بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate studies

Serodetection of Parvovirus B19 among Blood Donors Attending Haj Al Safi Teaching Hospital – Bahri (Khartoum State)

الانتشار المصلي لفيروس البارفو B19 لدى المتبرعين بالدم المترددين علي بنك الدم بمستشفى حاج الصافي التعليمي –(بحري)

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

By

Safa Ali Mohammed Osman
B.Sc (Honour)., Medical Laboratory SciencesSudan University of Sciences and Technology (2011)

Supervisor:
Dr.Wafa Ibrahim Elhag
Associate Professor of Microbiology- Al Neelain University

2018

الآيـــة

قال تعالى:

بسم الله الرحمن الرحيم

﴿سَنُرِيهِمْ آيَاتِنَا فِي الْآفَاقِ وَفِي أَنفُسِهِمْ حَتَّىٰ يَتَبَيَّنَ لَهُمْ أَنَّهُ الْحَقُّ ۗ أَوَلَمْ يَكْفِ بِرَبِّكَ أَنَّهُ عَلَىٰ كُلِّ شَيْءٍ شَهِيدٌ (53) أَلَا إِنَّهُمْ فِي مِرْيَةٍ مِّن لِّقَاءِ رَبِّهِمْ ۗ أَلَا إِنَّهُ بِكُلِّ شَيْءٍ مُّحِيطٌ (54)﴾

صدق الله العظيم

سورة فصلت الايات: (53-54)

Dedication

To my mother

To supportive cheerful father

Unique wonderful brothers

Sweet sisters

And all those who helped me

Acknowledgment

My greatest gratitude is to **ALMIGHTY AllAH** the Most compassionate and the most merciful.

Firstly I would like to thanks my supervisor **Dr.Wafa Ibrahim Elhag** -without her continous guidance this work could have not been a reality.

My thanks also extended to all members of **Microbiology Department of Sudan University of Siences and Technology** for provided good materials and equipments.

Great thanks to all blood donors who participated in the study. and to the doctors and technicians who helped me during sample collection in **Blood Bank of Haj Al Safi Hospital**.

Abstract

Human Parvovirus B19 (HPV) B19 is a small, non-enveloped, single-stranded DNA virus. The virus causes a variety of human diseases, including erythema infectiosum (fifth disease), acute or chronic erythritis, hydrops fetalis and spontaneous abortion in early pregnancy, a plastic crises and persistant pure red blood cells a plasia.This study was conducted to detect by serology parvovirus B19 among blood donors who attended Haj Al-Safi Teaching Hospital Blood Bank - (Bahri) during the period from Febreuary to Septemper 2016.

Ninety blood donors with different ages ranged between 19 - 48 years age were enrolled in this study, data was collected using questionare. Blood specimens were collected, serum was obtained and Enzyme Linked Immunosorbent Assay was used to detect IgG and IgM antibodies of Parvovirus B19 and the obtained results were analyzed statistically by SPSS. The study results demonstrated that 39(43.3%), 14(15.6%) were positive for Parvovirus B19 IgG, IgM antibodies respectively. Most blood donors were belonged to 19-28 years. Statistical analysis showed insignificant relation between Parvovirus and age(IgM *p*-value0.084, IgG *P*-value 0.743), residence (*P*-valuefor IgM 0.358and 0.183 for IgG) .The serofrequency of Parvovirus B19 among blood donors in this study was high and poses adverse transfusion risk especially in high risk group of patients. Serologic screening of blood donors is essential to determine their immunity to Parvovirus B19. Parvovirus B19 is not part of routine national wide immunization, blood donors were recommended to be target group for future immunization program in Sudan.

ملخص الاطروحة

فيروس البارفو B19 فيروس صغير؛ غير مغلف احادي الحمض النووي الريبي منقوص الاكسجين، الفيروس يسبب العديد من الامراض التي تصيب الانسان وتشمل متلازمة الخد المصفوع (الداء الخامس)، التهاب المفاصل الحاد والمزمن، الاستسقاء الجنيني، أزمة إنعدام التنسج في نقي العظم، عدم تصنيع كريات الدم الحمراء الدائم.

أجريت الدراسة للكشف عن الانتشار المصلي لفيروس البارفو B 19 لدي المتبرعين بالدم المترددين علي بنك الدم بمستشفي حاج الصافي التعليمي– بحري خلال الفترة من فبراير الي سبتمبر 2016م.

تسعون متبرع بالدم تتراوح اعمارهم مابين 19 الي 48 سنه تم إختيارهم للدراسه، تم جمع معلومات باستخدام الإستبيان، جمعت عينات الدم، تم استخلاص مصل الدم وفحصه بتقنية مقايسة الممتز المناعي المرتبط بالانزيم للكشف عن الاجسام المضاده IgM و IgG المصنعه ضد فيروس البارفوB19 وحللت النتائج احصائياً باستخدام برنامج التحليل الإحصائي SPSS.

أثبتت الدراسة أن 39 (43,3 %)، 14 (15,6 %) قد أعطو نتائج ايجابية للاجسام المضاده IgG، IgM علي التوالي واغلب المتبرعين بالدم كانوا من الفئة العمرية من 19 الي 28 سنه.

التحليل الاحصائي أثبت عدم وجود علاقة بين الاجسام المضادة للفيروس والعمر، والسكن (قيمة p أكثر من 0,05).

أعطى الإنتشار المصلي للفيروس البارفوB19 بين المتبرعين بالدم فى هذه الدراسة نتيجه عاليه وبالتالي يودي لخطوره عند نقل الدم بالاخص لدي المرضي ذوي الخطورة العاليه وبذلك فان الفحص المصلي للمتبرعين بالدم ذو اهميه عاليه وتوصي الدراسة باستخدام التطعيم لدى المتبرعيين بالدم ضد فيروس البارفوB19.

