CHAPTER ONE

1. INTRODUCTION

The common pediatric hematological malignancies include leukemia (30%) and lymphoma (12%), while acute lymphoblastic leukemia (ALL) accounts for one-fourth of all childhood cancers and three fourth (75%) of all newly diagnosed patients with leukemia. The incidence of ALL is 3-4 cases per 100,000 children below 15 years of age with a peak age group of 2-5 (Kishore *et al.*, 2011).

Complications from viral infections are common in children during chemotherapy for hematologic malignancies, including acute lymphoblastic Leukemia (ALL). Among these viral pathogens, parvovirus B19 is particularly ubiquitous; therefore, early screening for viral detection is essential (Lindblom *et al.*, 2008).

Human parvovirus is non-enveloped, single stranded DNA virus with icosahedral symmetry (Collier *et al.*, 2011).

The transmission of B19 occurs through respiratory droplets, contaminated blood, and organ transplants, as well as by vertical transmission from the mother to her fetus. B19 is easily transmitted through blood transfusion and therapy using plasma-derived products (Heegaard and Brown, 2002).

The pattern of clinical disease caused by B19 varies and is influenced by the hematological and immunological status of the infected individual. In healthy hosts, B19 virus generally causes self-limiting subclinical erythroid aplasia, followed by rash or arthralgia. However, in patients with diminished production or increased loss of erythrocytes, B19 infection results in a severe drop in hemoglobin levels and in anemia, which could be life-threatening (Heegaard and Brown, 2002).

Epidemiological and biological data suggest a role for infectious agents at some stage in the etiology of childhood ALL, studies have suggested in utero initiation of preleukemic clones which requires one or more subsequent genetic and/or proliferative event(s) to produce leukemia in ALL during early childhood. Although it is possible that infection may play a role in the initial genetic rearrangement, the host response to infection may play an additional role in allowing proliferation of the premalignant clone; therefore, the host response during B19 associated acute leukemia may provide insights into possible mechanisms by which the virus may at least participate in leukemia (Ibrahem *et al.*, 2014).

Children with hematological malignancies are anemic due to malignant infiltrations of bone marrow, cytotoxic drugs and further it may be complicated by infection with B19 virus. The effects of B19 infection have been studied mostly as sporadic cases usually in ALL patients

during the course of maintenance chemotherapy causing acute or chronic anemia, pure red-cell aplasia or pancytopenia. Pediatric patients with ALL receiving chemotherapy have also been reported to be at high risk for acquiring parvovirus B19 infection (El-Mahallawy *et al.*, 2004).

Since B19 has a great tropism for erythroid progenitor cells, it frequently leads to anemic conditions (Kishore *et al.*, 2011).

Clinical complications following parvovirus B19 infection in children with ALL were first described 20 years ago, after which a number of case reports linked the infection with ALL during either induction therapy or maintenance therapy or at the time of bone marrow transplantation (lindblom *et al.*, 2008).

In one study, 8% of B19-seronegative ALL patients seroconverted while on maintenance chemotherapy, and there was a cluster of cases coinciding with a well-established B19 epidemic. B19 infection was able to mimic a leukemic relapse or therapy-induced cytopenia and contributed to the development of chronic anemia and profound thrombocytopenia in the majority of infected individuals (Heegaard and Brown, 2002).

B19 can contaminate minipools, coagulation factors, and packed red cell concentrates, and there are at present no strict protocols for determining

B19 contamination of pooled plasma or blood products before product use and transfusion to those at risk (Heegaard and Brown, 2002).

Although parvovirus B19 infection is easily diagnosed and treated, it has often been overlooked in cases of unexplained cytopenia. The infection can be eliminated by the administration of intravenous immunoglobulin or blood transfusions and cessation of chemotherapy. The choice of intervention depends on the severity and type of cytopenia, symptoms, and host immune status (Lindblom *et al.,* 2008).

In addition, a few larger cohorts of parvovirus B19–infected patients have been reported, as a result, awareness of the complications associated with B19 infection in patients with ALL has increased, and diagnostic methods have improved. However, none of the studies systematically compared infected patients with uninfected patients in a longitudinal study lasting several years (Lindblom *et al.,* 2008).

1.2. Rationale

The etiology of childhood cancers has been studied for more than 40 years. However, most if not all cancers occurring in children are attributed to unknown causes.

Since there is no adequate information about prevalence of B19 virus among normal Sudanese children and those who suffering from acute

lymphoid leukemia, few studies that compared ALL children with normal one, in addition to importance of B19 virus as cause of anemia and aplastic crises in immunocompromised patients which may lead to interruption of chemotherapy.

This study aimed to determine association between B19 virus infection and acute lymphocytic leukemia, B19 virus infection and anemia in both study group (children suffering from ALL) and control group (healthy children).

1.3. Objectives

1.3.1. General objective

To determine seroprevalence of B19 virus among children with acute Lymphoplastic leukemia (study group) and compare it with seroprevalence among normal children (control group)

1.3.2. Specific objectives

To estimate parvovirus specific IgG antibodies among children with acute lymphoplastic leukemia ALL (cases) and control group.

To study association between B19 seropositivity and acute lymphocytic leukemia.

