Detection of Epstein Barr Virus among Renal Failure Patients Using ELISA and PCR, Gezira Hospital for Renal Disease and Surgery, Gezira State, Sudan (2017)

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Submitted to University of Gezira partial Fulfillment of the Requirements for the Award of the Degree of Master of Science in Medical Microbiology
Department of Medical Microbiology
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Declaration

The undersigned author hereby affirm that the dissertation entitled (Detection of Epstein Barr Virus among Renal Failure Patient Using ELISA and PCR, Some Teaching Hospital Gezira State, Sudan, 2017) which has been submitted for fulfillment of the requirements of MSc 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: RAZAZ ELZAIN HUSSEIN BADR
Dedication

I dedicate this work to the source of unconditional love

**My loving father**

Who made me proud to be his daughter.

**My mother**

To the piece of my heart

**My sister and my brothers**

To everyone who give something to light my way

RAZAZ ELZAIN HUSSEIN BADR

Place: Gezira State Sudan

Date: 5/2/ 2018
بسم الله الرحمن الرحيم

الآية

قال تعالى:

((نأولر ميعانك ولم يلمنا ولما مملكتنا لم يقلن وَنَذَّرُ وَدِينَكُمْ وَهُنَّ))

صدق الله العظيم

سورة البقرة الآية (32)
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.

I would like to express my gratitude to D. Elbadawi Abdalbagi Talha my co-supervisor for his care and guidance.

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I would like also to express my appreciation and gratitude to all persons who have volunteered to participate in this study.

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 Renal Failure Patient Using ELISA and PCR, Some Teaching Hospitals Gezira State, Sudan (2017)

RAZAZ ELZAIN HUSSEIN BADR

Abstract

Epstein Bar Virus is a virus from the Herpes family, the virus was named after Michael Epstein and Yvonne Barr 1940, has cosmopolitan distribution infection in almost all of world's population, EBV has wide range for transition by body secretion. This cross sectional laboratory based study was conducted during the period from May 2017 – December 2017. That purpose of the study was to screen and detect EBV infection among patients with renal failure by ELISA and PCR. A total of 75 blood samples from renal failure patients were collected from three hospitals of Gezira state (Wad Medani, El-Hasahisa and El-Managil). The samples included both gender, majority is male (69.3%) than female (30.7%), and their age ranged between 20-80 years, the majority in the category 41-60 (45.3%). The result show that there is 8 (10.7%) of total population in study is true positive EBV/VCA IgM, while from the 8 positive ELISA only 4 is positive by PCR (50%) , the overall infection rate by EBV is 7 (35.7%). Age, gender and diabetic showed strong link with EBV infection in renal failure patients. Epstein–Barr- virus induced Renal Failure patients is an important diagnosis to consider in the patient presenting with symptoms of Infectious Mononucleosis (IM), the sero-prevalence of the disease was found to be 10.7%. Detection of the virus by PCR give percentage of 35.0%. Usage of the PCR as confirmatory test during transplantation is strongly recommended.
الكشف عن فيروس إبستين بار بين مرضى الفشل الكلوي باستخدام المقايسة الامتصاصية للإنزيم المرتبط وتفاعل البلمرة المتسلسل، مستشفى ودمدني لجراحة وامراض الكلى، ولاية الجزيرة، السودان (2017)

رزاز الزين حسين بدر
ملخص الدراسة

بعد فيروس إبستين بار، فيروس من عائلة الهربس، تم تسميته بواسطة مايكل إبستين ويفون بار 1940، ويعتبر فيروس من عدوى التوزيع العالمية أي أنه يتوافق في جميع سكان العالم تقريبا، لديه طرق واسعة للانتقال من اهمها عن طريق جميع إفراز الجسم. أجريت هذه الدراسة المقطعية المستندة إلى المختبرات خلال الفترة من مايو 2017 - ديسمبر 2017. وكان الغرض من هذه الدراسة الكشف عن عدوى فيروس إبستين بار بين المرضى الذين يعانون من الفشل الكلوي بواسطة إجراء نوعين من الفحوصات المخبرية، مقايسة امتصاصيته للألزيم المرتبط (إليزا) وتفاعل البوليمير أز المتسلسل . تم جمع 75 عينة دم من مرضى الفشل الكلوي من ثلاثة مستشفيات في ولاية الجزيرة (ود مدني، الحصاحيصا، المناقل) ، وشملت العينات كلا الجنسين، كانت الأغلبية للذكور بنسبة (69.3٪) وكان عدد عينات الإناث (30.7٪) ، وتراوحت اعمار كل المرضى بين 20-80 سنة، كانت الغالبية في الفئة 41-60 (45.3٪). أظهرت نتائج الدراسة أن 8 حالات بنسبة (10.7٪) كانوا إيجابيين لفيروس إبستين بار من مجموع جميع المرضى في الدراسة عند تفحص عيناتهم بواسطة مقايسة امتصاصيته للألزيم المرتبط (إليزا) للأجسام المضادة ميو لفيروس إبستين بار. و 4 (50٪) فقط من مجموع الحالات الإيجابية هم إيجابيين لفيروس إبستين بار عند تفحص عيناتهم بواسطة تفاعل البوليمير أز المتسلسل حيث الحالات الموجبة الكلية من قبل تفاعل البوليمير أز المتسلاسل هو 7 حالات بنسبة (35.7٪) من عدد 20 حالة تم اختبارها . وأظهر العمر والجنس ومرض السكري ارتباطا قويا مع عدوى فيروس إبستين بار في مرضى الفشل الكلوي . توصي الدراسة بشدة استخدام تفاعل البوليمير أز المتسلسل كفحص تأكدي اثناء عملية زرع الكلى .
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* Sero positivity of PCR
* Distribution of ELISA * PCR
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<td>ELISA</td>
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<td>VCA</td>
<td>Viral capsid antigen</td>
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<td>CF</td>
<td>Complement-fixing soluble</td>
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<td>ncRNA</td>
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<td>IN</td>
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CHAPTER ONE
INTRODUCTION

