PREVALENCE OFPARVOVIRUS B19 ANTIGEN AMONG PREGNANT WOMEN AND SICKLE CELL PATIENTS IN YOBE STATE

BY

KALTUME BULAMA YUSUF
SPS/16/MML/00028

SUPERVISORS:
DR. LAWAL DAHIRU ROGO
PROFFESSOR MUHAMMAD YALWA GWARZO

A RESEARCH SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCE, FACULTY OF ALLIED HEALTH SCIENCES, COLLEGE OF HEALTH SCIENCES, BAYERO UNIVERSITY KANO, IN PARTIAL FULFILLMENT OF THE REQUIREMENTSFOR THE AWARD OF MASTER DEGREE IN MEDICAL LABORATORY SCIENCES (IMMUNOLOGY)

MAY, 2019.

DECLARATION

I hereby declare that this work is the product of my research efforts undertaken under the supervision of Dr. Lawal Dahiru Rogoand Prof. Muhammad Yalwa Gwarzo and has not been presented anywhere for the award of a degree or certificate. All sources have been duly acknowledged.

…….. ‘,,,,,,,,,,,,,,,,,,,,,,,,

KALTUME BULAMA YUSUF Date
(SPS/16/MML/00028)

CERTIFICATION

This is to certify that the research work titled 'PREVALENCE OF PARVOVIRUS B19 AMONG PREGNANT WOMEN AND SICKLE CELL PATIENTS IN YOBE STATE' meets the regulations governing the award of Masters Degree in Medical laboratory Science of the Bayero University Kano and is approved for its contribution to knowledge and literary presentation.

_____ _____

Dr. Lawal Dahiru Rogo Date
Supervisor

_____ _____

Prof. Muhammad Yalwa Gwarzo Date
Supervisor

_____ _____

Dr. Isah Abubakar Aliyu Date
Head of Department: Medical Laboratory Science

APPROVAL

This dissertationtitled**"Prevalence of Parvovirus B19 Antigen among Pregnant Women and Sickle Cell Patients in Yobe State"**has been examined and approved for the award of Master Degree in Medical Laboratory Sciences (Immunology)

_____	_____
Dr.Banda Jim Monday (External Examiner)	DATE
_____	_____
Dr. Hadiza Lawal Abdullahi (Internal Examiner)	DATE
_____	_____
Dr. Lawal Dahiru Rogo (Supervisor)	DATE
_____	_____
Prof. Mohammed YelwaGwarzo (Supervisor)	DATE
_____	_____
Dr. Isa Usman Lawal (School of Postgraduate Representative)	DATE
_____	_____
Dr. Isah Abubakar Aliyu (Head of Department/Chief Examiner)	DATE

ACKNOWLEDGEMENTS

All Praise is due to Allah, the most Beneficent and most Merciful. Who has always been the source of my strength, endurance, knowledge, wisdom and understanding. May peace be upon the most chosen servant of Allah, Muhammad (S.A.W), the best of mankind, his family, companions and those on the path of seeking knowledge till the last hour (Ameen).

I wish to record my sincere thanks to my supervisors Dr. L. D. Rogo and Prof. M. Y. Gwarzo for their guidance and cooperation during the supervision of this dissertation. I wish to acknowledge the motivation and contribution I got from all the lecturers of Department of Medical Laboratory Science. My sincere thanks go to my Dad, Late Alhaji Bulama Yusuf for all the support he gave me right from childhood. May Allah reward him abundantly Ameen.

DEDICATION

I dedicate this work to my lovelyfather.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
B19V	Parvovirus B19
SCD	Sickle Cell Disease
TAC	Transient Aplastic Crises
NIHF	Non Immune Fetal Hydrops
PRCA	Pure Red Cell Aplasia

LIST OF APPENDICES

ABSTRACT

Human parvovirus B19 has been implicated as a primary etiologic agent of Aplastic crisis in patient with sickle cell disease and also known to be associated with adverse effect on fetuses such as hydrops fetalis, intrauterine fetal death and spontenouse abortion in pregnant women.Human parvovirus B19 (B19V) is a small (22–24 nm) nonenveloped DNA virus belonging to the genus Erythrovirus and Family*Parvoviridae.*B19V Infection may have a different outcomein patients with inherited hemolytic anemia resulting in bone marrow suppression, triggering a life-threatening drop of hemoglobin values.This study determined the prevalence of *parvovirus* B19 among pregnant women and sickle cell disease patients in Yobe state. Atotal number of 372 participants were included in the study, consisting of 186 pregnant women and 186 sickle cell patients of age ranging from 1 to 50 years. Specific Antigen was measured using a ELISA kit according to manufacturers instruction (B19V ELISA antigen kit - Melsin Medical Company China).Antigen was observed to be prevalent (44%) among the study population. The antigen-prevalence of pregnant women was found to be 42% and that of sickle cell disease patient was 47%.Overall there was higher percentage of B19V in the lower age group 1 – 10 and minimal percentage of B19V antigen in the age group between 41 and 50. In conclusion, the presence of B19V antigen was found to be higher in sickle cell disease patients thanin pregnant women, this indicate that sickle cell disease patients has higher chance of B19V infections compared to pregnant women.Significant percentage of child-bearing aged women are at risk of primary infection with *parvovirus* B19 which could adversely affect their pregnancy.

It is thus important to include screening for parvovirus B19 in routine antenatal clinic and sickle cell disease clinic visits. Investigation for *parvovirus* B19 is recommended in cases of severe anemia.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Human Parvovirus B19 (B19V) is a small (22–24 nm) non enveloped DNA virus belonging to thefamily*Parvoviridae, subs* family *Parvovirinae* genus Erythrovirus. Although it generally causes self-limiting infection in immunocompitent individuals.B19 infection may have a different outcome in patients with sickle cell diseases and in pregnant women (Jegede*et al.,*2014).

The form of clinical disease associated with human Parvovirus B19 (B19V) varies and depends on both the haematological and immunological status of the infected individual (Slavov *et al.,* 2011). B19V infections are frequently associated with mild disease, but in immunocompromised and anemic patients, complications can arise (Hubschen *et al.,* 2009). Parvovirus B19 is associated with multiple conditions and Nigeria is among the highest ranked in the worldwide occurrence of sickle cell disease (SCD) with several etiologies and associated complications (Brown, 2015; Iwalokuna *et al.,* 2013; Landry, 2016). It was suggested that Parvovirus B19 can have significant marrow aplastic effects, even in immunocompetent individuals (Rajput *et al.,* 2012). There are currently no approved vaccines for the prevention of B19 virus*(*Jegede *et al.,*2014). However, virus like particlesbased Parvovirus B19 vaccine candidates have been produced by co-expressing VP2 and either wild-type VP1 or phospholipasenegative VP1 in a regulated ratio from a plasmid sin *Saccharomycescerevisiae* (Chandramouli *et al.,* 2013).

Although the outcome of transient red cell aplasia occurrences in children with SCD is mostly non-threatening but many are treated with red cell transfusions to minimize the threat of circulatory collapse due to severe anemia (Serjeant *et al.,* 1993). Hydroxyurea may reduce the requirements for blood transfusion and may attenuate symptoms during transient aplastic crisis episodes caused by Parvovirus B19 (Hankins *et al.,* 2016). In Nigeria, this virus is not routinely screened for during pregnancy and sickle cells diseases.

Human Parvovirus B19 was recently recognized as the cause of non-immune hydrops fetalis and intrauterine fetal death (Andrea *et al.,* 1999). Parvovirus B19 replication within erythroid progenitor cells leads to apoptosis, which ultimately results in inhibition of erythropoiesis (Heegaard *et al.,* 2002). Erythroblastopenia can then occur as a consequence of B19 replication, causing severe fetal anemia. Transmission occurs most commonly by personal conta ct via aerosol or respiratory secretions; however, contaminated blood products, such as clotting factor concentrates, are a source of iatrogenic transmission (Heegaard and Brown, 2002; Bdour, 2006). B19 can be transmitted transplacentally from an infected mother to the fetus, which leads to non-immune fetal hydrops (NIHF), spontaneous abortion, or intrauterine fetal death (Danil *et al.,* 2004). The fetus seems to be most susceptible to Parvovirus B19 infection during the first and second trimester of pregnancy and especially between weeks 10 and 20, which coincide with the major development of the erythroid precursors (Heegaard *et al.,* 2002). Parvovirus B19 has a propensity for infecting rapidly dividing cells, particularly erythroblasts (Jeanne *et al.,* 2001). Between the third and sixth months of pregnancy, the fetal red blood cell mass increases thirty times, with a risk of developing anemia if the fetus is infected by Parvovirus B19 (Elnifro*et al.,*2009). By the third trimester, the fetus is able to mount a more effective immune response to the virus, which may account for the decrease in fetal loss at this stage of pregnancy (Sukanya *et al.,* 2006).

1.2 Statement of Problem

There is paucity of information on the prevalence of B19 virus in the research area, thus warranting the need for a study of this nature to provide informed preventive strategies and management of the resulting complications in both pregnant women and sickle cell anemia.Most of the previous researchers employed the use of Antibody detection method, but this research, the researcher employed the use of Antigen detection which identifies the presence of the B19 virus and is also capable of detecting all the three (3) genotypes simultaneously.

1.3 Justification

Human Parvovirus B19 has been implicated as a primary etiologic agent of aplastic crisis in patients with sickle cell diseases and is also known to be associated with adverse effects on fetuses such as hydrops fetalis, intrauterine fetal death, and chronic anemia in pregnant women.(Iwalokuna *et al*,.2013). Parvovirus B19 is associated with multiple conditions and Nigeria is among the highest ranked in the world wide occurrence of sickle cell disease with several etiologies and associated complications (Iwalokuna *et al.,* 2013; Brown, 2015; Landry, 2016).