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Chapter One

Introduction

1. Introduction

1.1 Introduction:

Parvoviruses (Parvoviridae) are small, non- enveloped, single-stranded DNA viruses that are known to cause disease in a variety of mammalian species, although most parvoviruses are species specific. Parvovirus requires the host cell for replication, specifically the cell nucleus, and binds the host cell by the double-stranded ends of the genome. Viral replication occurs only in rapidly dividing cells such as intestinal crypt epithelial cells, precursor cells in the bone marrow, and myocardiocytes. Viral replication results in cell death and loss due to failure of mitosis. Not all rapidly dividing cell populations are equally affected, suggesting a viral tropism for certain target organs (Goddard and leisovits, 2010). Human blood and its components are widely used as life saving therapy in hospital practices however ,there is always an associated risk of transfusion via contaminated blood due to the high frequency of human Parvovirus B19 in blood donors and pooling of large number of blood donation used in plasma pool to produce a batch components like clotting factor concentrate ,a large number of batches could be potentially B19 infected(Kumar *et al.* , 2013) .

Parvovirus B19 infection is common worldwide, and most persons who contact the virus are infected by 15 years of age. Infection is most common in late winter or early spring. The virus is transmitted through exposure to infected respiratory droplets or blood products and vertically from mother to fetus (Jessiica, 2007).

Parvovirus B19 is the infectious agent of erythema infectiosum commonly known as slapped cheek syndrome or fifth disease (Broliden *et al*., 2006).

The disease in children and teenagers is usually mild, but infection with parvovirus B19 during pregnancy has been associated with miscarriage,

intrauterine fetal death, fetal anaemia and non-immune hydrops (Nyman *et al*., 2002).

Parvovirus B19 infection has also been associated with acute arthropathy in adults (Lindblom *et al*., 2005), with aplastic crisis in sickle-cell disease patients and with chronic anaemia in immunodeficient patients (Young *et al*., 2004).

Parvovirus B19 was discovered serendipitously in 1974 and is the only member of the family parvoviridae known to be pathogenic in humans (Agbandji *et al*., 1994).

Frequent transmission of parvovirus B19 infection by transfusion of factor VIII concentrates prior to the widespread use of viral inactivation technology has been well documented. Due to the relative resistance of parvovirus B19 to viral inactivation and to the high level of viremia in acutely infected persons, such transmission continued, though at lower levels (Kay and Gregory, 2002).

Even after the introduction of virally inactivated concentrates, data from the transfusion experience with solvent detergent (SD) treated plasma indicate that seroconversion in recipients occurred frequently when high viremia (107.5 to 108.5 copies /ml) was present but did not occur when viremia was present at < 104 copies /ml (Kay and Gregory ,2002).

1.2 Rationale:

Parvovirus B19 is a good model for new emergent viruses capable of infecting blood products because of their properties of physical resistance, in fact, B19 virus DNA is detectable in 50% to80% of non-virally inactivated factor 8 concentrates and in 30% to50% of solvent/detergent-inactivated factor 9 concentrates respectively (Norja *et al*., 2012).

B19 Parvovirus was also detected in in two of three unheated batches of clotting factor preparations and in 20% to25% of solvent/detergent-treated batches, while the fractionation process used to obtain albumin preparations is apparently more efficient at eliminating the virus (Yee *et al*., 1996).

Due to the high frequency of human parvovirus B19 in blood donors and pooling of large number of blood donations used in plasma pool to produce a batch of components like clotting factor concentrate a large number of batches could be potentially contaminated (Kumar *et al*., 2013)

Human erythrovirus parvovirus B19 caused a wide range of diseases such as erythema infectiosum of fifth disease a common illness in children, aplastic crisis, chronic pure red cell a plasia , fetal hydrops and fetal death (Jessica *et al.*, 2007).

The virus is associated with arthropathies ,hepatitis and various other syndromes and diseases (Jessica *et al.*, 2007).

So screening of blood donors is highly essential to reduce the virus transmission especially of high risk patients (immune compromised).

1.3 Objectives:

1.3.1 General objective:

-To determine seroprevalence of Parvovirus B19 antibodies among blood donors attending Haj Al-Safi Teaching Hospital during period from February to September 2016, using ELISA technique.

1.3.2 Specific objectives:

- To detect specific anti Parvovirus B19 IgM and IgG antibodies using Enzyme Linked Immune sorbent Assay (ELISA).

-To detect the relation between B19 seroprevalnce and other factors including age and residence.

Chapter Two

Literature Review

2. Literature Review

2.1 Discovery and brief history of Parovirus:

In 1974, Cossart *et al* first identified B19 while evaluating tests for hepatitis B virus surface antigen .The name originates from the coding of a serum sample, number 19 in panel B, that gave anomalous results when tested by counter immune electrophoresis and radioimmunoassay. Electron microscopy (E M) revealed the presence of 23-nm-diameter particles resembling animal Parvoviruses, B19 independently described in Japan 5 years later as (Nakatani) virus, but subsequent testing proved the two viruses to be identical (Heegaard and Brown ,2002). Extraction of DNA revealed complementary single strands of approximately 5.5kp encapsidated in separate virions (Heegaard and Brown , 2002). And the viral proteins were found to co purify with viral antigen at a density of 1.43g/ml, indicating that the virus was a member of the genus *Parvovirus*. Although originally labeled (serum parvovirus-like particle) or human parvovirus, it was officially recognized in 1985 as a member of *parvoviridae* and given the name B19 by the International committee on taxonomy of viruses (Heegaard and Brown , 2002).

In 1980 a brief uneventful febrile episode was noted in two soldiers, and B19 was detected in serum by Electron Microscopy (Heegaard and Brown, 2002). There was still no disease distinctly connected with the virus until an association with transient aplastic crisis in patients with sickle cell anemia was observed in 1981 (Heegaard and Brown , 2002).