1.1 General Introduction:

Epstein-Barr virus (EBV) is a human herpes virus that has cosmopolitan distribution infection in almost all of world's population. The virus has subclinical course during childhood and there after remains in the body for life (Henle and Henle, 1973). The virus colonizes antibody-producing (B) cells, which consider as an ideal site for long-term residence (Amon and Farrell, 2005). Here EBV evades recognition and destruction by cytotoxic T cells. EBV is passed to naïve hosts in saliva, but how the virus gains access to this route of transmission is not entirely clear (Henle and Henle, 1973). EBV carries asset of latent genes that, when expressed in resting B cells, induce cell proliferation and thereby increase the chances of successful virus colonization of the B-cell system during primary infection and the establishment of persistence. However, uncontrolled cell proliferation or complication by genetic events within the infected cells can lead to malignancy (Shannon and Rowe, 2014).

EBV has wide range for transition viz oral secretions, cervix, seminal fluid, sexual contact, blood transfusion, food and drinks or eating utensils with an infected person (Green et al., 2006). The incubation period between exposure and presentation of symptoms can range between 30 to 60 days. EBV has been associated with other viral infections such as Infectious Mononucleosis (IM), lymphomas in Immuno-comperomised individuals, nasopharyngeal carcinoma, gastric carcinoma (Amon and Farrell, 2005).

Renal failure is a condition in which the kidneys fail both to remove metabolic end products from the blood or to regulate the fluid, electrolyte, and pH balance of the extra cellular fluids. The underlying cause may be renal disease, systemic disease, or other infection of the renal system (KDIGO., 2008). Acute renal failure is abrupt in onset and often is reversible if recognized early and treated appropriately (Lei et al., 2000).

Acute infection with EBV causes fever, fatigue and pharyngitis (Amonand Farrell, 2005). Renal involvement in systemic EBV infections typically manifests as acute tubular necrosis or tubulointerstitial nephritis. Rarely, EBV infection causes nephritic syndrome due to minimal change disease. Nephritis associated with (IM) was first described in
1889 however, probably represented an acute post streptococcal glomerulonephritis. Post streptococcal on non-post streptococcal acute post infectious glomerulonephritis after (IM) has also been described (Dylewski, 2008).
1.2 Justification:-

- Epstein-Barr virus has wide cosmopolitan distribution through most of countries with reported health complications especially among Renal Failure Patients.

- Epstein-Barr 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 EBV among Renal failure patients 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 Renal Failure Patients using ELISA and PCR, Gezira State, Sudan.

1.3.2 Specific Objectives:

- To detect EBV in study population using IgM.

- To detect EBV in study population using polymerase chain reaction.

- To estimate the prevalence of EBV among the study population.

To validate molecular methods for detection of EBV.

- To assess Risk Factors associated with EBV infection among renal failure.
CHAPTER TWO
LITERATURE REVIEW

2.1 Definition:

The Epstein–Barr virus, also called human herpes virus 4 (HHV-4), is one of eight known human herpes virus types in herpes family, and is one of the most common viruses in humans. In the 1970s, IARC demonstrated that more than 90% of adults worldwide are infected with EBV, based on the detection of antibodies to EBV (especially antibodies to viral capsid "VCA" and complement-fixing soluble "CF/S" antigens), and it was considered by a previous IARC Working Group in 1997, new data have become available, these have been incorporated in the Monograph, and taken into consideration in the present evaluation (Green et al., 2006).

The virus is a three-part glycoprotein complexes of gHgL gp42 mediate B cell membrane fusion; although the two-part complexes of gHgL mediate epithelial cell membrane fusion. EBV that are made in the B cells have low numbers of gHgLgp42 complexes, because these three-part complexes interact with Human-leukocyte-antigen class II (HLA class II) molecules present in B cells in the endoplasmic reticulum are degraded. In contrast, EBV from epithelial cells are rich in the three-part complexes because these cells do not normally contain HLA class II molecules. As a consequence, EBV made from B cells are more infectious to epithelial cells, and EBV made from epithelial cells are more infectious to B cells. Viruses lacking the gp42 portion are able to bind to human B cells but unable to infect (Wang et al., 1998).

2.2 Taxonomy:

Two major EBV types have been detected in humans: EBV-1 and EBV-2 (also known as types A and B). EBV-1 and EBV-2 differ in the sequence of the genes that code for the EBV nuclear antigens (EBNA-2, EBNA-3A/3, EBNA-3B/4, and EBNA-3C/6) (Sample et al., 1990). EBV-2 immortalizes B cells less efficiently than EBV-1 in vitro, and the viability of EBV-2-infected lymphoblastic cell lines is less than that of EBV-1-infected lines (Rickinson et al., 1987). The differences in the immortalizing efficiency of the EBV subtypes may relate to a divergence in the EBNA-2 sequences (Cohen et al., 1989). In addition to type-specific polymorphism, significant DNA-sequence heterogeneity has been found when comparing selected regions of the EBV genome isolated in certain
geographic areas or even from the same area. These polymorphisms define different viral strains within both types (Aitken et al., 1994).