1.4 Aims and Objectives of the Study

1.4.1 Aims

The aim of the study is to determine the prevalence of parvovirus B19 among pregnant women and sickle cell patients in Yobestate.

1.4.2 Objectives of the Study

i. To determine the presence of human Parvovirus B19 antigen among pregnant women in Yobe State.

ii. To determine the presence ofhuman Parvovirus B19 antigen among patient with sickle cell anemia.

iii. To determine the relationship if any between the presence of the human Parvovirus B19 among pregnant women and sickle cell disease and the difference of age.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Parvovirus B19V

The form of clinical disease associated with human Parvovirus B19 (B19V) varies and depends on both the haematological and immunological status of the infected individual (Slavov *et al.,* 2011). B19V infections are frequently associated with mild disease, but in immunocompromised and anemic patients, complications can arise (Hubschen *et al.,* 2009). Parvovirus B19 is associated with multiple conditions with several etiologies and associated complications (Iwalokuna *et al.,* 2013; Brown, 2015; Landry, 2016).Rajput *et al.*(2012) suggest that Parvovirus B19 can have significant marrow aplastic effects even in immunocompetent individuals. There are currently no approved vaccines for the prevention of B19 virus, however, virus like particlesbased Parvovirus B19 vaccine candidates have been produced by co-expressing VP2 and either wild-type VP1 or phospholipasenegative VP1 in a regulated ratio from a plasmid in *Saccharomycescerevisiae* (Chandramouli *et al.,* 2013).

Although the outcome of transientred cell aplasia occurrences in children with SCD is mostly non-threatening, many are treated with red cell transfusions to minimize the threat of circulatory collapse due to severe anaemia (Serjeant *et al.,* 1993). Hydroxyurea may reduce the requirements for blood transfusion and may attenuate symptoms during transient aplastic crisis episodes caused by Parvovirus B19 (Hankins *et al.,* 2016). In Nigeria, this virus is not routinely screened for during pregnancy and sickle cells diseases.

Human Parvovirus B19 wasrecently recognized as the cause of non-immune hydrops fetalis and intrauterine fetal death (Andrea *et al.,*1999). Parvovirus B19 (B19 V) is a small, non-enveloped, single-stranded DNA virus in the family *Parvoviridae* (Amoda and Doyle, 2004).

B19 replication within erythroid progenitor cells leads to apoptosis, which ultimately results in inhibition of erythropoiesis (Heegaard and Brown, 2002). Erythroblastopenia can then occur as a consequence of B19 replication, causing severe fetal anemia (Bdour, 2006). Transmission occurs most commonly by personal contact via aerosol or respiratory secretions; however, contaminated blood products, such as clotting factor concentrates, are a source of iatrogenic transmission (Heegaard and Brown, 2002; Bdour, 2006).Parvovirus B19 can be transmitted transplacentally from an infected mother to the fetus, which leads to non-immune fetal hydrops (NIHF), spontaneous abortion, or intrauterine fetal death (Danil *et al.*,2004). The fetus seems to be most susceptible to Parvovirus B19 infection during the first and second trimester of pregnancy and especially between weeks 10 and 20, which coincide with the major development of the erythroid precursors (Heegaard *et al.,* 2002).Parvovirus B19 has a propensity for infecting rapidly dividing cells, particularly erythroblasts (Jeanne *et al.,* 2001). Between the third and sixth months of pregnancy, the fetal red blood cell mass increases thirty times, with a risk of developing anemia if the fetus is infected by Parvovirus B19 (Iwalokuna *et al,*. 2013). By the third trimester, the fetus is able to mount a more effective immune response to the virus, which may account for the decrease in fetal loss at this stage of pregnancy (Sukanya *et al.,* 2006).

Human Parvovirus B19 (B19V) is a small, non enveloped DNA virus belonging to the genus Erythrovirus,*Parvoviridae* family. Its capsid comprises 60 capsomers (VP1 and VP2) surrounding a single-stranded (ss) DNA genome (Kaufman *et al.,* 2004). VP1 protein is a dominant target for the neutralizing antibodies and is located on the external surface of the virion (B¨onsch *et al.*,2008). The mechanism of B19V replication is exceptional: DNA is replicated through high-molecular-weight intermediates linked through hairpin structures because of the presence of genomic palindromes (Ozawa *et al.,* 1986).

The pattern of clinical disease caused by B19V varies and is influenced by both the hematological and the immunological status of the infected individual. In healthy hosts, B19V generally causes self-limiting subclinical erythroid aplasia followed by a rash or arthralgia. (Heegaard and Brown, 2002; Zaki *et al.,* 2006; Krishnamurti *et al.,* 2007). However, in patients with diminished production or increased loss of erythrocytes, it is now clear that B19V infection results in a severe drop of hemoglobin values and anemia which could be life-threatening (Heegaard and Brown, 2002; Zaki *et al.,* 2006; Krishnamurti *et al.,* 2007; Toyokawa *et al.,* 2007). In 1981, B19V was implicated as the etiologic agent of severe a plastic crises in children with sickle-cell disease (SCD), in whom this infection can evolve into various life threatening conditions (Serjeant *et al.,* 1981) including acute encephalopathy (Bakhshi *et al.,* 2002), nephrotic syndrome (Quek *et al.,* 2010), splenic sequestration (Yates *et al.,* 2009) and fatal bone marrow embolism (Rayburg *et al.,* 2010).

2.2 Discovery and Brief History

B19V was discovered accidentally in 1974 in the serum of an asymptomatic blood donor by Yvonne Cossart's group in London (Cossart *et al.,* 1975). The viral name originated from the identification of the tested sample: number 19 of panel B. The electron microscopy studies which followed revealed 23-nm viral particles similar to those of the animal Parvoviruses (Heegaard and Brown, 2002; Brown, 2009). Characterized during 1983–1984, the viral genome of B19V proved to be a single _5.5 kb DNA molecule (Summers *et al.,* 1983) surrounded by superficial proteins copurifying at a density of 1.43 g cm^3 (buoyant density for Parvoviruses in CsCl: 1.38–1.45 g cm^3) (Clewley, 1984; ICTV, 2007). Description of B19V clinical entities coincided with its virological characterization.

The first connection between B19V and symptomatic disease was made in 1981, when it was demonstrated that it causes transient aplastic crisis (TAC) (Pattison *et al.,* 1981) in patients with SCD. *Erythema infectiosum* ('fifth disease') in children was consequently linked to acute B19V infection (Anderson *et al.,* 1983). Following studies described B19V-induced fetal loss (hydropsfetalis) (Brown *et al.,* 1984) during pregnancy and symmetrical arthropathy in adults (Reid *et al.,* 1985; White *et al.,* 1985).

2.3 Taxonomy

B19V is a member of the family *Parvoviridae,* subdivided into *Parvovirinae* and *Densovirinae* depending on the type of the infected host (vertebrate or invertebrate). Parvovirinae is further divided into five genera (Amdovirus, Bocavirus, Dependovirus, Erythrovirus and Parvovirus) due to differences in transcription, organization of their terminal repeats and host range. B19V is an autonomously replicating virus, as its life cycle is supported only in rapidly dividing erythroid cells. Therefore, B19V is classified within the genus Erythrovirus as a prototype. The genus Parvovirus comprises most of the Parvoviruses infecting vertebrates with a prototype member minute virus of mice (MMV). Dependovirus includes adeno-associated viruses and two avian viruses (duck and goose Parvovirus). Amdovirus has only one member, the Aleutian mink disease virus, which causes lethal infection among minks. The Bocavirus genus has as a prototype the bovine Parvovirus. Viral structure B19V is a non enveloped virus whose icosahedral virion is composed of two structural proteins (VP1 and VP2) surrounding a linear, ssDNA molecule with positive or negative polarity. Viral capsid proteins the virion consists of 60 structural subunits, 95% of which belong to VP2 (58 kDa) and 5% to VP1 (83 kDa) (Kaufman *et al.,* 2004; B¨onsch *et al.,* 2008).

VP1 and VP2 originate from overlapping reading frames and are identical with the exception of 227 additional amino acids at the N-terminus of VP1, called VP1 ‘unique region’ (VP1u) (Kaufman *et al.,* 2004). VP1u exhibits relatively high sequence variability in persistently infected individuals ranging from 3.5% (Heegard *et al.,* 2002) to 6.8/8.2% (Hemauer *et al.,* 1996). Such variation could cause nonconservative amino acid alterations (uncharged amino acid residues are replaced with polar amino acids). This region plays an important role in eliciting immune responses, with this type of mutations probably leading to a modified viral surface and inability of the infected organism to clear B19V, and the development of persistent infection (Hemauer *et al.,* 1996). In contrast to the mammalian Parvoviruses and despite its low concentration in the virion, VP1u is the major antigenic target for neutralizing antibodies. It also possesses phospholipase A2 (PLA2) motif, which takes part in the initiation of the infection. VP1u is thought to play an important role in the induction of autoimmune responses and chronic inflammation by mechanisms that are not fully understood (B¨onsch *et al.,* 2008).

