modern blood banking and transfusion medicine (AuBuchon and Whitaker et al 2007).

2.11 Blood types :-

Each person has an ABO blood type – either A, B, AB, or O – which means antigen A, antigen B, both antigens, or neither antigen is found on their red blood cells. Each person also is either Rh-positive or Rh-negative (either have Rh or don’t). These 2 factors can be combined into 8 possible blood types:

A positive, B positive, AB positive, O positive, A negative, B negative. AB negative or O negative. (Jones et al, 2017 )

2.12 Blood donor selection:-

The primary responsibility of a blood transfusion service is to provide a safe, sufficient and timely supply of blood and blood products. In fulfilling this responsibility, should be ensure that the act of blood donation is safe and causes no harm to the donor, build and maintain a pool of safe, voluntary non-remunerated blood donors and take all necessary steps to ensure that the products derived from donated blood are efficacious for the recipient, with a minimal risk of any infection that could be transmitted through transfusion.

All prospective blood donors should therefore be assessed for their suitability to donate blood, on each occasion of donation. The purpose of blood donor selection is to:

- Protect donor health and safety by collecting blood only from healthy individuals
- Ensure patient safety by collecting blood only from donors whose donations, when transfused, will be safe for the recipients
- Identify any factors that might make an individual unsuitable as a donor, either temporarily or permanently
Reduce the unnecessary deferral of safe and healthy donors
Ensure the quality of blood products derived from whole blood and apheresis donations
Minimize the wastage of resources resulting from the collection of unsuitable donations. (Jones et al, 2017)

2.13 The basic requiem for blood transfusion:

2.13.1 AGE:-

Blood donation is a voluntary procedure that may have untoward effects on the blood donor and, therefore, requires informed consent by the individual. It is necessary to establish a minimum age for blood donation to assure that the donor has both the competence and the capacity to provide informed consent (Klein et al, 2008).

2.13.2 Body Wight: (Blood to be collected):-

The amount of blood that circulates in the human body is proportional to body weight (70 mL per kg). To avoid untoward reactions in donors as a consequence of donating excessive blood volumes it is necessary to establish the minimum body weight for collection of a standard blood unit from an individual. A standard unit of blood usually corresponds to 450+/−50 mL, which should be no more than 12.5% of the total volume of blood circulating in the body (Lentner et al, 1984).

2.13.3 Body temperature (fever):-

Fever –elevated body temperature– is one of the body responses to injury and/or infection. Donors with elevated body temperature may be carrying infectious agents or may be suffering from a systemic inflammatory process. Making sure the prospective donor is fever–free protects both the donor and the patient who receives blood transfusions. Fever is 37.5°C or 99.5°F of oral temperature (Blatteis et al, 2005)

2.13.4 Blood pressure:-

The optimal readings for human adults are between 90 mm and 120 mm of mercury (mm Hg) for systolic pressure and 60–80 mm for diastolic pressure. Hypertension is associated with the concomitant occurrence of structural and functional changes in large arteries and small resistance arteries and with
other classic hallmarks of organ damage (left ventricular hypertrophy, renal dysfunction, microalbuminuria) (Byrne and Ditto, 2005).

2.13.5 Pulse:-

It is necessary to establish acceptable limits of heart beat rate (pulse) in order to assure that the donor’s heart is able to manage its cardiac output when blood is collected. The minimum heart rate established is 50 beats per minute. And a maximum heart rate of 100 beats per minute for donating blood. (Eckberg and Collaborators, 2000)

2.13.6 Blood volume to be collected:-

Standards require that no more that 10.5 mL of blood per kilogram of donor weight, including samples, be taken. The amount of blood collected should not exceed 10.5 mL per kilogram of body weight.

The minimum body weight for blood donors should be determined using the local information on adverse reactions to donation in relation to body mass. The blood volume collected from donors should be measured by means of the weight of blood entering the collection bag, 472 mL of blood weight, on the average, 500 grams. The use of balances to monitor the total weight of blood while being collected is highly recommended. The blood services should promote iron–reach diets among their donors. (Lentner et al, 1984)

2.13.7 Hemoglobin level / Hematocrit:-

Normal hemoglobin values fluctuate between 121 g/L and 151 g/L of blood in females, and between 138 g/L and 172 g/L in males. Hematocit refers to the proportion in volume of red blood cells to total blood volume. Normal values fluctuate between 36.1% and 44.3% in females, and between 40.7% and 50.3% in males. (Badami and Taylor, 2008).