2.3 Structure:

The structure of virus is approximately 122 - 180 nm in diameter, and is composed of a double stranded DNA which contain about 172kb DNA molecules that encodes more than 85 genes (Henle and Henle, 1973). Information on the structure of EBV is mainly based on its similarity to other herpes viruses, all visions of this family have a capsid with icosahedral symmetry that contains the viral dsDNA genome, surrounded by a membrane carrying various surface glycoproteins, Tegument proteins fill up the space between the membrane and the inner icosahedral capsid (Grünewald et al., 2003). EBV recombinant based molecular genetic analyses have demonstrated that LMP1 and EBNA2, -LP, and -3A are critical for B lymphocyte transformation (Cohen et al., 1989; Hammer Schmidt and Sugden, 1989; Mannick et al., 1991; Kaye et al., 1993; Tomkinsonet al., 1993). LMP1 is not only essential for primary B lymphocyte growth transformation but is also the only EBV gene that has transforming effects in nonlymphoid cells, In B lymphoblast's, LMP1 induces most of the phenotypic effects of EBV infection, including induced expression of activation markers and adhesion molecules, altered growth (Wang et al., 1998) In epithelial cells, LMP1 blocks differentiation, LMP1 is an integral membrane protein that may transform cells by constitutively activating a growth factor receptor pathway common to many cell types (Grünewald et al., 2003). LMP1 consists of a 23 amino acid amino-terminal cytoplasmic domain, 6 markedly hydrophobic trans membrane domains separated by short reverse turns, and a 200 amino acid carboxyl-terminal cytoplasmic domain (Swaminathan et al., 1991). The trans membrane domains enable LMP1 to posttranslational insert into membranes and to accumulate in aggregates in the plasma membrane (Grünewald et al 2003). Made of protein, which in turn is surrounded by an envelope containing both lipids (Kieff et al., 1979). And surface projections of glycoproteins which are essential to infection of the host cell (Odumade et al., 2011).
2.4 Pathogenesis:

2.4.1 Entry to the cell

EBV can infect both B cells and epithelial cells. The mechanisms for entering these two cells are different. Like other herpes viruses, EBV has a productive lytic cycle and a latent phase. To enter B cells, viral envelop glycoprotein gp350/220 binds to cellular receptor CD21. Then viral glycoprotein gp42 interacts with cellular MHC class II molecules. This triggers fusion of the viral envelope with the cell membrane, allowing EBV to enter the B cell (Kieff et al., 1979). Human CD35, also known as complement receptor 1 (CR1), is an additional attachment factor for gp350/220, and can provide a route for entry of EBV into CD21-negative cells, including immature B-cells. EBV infection down regulates expression of CD35 (Ogembo et al., 2013).

To enter epithelial cells, EBV spreads via the saliva entering the epithelium of the Waldeyer tonsillar ring situated in the oropharynx where it probably initiates a lytic infection that leads to amplification of the virus viral protein BMRF-2 interacts with cellular β1 integrin's. Then, viral protein gH/gL interacts with cellular αvβ6/αvβ8 integrin's, This triggers fusion of the viral envelope with the epithelial cell membrane, allowing EBV to enter the epithelial cell (Odumad, et al., 2011). Unlike B cell entry, epithelial cell entry is actually impeded by viral glycoprotein gp42. Once EBV enters the cell, the viral capsid dissolves and the viral genome is transported to the cell nucleus (Kieffet al., 1979).

2.4.2 Lytic replication:

The lytic cycle, or productive infection, results in the production of infectious virions. EBV can undergo lytic replication in both B cells and epithelial cells. In B cells; lytic replication normally takes place after reactivation from latency, In epithelial cells; lytic replication often directly follows viral entry (Odumad, et al., 2011). For lytic replication to occur, the viral genome must be linear. The latent EBV genome is circular, so it must linearize in the process of lytic reactivation, During lytic replication, viral DNA polymerase is responsible for copying the viral genome this contrasts with latency, in which host cell DNA polymerase copies the viral genome (Rickinson and Kieff., 1996). Lytic gene products are produced in three consecutive stages: immediate-early, early, and late (Odumadet al., 2011). Immediate-early lytic gene products act as trans activators, enhancing the expression of later lytic genes. Immediate-early lytic gene products include BZLF1 (also known as Zta, EB1, associated with its product gene ZEBRA)
and BRLF1 associated with its product gene Rta (Odumad et al., 2011). Early lytic gene products have many more functions, such as replication, metabolism, and blockade of antigen processing. Early lytic gene products include BNLF2. Finally, late lytic gene products tend to be proteins with structural roles, like VCA, which forms the viral capsid. Other late lytic gene products, such as BCRF1, help EBV evade the immune system (Kieff et al., 1979). Unlike lytic replication for many other viruses, EBV lytic replication does not inevitably lead to lysis of the host cell because EBV virions are produced by budding from the infected cell. Lytic proteins include gp350 and gp110 (Odumad, et al., 2011; Lockey et al., 2008).

2.4.3 Latency:
Unlike lytic replication, latency does not result in production of virions (Odumad et al., 2011). Instead, the EBV genome circular DNA resides in the cell nucleus as an episome and is copied by cellular DNA polymerase (Lockey et al., 2008). In latency, only a portion of EBV’s genes are expressed, Latent EBV expresses its genes in one of three patterns, known as latency programs, EBV can latently persist within B cells and epithelial cells, but different latency programs are possible in the two types of cell (Amon and Farrell, 2005).

EBV can exhibit one of three latency programs: Latency I, Latency II, or Latency III. Each latency program leads to the production of a limited, distinct set of viral proteins and viral RNAs (Calderwood et al., 2007; Hutzinger et al., 2009).