2.4 Nonstructural Proteins, NS1

B19V possesses several nonstructural (NS) proteins, the most abundant of which is NS1. It has a molecular mass of 77 kDa and multiple functions, including site-specific DNA-binding, DNA-nicking, helicase function and regulation of gene transcription. NS1 trans-regulates the viral P6 promoter as well as some cellular promoters and thus controls B19V replication (Zhi *et al.,* 2006). This protein is highly cytotoxic for erythroid precursors and can induce apoptosis via interaction with caspase 3 (Moffat *et al.,* 1998;Morita *et al.,* 2001).Most of the B19V-associated clinical manifestations are thought to be related to damage of the infected erythroid precursors by NS1 cytolytic or apoptotic activity (Morita *et al.,* 2001).

2.5 Viral Genome

B19V genome is composed of internal 4830 nt coding sequences flanked by 383 nt terminal repeats (Heegaard and Brown, 2002). The distal 361 nucleotides of each repeat form a palindrome, capable of assembling hairpins usually designated as ‘forks’ (Mori *et al.,* 1987). The hairpins exist in two distinct configurations, named ‘flip-flop’, which are found in all Parvoviruses (Deiss *et al.,* 1990) and serve as initiation sites for replication (Ozawa *et al.,* 1987). B19V genome is divided hypothetically into two main ORFs with NS1 protein encoded by the ‘left’ genome portion and the capsid proteins by the ‘right’ one (Heegaard and Brown, 2002). The coding potential of such a small genome is increased by the utilization of multiple reading frames that generate large, overlapping transcripts. B19V has approximately nine overlapping poly-(A) transcripts that differ from the other Parvoviruses (Ozawa *et al.,* 1987). A short ORF encoding protein X and located within the VP1 gene has been described recently. The functions of this hypothetical protein for the B19V infectious process have not been defined due to limitations of viral cultivation and lack of infectious clones (Zhi *et al.,* 2006). It is has also been proposed that the invasion of the single-stranded B19V genome during infection can induce DNA damage and cellcycle arrest (Chen and Qiu, 2010).

2.6 B19V Genetic Variability

At present, three B19V genotypes (1, 2 and 3) have been identified with an estimated divergence of more than 10% among them (Servant *et al.*, 2002). Genotype 1 is the most prevalent genotype worldwide with a prototype B19V. Because of genotype 1 inter-cluster variability of approximately 2% is additionally subdivided into two subgroups (1A and 1B) (Toan *et al.,* 2006).

Genotype 2 (prototypes A6 and LaLi) has been detected in patients from several European countries, the United States and Brazil (Toan *et al.,* 2006; Freitas *et al.,* 2008; Grabarczyk *et al.,* 2011).

Genotype 3 (prototypes V9 and D91.1) is divided into two subgenotypes: 3A and 3B. Circulation of genotype 3 has been described mainly in tropical countries such as Brazil (Freitas *et al.,* 2008) and Ghana (Parsyan *et al.,* 2007).

Recently, a new Parvovirus sequence PARV4 was discovered in clotting factor concentrates (Jones *et al.,* 2005). PARV4 differs genetically from the B19V genotypes described to date and is probably a new genetic type of the family Parvoviridae (Simmonds *et al.,* 2008). Although there is a significant divergence among the sequences of the viral genotypes, most of the nucleotide changes are at a third position and the amino acid variation is reduced. As a consequence, the structural proteins have approximately 96% similarity and low antigenicity, which is responsible for the high serological degree of cross-reactivity among the different B19V genotypes in clinical samples (Heegard *et al.,*2002).

2.7 B19V Life Cycle

The life cycle, tropism and transcriptional map of B19V are highly specific for this pathogen, distinguishing it from all other members of the Parvoviridae into a separate genus (Erythrovirus). B19V is an autonomously replicating Parvovirus. Compared with MMV, which produces three transcripts (R1, R2 and R3) terminating at one single polyadenylation site (Wan *et al.,* 2002), B19V utilizes at least 12 RNA transcripts, all processed by alternative splicing (Ozawa *et al.,* 1987).

B19V has multiple polyadenylation sites located in the middle of the genome and its right-hand portion (Amand St *et al.,* 1991). B19V replication occurs in the nucleus of the infected cell and includes common stages for the most DNA viruses: (1) attachment of the virus to the host cell receptors; (2) internalization; (3) translocation of the genome to the nucleus; (4) DNA replication; (5) RNA transcription; (6) assembly of the capsids; (7) packaging of the genome; and (8) cell lysis with release of infectious virions (Heegaard and Brown, 2002) (Fig. 1).

2.8 Tropism, Viral Receptors and Uncoating

B19V has exceptional tropism to human erythroid progenitors, fetal liver and umbilical blood erythroblasts (Heegaard and Brown, 2002). Factors responsible for this property include the possession by erythroid cells of blood group P antigen (globoside, Gb4), which is the main B19V receptor

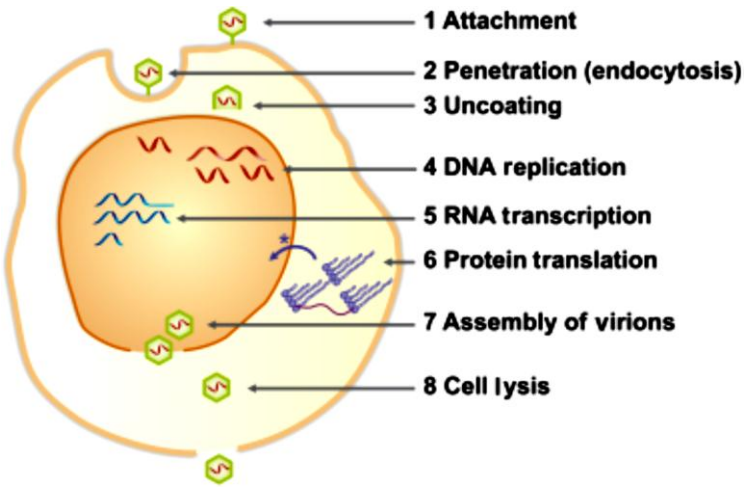

Fig. 1. Life cycle of B19V in an erythroid progenitor cell.

Source: https://academic.oup.com/femspd/article-abstract/62/3/247/598583

The attachment (1) is probably mediated by the P-antigen in combination with (a)-5 (b)-1integrins, after which B19V is translocated into the cytoplasm by endocytosis (2). The uncoating (3) occurs in the cytoplasm and the viral DNA is transported to the nucleus, where replication (4) and transcription (5) take place. After the translation (6) of the viral proteins in the cytoplasm, they are transported back to the nucleus (arrow with asterisk), where the assembly (7) of the virions into large inclusion bodies occurs. B19V leaves the cell by lysis (8) and causes a pronounced cytopathic effect in the bone marrow and the rapid division of erythroid cells, which provides indispensable intracellular factors for optimal B19V replication and transcription. Some individuals who genetically lack P antigen are naturally resistant to B19V infection (Brown, 2004). Nevertheless, other cellular types (especially those in kidney, liver tissues, synoviocytes, platelets, endothelium and vascular smooth muscle) that express P antigen do not allow B19V entry (Simeoni *et al.,* 2010). Thereby, P antigen is a necessary but not a sufficient factor for productive infection. That is why it is suggested that the cell entry is additionally mediated by (a)-5 (b)-1 integrins in high affinity confirmation.

Mature red blood cells that express high levels of P antigen, but not (a)-5 (b)-1 integrins, bindB19V but do not allow viral entry. In contrast, erythroid progenitors that possess high levels of P antigen and (a)-5 (b)-1 integrins allow P antigen/integrin-mediated entry. It is believed that in the natural course of the viral pathogenesis, red blood cells serve as efficient disseminators of B19V in the body (Weigel-Kelley *et al.,* 2003).

The globoside is also thought to mediate the capsid rearrangements required for the subsequent interactions leading to virus internalization. These findings may explain why virus tropism is restricted to erythroid progenitor cells that contain high levels of P antigen and coreceptors, and a number of P antigen-positive nonerythroid cells that do not permit efficient replication because of the lack of coreceptors (Simeoni *et al.,* 2010).

After binding to the receptors, B19V probably enters the target cell through clathrin-mediated endocytosis. The uncoating mechanism of B19V is not clear but the rigid organization of the capsid suggests that the genome is released without virion disassembly (Ros *et al.*, 2006).

2.9 Replication

Despite the small genome, B19V replication is a complex process because the DNA is both single-stranded and linear. B19V disrupts the functions of the synthetic machinery of the host cell for its own replication purposes using an ancient strand-displacement mechanism called rolling-circle replication. As a consequence, B19V replicates through DNA intermediates that are linked through their terminal hairpins. The 30 terminal end of eachpalindrome serves as a self-primer for the initiation of the synthesis for a plus sense DNA. During B19V replication, the imperfectly paired termini provide an energetically favorable environment for unfolding and refolding the hairpin.

B19V dimeric replicative forms are usually detected in infected bone marrow and therefore it is believed that the growing strand replicates back on itself to produce a tetrameric form, from which are generated two plus and two minus strands (White and Fenner, 1994).

2.10 Transcription

During B19V transcription at least 12 transcripts are generated by alternative splicing and polyadenylation from a single pre-mRNA. The polyadenylated transcripts are synthesized as spliced or unspliced mRNAs. The unspliced mRNAs encode the NS1 protein, and the transcripts polyadenylated internally at pA-p (proximal) position encode a 7.5-kDa protein with unknown function. All B19V transcripts polyadenylated at the distal polyadenylation site (pA-d) excise their first intron (D1 to A1-1 or D1 to A1-2).

Those that are not additionally spliced encode the capsid proteins VP1 and VP2. mRNAs further subjected to splicing with excision of the second intron (D2 to A2-1) encode one nonstructural 11-kDa protein thought to play a role in B19V transportation from the nucleus (Guan *et al.,* 2008).