To study association between B19 virus seropositivity and low HB levels.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Parvovirus

2.1.1 Discovery and Brief history

In 1974, *Cossart et al* first identified B19 while evaluating tests for hepatitis B virus surface antigen. The name B19 virus originates from the coding of a serum sample, number 19 in panel B that gave anomalous results when tested by counterimmunoelectrophoresis (CIE) and radioimmunoassay. Electron microscopy (EM) revealed the presence of 23-nm-diameter particles resembling animal parvoviruses (Heegaard & Brown, 2002).

B19 was independently described in Japan 5 years later as “Nakatani” virus, but subsequent testing proved the two viruses to be identical. Extraction of DNA revealed complementary single strands of approximately 5.5 kb encapsidated in separate virions indicating that the virus was a member of the genus Parvovirus. Although originally labeled as“serum parvovirus-like particle” or human parvovirus, it was officially recognized in 1985 as a member of the *Parvoviridae* and given the name B19 by the International Committee on Taxonomy of Viruses (Heegaard & Brown, 2002).

Antibodies to parvovirus B19 were found in a large proportion of normal adults, indicating that infection is common and probably occurs in childhood. A disease was linked to parvovirus B19 infection by John Pattison and colleagues, who found either virus-specific antibodies or the virus itself in samples from children who had a severe complication of sickle cell disease called transient aplastic crisis. The most common illness caused by parvovirus B19 was identified a few years later, during outbreaks in the United Kingdom of fifth disease, a highly contagious childhood exanthema long suspected of having a viral cause (Brown and Young, 2004).

In 1980 a brief and uneventful febrile episode was noted in two soldiers, and B19 was detected in serum by EM. 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. 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 (Heegaard & Brown 2002).

2.1.2. Structure and classification

The parvoviruses are the smallest of the DNA viruses (about 20 nm). They have single stranded linear genome and have been isolated from a wide range of organisms, from arthropods to humans. The family *Parvoviridae* is divided into two subfamilies: the *Parvovirinae* and the *Densivirinae*. The latter group infects only invertebrates. The *Parvovirinae* contains three genera: Parvovirus, Dependovirus and Erythrovirus (Kumar, 2012).

Erythrovirus contain only one parvovirus (B19) known to cause disease in humans and, together with closely related simian viruses, B19 has been, placed in a new genus, Erythrovirus (Kumar, 2012).

The B19 virion has a simple structure composed of only two proteins and a linear, single-strand DNA molecule. The nonenveloped viral particles are about 22 to 24 nm in diameter and show icosahedral symmetry, and often both empty and full capsids are visible by negative staining and EM. The limited DNA content and the absence of a lipid envelope make B19 extremely resistant to physical inactivation. The virus is stable at

56°C for 60 min, and lipid solvents have no effect. Inactivation of virus may be achieved by formalin, and gamma irradiation (Heegaard & Brown, 2002).

2.1.3. Transmission

Transmission of infection occurs via the respiratory route, through blood-derived products, and vertically from mother to fetus (Heegaard & Brown, 2002).

B19-specific DNA has been detected in respiratory secretions at the time of viremia, suggesting that virus is generally spread in the community by a respiratory route. Vertical transmission occurs in one-third of cases involving serologically confirmed primary maternal infections. nosocomial transmission has been described infrequently, and transmission has also been reported among staff in laboratories handling native virus (Heegaard & Brown, 2002).

2.1.4. Replication

The small genome of a parvovirus can encode only a few proteins, so the virus depends on its host cell (or another virus) to provide important proteins. Some of these cell proteins (a DNA polymerase and other proteins involved in DNA replication) are available only during the S phase of the cell cycle, when DNA synthesis takes place. This restricts the

opportunity for parvovirus replication to the S phase. Contrast this situation with that of the large DNA viruses, such as the herpesviruses, which encode their own DNA-replicating enzymes, allowing them to replicate in any phase of the cell cycle (Murray *et al.*, 2009).

2.1.4.1. Attachment and entry

A virion attaches to receptors on the surface of a potential host cell. In the case of B19 virus the host cell is a red blood cell precursor and the receptor is the blood group P antigen. The virion enters the cell by endocytosis and is released from the endosome into the cytoplasm, where it associates with microtubules and is transported to a nuclear pore. With a diameter of 18–26 nm, the parvovirus virion is small enough to pass through a nuclear pore, though there is evidence that the virion must undergo some structural changes before it can be transported into the nucleus. Nuclear localization signals have been found in the capsid proteins of some parvoviruses (Murray *et al.*, 2009).

2.1.4.2. Single-stranded DNA to double-stranded DNA

In the nucleus the single-stranded virus genome is converted to dsDNA by a cell DNA polymerase. The ends of the genome are double stranded as a result of base pairing, and at the 3 end the –OH group acts as a primer to which the enzyme binds (Murray *et al.*, 2009).

2.1.4.3. Transcription and translation

The cell RNA polymerase II transcribes the virus genes and cell transcription factors play key roles. The primary transcript(s) undergo various splicing events to produce two size classes of mRNA. The larger mRNAs encode the non-structural proteins and the smaller mRNAs encode the structural proteins.

The non-structural proteins are phosphorylated and play roles in the control of gene expression and in DNA replication (Murray *et al.*, 2009).

2.1.4.4. DNA replication

After and virion assembly conversion of the ssDNA genome to dsDNA, the DNA is replicated by a mechanism called rolling-hairpin replication. This is a leading strand mechanism and sets parvoviruses apart from other DNA viruses, which replicate their genomes through leading and lagging strand synthesis. Procapsids are constructed from the structural proteins and each is filled by a copy of the virus genome, either a (+) DNA or a (−) DNA as appropriate. One of the non-structural proteins functions as a helicase to unwind the dsDNA so that a single strand can enter the procapsid (Carter and Saunders, 2007).