Sera from jamaican children residing in London were observed to contain B19 antigen at the time of aplastic crisis, while convalescent –phase sera showed evidence of seroconversion. Two years later, erythema infectiosum was

seroepidemiologically linked to B19 infection in healthy children,and is now accepted as the etiological agent of this disease. Shortly thereafter, other clearly defined syndromes related to B19 infection were described, such as fetal loss in the midtrimester of pregnancy due to intrauterine transmission from an infected mother and post infectious symmetrical peripheral polyarthropathy or arthritis in adults In its chronic form B19 was found to be cause pure red cell aplasia (Heegaard and Brown , 2002).

2.2 Classification, structure ,and organization:

2.2.1 Taxonomy:

The classification of the family parvoviridae relies on morphology and functional characteristics. Parvoviruses are common animal and insect pathogen. Until the recent identification of the circoviruses and the related TT viruses, parvoviruses were among the smallest DNA-containing viruses able to infect mammalian cells; hence the name "parvum" (latin), meaning small (Siegl *et al*., 2000).

2.2.2 Morphology:

The B19 virion has a simple structure composed of only two proteins and a linear, single-strand DNA molecule, the non-enveloped viral particles are 22-24nm in diameter and show icosahedral symmetry, and often both empty and full capsids are visible by negative staining and electron microscope (Heegaard and Brown , 2002)

Mature infectious viral particles have a molecular weight of 5.**6**×10(Kerr, 2000). The virion is composed of 60 copies of capsomer, and both negative and positive strands of DNA are packaged. X- ray crystallography has shown that the surface

of B19 is significantly different from those of other parvoviruses by lacking prominent spikes on the three fold icosahedral axes involved in host recognition and antigenicity, the limited DNA content and the absence of a lipid envelope make B19 extremely resistant to physical inactivation. The virus is stable at 56 ℃ for 60 min, and lipid solvents have no effect. Inactivation of virus may be acheived by formalin, β-propiolactone, and gama irradiation (Heegaard and Brown , 2002).

2.3 Viral life cycle and blood group P receptor:

The life cycle of B19, like those of other nonenveloped DNA viruses, includes binding of the virus to host cell receptors, internalization, translocation of the genome to the host nucleus DNA replication, RNA transcription assembly of capsids and packaging of the genome, and finally cell lysis with release of the mature virions (Heegaard and Brown ,2002) .

B19 was initially shown to agglutinate human red cells (Heegaard and Brown , 2002) and it was hypothesized that the same agglutinin may act as the host cell receptor on erythroid progenitor cells. Thus, the haemagglutinin was identified as the glycolipid globoside, also known as the blood group p antigen, by using haemagglutination as a surrogate marker (Heegaard and Brown , 2002).

2.4 Culture:

There is no animal model for B19, and virus can only be grown in culture with difficulty. In vitro studies of B19 in explanted human BM cultures have confirmed the erythroid specificity of this virus (Heegaard and Brown , 2002).

2.5 Cytopathology:

The cytopathic effect of infection of erythroid progenitor cells with B19, both in vivo and in vitro, is manifested as giant pronormoblasts (alternately referred to as lantern cells), first recognized in 1948 in the bone marrow of patients with transient aplastic crisis, Electron microscope of cells reveals cytopathic ultrastructural changes that include psudopod formation, marginated chromatine, and virus particles in the neucleus (Chen and Qiu , 2010).

2.6 Pathogenesis and immune response:

Specific immunoglobulin M (IgM) and IgG antibodies are produced following experimental and natural B19 infection. Infection leads to a biphasic clinical phase: One week after intra nasal inoculation with B19 in healthy adult volunteers viremia was detected in seronegative individuals accompanied by a mild illness with pyrexia, malaise, myalgia, itching, and excretion of virus from the respiratory tract About 17-18 days after infection a second phase of symptoms commenced and was characterized by rash, itching, or arthralgia. Recovery involves production of IgM antibody 10 to 12 days post-infection, coinciding with a peak in virus level. IgM usually persists in serum sample for approximately 3 months but may be found for several months. IgG antibody is detectable in volunteers about two weeks after inoculation and presumbly persists for life and protects against secondary infections. IgA may also be detected and probably plays a role in protection against infection by the natural nasopharyngeal route (Heegaard and Brown, 2002).

During viremia reticulocyte numbers fall to undetectable levels, recovering 7 to 10 days later, resulting in a temporary drop in hemoglobin of 1 g/dl (0.6 mmol/liter) in a healthy person. Clinically non-significant lymphopenia,

neutropenia, and thrombocytopenia occur 6 to 10 days after inoculation. All hematologic parameters may exhibit a brief overshoot prior to stabilizing at pre-incubation levels. Viral replication in neutrophils has been proposed by one group, but this results have not confirmed by others. This may provide an explanation for the neutropenia sometimes observed, although B19 is apparently not linked to the development of clinically significant neutropenia (Heegaard and Brown , 2002).

the infrequently reported fulminant thrombocytopenia associated with B19 infection may consist of two types. In one type, thrombocytopenia precedes the onset of rash due to BM suppression, while the other type is probably mediated by immunologic mechanisms (Heegaard and Brown , 2002).

2.7 Epidemiology:

Infection with parvovirus B19 is global; infectivity rates, inferred from the presence of antiparvovirus IgG antibody in serum samples, are similar in the United States, Europe, and Asia. Some isolated Amazonian tribes and populations of remote islands off the coast of Africa have escaped exposure. Parvovirus B19 infection is common in childhood; half of 15-year-old adolescents have specific antiparvovirus B19 antibodies. B19 infection continues at a lower rate throughout adult life, and by the time, they are elderly, most persons are seropositive. In temperate climates, infections usually occur in the spring, and small epidemics at intervals of a few years are typical. The virus is spread by respiratory droplets, and secondary infection rates among household contacts are very high, Nosocomial infection has been described. Parvovirus B19 has also been transmitted by blood products, specially pooled factor eight and factor nine concentrates. (Neal *et al.*, 2004).