2.13.8 Diabetes:-

So individuals with diagnosis of diabetes can be blood donors if the disease is well controlled (absence of permanent thirst and polyuria) by oral medication or diet. Diabetic patients who require insulin or who have serious diabetes–related health issues such as kidney, heart, or eye disease, should not be allowed to donate blood (Ardigo et al, 2004).
2.14 Detection of transfusion transmitted infections:-

2.14.1 Human immune deficiency virus (HIV):-

Individuals with diagnosis of HIV infection should be deferred permanently. Those individuals who have engaged in behaviors that pose a risk for HIV infection should be deferred as blood donors for a period of 12 months after the last occurrence (Arreguin et al, 2008).

2.14.2 Hepatitis:-

Infections by hepatitis A, hepatitis B, hepatitis C, hepatitis D, hepatitis E.

Individuals may be accepted as blood donors at discretion of the appropriate medical authority, provided approved HBsAg and HCV tests are negative. Close household contact with hepatitis B (acute or chronic).

Prospective donors with history of hepatitis B or hepatitis C should be deferred permanently. Prospective donors who have been exposed to individuals with hepatitis B or hepatitis C should be deferred for six months after exposure. Individuals who have engaged in risky behaviors for hepatitis B and hepatitis C should be deferred for 12 months. Individuals with history of jaundice after their 11th birthday should be encouraged to be tested for HBV and HCV infection (Ballester et al, 2005).

2.14.3 Syphilis:-

Treponema pallidum is inactivated by low temperature and, therefore, is not transmitted by blood stored at 4–6°C for more than 72 hrs. Transmission of the infection by platelet transfusion is possible. Individuals who have a diagnosis of syphilis be deferred for 12 months (Azaria et al, 2008).

2.15 Personal health care issues:

- Vaccines/Immunization
- Medications:
- Travel:
- History of sever post donation reaction
2.16 Previous study :-

- In Burkina Faso Traore et al (2016) conducted study among 198 blood donors attending Regional Blood Transfusion Centre of Ouagadougou to diagnosis CMV, EBV and herpes virus 6 (HHV 6) using molecular technique (Multiplex real time PCR). Statistical analysis was performed with the software EpiInfo version 6 and SPSS version 17. P values ≤ 0.05 were considered significant, the donor age ranged between 18-56 years. Result of 198 samples tested, 18 (9.1%) were positive to at least one of the three viruses. In fact, 10 (5.1%) were positive for EBV Viral infections were higher in women than in men, EBV (8.6% versus 4.3%), EBV / CMV / HHV-6 co-infection was found in 3.5% (7/198) of blood donors and present of virus was observed in blood donors younger than 30 years.

- In Burkina Faso, West Africa Tao et al (2013) conducted study among 551 Voluntary non Remunerated Blood Donors (84.2% men and 15.8% women and the majority of donors (61.9%) was between 20 and 29 of age) to Screening of Hepatitis G and Epstein-Barr Viruses by molecular and serological technique (ELISA and PCR). The prevalence of EBV in 551 blood donors was 5.4%. EBV prevalence was higher among blood donors of <20 years age group. This study show the prevalence of HGV and EBV in blood donors in Burkina Faso and emphasizes the need for a general screening.

- Yap et al (1990) in their study carried at UK and intended for screening of infectious diseases by the administration of blood and its components or derivatives, they utilized serological test for several type of virus viz HIV, HBV, HCV, CMV, EBV. They recommend that EBV should not be screening during blood donation because the EBV is rarely transmitted by transfusion. This may be because the majority of adult transfusion recipients are already immune and even those who are not may receive passive transfer of EBV antibody with transfusion of plasma-containing products. Reduced viability of B lymphocytes infected with EBV in stored blood may also be a factor in reducing transmission. Nonetheless, Epstein Barr virus has been associated with post-transfusion mononucleosis, although the majority of such cases are probably due to CMV. Other cases of transfusion associated EBV have occurred in immunocompromised patients but due to the mildness of EBV related disease, there are no plans to introduce screening.
CHAPTER THREE
MATERIALS AND METHODS

3.1 Study design:
This was prospective analytical cross sectional laboratory–based study.

3.2 Study area:
This study conducted in Central blood bank in wad medani teaching hospital in Gezira state, Sudan. The study was conducted from May 2017 to December 2017.

3.3 Study population:
Donors in Central blood bank in wad medani teaching hospital, Gezira state.

3.4 study variable :-
3.4.1 study dependent :-
Epstein Barr virus /ELISA antibody IgM and Epstein Barr virus /DNA

3.4.2 study independent :-
Age, history and gender.