The two major non-structural proteins and the VP1 and VP2 structural capsid proteins are synthesized in the cytoplasm, and the structural

proteins go to the nucleus, where the virion is assembled. The VP2 protein is cleaved later to produce VP3. The nuclear and cytoplasmic membrane degenerates, and the virus is released on cell lysis (Murray *et al.*, 2009).

2.1.5. Pathogenesis

Human B19 virus is a small single-stranded DNA virus classified as a member of precursors. It is the only parvovirus that has been clearly linked with disease in humans. B19 virus replicates only in human cells and belongs to the family Parvoviridae, genus Erythrovirus, whose tropism is primarily for erythroid autonomous, that is, not requiring the presence of a helper virus. Specific antiviral antibody production is thought to represent the major defense against B19 virus, as human normal immunoglobulin frequently clears the virus from peripheral blood and results in clinical improvement in immunosuppressed persons.

The virus replicates in human erythroid progenitor cells of the BM and blood, inhibiting erythropoiesis. Tropism of productive B19 infection is mainly due to the restrictive cellular distribution of the P blood group antigen globoside (Gb4), which is found most commonly on cells of the erythroid lineage but also on platelets; on tissues from the heart, liver, lung, kidney, endothelium and on synovium (Murray *et al.*, 2009).

Studies performed in volunteers suggest that B19 virus first replicates in the nasopharynx or upper respiratory tract then spreads by viremia to the bone marrow and elsewhere, where it replicates and kills erythroid precursor cells (Murray *et al.*, 2009).

Individuals who lack erythrocyte P antigens are very rare (1 in 200000) and apparently cannot be infected by B19 virus. B19 virus capsids are composed of 2 structural polypeptides, the minor (4%) VP1 protein (83 kDa) and the major (96%) VP2 protein (58 kDa). VP1 immunoglobulin IgM and/or IgG antibodies are always associated with the clearance of PV-B19 virus from serum. The detection of anti-VP1 and anti-VP2 antibodies is the basis for the diagnosis of acute or past PV-B19 infections. The dominant humoral immune response is to VP2 during early convalescence and to VP1 during late convalescence. Anti-VP1 and anti-VP2 antibodies play a major role in limiting B19 infection in humans (Florea *et al.*, 2007).

2.1.6. Clinical features

The B19 virus causes many different syndromes.

2.1.6.1. Infections in immunocompetent

2.1.6.1.1. Erythema infectiosum

Infectious rashs ometimes known as ‘fifth disease’ (The original classification of the exanthematous illnesses of childhood comprised six diseases: the other five are measles, rubella, scarlet fever, exanthemsubitum, and Duke’s disease, a rash of obscure etiology). More picturesque name is ‘slapped cheek syndrome ‘, the presenting feature is often erythema over the malar areas, followed within the next 4 days by a maculopapular rash on the trunk and limbs, which may persist for 2 or 3 weeks (Heegaard & Brown 2002).

Volunteer and field studies show the incubation period to be 13–18 days. There may be some fever and malaise in the early stages, and mild febrile illness without rash is common. This is predominantly an infection of children but adults may also acquire it; in them, and particularly in women, the joints are much more likely to be involved (Heegaard & Brown 2002)

2.1.6.1.2. Joint disease

Symptoms and signs of joint involvement may occur with or without rash illness, and B19 infection should be considered in the differential diagnosis of acute arthritis. This is more common in adults, especially women, of whom 80% have joint symptoms, compared with about 10% in childhood. Like the rash, the arthropathy is very similar to that seen with rubella, being a symmetrical arthralgia or arthritis involving mainly the small joints of the hands, although feet, wrists, knees, ankles and other joints may be affected. In children, the arthropathy may be less symmetrical. Arthropathy usually resolves within 2–3 weeks, but may occasionally persist or recur for months and very rarely for years. Some of these patients may be classified clinically as having early benign rheumatoid arthritis. However, B19 arthropathy is not destructive; if rheumatoid factor is detected it does not persist, and B19 virus infection is not causally linked to rheumatoid arthritis (Green wood, 2012).

2.1.6.1.3. Parvovirus in pregnancy

Although B19 infection can cause fetal loss, most pregnancies result in the birth of a normal baby. There is no evidence of birth defects or developmental abnormalities in children exposed to B19 in uterus. Transplacental infection can occur during acute maternal infection, whether or not symptoms of B19 infection occur in the mother, before

maternal antibody has developed and crossed the placenta to protect the fetus. Infection in the first 20 weeks can lead to intra-uterine death (increased risk 9%) and non-immunological fetal hydrops (risk 3%), of which about half die and are included in the 9%. This is in contrast to hydrops which occurs following Rhesus incompatibility and is immune mediated (Green wood, 2012).

The greatest risk of fetal loss is during the second trimester, these effects are due to the immaturity of the fetal immune response and the shorter survival of fetal red cells. The large increase in red cell mass (in both the bone marrow and extramedullary sites of erythropoiesis) during the second trimester leads to the increased risk of developing anemia at this time. The anemia persists and becomes chronic because of ongoing viral replication, Infection of fetal myocardial cells can also occur causing myocarditis. Both the anemia and the myocarditis contribute to the development of cardiac failure, leading to the development of ascites and fetal hydrops (Fetal loss is usually 4–6 weeks after maternal infection, but can be as long as 12 weeks later. Overall, B19 probably accounts for 10% of cases of fetal hydrops. A more effective fetal immune response reduces the risk of fetal loss in the third trimester; very rarely chronic anemia in the newborn has followed intra-uterine infection (Green wood, 2012).