2.8 Clinical syndromes commonly associated with Parvovirus B19:

The development of Parvovirus B19 disease is influenced by the hosts hematological and immuonological status. Healthy children usually develop asymptomatic infection, nonspecific illness or benign erythema infectiosum. But in patients suffering from decreased production or increased loss of erythrocytes, B19 can cause a severe drop in hemoglobin values, leading to aplastic crisis and anemia, which can be fatal. Immunocompromised patients can develop a state of chronic anemia due to their inability to clear the persistent Parvovirus B19 replication (Heegaard and Brown , 2002).

2.8.1 Erythema infectiosum:

Erythema infectiosum (fifth disease) is the major manifestation of B19 infection in children. It is a self-limiting contagious exanthema, that has been recognized by pediatrician for over a century. Typically, the rash involves the cheeks, hence its other name slapped cheek syndrome, as the child has the appearance of having been slapped on the both cheeks. Approximately 25-50% of such infections may be asymptomatic (Kudesia and Wreighitt ,2005).

Erythema infectiosum, initially presenting with flu-like symptoms, fever, and headache, and flowed 1 to 4 days later by (slapped cheeks) rash that becomes lacy in appearance and after about one week may spread to the trunk and limbs , Adults with parvovirus B19 infection usually do not have extensive rash. The onset of the rash usually coincides with the appearance of parvovirus B19antibodies (IgM), suggesting that this symptom is immune-mediated Other dermatologic syndromes associated with parvovirus infection in adult include popular-purpuric (gloves and socks) syndrome (Jessica *et al.*, 2007).

2.8.2 Arthropathy:

For those adults with symptoms, the most common symptom is arthropathy. It affects up to 50% of pregnant women with parvovirus infection and may last several weeks to months. The arthropathy usually present as polyarthralgia, affecting the hands, wrists, ankles, and knees. The onset of the arthritis is coincident with the increase in parvovirus B19 antibodies (IgM), suggesting that, similar to erythema infectiosum, it is immune-mediated. It is supposed that the antibodies developed against B19 in such cases are deposited in the synovial fluid of the joints and thus contribute to the pathogenesis of arthralgia. B19 V arthralgia is a self-limiting condition (Guilaume *et al.*, 2002).

2.8.3 Anemia and transient aplastic crisis:

Parvovirus B19 has an affinity for hematopoietic system cells, including erythroid progenitor cells and to a lesser degree, leukocyte and megakariocyte cell lines, notably through the P antigen (Pawelec *et al.*, 2016).

The virus attacks cells of the red blood cell lines in the bone marrow, causing hemolysis and red blood cell aplasia , the decline in hemoglobin level is usually minimal in healthy children and adults because the red cell aplasia lasts only 7 to 10 days and red blood cells have along half-life of 2 to 3 months (De jong *et al.*, 2011).

The anemia, however, may be significant in those with underlying hematologic disorders including sickle cell disease, thalassemia, hereditary spherocytosis, pyruvate kinase deficiency, and autoimmune hemolytic anemia, who have low hemoglobin level prior to infection. Presentation of transient nonspecific prodromal symptoms followed by aplastic crisis includes paller and fatigue and is usually not associated with rash (Pawelec *et al,*. 2016).

2.8.4 Immunocompromised patient:

Chronic bone marrow suppression after Parvovirus B19 infection leading to chronic severe anemia has been described in immunodeficient patients including those with HIV, acute lymphocytic leukemia on chemotherapy, and congenital immunodeficiency (Terri *et al*., 2000).

2.8.5 Infection in pregnancy (hydrops fetalis):

Hydrops fetalis is a condition defined by the presence of generalized fetal subcutaneous tissue accumulation of fluids (edema) in at least two fetal compartments. Other location of edema can include pleura (pleural effusion) pericardium (pericardial effusion) and abdomen (ascites). The multiple causes of the development of fetal hydrops are divided into immune (Rh disease) and non-immune ones (Georgy and Antonietta, 2010).

Among all non-immune causes, Parvovirus B19V is of principal importance. As the fetus is highly dependent on the increased erythropoiesis rates and B19V arrests erythropoiesis, aplastic crisis, profound cardiac failure and edema are often developed (Georgy and Antonietta, 2010). Cardiac failure may be result of the severe anemia but may also be associated with myocarditis; which can cause arrhythmias or even cardiac arrest without evidence of anemia, cardiac failure or hydrops (Lamont, 2010).

Fetal transmission may occur in up to 30% of the cases with maternal infection but is not always associated with congenital defects. It is estimated that fetal loss can occur in 7-10% of infected women. The greatest risk is between the 11th and 20th week of gestation coinciding with the increased activity of the fetal liver and

the shortened half-life of red blood cells. After the 20th week of gestation, although fetal transmission may occur, it is believed that it is not associated with unfavorable outcome. Evidence also suggests that the asymptomatic infection in pregnancy carries a higher risk of transmission because it can be connected with weak immune response unable to clear B19V replication (Kudesia and Wreighitt, 2005).

2.9 Parvoirus B19 and blood transfusion:

Previously it was thought that the antivirus antibodies in plasma make B19 less infectious and, as a consequence, the transfusion of positive B19 DNA plasma was a safe procedure. This has been contradicted by the discovery of the different viral genotypes, novel human parvoviruses (PARV4), the transfusion transmission of B19 in spite of the presence of antiviral antibodies and the severity of the clinical picture in predisposed patients(Modrow *et al*., 2010).

Parvovirus B19 is easily transmitted by blood transfusion and therapy with plasma-derived products. Moreover, its viral load in plasma-derived products varies between 2 and 1.3gEq\ml (Modrow *et al*., 2010).