- **Latency I** (Gene Expressed is EBNA-1 protein and EBER ncRNA).
- **Latency II** (EBNA-1, EBNA-LP, LMP-1, LMP-2A, LMP-2B protein and EBER ncRNA).
- **Latency III** (EBNA-1, EBNA-2, EBNA-3A, EBNA-3B, EBNA-3C, EBNA-LP, LMP-1, LMP-2A, LMP-2B protein and EBER ncRNA). Within B cells, all three latency programs are possible (Amon and Farrell, 2005). EBV latency within B cells usually progresses from Latency III to Latency II to Latency I, Each stage of latency uniquely influences B cell behavior (Hutzinger et al., 2009). Upon infecting a resting naive B cell, EBV enters Latency III, The set of proteins and RNAs produced in Latency III transforms the B cell into a proliferating blast (also known as B cell activation. Later, the virus restricts its gene expression and enters Latency II, The more limited set of proteins and RNAs produced in Latency II induces the B cell to differentiate into a memory B cell, Finally; EBV restricts gene expression even further and enters Latency I, Expression of EBNA-1 allows the EBV genome to replicate when the memory B cell divides (Amon and Farrell, 2005;
Odumad et al., 2011). Within epithelial cells, only Latency II is possible. In primary infection, EBV replicates in oro-pharyngeal epithelial cells and establishes Latency III, II, and I infections in B-lymphocytes, EBV latent infection of B-lymphocytes is necessary for virus persistence, subsequent replication in epithelial cells, and release of infectious virus into saliva, EBV Latency III and II infections of B-lymphocytes, Latency II infection of oral epithelial cells, and Latency II infection of NK- or T-cell can result in malignancies, marked by uniform EBV genome presence and gene expression (Robertson, 2010).

2.4.4 Reactivation:
Latent EBV in B cells can be reactivated to switch to lytic replication, This is known to happen in vivo, but what triggers it is not known precisely. In vitro; latent EBV in B cells can be reactivated by stimulating the B cell receptor, so reactivation in vivo probably takes place when latently infected B cells respond to unrelated infections, In vitro; latent EBV in B cells can also be reactivated by treating the cells with sodium butyrate or TPA(Odumad, et al., 2011).

2.4.5 Transformation of B-lymphocytes:
When EBV infects B cells in vitro, lymphoblastoid cell lines eventually emerge that are capable of indefinite growth, The growth transformation of these cell lines is the consequence of viral protein expression, EBNA-2, EBNA-3C and LMP-1 are essential for transformation, whereas EBNA-LP and the EBERs are not(Yates et al., 1984 ).It is postulated that following natural infection with EBV, the virus executes some or all of its repertoire of gene expression programs to establish a persistent infection, Given the initial absence of host immunity, the lytic cycle produces large amounts of virus to infect other (presumably) B-lymphocytes within the host, The latent programs reprogram and subvert infected B-lymphocytes to proliferate and bring infected cells to the sites at which the virus presumably persists, Eventually; when host immunity develops the virus persists by turning off most (or possibly all) of its genes, only occasionally reactivating to produce fresh virions, A balance is eventually struck between occasional viral reactivation and host immune surveillance removing cells that activate viral gene expression, The site of persistence of EBV may be bone marrow, EBV-positive patients who have had their own bone marrow replaced with bone marrow from an EBV-negative donor are found to be EBV-negative after transplantation (Gratama et al., 1988).
2.4.6 Latent antigens:
All EBV nuclear proteins are produced by alternative splicing of a transcript starting at either the Cp or Wp promoters at the left end of the genome (in the conventional nomenclature). The genes are ordered EBNA-LP/EBNA-2/EBNA-3A/EBNA-3B/EBNA-3C/EBNA-1 within the genome. The initiation codon of the EBNA-LP coding region is created by an alternate splice of the nuclear protein transcript. In the absence of this initiation codon, EBNA-2/EBNA-3A/EBNA-3B/EBNA-3C/EBNA-1 will be expressed depending on which of these genes is alternatively spliced into the transcript (Robertson, 2010).

2.5 Epidemiology:
EBV known as the cause of infectious mononucleosis (glandular fever), and also associated with particular forms of cancer, such as Hodgkin's lymphoma, Burkitt's lymphoma, gastric cancer, nasopharyngeal carcinoma, and conditions associated with human immunodeficiency virus (HIV), such as hairy leukoplakia and central nervous system lymphomas (Maeda et al., 2009). Some 200,000 cancer cases per year are thought to be attributable to EBV (Amon and Farrell, 2005). There is evidence that infection with EBV is associated with a higher risk of certain autoimmune diseases. Most people become infected with EBV and gain adaptive immunity (Shannon and Rowe, 2014). In the United States, about half of all five-year-old children and about 90 percent of adults have evidence of previous infection (Odumade et al., 2011). Infection with EBV mainly occurs by the oral transfer of saliva and genital secretions (Amon and Farrell, 2005). The oral route is the primary route of transmission of the virus; however, transmission by transfusion has been documented. In developing countries, infection is acquired in the first few years of life. Crowding and/or the practice of pre-chewing food for infants may be contributing factors. In the developed world, infection is often delayed to adolescence. About 50% of primary EBV infections during young adulthood result in clinical infectious mononucleosis (Henle and Henle, 1973). The age at primary infection varies substantially worldwide, EBV infection usually occurs in individuals of a young age, with low socioeconomic status or development, from a larger than average family and with poor hygienic standards. By their third decade of life, 80–100% of these individuals become carriers of the infection (Day et al., 1975). Although primary EBV infection during early childhood is usually subclinical or has symptoms that are similar to other respiratory illnesses, a delay in acquiring a primary EBV infection at an older age is associated with an increased risk of certain cancers.
age in childhood or adolescence, which usually occurs in more developed countries (Rickinson and Kieff, 1996). can manifest itself as infectious mononucleosis occurring in approximately 25–75% of EBV-infected persons (Evans., 1971), are ubiquitous in nature, humans serve as the only natural host for EBV (Kieff et al., 1979). EBV probably the most potent transforming human virus in culture, is nonetheless known to infect and persist for life in > 90% of human adults without causing disease (Henle and Henle., 1973). Two major types of EBV – EBV-1 and EBV-2 – have been identified and differ in geographic distribution. EBV-2 may be more common in Africa (Gratama and Ernberg., 1995), and in homosexual men (van Baarle et al., 2000). There are few or no symptoms noticeable when a person contracts EBV at the adolescence stage of life but when EBV is contracted as an adult it may cause fatigue, fever, inflamed throat, swollen lymph nodes in the neck, enlarged spleen, swollen liver, or rash (Evans., 1971). EBV can infect different cell types, including B cells and epithelial cells (Shannon and Rowe., 2014).