2.1.6.1.4. Other infections

In healthy people, case reports suggest that the manifestations of B19 may on occasion be very wide (e.g. meningitis, hepatitis, haemophagocytic syndrome or myocarditis). However, as both IgM and DNA may persist for months, some associations may be casual rather than causal (Heegaard & Brown 2002).

2.1.6.2 Infection in the Immunodeficient Host

2.1.6.2.1 Chronic pure red cell aplasia

In immunocompromised patients unable to mount a neutralizing antibody response due to a persistent bone marrow (BM) insufficiency, B19 infection may cause chronic anemia. Predisposing conditions include Nezelof's syndrome, acute lymphatic leukemia(ALL), acute myloid leukemia, chronic myeloid leukemia, myelodysplastic syndrome, Burkitt's lymphoma, lymphoblastic lymphoma, astrocytoma, Wilms'stumor, HIV infection, SCID, BM transplantation, organ transplantation, class switch defect, steroid and cancer chemotherapy treatment.

Clinical hallmarks include fatigue and pallor, while immune-mediated symptoms (rash and arthralgia) are generally not present, Infection may serve as a prodromal of an underlying disease, and anemia may promptly

remit after immunoglobulin or antiviral chemotherapy (in HIV patients). Temporary cessation of maintenance chemotherapy has also led to resolution of anemia (Heegaard & Brown 2002).

2.1.7. Epidemiology

B19 infection is common and worldwide in distribution. Serological studies indicate that infection is most commonly acquired between 4 and 10 years of age, and by 15 years approximately 50% of children have antibody. Infection occurs throughout adulthood and up to 90% of elderly people are seropositive. B19 virus infections are endemic throughout the year in temperate climates, with a seasonal increase in frequency in late winter, spring and early summer months. There are also longer-term cycles of infection with a periodicity of about 4–5 years. Most infections are transmitted by close contact by the respiratory route, with a seroconversion rate of 20–30% for day-care personnel in close contact with infected children. Asymptomatic blood donors with high-titer viraemia enable transmission by blood or blood products, and the stability and heat resistance of B19 enables transmission by heat-treated factor VIII and IX to haemophiliacs (Green wood, 2012).

2.1.8. Laboratory diagnosis

Parvovirus B19 produces diverse clinical manifestations, depending on the stage of infection and the immune status of the host. The diagnostic approach is different for the different clinical manifestations. The virus cannot presently be cultured in routine diagnostic virology laboratories and, therefore, diagnosis is based on serologic tests to detect parvovirus B19-specific antibodies and on nucleic acid amplification testing (Knipe *et al.*, 2007).

Acute parvovirus B19 infection causes fifth disease, also known as exanthemsubitum. Because patients with fifth disease are usually viremic, the diagnosis can be established by using PCR to detect parvovirus B19-specific DNA in serum. An alternative approach is to test for parvovirus B19-specific IgM antibodies, which are present within several days of the onset of symptoms. A disadvantage of this approach is that tests for parvovirus B19 IgM may cross-react with other viruses, especially rubella, measles, EBV, and CMV. Serologic diagnosis of acute parvovirus B19 infection can also be achieved by comparing parvovirus B19-specific antibody titers in acute and convalescent specimens, with seroconversion or a fourfold rise taken as diagnostic of recent infection. Parvovirus B19 is also the main cause of hemolytic crisis in individuals with chronic hemolytic anemia, especially sickle cell disease (Knipe *et al.*, 2007).

Hemolytic crisis occurs earlier after infection than fifth disease, and the role of parvovirus B19 is best confirmed using nucleic acid amplification, because antibodies may not be detectable for approximately 1 week after the onset of crisis. Parvovirus B19 infection can cause chronic hypoplasticanemia in immunocompromised patients. These patients are chronically viremic and, thus, PCR is the best approach for making a virologic diagnosis. Serologic testing is usually not helpful because patients with chronic parvovirus B19 infection may fail to develop parvovirus B19-specific antibodies. The possibility of using parvovirus nucleic acid amplification assays to screen blood donors is under discussion, especially for pooled plasma products. Finally, parvovirus B19-specific immunity can be demonstrated by testing for parvovirus B19-specific IgG antibodies (Knipe *et al.*, 2007).

2.2. Childhood leukemia

The leukemia's are a group of disorders characterized by the accumulation of malignant white cells in the bone marrow and blood. These abnormal cells cause symptoms because of bone marrow failure (i.e. anemia, neutropenia and thrombocytopenia) and infiltration of organs e.g. liver, spleen, lymph nodes, meninges, brain, skin or testes(Hoffbrand *et al.*, 2006).

2.2.1. Classification of leukemia

The main classification is into four types: acute and chronic leukemias, which are further subdivided into lymphoid or myeloid leukemia (Hoffbrand *et al.*, 2006).

2.2.1.1. Acute leukemia

Characterized by presence of over 20% of blast cells in the blood or bone marrow at clinical presentation. It can be diagnosed with even less than 20% blasts if specific leukemia-associated cytogenetic or molecular genetic abnormalities are present. It is further subdivided into acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) on the basis of whether the blasts are shown to be myeloblasts or lymphoblasts **(**Hoffbrand *et al.*, 2006).