2.11 Pathogenetic and Clinical Aspects of B19V Infection Transmission

The transmission of B19V occurs mainly via respiratory droplets but it can also be spread by contaminated blood, organ transplantation and vertical transmission from mother to fetus (Heegaard and Brown, 2002). After respiratory acquisition of B19V, a short replication occurs in the nasopharyngeal lymphoid tissue, followed by a massive viremia with a viral load that can exceed 1013 copies (Anderson *et al.,* 1985). Subsequently, B19V is disseminated throughout the body and enters the bone marrow microenvironment, producing generalized erythroblast infection (Anderson *et al.,* 1985; Heegaard and Brown, 2002). The lymphopenia, neutropenia and thrombocytopenia that occur during the acute viremic phase are not significant. The etiology of the thrombocytopenia found in the productive B19V infection can be partly explained by the existence of viral replication in the thrombocytes, without synthesis of structural proteins (Pallier *et al.,* 1997).

2.12 Transmission Via Blood Products

Transmission of B19V by blood products is favored by two important viral characteristics: (1) persistent infection in the bone marrow of asymptomatic individuals (Cassinotti *et al.,* 1997), and (2) prolonged replication (up to several years) after initial infection/reinfection (Lefrere *et al.,* 2005). The prevalence of B19V DNA in asymptomatic blood donors (1%) is sufficient to contaminate the plasma pools used for fractionation (Azzi *et al.,* 1999; Ke *et al.,* 2011).

During epidemics the asymptomatic donors frequently show high viral titers 4106 IUmL 1 without concurrent antibodies that prompt unrestrained contamination of blood collections (Kooistra *et al.,* 2011). As a consequence, B19V is easily transmitted by blood transfusion or hemotherapeutic procedures. Acute B19V infection was reported after transfusion of heat-treated factor IX concentrates (Lyon *et al.,* 1989), high-purity heated or solvent–detergent-treated factor VIII concentrates (Azzi *et al.,* 1992) and factor VIII or prothromb in complex concentrates (Williams *et al.,* 1990). Such types of transmission may have serious consequences in several categories of high-risk patients: (1) individuals with shortened red cell survival (chronic hemolytic conditions); (2) pregnant women; and (3) immune compromised patients (Lefrere *et al.,* 2005). In immune compromised patients B19V infection can result not only from blood transfusion or airway transmission but also from reinfection and reactivation. It is difficult to distinguish reinfection and reactivation in such patients but both mechanisms can lead to persistent reticulocytopenia and chronic anemia (Broliden, 2001). The presence of viral DNA in blood donations is usually associated with the presence of anti-B19V immunoglobulin G (IgG) (Daly *et al.,* 2002), but whether these antibodies eliminate or reduce the infectivity in the transfused fraction is controversial. Nevertheless, transfusion transmission of B19V in the presence of neutralizing anti-B19V IgG antibodies is possible and is explained by theirqualitative inability to inhibit the virus infectivity (qualitative defects) (Kurtzman *et al.,* 1989). Transplacental transmission B19V vertical transmission occurs in approximately 30% of the cases of maternal infection and in 2–5% of these cases, results in fetal hydrops or fetal death. The exact mechanism of B19V fetal transmission and the consequent development of fetal infection have not been elucidated fully.

Recent studies indicate that fetal capillary endothelium in the placental villi can support B19V replication. Infection of the placental endothelium may lead to structural and functional destruction of the blood exchange between the mother and the fetus and facilitate the fetal involvement of B19V (Pasquinelli *et al.,* 2009). The highest risk of transplacental transmission is between the first and second trimesters. Termination of pregnancy is not indicated because B19V has no oncogenic properties. Intravascular and intraperitoneal transfusion of high doses of intravenous globulin may help to clear B19V and normalize the blood Circulation and anemia (Hsu *et al.,* 2007).

2.13 Immune Response to B19V Infection and Mechanisms of Autoimmunity

B19V infection is a common event in children and adults and more than 50% have lifelong immunity (Tolfvenstam and Broliden, 2009). Acute B19V infection is controlled by the neutralizing antibodies of the humoral immune response: the viremia declines with the appearance of IgM, followed by synthesis of lifelong anti-B19V IgG (Lefrere *et al.,* 2005). IgM antibodies are directed mainly against the conformational VP2-specific epitopes. They persist up to several weeks after acute viremia but in some patients can be found for up to several months. In the natural course of the humoral response, IgM antibodies are substituted by IgGdirected to VP1 and VP2 conformational and, to some extent, VP1 linear epitopes (Gray *et al.,* 1993). Cell-mediated immunity is also developed during B19V infection, as demonstrated by Th1 and Th2 clonal proliferation in B19V-seropositive patients, but this is difficult to analyze (Franssila *et al.,* 2005). Following VP1/VP2 antigen stimulation in previously infected individuals, an elevated secretion of interferon-g and interleukin (IL)-2 is observed, indicating the existence of Th1 cell-mediated response (Corcoran *et al.,* 2000). B-cell memory can also be established and is maintained against the nonlinear conformational VP2 epitopes and the linear VP1 ones (Corcoran *et al.,* 2004).

B19V persistence probably contributes to the development of autoimmune responses (Lunardi *et al.,* 2008). PLA2 activity belonging to the VP1 region as well as molecular mimicry events (Reitblat *et al.,* 2000) are probably involved in the development of antiphospholipid antibodies (autoimmunity). Another possible cause may be increased gene expression of tumor necrosis factor-a and IL-6, provoked by the NS1 cytotoxic properties, which transactivate the promoter regions of these two genes (Lunardi *et al.,* 2008).

2.14 Clinical Syndromes Commonly Associated with B19V

The development of B19V disease is influenced by the host's hematological and immunological 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 B19V 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 B19V replication (Heegaardand Brown, 2002; Broliden *et al.,* 2006). Erythema infectiosum ('fifth disease') is the major manifestation of B19V infection in children. It is a self-limiting, contagious exanthema (Anderson *et al.,* 1983) that has been recognized by pediatricians 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 (White and Fenner, 1994; Kudesia and Wreighitt, 2005).

2.15 Transient Aplastic Crisis

TAC is described as a precipitous drop of the hemoglobin associated with cessation of reticulocyte production, it is a temporary but potentially life-threatening condition in patients with various forms of chronic hemolytic anemias (Lefrere *et al.,* 1986a) such as SCD (Pattison *et al.,* 1981), a and b-thalassemia (Lefrere *et al.,* 1986b), iron deficiency anemia, or hereditary spherocytosis (Lefrere and Bourgeois, 1986). The patient presents with severe anemia associated with pallor, weakness and lethargy. The sudden drop of hemoglobin values is due to the disappearance of the erythroid progenitors in the bone marrow and the reticulocytes in the blood. Recovery normally occurs within 1 week but it is maintained by intensive blood transfusions, which are lifesaving in such cases (White and Fenner, 1994).

2.16 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 locations 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 nonimmune ones. Among all nonimmune causes, B19V is of principal importance. As the fetus is highly dependent on the increased erythropoiesis rates and B19Varrests erythropoiesis, aplastic crisis, profound cardiac failure and edema are often developed (Kinney *et al.,* 1988; Van Elsacker-Niele *et al.,* 1989). Cardiac failure may be the 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 *et al.,* 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 (Yaegashi *et al.,* 1998). 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.

2.17 Chronic Anemia [Pure Red Cell Aplasia (PRCA)

Patients with various types of immune suppression may not be able to clear B19V effectively, which can result in persistent low-titer viremia accompanied by PRCA and chronic anemia. PRCA has normally been observed when patients with disturbed cell-mediated immunity are infected with B19V, especially patients with acute lymphoblastic leukemia (Weiland *et al.,* 1989), HIV-positive individuals (Sanphasitvong *et al.,* 2005; Watanabe *et al.,* 2010), bone marrow transplant recipients (Cohen *et al.,* 1997) or children with congenital immune deficiencies (Reed *et al.,* 2000). Such patients may develop persistent anemia due to the continuous and uncontrollable B19V replication and the constant involvement of the erythroid progenitors (Soliman *et al.,* 2009). Studies involving large cohorts of HIV-infected patients demonstrated low titers of circulating B19V DNA (105 copiesmL1) but concluded that there was a minimal impact of the virus and the viral genotype for the development of severe anemic conditions (Ferry *et al.,* 2010); however, such a possibility may exist due to the nature of HIV-infection and the side effects of the antiretroviral therapy Approximately 5% of adults and 10% of children undergoing chemotherapy for hematological malignancies may be persistently infected with B19V, which can result in severe and even fatal cytopenias (Broliden *et al.,* 2006).

Moreover, the presence of B19V can be associated with lymphadenopathy or splenomegaly, which is attributed to the B19Vassociated erythroid suppression and immune cell proliferation and can be important for the pathogenesis of leukemic disease(Broliden *et al.,* 2006). Cases of B19V-associated pancytopenias of the different hematological cells could be misinterpreted as a relapse of the underlying malignancy or drug toxicity (Zaki and Ashray, 2010). At the same time, B19V infection can seriously worsen the course of the neoplastic disease and increase the number of relapses (Jitschin *et al.,* 2010). B19V-induced cytopenias in patients with hematological malignancies can significantly prolong the periods of unwanted interruption of chemotherapy, and can require additional bone marrow investigations and more erythrocyte and thrombocyte transfusions (Gustafsson *et al.,* 2010; Jitschin *et al.,* 2010).