2.6 Diagnosis:

Various laboratory tests have been used to diagnose EBV infection. In addition to tests for other diagnostically useful parameters (leukocytosis, lymphocytosis with atypical lymphocytes, abnormal liver function test, etc.), there are tests for detecting non-specific heterophile antibodies and specific anti-EBV antibodies, as well as molecular biology methods used to detect EBV DNA (Yates et al., 1984).

2.6.1 Serological diagnosis:

Serological tests for antibodies specific for Epstein-Barr virus (EBV) antigens are frequently used to define infection status and for the differential diagnosis of other pathogens responsible for mononucleosis syndrome. Using only three parameters [viral capsid antigen (VCA) IgG, VCA IgM and EBV nuclear antigen (EBNA)-1 IgG], it is normally possible to distinguish acute from past infection: the presence of VCA IgM and VCA IgG without EBNA-1 IgG indicates acute infection, whereas the presence of VCA IgG and EBNA-1 IgG without VCA IgM is typical of past infection. However, serological findings may sometimes be difficult to interpret as VCA IgG can be present without VCA IgM or EBNA-1 IgG in cases of acute or past infection, or all the three parameters may be detected simultaneously in the case of recent infection or during the course of reactivation. A profile of isolated EBNA-1 IgG may also create some doubts. In order to interpret these patterns correctly, it is necessary to determine IgG avidity,
identify anti-EBV IgG and IgM antibodies by immunoblotting, and look for heterophile antibodies, anti-EA (D) antibodies or viral genome using molecular biology methods. These tests make it possible to define the status of the infection and solve any problems that may arise in routine laboratory practice (De Paschale and Clerici., 2012).

2.6.2 molecular Diagnosis:
A number of different methods, techniques and protocols have been used to determine the presence of EBV DNA and measure viral load (Kimura et al., 1999; Stevens et al., 2002). Dot blotting, Southern blotting, PCR and in situ hybridization have all been applied to various materials, but their differences in sensitivity and specificity have led to the results that need to be considered cautiously (Jenson., 2004).

2.7 Renal Failure Associated with Epstein Barr Virus:
Renal involvement in systemic EBV infections typically manifests as acute tubular necrosis or tubulointerstitial nephritis. Rarely, EBV infection causes nephrotic syndrome due to minimal change disease (Dylewski ., 2008). Over 95% of adults worldwide become infected with EBV in their lifetime. IM is usually a clinical syndrome characterized by a self-limiting illness with fever, pharyngitis patients with IM have abnormalities in urinary sediment (Blowey ., 1996). Acute kidney injury with significant parenchymal dysfunction is rare. This usually manifests as acute renal failure, rhabdomyolysis or immune complex glomerulonephritis and rarely minimal change disease, membranous nephropathy and cholemicnephrosis (Lei et al., 2000). In 1996, nephrotic syndrome was reported in a 19 month old infant with acute EBV infection. Proteinuria resolved after resolution of the viral infection. A renal biopsy was not done (Blowey., 1996). Two cases of EBV-associated renal failure were reported in 2000 and 2002 associated with interstitial nephritis and one of these also had minimal change disease (Leietal., 2000). The pathogenesis of IM induced renal failure remains unclear. T lymphocytes may be activated having recognized EBV antigens in kidney tissue or there may be direct renal injury due to EBV (Lei et al., 2000). There was also evidence of EBV DNA detected by polymerase chain reaction (PCR) in renal tissue in patients with interstitial nephritis favoring direct virally mediated renal toxicity (Dylewski ., 2008). EBV receptors (CD21) were detected in proximal tubule cells and were up-regulated in the EBV-infected tissues (Okadaet al., 2002). Epstein-Barr virus (EBV) DNA in renal tissue in acute interstitial nephritis (IN) has not been previously reported. An 18-year-old male presented with a sore throat, fever, cervical lymphadenopathy, and oliguric renal
failure. The rapid slide test for heterophile antibodies associated with infectious mononucleosis was positive, and a renal biopsy showed an acute interstitial nephritis. A polymerase chain reaction (PCR) assay identified EBV DNA in the renal biopsy. In situ hybridization (ISH) for EBV RNA and immunohistochemistry for latent membrane protein 1 of EBV were negative. Evidence of mild renal involvement may be present up to 16% in patients with IM, but severe renal failure is rare. Hematuria or proteinuria can be found in 2% and 18% of IM cases respectively, whereas azotemia has been reported in only 8% (Okada et al., 2002). Interstitial nephritis is the most common histologic abnormality. EBV is believed to play a vital role in the pathogenesis of IgA nephropathy. The possibility of IM should be considered when patients present acute renal failure, particularly if other features such as fever, hemolytic anemia, hepatitis, or thrombocytopenia are present (Lei et al., 2000).

2.8 Previous Study:

According to Mikhalkova et al (2012) the study is done on one case A 22-year-old male presented to his primary care physician with 1 week of fatigue, fever, sore throat, nausea and vomiting. Physical examination revealed tonsillitis. A rapid streptococcal throat test was negative and a diagnosis of IM was confirmed with a positive IgM antibody against the EBV capsid antigen, IgG antibodies against the anti-EBV-viral-capsid-antigen were negative suggesting acute EBV infection. A urine analysis revealed normal microscopy but was significant for a random urine protein of 620 mg/Dl, A kidney biopsy was performed to further evaluate his proteinuria, there is no injury and change in the kidney of patient.