2.2.1.1.1. Acute lymphoblastic leukemia

Characterized by an accumulation of lymphoblasts in the bone marrow and is the most common malignancy of childhood.

2.2.1.1.2. Differentiation of ALL from AML

In most cases the clinical features and morphology on routine staining distinguish ALL from AML. In ALL the blasts show no differentiation (with the exception of B-cell ALL (B-ALL)) whereas in AML some evidence of differentiation to granulocytes or monocytes is usually seen in the blasts or their progeny. Specialized tests are needed to confirm the diagnosis of AML or ALL and to subdivide cases of AML or ALL into their different subtypes (Hoffbrand *et al.*, 2006).

2.2.1.1.3. Incidence and pathogenesis of ALL

ALL is the most common form of leukemia in children; its incidence is highest at 3-7years, falling off by 10 years with a secondary rise after the age of 40 years. The common (CDI0+) precursor B type which is most usual in children has an equal Gender incidence; there is a male predominance for T-cell ALL (T-ALL). The pathogenesis is varied. In a proportion of cases the first event occurs in the fetus in utero, with a secondary event possibly precipitated by infection in childhood. In other cases, the disease seems to arise as a mutation in an early lymphoid progenitor cell (Hoffbrand *et al.*, 2006).

2.2.1.1.4. Clinical features of ALL

Clinical features are a result of the following:

2.2.1.1.4.1. Bone marrow failure

Anemia (pallor, lethargy and dyspnoea); neutropenia (fever, malaise, features of mouth, throat, skin, respiratory, perianal or other infections); and thrombocytopenia (spontaneous bruises, purpura, bleeding gums and menorrhagia) are major signs (Hoffbrand *et al*, 2006)

2.2.1.1.4.2. Organ infiltration

Tender bones, lymphadenopathy, moderate splenomegaly, hepatomegaly and meningeal syndrome (headache, nausea and vomiting, blurring of vision and diplopia). Many patients have a fever which usually resolves after starting chemotherapy. (Hoffbrand *et al*, 2006).

2.2.1.1.5. Classification of ALL

This maybe on the basis of morphology or immunological markers.

Morphology the French-American-British (FAB) group sub classifies ALL into three subtypes:

2.2.1.1.5.1 L1 type

Show uniform, small blast cells with scanty cytoplasm (Hoffbrand *et al.*, 2006).

2.2.1.1.5.2L2 type

Comprise larger blast cells with more prominent nucleoli and cytoplasm and with more heterogeneity (Hoffbrand *et al.*, 2006).

2.2.1.1.5.3. L3 type

Blasts are large with prominent nucleoli, strongly basophilic cytoplasm and cytoplasmic vacuoles (Hoffbrand *et al.*, 2006).

2.2.1.1.6. Investigations

Hematological investigations may reveal a normochromic, normocytic anemia with thrombocytopenia in most cases. The total white cell may be decreased, normal or increased to 200 x 109/L or more.

Blood film examination typically shows a variable numbers of blast cells. The bone marrow is hypercellular with>20% leukaemic blasts.

The blast cells are characterized by morphology, immunological tests and cytogenetic analysis. Identification of the immunoglobulin IT-cell receptor (TCR) gene rearrangement, (aberrant) immmnophenotype and molecular genetics of the tumor cells is important to determine treatment and to detect minimal residual disease during follow-up. Lumbar

puncture for cerebrospinal fluid examination should be performed and may show that the spinal fluid has an increased pressure and contains leukemic cells (Hoffbrand *et al.*, 2006).

Biochemical tests may reveal a raised serum uric acid, serum lactate dehydrogenase or, less commonly, hypercalcaemia. Liver and renal function tests are performed as a baseline before treatment begins (Hoffbrand *et al.*, 2006).

2.3. B19 infections and acute lymphatic leukemia

The reported cases indicate that ALL patients with B19 infection typically present with persistent anemia, while rash or arthropathy is commonly absent. Children with leukemia share the chief age of B19 infections and may be particularly vulnerable to the ill effects of B19 due to immunosuppression. In one study, 8% of B19-seronegative ALL patients seroconverted while on maintenance chemotherapy, and there was a cluster of cases coinciding with a well-established B19 epidemic. B19 infection was able to mimic a leukemic relapse or therapy-induced cytopenia and contributed to the development of chronic anemia and profound thrombocytopenia in the majority of infected individuals. Infrequently, isolated thrombocytopenia or transient pancytopenia preceding ALL has also been observed (Heegaard & Brown 2002).

CHAPTER THREE

Materials and Methods

3.1. Study design

This study is case control study.

3.2. Study area

Children enrolled in this study (cases) are from different locations in Sudan attending National Institute for Cancer-University of Al Gazira, Al Gazira State.

3.3. Study population

Sudanese children (less than 15 years), 40 diagnosed with acute lymphocytic leukemia as study group (case) and 50 normal children serve as control matched by age and Gender.

3.4. Sample size

In this study 90 participants were enrolled.

3.5. Data collection techniques and tools

Data were collected from available information in the patients files were shown in (Appendix1).

3.6. Data processing

All data were coded then computerized. Statistical package for social science (SPSS) was used for data entry and analysis.

3.7. Study duration

This study was conducted during the period from January to June 2016.

3.8 Ethical consideration

This study was approved by National Ethical Committee.