3.5 Selection criteria :-
3.5.1 Inclusion criteria:
All donor attending Wad-medani teaching hospital.

3.5.2 Exclusion criteria:
All other donors not attending Wad-medani teaching hospital.

3.6 Study sample size:
The study sample calculated according to the following formula:
\[ n = \frac{P (1-P)^2}{ME^2} \]
Where \( n \) = required sample size
\( P \): pervious estimated prevalence.
\( Z \): confidence level which is equal to 1.96 at 95%.
\( ME \): margin of error = 0.05.
\[ N = 0.32 \times (1-0.32)1.96^2 / 0.05^2 = 334 \]
Due to high cost the sample size was adjusted to 25.
3.7 **Data collection:**
Structured tested questionnaire will be used to collect data from study population.

3.8 **Statistical analysis:**
This study will be analyzed by using statistical package for social sciences (SPSS) software. Descriptive analysis, correlation analysis, sensitivity, specificity and predictive value of every diagnostic test will be done. Also ROC or AUC analysis will be done. The results will be considered significant when $p < 0.05$.

3.9 **Ethical Consideration:**
Ethical approval to conduct this study will be obtained from State Ministry of health Gezira state. Permission was obtained from head direction wad medani teaching hospital. Also obtained consent of study population, information were collected from the subjected under privacy and were used for research study.

3.10 **Method:**

3.10.1 **Sample collection and preparation:**
One Blood sample was collected by clean vein puncture technique and about 3-5ml of venous blood was withdrawn and dispensed in plain container and EDTA container the containers was labeled. Plain tube was allowed to clot then centrifuged to obtain serum then was transferred to epindroff tube and stored with EDTA container at frozen at $-20 \, ^\circ C$.

3.10.2 **Enzyme linked Immunoassay:**
Use EUROIMMUN Anti-EBV-CA-ELISA (IgM) kits by following these steps:

1. Patients samples were diluted with sample buffer (add 10µl sample to 1ml sample buffer) and mixed well by vortexing. Incubate for 10min at room temperature.
2. Transferred 100µl of the calibrator, positive and negative controls, diluted patient samples into the individual microplate wells. Incubated for 30min at room temperature.
3. The wells were empty and subsequently washed 3 times using 300µl of working strength wash buffer for each wash.
4. Added 100µl of enzyme conjugate (peroxidase-labelled anti-human IgM) into each microplate wells. Incubated for 30min at room temperature.
5. Repeated the step 3.
6. 100µl of chromogen/substrate solution was added to each microplate wells Incubated for 15 min at room temperature.
7. 100µl of stop solution was added to each microplate wells.

8. Detected of the color intensity by using Photometric measurement at wavelength of 450 nm and reference wavelength between 620 nm and 650 nm within 30 min of added the stop solution.

3.10.3 DNA extraction procedure:

Use FAVORGEME BIOTECH CORP by following these steps:

1. 200µl of whole blood sample was transferred into a micro centrifuge tube (Eppendorf tube).

2. 570µl of VNE Buffer was added (Carrier RNA added) to the sample, mixed well by vortexing and incubated for 10 min at room temperature.

3. 570µl of ethanol (96-100%) was added to the sample mixture, mixed well by plus-vortexing.

4. A VNE column was combined with a collection tube. Up to 700µl of sample mixture transferred to VNE column, centrifuged at 8,000 × g for 1 min then discarded the flow-through. VNE column then was combined with a new collection tube.

5. The rest of sample mixture was transferred to the VNE column, centrifuged at 8,000 × g for 1 min. discarded the flow-through and the collection tube. The VNE column was combined with a new collection tube.

6. 500µl of Wash Buffer 1 was added (ethanol added) to the VNE column, centrifuged at 8,000 × g for 1 min then discarded the flow-through. The VNE column was combined with the used collection tube.

7. 750µl of Wash Buffer 2 was added (ethanol added) to VNE column, centrifuged at 8,000 × g for 1 min then discarded the flow-through. The VNE column was combined with the used collection tube.

8. Step 7 was repeated.

9. Centrifuged at full speed 13,000 × g for an additional 3 min to dry the VNE column. Discarded the flow-through and the collection tube (this step was done to avoid the residual liquid to inhibit the subsequent enzymatic reactions).

10. The VNE column was combined with a elution tube. 50 µl of RNase-free water was added to the membrane center of the VNE column. VNE column was stand for 2 min.