A review of 27 cases of acute renal failure in heterophile positive IM by Meyer et al (1996), showed that 18% had rhabdomyolysis and myoglobinuria and one patient had minimal change disease. Only 13 of the 27 patients underwent kidney biopsies, 10 of whom had interstitial nephritis. Another two had immune-complex glomerulonephritis, review demonstrated a predominance of cases with cytotoxic T cells in the interstitium.

In 1996 study done by Blowey, was reported on nephritic syndrome in a 19 month old infant with acute EBV infection. Proteinuria resolved after resolution of the viral infection. A renal biopsy was not done.
According to Lei (2000), was reported to A 17-year-old boy with juvenile rheumatoid arthritis presented with jaundice, confusion, hemolytic anemia, thrombocytopenia, and acute renal failure secondary to titer-confirmed acute Epstein-Barr virus (EBV). Renal biopsy specimen revealed interstitial nephritis with an inflammatory infiltrate composed of cytotoxic/suppressor T cells, and interstitial mononuclear cell nuclei expressed EBV encoded RNA-1 (EBER-1) mRNA. Methylprednisolone treatment resulted in rapid improvement.

In 2002, Okada et al., reported a case of a patient with chronic active EBV infection who developed both acute tubulointerstitial nephritis and minimal change disease. Renal biopsy showed papillary inholdings of atypical tubular epithelium and lymphocytic interstitial infiltrates. EBV DNA was detected by PCR in some infiltrating lymphocytes but not in the tubular epithelial cells. It was proposed that EBV-infected T-cells activated other cells (educated T cells), which infiltrated into the interstitial and secreted cytokines and promoted tubular epithelial atypical and minimal change disease. In our case, the kidney biopsy tissue was negative for EBV mRNA by in situ hybridization and there were no interstitial infiltrates.

In (1992) by Nadasdy et al., Two patients (3-year girl and 28-year male) with renal disease who, despite repeatedly negative serologies, were shown by molecular hybridization techniques-in situ hybridization (ISH) and polymerase chain reaction (PCR)-to have EBV infection are reported here. Results from this two patients in study indicate that renal disease can be associated with EBV infection, the relationship of EBV infection with glomerular diseases is less well established. They were believed that the glomerular disease of him first patient was related to her chronic, devastating EBV
CHAPTER THREE
MATERIALS AND METHODS

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

3.2 Study area:
This study was conducted in Wad Medani, Elhassahisa, Elmanagil Renal teaching hospitals, Gezira state, Sudan. The study was conducted May 2017 December 2017.

3.3 Study population:
Renal failure patients admitted to Wad Medani, Elmanagil, and Elhasahisa Renal teaching hospital, Gezira state, Sudan.

3.4 Study Variable :
3.4.1 Variable Depended :
Epstein Barr Virus / Antibody, Epstein Barr Virus.

3.4.2 Variable Independent :
Age, Gender, History.

3.5 Selection criteria :
3.5.1 Inclusion criteria:
All Renal failure Patients attending Wad-Medani teaching hospital, Elmanagil and Elhasahisa hospital and accept to participate.

3.5.2 Exclusion criteria:
Other renal failure patients not attending Wad-Medani teaching hospital, Elmanagil and Elhasahisa hospital.

3.6 Study sample size:
The study sample was calculated according to the following formula:

\[ n = \frac{P (1-P) Z^2}{M^2} \]

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.

\[ N = 0.32 \times (1 - 0.32) \times 1.96^2 / 0.05^2 = 334 \]

Due to high cost the sample size was adjusted to 75.

### 3.7 Data collection:

Structured tested questionnaire was used to collect data from study population.

### 3.8 Statistical analysis:

This study data was analyzed by using statistical package for social sciences (SPSS) software. Descriptive analysis, correlation analysis, sensitivity, specificity and predictive value of every diagnostic test was done. Also ROC or AUC analysis was done.

The results is considered significant when \( p < 0.05 \).

### 3.9 Ethical Consideration:

Ethical approval to conduct this study was obtained from Ministry of health Gezira state. Permission was obtained from the head direction in same teaching hospitals in Gezira state. Also obtain consent of study population, Information was collected from the subjects under privacy and is used for research study only.

### 3.10 Methods:

#### 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 c.

#### 3.10.2 Enzyme linked Immuno sorbent assay:

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-labellel 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 FAVORGEN 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 polymerase chain reaction (PCR) is a laboratory technique for DNA replication that allows a “target” DNA sequence to be selectively amplified. PCR can use the smallest sample of the DNA to be cloned and amplify it to millions of copies in just a few hours. Discovered in 1985 by Kerry Mullis, PCR has become both an essential and routine tool in most biological laboratories.

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 94º c to separates 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 58º c). Finally, DNA polymerase extend the primers by its polymerase activity, this is done in a temperature optimal for the Taq polymerase which is 72ºc. These steps are repeated for 35 times. (# In sterile 0.2 ml micro centrifuge tubes the PCR ingredients were added in the ratio shown in the (Table 1).
Table 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 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:

3.10.5.1 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 10 ml TBE) in a microwavable flask.
3. Then was microwaved for 1 min 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 20-30 mins until it has completely solidified.

3.10.5.2 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 the DNA fragments was visualized.
CHAPER FOUR
RESULT & DISCUSSION

This study was carried at same teaching hospitals Gezira state, conducted in the period from May to December 2017. 75 blood sample were collected from renal failure patients.

4.1 Sociodemographic characteristics of study population:

*Age: The age groups of the study population were 24(32%) aged between 20 – 40 years, 34 (45%) aged between 41 – 60 years, aged between 61 – 80 years, (Figure 4-1)

![Frequency Distribution of study population according to age.](image)

**Figure 4.1:** Frequency Distribution of study population according to age.
*Gender:* From the 75 individual included in this study, 52 (69%) were males and 23 (32%) were females (Figure 4.2).