B19 can contaminate mini-pools, coagulation factors and patched red cells concentrates. Such contamination is highly problematic because, depending on the transfused group, the virus may impact high risk patients. Despite the widely known fact that Parvovirus B19 infection can be transmitted via contaminated blood, there are at present no strict regulatory requisites regarding Parvovirus B19 contamination of pooled plasma or blood products before product use and transfusion (Corcoran and Doyle, 2004)

In relation to this, the first evidence of blood-transmitted Parvovirus B19 V infection was described in 1995 in 22-year-old patient suffering from thalassemia

major, followed by the development of transient aplastic crisis (A T C) and transitory heart failure, Sustained anemia and pure red cell aplasia as well as pancytopenia were observed in patients with hematological malignancies and hemolytic diseases after transfusion of Parvovirus B19 contaminated blood in Japan (Satake *et al.*, 2011).

Hypothetical B19 transmission was also reported by (Hourfar *et al*., 2011).

As Parvovirus B19 sequences detected in transfused patients were identical to the sequences obtained from the red cell concentrates used for their hemotherapy (Stramer *et al*., 2009).

According to the importance in transfusion medicine, transfusion-transmitted infectious agent are divided into several group :"red" –potential for severe clinical outcome;"orange" –higher priority for the future;"yellow" – agents of public and regulatory concern; and "white" – agents which do not appear to need higher prioritization at present. Parvovirus B19 virus falls in to the "yellow" priority and is considered an emerging infectious agent in blood transfusion (Stramer *et al*., 2009).

Nowadays, the actual frequency B19V transmission via blood products is not assessed prospectively and the complete picture of transfusion-transmitted case could not be assessed. As the group at major risk of Parvovirus B19 infection| reinfection includes patients who have inherited anemias and frequent need of transfusion, it is necessary that routine quantitative PCR diagnostic techniques are implemented, as optimized by the WHO standards, not only in clinical virology laboratories but also in transfution centers worldwide (Starmer *et al.*, 2009) .

2.10 Parvovirus B19 infection in malaria patients:

The high-titer of Parvovirus B19 viremia causing a significant drop of hemoglobin and reticulocytes could have serious consequences in patients, especially children with underlying malaria or individuals living malaria region of the world (Africa, Latin America, South and South-East Asia). Although it is generally accepted that the anemic state in such areas is caused mainly by the malaria, iron deficiency, hook worm infection and sickle cell disease, as B19 is a pathogen with significant impact on anemic patients, its importance for aggravating plasmodium species. Infection is notable. Such process could be easily facilitated by 3-7 days cessation of erythropoiesis induced by B19 and the dramatic hemolytic potential of malaria (Pasvol, 2006).

Recently, B19 co-infection was reported in patients with malaria leading to severe anemia, renal failure and hepatosplenomegaly (Ingrassia *et al*., 2010)

2.11 Laboratory diagnosis of Parvoirus B19 infections:

2.11.1 Cytopathology, Electron Microscopy and Immunohistochemistry:

The cytopathological abnormalities caused by B19 are characterisitic but they are not sufficient for diagnostic purposes. Active B19 infection induces the formation of giant pronormoblasts in the bone marrow. They are characterized by cytoplasmic vacuolization, ground-glass appearance of the nucleus and clear perinuclear halo, the chromatin is often immature and appears as a thin rim around the viral inclusion. This method is useful for cytopathological evaluation of suspected hydrops fetalis (Svetoslav *et al*., 2011).

2.11.2 Serological methods:

The precise diagnosis of recent or past infection with B19V depends on the use of enzyme immunoassays to detect anti-B19V IgM and IgG in plasma. Commercial assays have been developed using expression of B19Vcapsid proteins (Vp1 and Vp2) in the baculovirus system (Michel *et al*., 2008). The general advantages of baculovirus system is its ability to induce post-translational protein folding, which is necessary for the production of soluble and conformationally complete capsid proteins. This is of critical importance for the sensitivity of the immunoassays because the proteins retain their conformational epitopes and there is less of a risk of false-negative results (Svetoslav *et al*., 2011).

To confirm acute B19V infection, IgM antibodies must be detected in plasma or serum. These antibodies are synthesized approximately 7- 10 days after the high-titer viremia and are directed against linear and conformational epitopes of Vp1 and Vp2. The IgM response against Vp1 coformational epitopes predominant, where as IgM response against the linear Vp2antigen is found less frequently. IgM antibodys against Vp1 and Vp2 conformational epitopes and Vp1 linear epitopes appear at the same time but IgM antibodys against linear Vp2 epitopes diminish rapidly thus utilization of Vp1 and Vp2 linear antigens alone for diagnosis of recent B19 virus infection could give false-negative results (Manaresi *et al.,* 2001). Decline of IgM production is followed by development of IgG antibodies. They have lifelong persistence and protective function but an important feature is that IgG directed against the linear epitopes of VP2 fades rabidly (Svtovlav *et al*., 2011)In contrast to IgM limitations, there is international standard for IgG which is a valuable tool for the accurate confirmation of past B19 infection among different laboratories that use variable test systems (Svetoslav *et al*., 2011).

2.11.3 Viral DNA Detection (PCR):

Nucleic acid amplification to detect B19 DNA is an extremely sensitive means (most published PCR are able to detect viral DNA at 1-100 copies/ml) to detect viral DNA in sample. This method is especially useful in patient lacking an adequate antibody-mediated immune response, immunocompromised or immunosuppressed individuals and fetuses. in such cases serological testing for B19 is unreliable (Jordan., 2001).

Using standard procedure detection of B19V specific IgM in fetal blood has sensitive of 29% compared to almost 100% for PCR (Beersma *et al.*, 2005).however low B19 DNA levels may persist for years after infection and therefor low-positive PCR results for B19 do not prove recent infection (lindblom *et al*., 2005).