11. Was centrifuged for 2 min to elute the nucleic acid.

12. Nucleic acid was stored at -70 ºc.
3.10.4 DNA amplification using polymerase chain reaction (PCR):

The PCR reactions were performed to replicate EBV LMP1 gene using purified genomic DNA as template. This PCR reaction was done by using MyTaq™Red Mix Kit. The PCR reaction is consisting of three steps: denaturation, annealing and extension, with different temperature for each one. The first step of PCR is the denaturation in which the DNA sample is heated in 94°C to separate the double-strands. The high temperature breaks down the hydrogen bonds that bind between the nucleotides that form the DNA code. The second step is annealing in which the two primer (forward and reverse) bind to appropriate complementary strand. The temperature of this step various depending on the size of the primer and its homology to target DNA (the appropriate temperature for LMP1 is 58°C). Finally, DNA polymerase extend the primers by its polymerase activity, this is done in a temperature optimal for the Taq polymerase which is 72°C. These steps are repeated for 35 times. (#In sterile 0.2 ml microcentrifuge tubes the PCR ingredients were added in the ratio shown in the (Table).)

Table3.1: PCR ingredients and concentration used in the reactions

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>1X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward Primer</td>
<td>1 µl</td>
</tr>
<tr>
<td>Reverse Primer</td>
<td>1 µl</td>
</tr>
<tr>
<td>Ready Master Mix</td>
<td>5 µl</td>
</tr>
<tr>
<td>DW</td>
<td>14 µl</td>
</tr>
<tr>
<td>DNA</td>
<td>4 µl</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>25 µl</td>
</tr>
</tbody>
</table>
The conditions for the PCR were as follows:

**Table 3.2:** Stages, temperature and time used for PCR for EBV LMP1.

<table>
<thead>
<tr>
<th>Stages</th>
<th>Temperature</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initiation denaturation</td>
<td>94 °C</td>
<td>5 min</td>
</tr>
<tr>
<td>Denaturation</td>
<td>94 °C</td>
<td>30 sec</td>
</tr>
<tr>
<td>Annealing</td>
<td>58 °C</td>
<td>30 sec</td>
</tr>
<tr>
<td>Elongation</td>
<td>72 °C</td>
<td>45 sec</td>
</tr>
<tr>
<td>Final elongation</td>
<td>72 °C</td>
<td>10 min</td>
</tr>
<tr>
<td>Refrigerator</td>
<td>4 °C</td>
<td>Infinity</td>
</tr>
</tbody>
</table>

This step was followed by 35 cycles of the three stages.

**3.10.5 Electrophoresis of DNA:**

- **Preparation of Agarose gel:**
  1. 1g of Agarose powder was measured by sensitive balance.
  2. Agarose powder was mixed with 10ml TBE (Tris-borate EDTA) buffer 10X (90ml of DW to 10ml TBE) in a microwavable flask.
  3. Then was microwaved for 1min and 30 sec until the Agarose is completely dissolved.
  4. Agarose solution was lifted to cool down.
  5. 4ul of the ethedium bromide dye was added to final concentration.
  6. The Agarose was poured into a gel tray with the well comb in place.
  7. Newly poured gel was placed at room temperature for 20-30mins until it has completely solidified.

- **Loading samples and running an Agarose gel:**
  1. The running buffer was prepared by add 90ml of DW to 10ml of TBE (10X) buffer to prepare TBE 1X buffer.
  2. Once solidified, the Agarose gel was placed into the gel box (electrophoreses unit.)
  3. Gel box was filled with 1X TBE until the gel is covered.
  4. 4ul of each PCR product carefully was loaded into the additional wells of gel.
5. The gel was ruined at 100 voltages 100 current for 25 min until the dye line is approximately 75-80% of the way down the gel.

6. The power was turned off, the electrodes were disconnected from the power source and then the gel was removed carefully from the gel box.

7. By using UV transilluminator to the DNA fragments was visualized.
CHAPTER FOUR
RESULT AND DISCUSSION

4.1 RESULT :-

4.1.1 Socio demographic characteristic of study population :-

Table 4.3 frequency distribution of study population according to age :-

This table shows the distribution of study population, the age follow: from (21 - 30) years (72%) and from (31 - 39) years (28).