**Figure 4.2:** Frequency Distribution of study population according to Gender.

*Locality:* was collected 25 samples from Wad Medani, Elhasahisa and ELmanagil (Figure 4.3).

**Figure 4.3:** Frequency Distribution of study population according to Locality.
*History: *According the history of disease 19(25%) was Diabetes ,14(18.7%) was hypertension patients and 7 (9%) was thyroid ( Table 4-3).

**Table (4.3)**: Frequency Distribution of study population according to History:

<table>
<thead>
<tr>
<th>Diseases</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>14</td>
<td>18.7</td>
</tr>
<tr>
<td>Diabetes</td>
<td>19</td>
<td>25.3</td>
</tr>
<tr>
<td>Thyroid</td>
<td>7</td>
<td>9.3</td>
</tr>
<tr>
<td>Normal</td>
<td>35</td>
<td>46.7</td>
</tr>
<tr>
<td>Total</td>
<td>75</td>
<td>100.0</td>
</tr>
</tbody>
</table>

*Sero positivity of ELISA :* According to the sero positivity of ELISA there were 8 (10.7%) positive from the total of study population(Figure 4-4).

**Figure 4.4:** Distribution of study population according to ELISA.
*Sero positivity of PCR*: According to the sero positivity of PCR there were 7 (35%) positive from the total of study population(Figure 4-5).

Figure 4.5: Distribution of study population according to PCR.

Figure 4.6: PCR running in gel electrophoresis.
**Distribution of ELISA * PCR**: According to the Frequency Distribution of study population to ELISA and PCR, they were 4 cases positive by ELISA and PCR and 3 cases positive only by PCR (Table 4-4).

**Table 4.4**: Frequency Distribution of study population according to ELISA * PCR Cross tabulation.

<table>
<thead>
<tr>
<th>Count</th>
<th>PCR</th>
<th>Total</th>
<th>P. Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>ELISA Positive</td>
<td>4</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Negative</td>
<td>3</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>7</td>
<td>13</td>
<td>20</td>
</tr>
</tbody>
</table>

**Figure 4.7**: Chart shows ELISA sensitivity was 57% and specificity was 69% comparing with PCR that considered golden standard for detection of EBV.
**ELISA * Age Cross tabulation**: The positive ELISA According to Age, there were four positive in the category 20 – 40 years, one in category 41 – 60 years, and three in category 61 – 80 (Table 4-5).

**Table 4.5**: Frequency Distribution of study population according to ELISA * Age Cross tabulation

<table>
<thead>
<tr>
<th>ELISA * Age Group Cross tabulation</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Count</th>
<th>Age Group</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20 - 40Year</td>
<td>41 - 60Year</td>
<td>61 - 80Year</td>
<td>Total</td>
</tr>
<tr>
<td>ELISA</td>
<td>Positive</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>20</td>
<td>33</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>24</td>
<td>34</td>
<td>17</td>
</tr>
</tbody>
</table>

**ELISA * Gender Cross tabulation**: The positive ELISA According to Gender, there was 7 positive male and only one female (Table 4-6).

**Table 4.6**: Distribution of study population according to ELISA * Gender Cross tabulation

<table>
<thead>
<tr>
<th>Count</th>
<th>Gender</th>
<th></th>
<th></th>
<th>P. Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>ELISA</td>
<td>Positive</td>
<td>7</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>45</td>
<td>22</td>
<td>67</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>52</td>
<td>23</td>
<td>75</td>
</tr>
</tbody>
</table>
**ELISA and history Cross tabulation**: According to the positive of ELISA, from Diabetes patients there was three positive EBV infection, one positive hypertension, and one positive thyroid patient (Table 4-7).

**Table (4.7)**: Frequency Distribution of study population according to ELISA * history Cross tabulation.

<table>
<thead>
<tr>
<th>Diseases</th>
<th>Hypertension</th>
<th>Diabetes</th>
<th>Thyroid</th>
<th>Normal</th>
<th>Total</th>
<th>P. Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELISA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>8</td>
<td>0.810</td>
</tr>
<tr>
<td>Negative</td>
<td>13</td>
<td>16</td>
<td>6</td>
<td>32</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>19</td>
<td>7</td>
<td>35</td>
<td>75</td>
<td></td>
</tr>
</tbody>
</table>

**PCR and History Cross tabulation**: According to the positive of PCR, from Diabetes patients there was four positive EBV infection and two positive thyroid patient (Table 4-8).

**Table (4.8)**: Frequency Distribution of study population according to PCR * History Cross tabulation.

<table>
<thead>
<tr>
<th>Diseases</th>
<th>Hypertension</th>
<th>Diabetes</th>
<th>Thyroid</th>
<th>Normal</th>
<th>Total</th>
<th>P. Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>0</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>7</td>
<td>0.021</td>
</tr>
<tr>
<td>Negative</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>7</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>8</td>
<td>20</td>
<td></td>
</tr>
</tbody>
</table>
**Discussion:**

Epstein-Barr virus is an antique virus. With the ability to establish lifelong latency and intermittent reactivation after primary infection and with limited clinical symptoms in the majority of infected individuals, EBV has become ubiquitous in all human populations.

EBV infection occurs in the first few years of life, and universal sero conversion is often seen by ages 3–4 years, whereas infection in developed countries often is delayed until adolescence (Green et al., 2006).

The study was done to screen and detect EBV among renal failure patients in same Gezira state cities teaching hospitals, by using serological and PCR base analysis and confirm the accurate analyzed method of them to detect the EBV.

Out of 75 screened patients, 8 of them found positive for IgM/VCA by ELISA (10.7%). Am confirm that high levels of EBV antibody titers in accordance with the findings of Guiserix et al. (1996), which is reported that 14 and 40% Among chronic hemodialyzed patients, a higher incidence of EBV infection and activity assessed by high titers of VCA-IgG antibody has been observed, and he confirm his report by Yamamoto et al. 1995. EBV is common posttransplant and increases with time from transplantation (Morton et al. 2014).