The frequent blood transfusions and bone marrow aspirates are not only associated with higher costs but also imply an additional risk of infections or complications transmitted by the transfusions. For these reasons, measures avoiding iatrogenic or nosocomial spread of B19V infection among patients with hematological malignancies must be taken (Jitschin *et al.,* 2010). The early molecular detection of B19V in bone marrow samples or plasma is a clinically important tool for all children with malignancies, not only because of the early diagnosis and appropriate treatment but also to limit the effects of the associated complications (Broliden *et al.,* 2006).

2.18 Arthropathy

Reid et al. (1985) were the first to associate B19V and the development of arthritis. Arthralgias and arthritis are described as major symptoms of B19V infection in adults (Cassinotti *et al.,* 1995).

It is supposed that the antibodies developed against B19V in such cases are deposited in the synovial fluid of the joints and thus contribute to the pathogenesis of arthralgia. B19V arthralgia is a self-limiting condition but it may recur after several months and may involve different joints (Guillaume *et al.*, 2002).

2.19 Virological Diagnosis of B19V

The cytopathological abnormalities caused by B19V arecharacteristic but they are not sufficient for diagnosticpurposes. Active B19V infection induces the formation of giant pronormoblasts in the bone marrow(Iwa and Yutani, 1995). They are characterizedby their cytoplasmic vacuolization, ground-glass appearance of the nucleus and clear perinuclear halo(Pasquinelli, *et al.,* 2009). The chromatin is often immature and appears as a thin rim around the viral inclusion. The cytological methods can be useful for cytopathological evaluation of suspected hydrops fetalis (Iwa and Yutani, 1995). B19V can also be detected using electron microscopy in plasma and fetal tissues, particularly in cases of high-titer viremia during acute infection (Pasquinelli*et al.*, 2009).

However, electron microscopy is a technically sophisticated method and cannot be used for routine purposes. Immunohistochemistry can be used for diagnostic purposes but is a time-consuming method. In patients with dilated cardiomyopathy or inflammatory myocardial diseases, when B19V is suspected as a causative agent, immune his to chemistry enables visualization of B19V VP1/VP2 within the myocardium (Escher *et al.*,; 2008Pankuweit *et al.*, 2005). Immunohistochemistry can also be used for the pathological examination of different tissue materials from hydropic fetuses (lungs, liver, thymus, kidneys, heart, placenta) (Landolsi *et al.*, 2009).

2.20 B19V Infection in Patients with SCD and Thalassemia

B19V is a common pathogen worldwide; serological studies indicate that more than 50% of people are infected during childhood but higher prevalence rates are observed among children with SCD in some tropical regions: Brazil (80% among children of 5–15 years) (Amaku *et al.,* 2009) and Australia (55% of the same year range) (Kelly *et al.,* 2000). B19V outbreaks occur repetitively, generally at intervals of 3–4 years (Serjeant *et al.,* 1993). These outbreaks as well as ongoing sporadic cases of B19V infection have a major impact on the development of TAC among patients with various forms of hereditary anemias. TAC in a patient with hereditary anemia is defined as a transient episode of PRCA, with absence of erythroid precursors in the bone marrow and of reticulocytes in the blood circulation. Because of the shortened red cell life span in such patients, a temporal interruption of erythropoiesis leads to a severe fall in hemoglobin and hematocrit levels and, as a consequence, acute life-threatening anemia (Saarinen *et al.,* 1986).

It was discovered in 1981 that B19V causes TAC in children with SCD and it is now clear that almost 70% of all B19V infections in this cohort result in this disease (Serjeant *et al.,* 1981). B19V can trigger acute cessation of erythrocyte production, causing TAC in patients with hereditary anemias (iron deficiency anemia, SCD, thalassemia, hereditary spherocytosis) that already have a shortened red blood cell lifespan (Servey *et al.,* 2007). Manifestations of B19V infection in SCD patients can range from transient and isolated anemia to a life-threatening drop in hemoglobin levels (Biesma and Nieuwenhuis, 1997; Fartoukh *et al.,* 2006).

In children with SCD the B19V anemia is characterized by profound reticulocytopenia and frequent splenic sequestration (Yates *et al.,* 2009). Moreover, in patients with SCD, B19V can be a causative agent of massive virally induced bone marrow necrosis, complicated by systemic fat embolism, fungal superinfections (Fartoukh *et al.,* 2006), and even fatal bone marrow embolism. Bone marrow necrosis is a common complication of sickle-cell vasoocclusion and is frequently found at autopsy in patients dying from such episodes. B19V infection can provoke bone marrow necrosis, triggering embolic syndrome (Godeau *et al.,* 1991; Rayburg *et al.,* 2010). Regarding superinfections, B19V and herpes simplex superinfection were described in a child with life-threatening myocardial dysfunction (Krishnamurti *et al.,* 2007) but the interactions between these two pathogens in aggravating the cardiac functions are not clear. B19V may be involved in the etiology of myocarditis and acute myocardial infarction in children and adults with SCD (Assanassen *et al.,* 2003; Munro *et al.,* 2003).

Unlike enteroviruses that damage the heart muscle via direct lysis of the infected myocytes, B19V does not infect myocytes but infects endothelial cells of small intracardiac arterioles and venules, which results in impairment of myocardial microcirculation with secondary myocyte necrosis during acute infections (Krishnamurti *et al.,* 2007). Another severe clinical condition among patients with SCD is glomerulonephritis accompanied by proteinuria. The involved kidney tissues demonstrate focal proliferative glomerulonephritis, progressing to focal or segmental glomerulosclerosis. What is more important, the renal tissue tested by PCR for B19V DNA is positive, whereas the blood is negative. This indicates a direct relationship between B19V replication and kidney involvement but the role of the kidney in supporting the persistent infection is doubtful (Wierenga *et al.,* 1995; Tolaymat *et al.,* 1999).

Acute chest syndrome (ACS) continues to be a major source of morbidity and mortality among patients with SCD. It is characterized by the presence of pleuritic chest pain, fever, raises on lung auscultation and pulmonary infiltrates. The pathophysiology of this disease remains poorly understood, leading to the descriptive term 'acute chest syndrome' designated by Charache et al. (1979). B19V infection is recognized as one of the causes in the development of ACS, as viral replication is often detected in patients with SCD and ACS. Thus, B19V might contribute not only to the development of TAC but also to various spectra of severe conditions including ACS (Lowenthal *et al.,* 1996). Acute B19V infection in SCD patients is also involved in the development of thrombocytopenia associated with hemocytic histiocytosis (transient blood plasmacytosis), which is probably an immunological response to B19V infection (Koduri and Naides, 1996).

There are some reports that B19V can induce encephalopathy in patients with b-thalassemia (Sb1) due to the abnormal immune reaction to B19V with central nervous system (CNS) autoantigens. B19V-induced CNS hypersensitivity vasculitis should therefore be considered in the differential diagnosis of the encephalopathy in patients with thalassemia (Bakhshi *et al.,* 2002). Cerebrovascular complications in close temporal association with aplastic crisis among patients with SCD have also been described. The crude risk of cerebrovascular episodes in the 5-week interval after B19V infection was calculated as 58 times greater than expected, evidence of a causal association between B19V infection and cerebrovascular complications (Wierenga *et al.,* 2001). The outcome of the TAC episodes in children and adults with SCD is generally benign but most of the patients must be treated by frequent blood transfusions to reduce the risk of circulatory collapse.

Furthermore, as B19V induces various complications (acute splenic sequestration, hepatic sequestration, acute chest syndrome, nephritic syndrome, meningoencephalitis and stroke) the development of a vaccine or other prevention strategies that would reduce B19V morbidity and mortality among patients with hemoglobinopathies and SCD in particular is of high priority (Smith-Whitley *et al.*, 2003).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was carried out in Yobe State, Nigeria. Yobe State has an estimated population of 2,321,339. It lies between $12^{0}00$'N and $11^{0}30$'E coordinates. Yobe State occupies 45,502 square kilometers, while the inhabitants are Kanuri, Fulani, Kare-kare, Bade and Hausa. The people of Yobe State are predominantly farmers, civil servants and some percentage being traders.

3.2 Study Population

The study population consists of pregnant women attending antenatal clinic and sickle cell patients attending sickle cell clinics in the state.

3.2.1 Inclusion Criteria

All registered and consenting pregnant women attending antenatal clinics and sickle cell patients attending sickle cell clinic were included in the study.

3.2.2 Exclusion Criteria

All registered non consenting pregnant women attending antenatal clinics and sickle cell patients attending sickle cell clinic were excluded in the study.

3.3 Study Design

The study is a cross-sectional descriptive study.

3.4 Sample Size Determination

For the purpose of this study, a prevalence of 41.5%asreported among pregnant women in Kano State by Jegede *et al.*,2014.

Formulae

Calculation of sample size

$$n = \frac{Z^2pq}{d^2}$$

Where $n = \frac{Z^2pq}{d^2}$

z = Standard normal deviate at 95% confidence level 1.96

p = Prevalence

q = 1 – p

d = precision

$$n = \frac{1.96^2 x 0.41 x\ 0.59}{0.0025}$$

$$= \frac{0.929228}{0.0025}$$

$$= 371.7$$

3.5 Sampling Technique

The subject of the study was obtained using random sampling technique among the study population. The samples was collected based on Yobe State population and will be shared among the three senatorial zones; zone A (74), zone B (170) and zone C (118).

The demographic data of the participants was collected using simple questionnaire.

3.6 Ethical Consideration

An ethical approval was obtained from Yobe State Hospital Management Board (YSHMB) Ethical Committee before the commencement of the study.

A consent form containing the research topic and purpose of the study was administered to the subjects for their consent.