3.9. Methods

3.9.1 Collection of blood specimens

Five milliliters of blood were collected on EDTA container after decontamination with ethyle alcohol from the vein, complete blood count (CBC) were done.

The plasma were separated from the cells by centrifugation 3000 rpm for 5 minutes then stored at -70°C until it was used.

3.9.2. Complete blood count (CBC)

All blood samples were firstly analyzed by using Sysmex KX-21N™ Automated Hematology analyzer to estimate HB level before it was separated to obtain plasma to be used for ELISA technique.

3.9.2 Enzyme linked immunosorbent assay (ELISA)

3.9.2.1 Principle

The ELISA test kit (EUROIMMIN, Germany) provides a quantitative *in vitro* assay for human antibodies of the IgG class against Parvovirus B19 in a serum or plasma. The test kit contains microtiter plate (96 wells) each coated with parvovirus antigen (the antigen source is recombinant viral structural protein expressed in eukaryotic cell) in the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgG antibodies will bind to the antigen. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalyzing a color reaction.

3.9.2.2. Storage and precautions of ELISA kits

All kit contents were stored at 8ºC and used before expired date on the label.

The assay standards and controls were manufactured from diluted non-infectious human serum. Clinical laboratory safety procedures were maintained all times.

3.9.2.3. Procedure

The following steps were done according to the instructions of the manufacturer (appendix 3)

3.9.2.3.1. Specimens preparation and dilution

The specimens (cases and control) were treated at the same manner, both were diluted 1:101 in buffer (provided with EUROIMMUN ELISA Kits) and mixed well by using vortex.

3.9.2.3.2. Sample incubation

From each 3 calibrators, positive control, negative control and diluted patient samples 100 µl were added into the individual microplate wells according to pipetting protocol.

The reagent wells were covered with protective foil and incubated at 37 °C for 60 minutes.

3.9.2.3.3. Washing

After the incubation, reagent wells were washed 3 times with 450µl of wash buffer (30-60 seconds per washing cycle) by using of TECAN Columbus washer.

After the final washing cycle, the strip plate was turned onto clean towel, and tapped to remove any remainders.

3.9.2.3.4. Conjugate incubation

From enzyme conjugate (peroxidase-labelled anti-human IgG), 100µl was added into each of the microplate wells. The reagent wells were covered with protective foil and incubated for 30 minutes at room temperature.

3.9.2.3.5. Washing

After the incubation, reagent wells were washed 3 times with 450µl of wash buffer (30-60 seconds per washing cycle) by using of TECAN Columbus washer.

At the end of washing cycle, the strip plate was turned onto clean towel, and tapped to remove any remainders.

3.9.2.3.6 Substrate incubation

From chromogen/substrate (TMB/H_2O_2) 100µl was added into each of the microplate wells and incubated for 15 minutes at dark area.

3.9.2.3.7 Stopping of the reaction

Stop solution (0.5 M sulphuric acid) was added into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

3.9.2.3.8 Measurement

Photometric measurement of color intensity was made at a wavelength of 450nm and reference wavelength between 620 nm and 650 nm within 30 minutes of adding the stop solution.

3.9.2.3.9 Calculation of the results

Results were evaluated semi quantitatively by calculating a ratio of the extinction value of the patient sample over the extinction value of calibrator 3 according to following formula:

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

3.9.2.3.10 Interpretation of the results

Ratio <0.8: negative

Ratio ≥0.8 to <1.1: borderline

Ratio ≥1.1: positive

3.9 Data analysis

The data were analyzed using SPSS computer program (software version 16).

CHAPTER FOUR

4. RESULTS

4.1 Study population

A total of 90 children, 40 were suffering from ALL (cases) and 50 were healthy as control from different areas in Sudan were enrolled in this study. The distribution of samples are represented in table 1.

Table 1: **Distribution of specimens**

Participants	**n**	**%**
Leukemic children (Cases)	40	44
Healthy children (Control)	50	56
Total	90	100

n: Number

4.2 Seroprevalence of B19 virus among participants

The overall distribution of B19 IgG antibodies among study and control groups were shown in table 2. The highest seropositivity was noticed among study group (cases) was 16 (40%) compared with control group which was 13 (26%**).** No significant statistical difference between case and control group in prevalence of B19 virus.

Table 2. Frequency of B19 virus among participants

B19 virus	**Participants**					
	Cases		Control		**Total**	
	n	%	n	%	n	%
Positive	16	40	13	26	29	32
Negative	24	60	37	74	61	68
Total	40	100	50	100	90	100

P value= < 0.05

4.3 B19 virus and Gender

The distribution of specimens according to Gender were shown in table 3, and the distribution of B19 IgG antibodies according to Gender are shown in Fig 1.

22% of males were positive to B19 virus while 10% of females were positive to same virus but there is no significant association between B19 seropositivity and Gender (*P* value = 0.491).