In the current study, sero-positivity of EBV was observed to be higher among age group ranging between 41-60. This finding goes well with that reported by Ibrahim, et al. (2016), showed that the percentage of renal transplant patients co-infected with EBV was increased with age.

Regarding the gender of participant in this study, 35% of males were positive for EBV infection when screen for active infection. In contrast, Ibrahim, et al. (2016) reported that (3%) of the screen males were positive active infection and (94%) of them were positive for IgG.

The true positive samples detected by ELISA is 8 samples (10.7%), and the true negative is 67 samples (89.3%).

Out of 20 samples detected by confirmatory PCR was contain 8 true positive and 12 true negative screening by ELISA, confirmed that 7 (35.0%) of them found true positive, and
13 (65.0%) true negative. The study observed that molecular analysis method (PCR) is more confirm and accurate to detect event the true negative result by serological screening (ELISA), this result was supported by previous studies (Nadasdy et al., 1992). The author and his colloquies reported that two patients with renal disease who were negative serologically, are shown positive EBV infection by molecular hybridization techniques—in situ hybridization (ISH) and polymerase chain reaction (PCR).

The study taking in consideration a history disease as cofactor to renal failure, the diabetic patients (19) represent the majority where they 4 true positive detect by ELISA. According to Sairenji et al., (1991), a homology between EBV and diabetes when tested their samples serologically by ELISA. In other hands, Hyooty et al., (1991) confirmed the circulating of EBV/VCA and EBV/EA antibody when analyzed by ELISA in 54 newly diagnosed type I diabetic children and in matched controls. The patients had significantly lower EBV VCA IGG-class antibody levels, and VCA IGM-class antibodies were observed in two of the patients only.

The effect if hypertension is doubtful in this study, among 14 hypertensive renal failure patients, only one participant showed positive reaction to EBV when screen by ELISA the same result could be generalized for the co-exitant of EBV and thyroid, as the result indicated only one thyroid patient was positive for the virus infection.
CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion:

Epstein–Barr- virus induced Renal Failure patients is an important diagnosis to consider in the patient presenting with symptoms of Infectious Mononucleosis(IM) the seroprevalence of the disease was found to be 10.7% . Detection of the virus by PCR give percentage of 35.0% . The PCR is a technique with high sensitivity , specificity , imperceptible and purport for confirm Epstein–Barr infection in cases where clinical presentation is atypical or serology in conclusive. The data in this study also demonstrate the elderly age ranged between 20-40 years is more fair to exposure to EBV, and the male is more exposure than female. on the other hand, diabetic patients among Renal failure is a majority cofactor observed in this study.

5.2 Recommendations:

5.2.1 Serological techniques (ELISA) should be included as routine screening test for all renal failure patient in Gezira state to detect and analyzed EBV.

5.2.2 Use of the PCR as confirmatory test during transplantation and for patient who have symptoms of infectious mononucleosis.

5.2.3 Recommendations of the trainees in the next studies , taking into account the increase in sample size and that the study includes a wider area.

5.2.4 Health education program should be done to increase the awareness of EBV to reduce the way of transmission.
5.3 REFERENCES


Yamamoto T, Nakajima Y, Yamamoto M, Hironaka T, Hirai K, Nakamura Y: Epstein-Barr virus activity in patients on chroni

APPENDIX (1)

Questionnaire form

1. Name ........

2. Age ....... Locality ............

3. Sex: Male .... Female....

4. Do you have fever, sore throat or other infection as tonalities or rach ....etc ?
   Yes ....... No.......

5. Do you have suffer from any problem related with oral as in teethes or gum ?
   Yes....... No.....

6. Are you pregnant ?
   Yes ...... No ........

7. Did you had Graft, jaundice, cancer, or contact with hepatitis patient ?
   Yes ........ No ........

8. Have you ever had a history of blood disease?  Yes.....
   No.....

9. IF previous question answered yes what any type you had:
   1- Anemia ..... , 2-Myelofibrosis ..... , 3-Hemophilia....

10. Did you have contact with contaminated sharp or, needle prick ?  Yes...... No ............
11. Do you have inherited disease in your family? Yes..... No......

12. If answer yes the disease as;
   1-Rheumatoid ......, 2-Thyroid......, or 3-Diabetes......

13. Are you recipient in blood transfusion process? Yes ...... No......

14. If your answer yes in previous question, do you have symptoms? as;
   1-fatigue ......... 2-fever ......... 3-inflamed thorat...... 4-swollen lymphnode in neck ......... 5-enlarged spleen and liver ...... 6-rash.... 7-malasia.....
Appendix (2)

Informed consent

بسم الله الرحمن الرحيم

جامعة الجزيرة
كلية علوم المختبرات الطبية
برنامج ماجستير علوم المختبرات الطبية
تخصص أحياء دقيقة

الاسم: ..............................................

أوافق أنا المذكور أعلاه على التبرع بعينة الدم لإجراء هذه الدراسة.

الإمضاء: ..............................................

التاريخ: ..............................................

سوف يتم اخذ عينة دم من الوريد بحجم 5 مل بواسطة حقنة طعن, وذلك بعد مسح منطقة اخذ العينة بواسطة المطهر. كل الأدوات المستخدمة لأخذ العينة معقمة ومتبعة فيها كل وسائل السلامة المعملية, وليس هناك آثار جانبية للعملية.

ربما يحصل تورم بسيط في منطقة اخذ العينة وسوف يزول بعد فترة قصيرة.

أوافق أنا المذكور أعلاه على التبرع بعينة الدم لإجراء هذه الدراسة.

الإمضاء: ..............................................

التاريخ: ..............................................