3.7 Sample Collection

Blood sample (5ml) was collected in plain container from study subjects, spun at 3000r/min for 5min, separated with micropipettes and stored at -20^{0}C until further analysis

3.8 Test Procedure

ELISA was carried out using Human Parvovirus B19 Antigen ELISA KIT (Melsin Medical Company, China) by following the manufacturers' instruction as follows:

First reagents were prepared before starting the assay procedure and test were run into the micro titer plate.

Secondly, fifty micro litres (50 µl) of positive and negative control were dispensed into separate wells. 10 µl of sample and 40 µL of sample diluents were added to the test wells. Nothing was added to the blank well.

Thirdly one hundred micro litres (100 µl) of HRP-conjugate reagent was added to each well, covered with an adhesive strip and incubated for 60minutes at 37°C.

Fourthly, wells were washed five times by adding 400 µL of washed solution and the plate was inverted and blotted against clean paper towel.

Fifthly, Chromogen solution A and B was added to each well, mixed and incubated in dark at 37°C for 15minutes.

Then, fifty micro litre (50 µL) of stop solution was added to each well, the well was tapped to ensure through mixing. Colour changed from blue to yellow.

Finally, Optical density was read at 450 nm within 15minutes using microtiter plate reader.

3.9 Statistical Analysis

The data collected from the subjects was coded using Microsoft excel 2007 and further analyzed using Statistical Package for Social Sciences (SPSS) version 21. The association between variables was calculated using correlation.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

Table 1 shows that 78(42%) out of 186 pregnant women were positive and 108 (58%) of them were negative for B19V.

The finding in table 2 also indicates that sickle cell disease patients show the highest percentage (47%) of Parvovirus B19.

Table 3 shows that out of 186 pregnant women in the study subjects, the highest B19V antigen was found among age group 21-30. The lowest B19V antigen was found among the old age (41-50). The prevalence of B19V decreased with age.

Table 4 indicates highest B19V among small children (1-10) years in the study population. Whereas the lowest B19V was seen among the old age (41-50) years. The age range of the respondent was from 1 year to 50 years with a mean age of 18.2 years, and standard deviation of 10.4. Out of the 372 sickle cell patients and pregnant women tested, 165 (44%) of the respondent were tested positive indicating presence of Parvovirus B19 while 207 (56%) where tested negative indicating absence of Parvovirus B19.

Parvovirus B19 tends to have a significant difference in sickle cell patient than in pregnant women. Statistically, at significant difference of $p= 0.039$, 95% confidence interval (level) 0.6- 0.8 and standard error of 1.29 about 95.7% of the tested samples have history of preterm labour (Table 5)

Less than 45% indicate history of anemia which is present in most sickle cell patients.However, Parvovirus B19 antigen is found to be present among most of the people who have non-formal education (Table 6)

From table 7 below describe the situation based on level of education, among the study population recruited for this study individuals with primary level of education shows the higher percentage (49%) of Parvovirus B19 infections. This is followed by secondary school level of education having 47%. Lower Parvovirus B19 infections was observed among individual with tertiary level of education of 43%, which implies that Parvovirus B19 infections decrease with increase in level of education.

Table 8 shows that out of 32 respondents that have history of fetal death, 28 (80%) of them are positive, while 136 (40%) are positive among women with no history of fetal death.In the case of people with history of fetal death, out of 339 respondents 203 which represent 60% are tested negative. This indicate the strong relationship between fetal death and B19V infections where higher percentage of fetal loss was observed among individual with positive Parvovirus B19 infections, thus Parvovirus B19 is associated with fetal loss.

Table 1: Parvovirus B19 Status among Pregnant Women.

Status	Positive		Negative		Total	
	Number	**%**	**Number**	**%**	**No**	**%**
Pregnant Women	78	42	108	58	186	100

Table 2: Parvovirus B19 Status among Sickle Cell Disease Patients

Status	Positive		Negative		Total	
	Number	**%**	**Number**	**%**	**No**	**%**
Sickle Cell Disease	87	47	99	53	186	100

Table 3: Parvovirus B19 Antigen among Pregnant Women Stratified by Age.

Age(years)	Positive		Negative		Total
	Number	%	Number	%	
1-10	0	0	0	0	0
11-20	30	41	43	59	73
21-30	38	43	50	57	88
31-40	6	25	18	75	24
41-50	1	100	0	0	1
Total	**75**		**111**		**186**

Table 4: Parvovirus B19 Antigen among Sickle Cell Disease Patient Stratified by Age

	Positive		Negative		
Age(years)	Number	%	Number	%	Total
1-10	55	50.46	54	49.54	109
11-20	18	42.86	24	57.14	42
21-30	12	52.17	11	47.83	23
31-40	3	37.50	5	62.50	8
41-50	2	50.00	2	50.00	4
Total	**90**		**96**		**186**

Table 5: Statistical Analysis of Pregnant Women with B19 Parvovirus

Variables	Mean	Std. Deviation	N
Age	18.25	10.417	372
Level of Education	3.64	.696	372
Previous History of Abortion	1.79	.407	372
History of Fatal Death	1.92	.279	372
History of Preterm Labour	1.96	.199	372
Result Status	1.55	.498	372

Table 6: Statistical Analysis of Sickle Cell Disease Patients with B19 Parvovirus

Variables	Mean	Std. Deviation	N
Age	18.25	10.417	372
Level of Education	3.64	.696	372
History of Anemia	1.55	.499	372
Symtomatic	1.81	.394	372
History of Join Pain	1.52	.500	372
Result Status	1.55	.498	372

Table 7: Status Base on Level of Education for Pregnant Women

Status	Positive		Negative		Total%
	Number	%	Number	%	
Primary school.	21	49	22	51	100
Secondary School.	15	47	17	53	100
Tertiary Education	9	43	12	57	100
Others	119	43	157	57	100
Total	**164**		**208**		

Table 8: Status Based on History of Fetal Death among Pregnant Women

Status	Positive		Negative		Total
	Number	**%**	**Number**	**%**	
Having history of fetal death	28	87	5	13	**33**
No history of fetal death	136	40	203	60	**339**

4.2 Discussion

In determining the prevalence of B19 virus among pregnant women in Yobe State, Nigeria, the study found out that 42% of the pregnant women tested positive to B19V. This is in agreement with the findings of Adams et'al in a study in Sudan, which found out that 61.4% of the 500 pregnant women tested were immune to the parvovirus B19. Similarly, in another study carried out in Norway where the prevalence of parvovirus B19 in 200 pregnant women tested was 59.7% (Adam et'al 2015). In Iran, Khameneh et'al (2014) suggests that the parvovirus B19 sero-prevalence in 86 pregnant women tested was slightly higher was 75, 6%.It can also be noted that, in a study conducted in Saudi Arabia, in which the prevalence of parvovirus B19 among 1200 pregnant women tested, was 46.6% (Ghazi, 2007) this figure is lower than the reports from Sudan, Norway and Iran were the parvovirus B19 prevalence was found to be 61.4%, 59.7% and 75.6% respectively.

Secondly, in determining the prevalence of parvovirus B19 among sickle cell disease patients in Yobe State, Nigeria, the study found out that 47% of sickle cell disease patients were tested positive. This study supported the previous findings by Kleiboeker, et'al (2013) and Iwalokun, et'al (2013) were they suggested that higher numbers of cases were observed among sickle cell patients than in pregnant women. Human parvovirus B19 is known to show singular tropism and lytic infection of erythroid progenitor cells which may consequently result in transient red cell aplasia and chronic anemia (Kleiboeker *et al.*, 2013). Nigeria has the highest prevalence of sickle cell anemia (SCA) in Sub-Saharan Africa according Kleiboeker *et al.* (2013).Unfortunately; the role of B19V in the pathogenesis and clinical complications among SCA patients remains unclear in Nigeria, especially in the northern part of the country. Considering the significantly high transmissibility and prevalence of Parvovirus B19, many

SCA patients could have been transfused with red cells infected with Parvovirus B19. Unfortunately, these donated bloods are neither tested nor treated for Parvovirus B19 as pointed out by Serjeant*et al.* (1981).

This consequently increases the risk of circulatory collapse due to severe anemia. Despite being a country that is highly burdened by SCA, data defining the burden of parvovirus B19 infections in the general population and SCA subpopulation are scanty (Iwalokun*et al.,* 2013).

Finally, in determining the relationship between the presence of B19V among pregnant women and sickle cell disease patients and the differences in age, it was found out that the highest prevalence of B19V antigen was among children between the ages of 1 – 10 years, 55 children (50.46%) were positive in the study population. Whereas the lowest prevalence of B19V antigen was observed among adults between the ages of 41 – 50 years, 2 adults (50%) were positive. This is in line with that of Slavov, et'al (2011) who obtained the prevalence of 55% for children between the ages of 5 – 15 years in Australia.

Globally, Parvovirus B19 is a common viral pathogen. Sero-surveys indicate that >50% of people were infected during childhood, but higher number of cases are observed among SCA children in some tropical countries(Slavov *et al*., 2011).For instance, a Brazilian study reported 80% seroprevalence of B19V in children between 5 and 15 years. Parvovirus B19 outbreaks occur repetitively at 3-4-year intervals (Slavov *et al.,* 2011). These outbreaks have a major impact on the development of Transient red cell aplasia (TRAC) among patients with various forms of hereditary hemoglobinopathiesSlavov *et al.*,2011).