Table 3. Specimens distribution according to Gender

Participants	Gender			
	Male		Female	
	n	%	n	%
Cases	29	72.5	11	27.5
Controls	28	56	22	44
Total	57	63	33	37

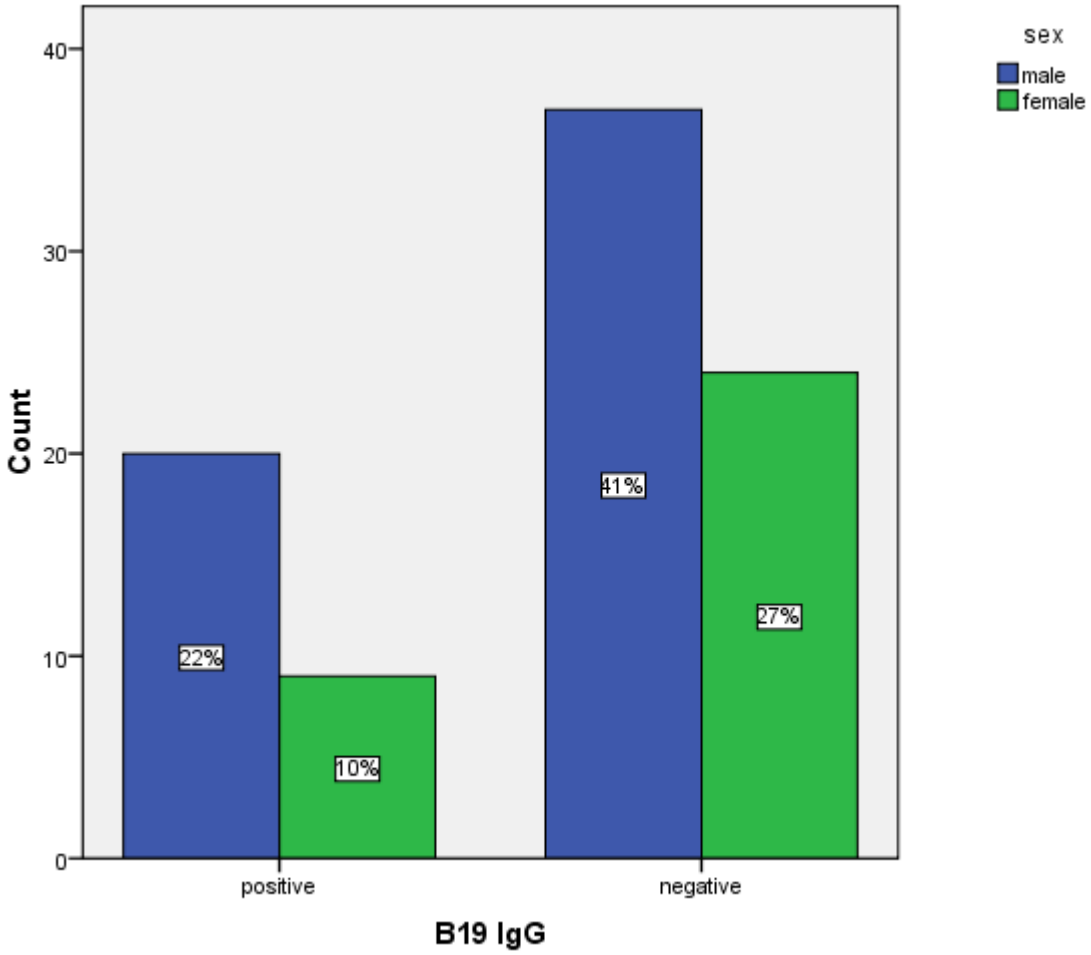

Fig 1. B19 virus according to Gender

4.4 B19 IgG and Acute lymphocytic leukemia

The prevalence of B19 IgG antibodies among children suffering from ALL (cases) were 40% and 26% in control group.

No significant association between B19 seropositivity and ALL (*p* value =.179).

4.5 B19 and HB level

HB levels (gm/dl) among case and control group are shown in table 4. There was significant difference between HB levels among cases and controls *(p* value = .03).

Analysis of the effect of B19 infections on the level of hemoglobin (HB) is presented in table 5, it shows that 37.9% of patients who were positive to B19 antibodies had HB levels range of 5-10 gm/dl, 58.6% had HB level more than 10 gm/dl and 3.4% had HB level less than 5 gm/dl as compared to the B19 seronegative group which showed HB levels of 5-10gm/dl among 25.5%, 2.2% with HB level less than 5 mg/dl and 72.2% with greater than 10 mg/dl.

No significant association between B19 IgG and HB levels (*p* value =.228)

Table 4. **HB levels (gm/dl) among case and control group**

Participants	HB level gm/dl							
	5>		5-10		10<		Total	
	n	%	n	%	n	%	n	%
Cases	2	5	14	35	24	60	40	100
Controls	0	0	9	18	41	82	50	100

Table 5. **Effect of B19 virus on HB level**

B19 IgG	HB level gm/dl							
	5>		5-10		10<		Total	
	n	%	n	%	n	%	n	%
Positive	1	3	11	38	17	59	29	100
Negative	1	2	12	20	48	79	61	100

CHAPTER FIVE

DISCUSSION

Certain populations are vulnerable to complications from depressed erythropoiesis that arises with parvovirus B19 infection. These populations include patients with hematological disorders that cause an increase in erythrocyte destruction (Azzazy *et al.*, 2013).

In this study, there was difference in the prevalence of B19 virus IgG among ALL cases compared to control group (normal children) which was 40% vs 26% respectively. These results are considered relatively high because all of the patients involved in this study were in high risk population (leukemic). This result is similar to that reported by (EL-Mahallawy *et al*., 2004) which was 38%.

(Zakie *et al*., 2006) in Egypt, reported that B19 IgG in ALL children under chemotherapy was 45% and (Ibrahem *et al*., 2014) in Basrah, Iraq which was 47.5%

Higher prevalence of B19 virus reported by (Azzazy *et al*., 2013), Egypt was 50%.