<table>
<thead>
<tr>
<th>Age</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>21 – 30Year</td>
<td>18</td>
<td>72.0</td>
</tr>
<tr>
<td>31 – 39Year</td>
<td>7</td>
<td>28.0</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 4.1.2 frequency distribution of study population according to Gender :-

<table>
<thead>
<tr>
<th>Gender</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valid Male</td>
<td>25</td>
<td>100.0</td>
</tr>
</tbody>
</table>
Table 4.1.3 Result of ELISA :-
This table the frequency of positive and negative detection of EBV VCA – IgM by ELISA among study population , All detection is negative .

<table>
<thead>
<tr>
<th></th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Examined No</td>
<td>25</td>
<td>100</td>
</tr>
<tr>
<td>Valid Negative</td>
<td>25</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 4.1.4 Result of PCR :-
This table the frequency of positive and negative detection of EBV by PCR among study population , one out of 10 was positive (10%).

<table>
<thead>
<tr>
<th></th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valid Positive</td>
<td>1</td>
<td>10.0</td>
</tr>
<tr>
<td>Negative</td>
<td>9</td>
<td>90.0</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 4.1.7 :ELISA * PCR Cross tabulation :-
This table shows the cross tabulation between ELISA and PCR.

<table>
<thead>
<tr>
<th></th>
<th>PCR</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Total</td>
</tr>
<tr>
<td>ELISA Negative</td>
<td>1</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>1</td>
<td>9</td>
<td>10</td>
</tr>
</tbody>
</table>

Using Ultra Violet trans illuminator to visualize the DNA bands on a garose gel:
Length of band of Epstein Barr Virus (LMP1 gene) 770 bp
4.2 Discussion :-

In blood bank to ensure the safety of blood donation for both donors and recipients, all volunteer blood donors must be evaluated to determine their eligibility to give blood. The final determination will be made on the day of the donation, the age of donation male or female must be at least 17 years old.

The donation process is discussed the donor heath history before any blood is collected. each donor receives a brief examination during which temperature, pulse, blood pressure, hemoglobin and blood volume are measured.

Detection the presence of transfusion transmitted infections (TTIs) such as HBV, HCV, HIV and syphilis, among blood and plasma donors, permits an assessment of the occurrence of infections in the blood donor population and consequently the safety of the collected donations. It also gives an idea of the epidemiology of these diseases in the community.

Epstein Barr Virus is wide spread in all human population as lifelong a symptomatic infection of the B-lymphocyte pool ,can be spreads most commonly through bodily fluids, primarily saliva and the virus was reported to be spread by blood transfusion, EBV can cause infectious mononucleosis and other illnesses and also associated with a wide a variety of malignant and nonmalignant conditions.

The current study aim to detect the EBV infection among blood donors attending to central blood bank in wad medani teaching hospital by using Anti-EBV ELISA (IgM) and virus DNA amplification using polymerase chain reaction.

The study population consisted of 25 blood donors attended to central blood bank in wad medani teaching hospital and all of them were males. Their age range between( 21-39), they were tested by ELISA and all of them are negative for EBV infection. further,10 of randomly selected blood donors were tested by PCR and one of them is positive for EBV infection (10%).

Wide literature survey regarding the prevalence of EBV infection among blood donors indicated that the virus has a very low infection rate. the result of this study strongly supported by finding of Traore et al (2016). the author and his collages when using real time PCR to diagnose EBV among 198 of blood donors they found that 5.1% of them were positive for EBV infections.
In other study carried at UK by Yap et al (1990) for screening of infectious diseases by the administration of blood using serological diagnostic test for several type of virus viz. HIV, HBV, HCV, HBV, CMV, EBV and recommend that EBV should not be screening because is rarely transmitted by transfusion.

Despite sensitive antibody-based blood-donor screening, a residual risk of transfusion-transmitted viral infections exists. Only direct monitoring by sensitive nucleic-acid tests would provide data accurately to measure the risk and to assess risk-reduction procedures.
CHAPTER FIVE
CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion:-
Main result of this study revealed that Screening of 25 blood donors males by ELISA, all of them are negative for EBV infection. Further, PCR detection give 10% infection rate by EBV among study population. Despite sensitive antibody-based blood-donor screening, the nucleic-acid tests by PCR is more sensitive in detection of virus and provide data accurately to measure the risk.

5.2 Recommendations :-

1. Virus DNA amplification using polymerase chain reaction is more sensitive than antibody-based screening(ELISA) for detection of virus among blood donors.

2. Due to very limited sample size of this study, further studies including a larger number of participles is highly recommended to confirm the transfusion of EBV through blood donations.

3. Due to high cost of molecular diagnostic test (PCR), the test could be only us as a confirmatory test if the donors suspected by EBV infection.
Reference