CHAPTER FIVE

5.0 SUMMARY, CONCLUSION AND RECOMMENDATION

5.1 Summary

- In this research, a total number of three hundred and seventy two (372) samples were analyzed. One hundred and eighty six (186) samples from pregnant women and the remaining one hundred and eighty six (186) from sickle cell disease patients.
- From the total population of this study 44% tested positive indicating the presence of B19V, while the remaining 56% tested negative.
- Parvovirus B19 status among pregnant women was found to be 42% which is slightly lower than that of sickle cell disease patients which was found to be 47%.
- Higher percentage of B19V was observed among middle aged pregnant women between 21 to 30 years while for sickle cell disease patients, higher percentage of B19V was found among small children between the ages of 1 to 10 years.
- In both pregnant women and sickle cell disease patients, the minimal percentage was found among the age range of 41 to 50 years indicating that B19V infections decrease with age.

5.2 Conclusion

- Maternal Parvovirus B19 infections during pregnancy increase the risk of fetal loss, spontaneous abortion and stillbirth. A high incidence of abortion and preterm labour was observed in pregnant women with Parvovirus B19 infection in this study.
- The result reinforced the relevance of Parvovirus B19 asan infectious agent responsible for abortion in pregnant women,

- And also a cause of anemia in sickle cell disease. It is thus important to include screening for Parvovirus B19 in routine antenatal clinic and sickle cell disease clinic visits. Parvovirus infection presents a serious complication in children with sickle cell disease.

5.3 Recommendation

- Significant percentage of child-bearing aged women are at risk of primary infection with parvovirus B19 which could adversely affect their pregnancy.It is thus important to include screening for parvovirus B19V in routine antenatal clinic.
- It is also important to include B19V screening in sickle cell disease clinic visit to reduce its prevalence in this susceptible population.
- Strategies to prevent and monitor parvovirus B19 infection should be implemented through extended awareness and definition of the actual pathogenic role of this virus

REFERENCES

Adam, O., Makkawi, T., Reber, U., Kirberg, H., Eis-Hübinger, A.M.(2015). The seroprevalence of parvovirus B19 infection in pregnant women in Sudan. *Epidemiology of Infection,*143:242-8.

Amaku, M., Azevedo, R., Morgado de Castro, R., Massad, E. and Coutihno, F. (2009). Relationship among epidemiological parameters of six childhood infections in a non-immunized Braziliancommunity. *Memorias Instituto Oswaldo Cruz*, 104: 897–900.

Amoda, C., Doyle, S. (2004). Advances in the biology, diagnosis and host pathogen interaction of Parvovirus B19. *Journal of Microbiology*, 3: 459-475.

Anderson, A., Higgins, P.G., Davis, L.R., William, J.S., Jones, S.E., Kidd, I.M., Pattison, J.R. and Tyrrell, D.A. (1985). Experimental parvoviralinfection in humans. *Journal of Infectious Disease*, 152: 257–265.

Anderson, M., Jones, S., Fisher-Hoch, S., Lewis, E., Hall, S., Bartlett,C., Cohen, B., Mortimer, P. and Pereira, M. (1983). Human Parvovirus, the cause of erythema infectiosum (fifth disease)?*Lancet,*:1378

Andrea, H., Andrea, G., Ralph, G., Albret, R., Hanswol fand Susanne, M. (1999). Infection of aphersis by Parvovirus B19. *Journal ofGeneral Virology*, *80: 627-630.*

Assanassen, C., Quinton, R.A. and Buchanan, G.R. (2003). Acute myocardial infarction in sickle-cell anemia. *Journal of Pediatric Hematology and Oncology*, 25: 978–981.

Azzi, A., Ciappi, S., Zakarewska, K., Morfini, M., Mariani, G. and Mannucci, P.M. (1992). Human Parvovirus B19 infection in hemophiliacs first infused with two high purity, virally attenuated factor VIII concentrates. *Journal of Hematology*, 39: 228–230.

Azzi, A., Morfini, M., and Mannucci, P.M. (1999). The transfusion associated transmission of Parvovirus B19. *Transfusion Medicine Review*, 13: 194–204.

B¨onsch, C., Kempf, C.and Ros, C. (2008). Interaction of Parvovirus B19 with human erythrocytes alters virus structure and cell membrane integrity. *Journal of Virology*, 82: 11784–11791.

Bakhshi, S., Sarnaik, S., Becker, C., Shurney, W., Nigro, M. and Savasan, S. (2002). Acute encephalopathy with Parvovirus B19 infection in sickle cell disease. *Archives of Disease in Childhood,*87:541–542.

Bdour, S., (2006). Risk of prenatal transmission of rubella and Parvovirus B19 in Jordanian pregnant women. *Vaccine*, 12: 3309-3312.

Biesma, D. and Nieuwenhuis, H. (1997). Life-threatening anemia caused by B19 Parvovirus in a non-immunocompromised patient. *Journal of Medicine,* 50: 81–84.

Broliden, K., Tolfvenstam,T. and Norbeck, O. (2006). Clinical aspects of Parvovirus B19 infection. *Journal of Internal Medicine,* 260: 285–304.

Brown, K. (2004). Human Parvoviruses. Principles and Practice of Clinical Virology (Zuckerman, A., Bantavala, J., Pattison, J., Griffiths, P. and Schoub, B., eds), pp. 703–721. *JohnWiley and Sons, Chichester.*

Brown, K.E. (2015). Parvovirus B19. In: Bennet, J.E., Dolin, R. and Blaser, M., editors. *Mandell, Douglas and Bennett's principles and practice of infectiousdiseases*. 8th ed. Philadelphia: *Churchill Livingstone Elsevier, p. 1840-7.*

Brown, T., Anand, A., Ritchie, L., Clewly, J. and Reid, T. (1984). Intrauterine Parvovirus infection associated with hydrops fetalis. *Lancet,* 2: 1033–1034.

Cassinotti, P., Bas, S., Siegl, G. and Vischer, T.,L. (1995). Association between human Parvovirus B19 infection and arthritis. *Annals of Rheumatic Disease,* 54: 498–500.

Cassinotti, P., Burtonboy, G., Fopp,M.and Sigel, G. (1997). Evidence for persistence of human Parvovirus B19 DNA in bone marrow. *Journal of Medical Virology,* 53: 229–232.

Chandramouli, S., Medina-Selby, A., Doris, C., Schaefer, M., Spencer, T, and Brito, L.A. (2013). Generation of a Parvovirus B19 vaccine candidate. *Vaccine*,31(37): 3872-8.

Charache, S., Scott, J.C. and Charache, P. (1979). 'Acute chest syndrome' in adults with sickle cell anemia. Microbiology, treatment, and prevention. *Archives of Internal Medicine,* 139: 67–69.

Chen ,A.Y. and Qiu, J. (2010). Parvovirus infection-induced cell death and cell cycle arrest. *Future Virology*, 5: 731–743.

Clewle, J. (1984). Biochemical characterization of a human Parvovirus. *Journal of General Virology,*65: 241–245.

Cohen, B.J., Beard, S., Knowles, W.A., Ellis, J.S., Joske, D., Goldman, J.M., Hewitt., P. and Ward, K.N. (1997). Chronic anemia due to Parvovirus B19 infection in a bone marrow transplant patient after platelet transfusion. *Transfusion,* 37: 947–952.

Corcoran, A. and Doyle, S. (2004). Advances in the biology, diagnosis and host–pathogen interactions of Parvovirus B19. *Journal of Medicineand Microbiology,* 53: 459–475.

Corcoran, A., Doyle, S., Waldron, D., Nichholson. and Mahon, B.P. (2000). Impaired gamma interferon responses against Parvovirus B19 by recently infected children. *Journal of Virology*, 74: 9903–9910.

Corcoran, A., Mahon ,B.P. and Doyle, S. (2004). B cell memory is directed toward conformational epitopes of Parvovirus B19 capsid proteins and unique region of VP1. *Journal of Infectious Disease*, 189: 1873–1880.

Corcoran, A., Kerr, S., Koppelman, M., and Doyle, S. (2007). Improved detection of acute Parvovirus B19 infection by IgM EIA in combination with a novel antigen EIA. *Vox Sang*,93, 216–222.

Cossart, Y.,, Field A., Cant, B. and Widdows, D. (1975). Parvovirus-like particles in human sera. *Lance*, 1: 72–73.

Daly, P., Corcoran, A., Mahon, P.B. and Doyle, S. (2002). High-sensitivity PCR detection of Parvovirus B19 in plasma. *Journal of Clinical Microbioogy*,40: 1958–1962.

Danil, E.N., Padilla, M.L., Ramos, M.G., Zyas, P. and Perez, B. (2004). Parvovirus B19 infection in medical student during Hospital outbreak. *Journal of Medical Microbiology, 55: 46-141*.

Deiss, V., Tratschin, J., Weitz, M. and Siegl, G. (1990). Cloning of the human Parvovirus B19 genome and structural analysis of its palindromic termini. *Virology*,175: 247–254.

Elnifro, E., Nisha, A. K., Almabsoot, M., Daeki,A., Mujber,N. and Muscat,J. (2009).Seroprevalence of Parvovirus B19 among pregnant women in Tripoli, Libya.*Journal of Infections in Developing Countries*,3(3):218-220.

Fartoukh, M., Prigent, H., Thioliere, B., Enache-Angoulvant, A., Garbarg-Chenon, A. and Girot, R. (2006). Fatal fungal superinfection complicating B19-virus induced massive bonemarrow necrosis in sickle-cell disease. *Haematology*, 91: ECR18.

Ferry ,T., Hirshel, B., Dang ,T., et al. (2010). Swiss HIV cohort study infrequent replication of Parvovirus B19 and erythrovirus genotype 2 and 3 among HIV infected patients with chronic anemia. *Clinical Infectious Disease*, 50: 115–118.