2.12 Back ground studies:

Two recent studies at Germany at 2001, 2002 of plasma donations and serum samples of asymptomatic blood donors demonstrated a prevalence of Parvovirus B19 of 1:800 and 1:3915 respectively (Weimer *et al*, 2001) (Hitzler and Runkel, 2002).

Another study in Germany in 1999 showed that Parvovirus B19 associated infections might be more common in immune compromised persons than previousely anticipated, for their cohort of 60 bone marrow graft recipients, the authors reported an incidence of B19 infections of 15% and a B19- associated mortality rate of 7% (Hitzler and Runkel, 2002).

Other study in Munich in 2005 showed that the prevalence of Parvovirus B19 Parvovirus specific antibodies was much higher in groups receiving clotting factor (Plentz *et al*., 2005).

2.13 Vaccine Development:

Effective vaccines are available for animal Parvoviruses, and it is likely that parvoviruses B19 infection can also be prevented. The recombinate immunogen that is being developed as a vaccine for the human virus lacks DNA and is therefore noninfectious empty capsids have been engineered to overexpress the highly immunogenic VPI and single dose of 2.5 mg capsids elictited neutralizing antibody response in normal volunteers (Ballou *et al.,* 2003).

As with many other vaccines commercial interest rather than lack of efficacy or safety has limited the development of Parvovirus B19 vaccine could prevent transient crisis in patients with sickle cell disease or other hemolytic anemias and pure-red cell aplasia in some immune-deficient persons, as well as hydrops fetalis, if seronegative women were inculated early in pregnancy. Chimeric viral capsids have been proposed as more general vehicles for the delivery of antigens and Parvovirus B19 especially attractive for this purpose ,because VPI unique region can be entirely replaced with other protein sequences, allowing for example the presentation of conforationally and functionally intact enzyme on the surface of the empty viral capsids .this method is now being adapted for protection against an agent of bioterrorism a domain of protective antigen is being incorparted on Parvovirus B19 particle (Young *et al.,* 2004).

Chapter Three

Materials and Methods

3. Materials and Methods

3.1 Study type and design:

This study was a descriptive cross sectional study.

3.2 Study area and duration:

This study was conducted in Blood Bank Department at Haj Al Safi Teaching Hospital Khartoum North From in period from February to September 2016.

3.3 Study population:

Blood donors who attending the blood bank for donation of blood during the study period were included in the study.

Irrespective of age and residence subjects were selected from apparently healthy individual.

3.4 Sample size:

n→ sample size

z→ statistc corresponding to level of confidence

p→ expected prevalence

d→ corresponding to effect size

A total of 90 blood donors were included.

3.4 Sampling technique:

The study was based on non-probability convenience sampling technique.

3.5 Method of data collection:

Data was collected using direct structured interviewing questionnaire (appendix I).

3.6 Ethical considerations:

Permission to carry out the study was taken from the college of Graduate Studies, Sudan University of Science and Technology.

The blood donors were informed for the purpose of the study and its objectives, before taking their permission with protection of their privacy.

3.7 Experimental work:

3.7.1 Specimen collection:

Five ml of venous blood was collected by venous puncture after disinfecting the site of collection, the collected blood was drown into plain containers, allowed to clot and then centrifuged at 3000 rpm for 5 minutes ,sera were separated into new sterile eppendorf tubes preserved at -20°C until used, hemolytic, lipemic or icteric specimen was excluded.

3.8 Specimen processing:

Specimens were analyzed for Parvovirus B19 IgM and IgG antibodies by ELISA (EUROIMMUN-Germany), principle of ELISA (appendix III).

3.9 Procedures:

Sample dilution: samples were diluted 1:101in sample buffer

Preparation of the washing buffer:

The wash buffer was a 10× concentrate (one part reagent plus 9 parts distilled water).

Adding samples:

In ELISA microtiter wells 100 µL of calibrator, positive control, negative control, and samples were added in specific order in the well according the protocol of the test and were Incubated 60 minutes at 37 °C.

Washing (1):

Wells were discarded and subsequently washed 3 times using 300µL of working strength wash buffer for each wash. Wash buffer were left in each well for 30 to 60 seconds per washing cycle, and then emptied the wells. After washing thoroughly all liquid were disposed from the microplate by tapping it on absorbent paper with openings facing downwards to remove all residual wash buffer.

Conjugate incubation:

Enzyme conjugate (100µL) was pipetted (peroxidase-labelled anti-human IgG and peroxidase- labelled anti-human IgM) into each of the microplate wells. Incubated for 30 minutes at room temperature.

Washing (2):

The wells were discarded and washed as described above.

Substrate incubation:

Chromogen/substrate (100µL) was pipetted into each of the microplate wells. Incubated for 15 minutes at room temperature (protect from direct sunlight).

Stopping the reaction:

Stop solution (Sulfuric acid) 100µL was pipetted into each microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:

Photometric measurement of the colour intensity was made at a wavelength of 450 nm and a reference wavelength between 620 nm and 650 nm within 30 minutes of adding the stop solution prior to measuring. The microplate was shaked slightly to insure a homogeneous distribution of the solution.

Calculation of the result of IgG antibodies:

Results were evaluated by calculating a ratio of the extinction value of the control or patients sample over the extinction value of calibrator 3 the ratio was calculated according to the following formula:

$$\frac{\text{Extinction of the control or patient sample}}{\text{Extinction of calibrator 3}} = \text{Ratio}$$

Calculation the result of the IgM antibodies

Results was evaluated by calculating a ratio of the extinction value of the control or patient sample over the extinction value of calibrator. The ratio was calculated according to the following formula:

$$\frac{\text{Extinction of the control or patient sample}}{\text{Extinction of calibrator}} = \text{Ratio}$$

3.10 The result was interpreted as follow:

Ratio<0.8: negative

Ratio>0.8 to <1.1: borderline

Ratio>1.1: positive

3.11 Data analysis:

Data were analyzed by using SPSS software version (11.5). Significance of differences was determined using chi-square test and statistical significance was set at $p<0.05$.