Our results show that 37.9% of patients who were positive to B19 antibodies had HB levels range of 5-10 gm/dl, 58.6% had HB level more than 10 gm/dl and 3.4% had HB level less than 5 gm/dl

Our results differ from which reported by (Ibrahem *et al*., 2014) which was 63.2%, 26.3% and 26.3 respectively.

B19 seronegative group showed HB levels of 5-10gm/dl among 25.5% and 72.2% with greater than 10 mg/dl. It is similar to that reported by (Ibrahem *et al*., 2014) which was 19%, 71.4% respectively.

2.2% of B19 seronegative group have HB level less than 5 mg/dl.

No significant association between B19 IgG and HB level (*p* value =.228) and this results is differ from (Ibrahem *et al*., 2014) in which there is significant association between low HB level and B19 seropositivity.

These variations could be attributed to different environmental conditions between our study area and other study areas reported.

5.2 Conclusion

B19 infection is highly prevalent among children with ALL in comparison to normal ones. ALL patients are at high risk group, they are susceptible to many viral infections.

Although, in this study there is no significant association between low HB levels and B19 virus seropositivty (may due to small sample size) but B19 virus must be suspected and screened for when anemia is present in children suffering from ALL.

5.3 Recommendations

• In patients with acute lymphocytic leukemia under chemotherapy who have unexpected anemia or cytopenia, B19 infection should be considered before a change in chemotherapy protocol.

• The serological testing needs to be coupled with direct detection of B19 DNA by using PCR for more reliable diagnosis of B19 infections in those children

• Measures to avoid nosocomial transmission must be implemented, including screening of donated blood for B19 DNA, especially blood given to patients with ALL and introduction of an approved B19 vaccine

• Further studies involving serial bone marrow examination for B19 DNA and other genetic studies to establish or deny the association between B19 infection and ALL are also recommended.

References

1. **Azzazy**, **E.A., Shaheen, A.A., Mousaad, A.A., Abdel, M.M., Ibrahim, R.A. (2013**). The prevalence of human parvovirus B19 infection in children with a variety of hematological disorders. *Egyptian Journal of Hematology* **38** (3). p.115-121.

2. **Carter, J. B., & Saunders, V. A. (2007).** *Virology: Principles and Applications.* 2ed edition**.** Chichester, England: John Wiley & Sons 253-254.

3. **Collier, L., Kellam, P., Oxford, J. (2011**). *Human virology*. Oxford University Press. **4.** 259-263.

4. **David Greenwood, Barer, Richard Slack & wil Irving. (2007).** *Medical Microbiology: A Guide to Microbial Infections: Pathogenesis, Immunity, Laboratory Diagnosis and Control*. 18 edition. Churchill livingstone.154-166.

5. **El-Mahallawy, H. A., Mansour, T., El-Din, S. E., Hafez, M., & Abd-El-Latif, S. (2004).** Parvovirus B19 Infection as a Cause of Anemia in Pediatric Acute Lymphoblastic Leukemia Patients during Maintenance Chemotherapy. *Journal of Pediatric Hematology and Oncology*. **26**(7). 403-406.

6. **Erik D. Heegaard and Kevin E. Brown (2002).** Human Parvovirus B19: Pathogenesis of Disease. *Clinical Microbiology review*. **15** (3). 485-505.

7. **Florea, A. V, Ionescu, D.N., Melhem, M.F. (2007**). Parvovirus B19 Infection in the Immunocompromised Host. *Arch Pathol Lab Med.* **131** (5).799-804.

8. **Hoffbrand, A. V., Moss, P. A., & Pettit, J. E. (2006)**. Essential Hematology. 5th ed. Malden, MA: Blackwell Pub. 129-174.
9. **Ibrahem, W.N., Hasony, H.J., Hassan, J.G. (2014).** Human parvovirus B19 in childhood acute lymphoblastic leukemia in Basrah. *J. Pak. Med. Assoc.* **64** (1). 9-12.

10. **Kishore, J., Sen, M., Kumar, A., Kumar, A. (2011**). A pilot study on parvovirus B19 infection in paediatric haematological malignancies. *Indian J Med Res*. **133** (3). 432-434.

11. **Knipe, D. M., Howley, P. M., Fields, B. N., & Chanock, R. M. (2007).** Field virology. 5th ed., Vol. 1, Philadelphia: Lippincott

12. **Lindblom, A., Heyman, M., Gustafsson, I., Norbeck, O., Kaldensjö, T., Vernby, A., Henter, J.-I., Tolfvenstam, T., Broliden, K. (2008)**. Parvovirus B19 infection in children with acute lymphoblastic leukemia is associated with cytopenia resulting in prolonged interruptions of chemotherapy. *Clin Infect Dis.* **46** (4). 528-536.

13. **Patrick R. Murray, Ken S. Rosenthal & Michael A. Pfaller.** *Medical Microbiology* **(2008).** 6th ed. Mosby. 203-219.
14. **Surinder Kumar (2012).** *Textbook of Microbiology*. Jaypee Brothers Medical Pub. 311-321.

15. **Zaki, M. E., Hassan, S. A., Seleim, T., & Lateef, R. A. (2006).**

Parvovirus B19 infection in children with a variety of

hematological disorders. *Journal of Hematology*. **11**(4). 261-266.

بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate studies

Appendix 1

…… Name

……Age

……Gender

.……Residence

..……Diagnosis

HB level: …….gm/dl

96 wells ELISA microtiter plate