Chapter Four

Results

4.Results

Atotal of 90 blood donors who attended Haj Al-safi Teaching Hospital, (Khartoum), were included in this study to determine seroprevalence of Parvovirus B19.

The result showed that 14(15.6%), 39(43.3%) were positive for Parvovirus B19 IgM and IgG respectively, while 76 (84.4%), 51(56.7%) were negative to anti Parvovirus B19 IgM and IgG respectively (Table 1).

The study population age ranged between19 - 48 years age range with 34 years mean, all of them were males.

Most of Parvovirus seropositivity was observed among 19-28 age range and 14(15.6%), 39(43.3%) of them were positive for IgM, IgG respectively.

Statistical analysis showed insignificant relation regarding age, (P-value 0.084 and 0.74) for IgM and IgG respectively (Table2).

Regarding residence study group where equaly from Bahri Omdurman and Khartoum. However, high seroprevalence was observed among Khartoum residenses 6(42.8%) then Bahri 5(35.7%) regarding to IgM antibodies, while IgG antibodies were observed high among Omdurman residenses 16(41.0%) then in Khartoum 14(36.0%), Statistical analysis showed insignificant relation P-value 0.358 and 0.183 for IgM and IgG respectively (Table 3).

Table 1: Seroprevalence of Parvovirus B19 among study population (n=90)

Parvovirus B19	IgM	IgG
Positive	14(15.6%)	39(43.3%)
Negative	76(84.4%)	51(56.7%)
Total	90(100%)	90(100%)

Table 2: Seroprevalence of Parvovirus B19 among study population in relation to the age.

Age Groupe in years	IgM		IgG	
	Positive	Negative	Positive	Negative
19-28	10 71.4%	33 43.4%	19 48.7%	24 47.0%
29-38	2 14.3%	33 43.4%	16 41.0%	19 37.8%
39-48	2 14.3%	10 13.2%	4 10.2%	8 15.2%
Total	14 100%	76 100%	39 100%	51 100%

IgM: Pearson chi-square 4.533, p value 0.084.

IgG: Pearson chi-square 0.582, p value 0.743.

Table 3: Seroprevalence of Parvovirus B19 among study population in relation to the residence.

Residense	IgM		IgG	
	Positive	Negative	Positive	Negative
Bahri N=30	5 35.7%	25 32.8%	9 23.0%	21 41.2%
Omdurman N=30	3 21.5%	30 39.5%	16 41.0%	17 33.3%
Khartoum N=30	6 42.8%	21 27.7%	14 36.0%	13 25.5%
Total	14 100%	76 100%	39 100%	51 100%

IgM: Pearson chi-square 1.992, p value 0.358.

IgG: Pearson chi-square 3.326, p value 0.183.

Chapter Five

Discussion

5. Discussion

5.1 Discussion

Infection with Parvovirus B19 are quite common, particularly in children. In other wise healthy individuals, B19 infections can result in serious complications in certain high-risk population. The major high- risk groups are pregnant women, patients with underlying haematological problems and immune-deficients patients who constantly are multi-transfused. But yet donor screening is not mandatory (Bell *et al*., 1989).

This study was aimed to determine the seroprevalence of Parvovirus B19 among blood donors. The overall results revealed that 14(15.6%), 39(43.3%) were positive for IgM, IgG respectively.

In this study, there were variations in the seroprevalence of B19 parvovirus antibodies compared to other studies.

In comparison with an earlier studies in parvovirus B19 in Virgenia 2014 in blood donors, our study showed lower result than they reported (66%) was positive for anti parvovirus B19 IgG. in Nigeria,. However, anti parvovirus B19 IgM in our study was higher than that reported by the same study (1.3%) (Iheanacho *et al*., 2014).

In comparison with an earlier studies in south india (2002) in healthy blood donors more than 15 years , our study showed lower results than they reported (70%) positive to IgG antibodies which reported by Abraham 2002 (Abraham and Ruddraruju, 2002).

In this study there was diffrence in the prevalence of anti parvovirus B19 IgG which was higher than that reported by Spanish study in 1998 in which 9.8% were positive (Mata *et al*., 1998) this showed that there were geographical variations in the seroprevalence of Parvovirus B19 infections. Also in their

study there was an increasing in seroprevalence of Parvovirus B19 with age wich was differ than that reported by this result (Mata *et al*., 1998) .

In Anatolia in 2010 the ratio of Parvovirus seropositivity were higher than that reported by the present study in comparison with different age groups (Ozdemir *et al*., 2010).

These results were compared with the previous resulte in North African (Tunisian) population and western European (Belgian) population of blood donors in 1997,the seroprevalence of Parvovirus B19 IgM were less than 2% in these countries, so this result was higher than results of this countries, where as the seroprevalence of Parvovirus B19 IgG were 74% in these countries ,and our result was lesser than that reported in these countries (Mletale and Mertons, 1997).

Comparing with previous studies to regarding parvovirus B19 IgM it was lower than result reported by Elsedig *et al.*, 2014 in Sudan in Sudanese patients infected by Rhumatoid Arthritis wich were differ than population of this study and this may suggest the diffrences in results obtained in which it was 34.4%, in other wise anti parvovirus B19 IgG in our study was lower than that reported by Elsedig *et al.,* 2014 in which was 54.4%(Elsedig *et al*., 2014).

5.2 Conclusion:

Seroprevalence of human Parvovirus B19 among blood donors population in this study was high and poses an adverse transfusion risk especially in high-risk group of patients.

5.3 Recommendations

- Mini pool whole-blood screening may be planned for the future in blood centers.
- Screening of source plasma for parvovirus DNA is recommended.

- Investigation for Parvovirus B19 infection is recommended as part of the standard work up for fetal hydrops or intrauterine fetal death.
- Further studies with prolonged time and more effort should be done to get solid results.

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.

Appendices

Appendix I

Questionnare

Sudan Unversity of Sceience and Technology

College of graduate studies

Seroprevalence of Parvovirus B19 among Blood Donors

Attending Haj Al Safi Teaching Hospital Bahri

1- Date……..

2- Name ……..

3- Sample No…….....

4- Age …….

5- Residence……

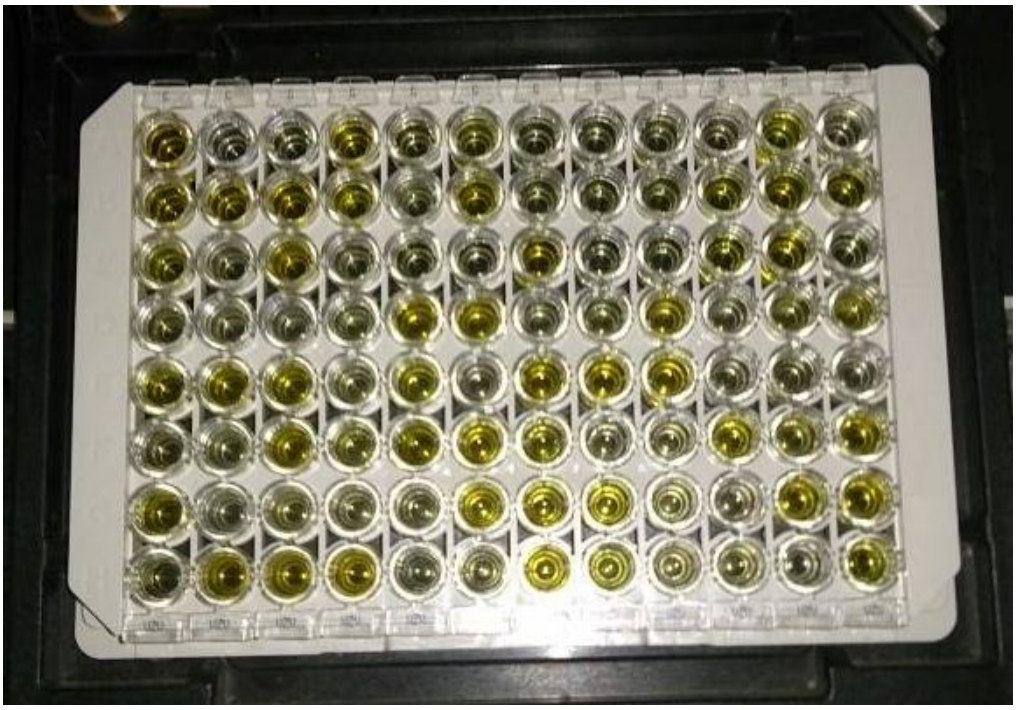
6- Result ……

- B19 Igm: Positive ☐ Negative ☐
- B19 IgG: Positive ☐ Negative ☐

Appendix II

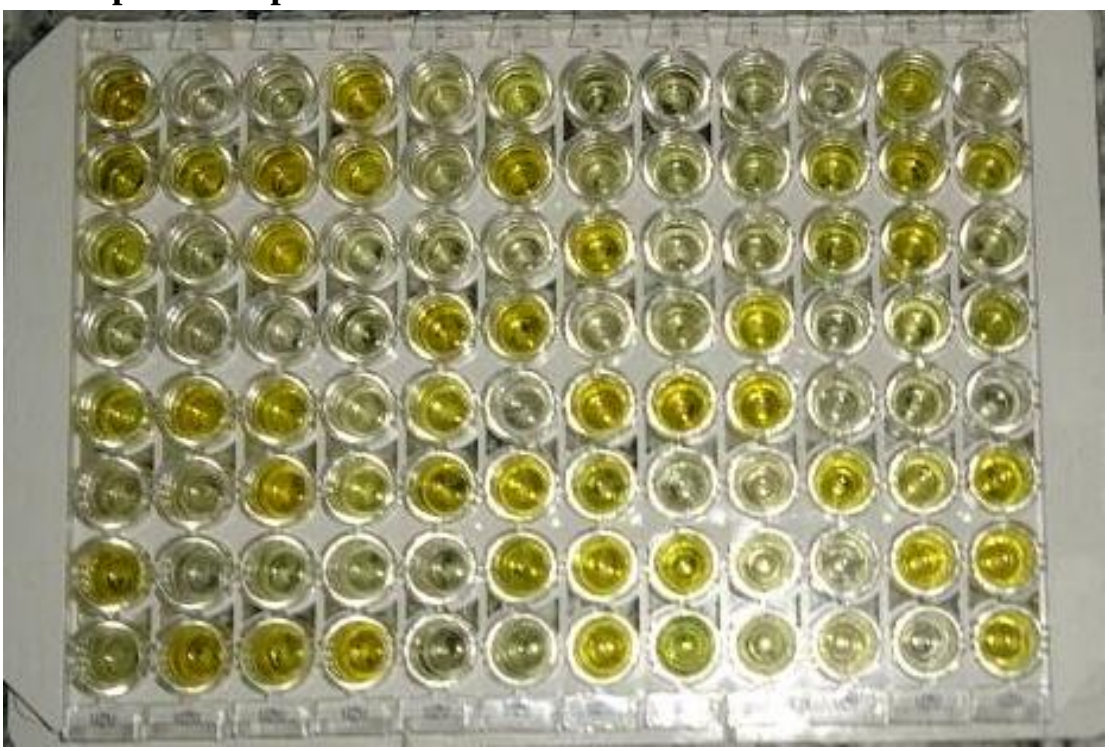
Result (IgM) antibody

14 Samples were positive



Result (IgG) antibody

39 Samples were positive



ELISA washing