Franssila, R., Auramo, J.,Modrow ,S.,Mobs ,M., Oker-Blom, C., Kapyla, P., Soderlund-Venermo, M. and Hedman, K. (2005). T-helper cellmediated interferon-gamma expression after human Parvovirus B19 infection: persistent VP2 specific and transient VPu-specific activity. *Clinical Experimental Immunology*,142: 53–61.

Freitas, R.B., Melo, F.L., Oliveira, D.S., Romano C.M., Freitas, M.R., Maceˆdo, O., Linhares, A.C., de. A., Zanotto, P.M., Durigon, E.L. (2008). Molecular characterization of human erythrovirus B19 strains obtained from patients with several clinical presentations in the Amazon region of Brazil. *Journal of Clinical Virology,* 43: 60–65.

Ghazi, H.O.,2007 . Prevalence of antibodies to human parvovirus B19 in Saudi women of childbearing age in Makkah. *Journal of Family and Community Medicine,* 2007;14:15-7.

Godeau, B., Galacteros, F., Schaeffer, A., Morinet, F., Bachir, D., Rosa, J. and Portos, J.L. (1991). Aplastic crisis due to extensive bonemarrow necrosis and human Parvovirus infection in sickle cell disease. *Journal of Medicine,* 91: 557–558.

Grabarczyk, P., Kalinska, A., Kara, M., Wieczorek, R., Ejduk, A., Sulkowska, E., Gołebiowska-Staroszczyk, S., Matysiak, M., Baylis, S. and Brojer, E. (2011). Identification and characterization of acute infection with Parvovirus B19 genotype 2 in immunocompromised patients in Poland. Journal of *Medicine and Virology,* 83: 142–149.

Gray, J., Cohen, B. and Desselberger, U. (1993). Detection of human Parvovirus B-19-specific IgM and IgG antibodies using a recombinant viral VP1 antigen expressed in insect cells and estimation of time of infection by testing antibody avidity. *Journal of Virology*, 44: 11–23.

Guan, W., Cheng, F., Yoko, Y., Kleiboeker, S., Wong, S., Zhi ,S., Pintel, D.J. and Qiu, J. (2008). Block to the production of full length B19 virus _c 2011 Federation of European Microbiological Societies FEMS *Immunology and Medical Microbiology,* 62 (2011) 247–262 transcripts by internal polyadenylation is overcome by replication of the viral genome. *Journalof Virology* 82: 9951–9963.

Guillaume, M.P., Hermanus, M.and Peretz, A. (2002). Unusual localization of chronic arthropathy in lumbar facet joints after Parvovirus B19 infection. *Clinical Rheumatology,* 21: 306–308.

Gustafsson, I., Kaldensjo ,T., Lindblom, A., Norbeck, O., Henter ,J., Tolfvenstam, T. and Broliden, K. (2010). Evaluation of Parvovirus B19 infection in children with malignant or hematological disorders. *Clinical Infectious Disease,* 50: 1426–1427.

Hankins, J.S., Penkert, R.R., Lavoie, P., Tang, L., Sun, Y. and Hurwitz, J.L. (2016). Parvovirus

B19 infection in children with sickle cell disease in the hydroxyurea era. *Experimental Biology Medicine, ; 241(7): 749-754.*

Heegaard, E. and Brown, K.E. (2002). Human Parvovirus B19. *Clinical Microbiology Review, 15: 485-505.*

Heegaard, E.D., Peterson, B.L., Heilmann, C.L. and Horsleyh, A. (2002). Prevalance of Parvovirus B19 and Parvovirus V9 DNA and antibodies in paired bone marrow and serum samples from healthy individuals. *Journal Clinical Microbiology, 40: 933-936.*

Hemauer, A., von Poblotzki, A., Gigler. A., Cassinotti ,P., Siegl, G.,Wolf, H. and Modrow, S. (1996). Sequence variability among different Parvovirus B19 isolates. *Journal of General Virology,*77: 1781–1785.

Hsu, S., Chen, Y., Huang, Y., Yeh, T.T., Chen, W.C., Ho, E.S. and Chou, M.M. (2007). Prenatal diagnosis and perinatal management of maternal–fetal congenital Parvovirus B19 infection. *Taiwan Journal of Obstetrics and Gynecology,* 46: 417–422.

Hubschen, J.M., Zefira, M., Andreas, F.M., Francois, S., Yair, A. and Zehava, G. (2009). Phylogenetic analysis of human Parvovirus B19 sequences from eleven different countries confirms the predominance of genotype 1 and suggests the spread of genotype 3b. *Journal of Clinical Microbiology*,47: 3735-3738.

Iwa, N. and Yutani, C. (1995). Cytodiagnosis of Parvovirus B19 infection from ascites fluid of hydrops fetals: report of a case. *Diagnostic Cythopathology,* 13: 139–141.

Iwalokuna, B.A., Iwalokun, S.O. and Hodonu, S.O. (2013). Seroprevalence of Parvovirus B19 antibodies and evidence of viremia among Nigerian patients with sickle cell anemia. *Journal of Biomedical Research,* **27**(4): 272-282.

Jeanne, A.J,, DaleHuff and Julie, A.D. (2001). Placental cellular Response in women infected B19 during pregnancy. *Clinic Diagnostics Laboratory Immunology, 8: 288-292.*

Jegede,A., Aminu, M. and Ella, E.E. (2014). Seroprevalence of human parvovirus Among patients Attending some hospitals in kano Metropolis, Nigeria. *African Journal of Clinical and Exprimental Microbiology, 15(1):21-26.*

Jitschin, R., Peters, O., Plentz, A., Turiwski, P., Segerer, H. and Modrow, S. (2010). Impact of Parvovirus B19 infection on paediatric patients with hematological and/or oncological disorders. *Clinical Microbiology of Infections,* DOI: 10.1111/j.1469-0691.2010.03355.x.

Johargy, A(2009). Seroprevalence of erythrovirus B19 IgG antibody among paediatric patients in Makkah and Jeddah, Kingdom of Saudi Arabia. *Medical Principle and Practice,* 2009;18:339-41.

Jones, M.S., Kapoor, A., Lukashov, V.V., Simmonds, P., Hecht, F. and Delwart, E. (2005). New DNA viruses identified in patients with acute infection syndrome. *Journal of virology, 79:* 8230–8236.

Kaufman, B., Simpson, A. and Rossmann, M. (2004). The structure of human Parvovirus B19. *Proceedings of the National Academy of Sciences USA,* 101: 11628–11633.

Ke, L., He, M., Li, C. et al. (2011). The prevalence of human Parvovirus B19 DNA and antibodies in blood donors from four Chinese blood centers. *Transfusion, DOI*: 10.1111/j.1537- 2995.2011.03067.x.

Kelly, H., Siebert, D., Hammond, R., Leydon ,J., Kiley, P. and Maskill ,W. (2000). The age-specific prevalence of human Parvovirus immunity in Victoria, Australia compared to other parts of the world. *Epidemiology of Infections,* 124: 449–457.

Khameneh, Z.R., Hanifian, H., Barzegari, R., Sepehrvan,N. Human parvovirus B19 in Iranian pregnant women: A serologic survey. *Indian Journal of Pathology and Microbiology,* 2014;57:442-4.

Kinney, J.S., Anderson, L.J., Farrar, J., Strikas, R.A., Kumar, M.L., Kliegman, R.M., Sever, J,L., Hurwitz. E.S. and Sikes ,R.K. (1988). Risk of adverse outcomes of pregnancy after human Parvovirus B19 infection. *Journal of Infectious Diseases,* 157: 663–667.

Koduri, P.and Naides, S. (1996). Transient blood plasmacytosis in Parvovirus B19 infection: a report of two cases. *Annals of Hematology,* 72: 49–51.

Kooistra, K., Mesman, H., de Waal, M., Koppelman, M. and Zaaijer, H. (2011). Epidemiology of high-level Parvovirus B19 viraemia among Dutch blood donors, 2003–2009. *Vox Sang,* 100: 261–266.

Krishnamurti, L., Lanford, L. and Munoz, R. (2007). Life-threatening Parvovirus B19 and herpes simplex virus associated acute myocardial dysfunction in a child with sickle cell disease.

Kudesia, G .and Wreighitt, T. (2005). Parvovirus B19. Clinical and Diagnostic Virology (Kudesia G & Wreighitt T, eds), pp. 90–93. *Cambridge University Press, Cambridge*.

Kurtzman, G.J., Cohen, B.J., Field. A.M., Oseas, R., Blaese, R.M. and Young, N.S. (1989). Immune response to B19 Parvovirus and an antibody defect in persistent viral infection. *Journal of Clinical Investigation,* 84: 1114–1123.

Lamont, R., Sobel, J., Vaisbuch, E., Kusanovic, J., Mazaki-Tovi, S., Kim, S., Uldbjerg, N. Romero, R. (2010). Parvovirus B19 infection in human pregnancy. *Journal of Obstetrics and Gynaecology,*118: 175–186.

Landolsi, H., Yacoubi, M.T., Bouslama, L., Lahmar. A., Trabelsi, A., Hmissa. S., Aouni, M. and Korbi, S. (2009). Detection of the human Parvovirus B19 in nonimmune hydrops fetalis using immunohistochemistry and nested-PCR in formalin fixed and paraffin embedded placenta and fetal tissues. *Pathology and Biology,* 57: e1–e7.

Landry, M.L. (2016*). Parvovirus B19. Microbiology Spectrum,* **4**(3): 1-13.

Lefrere, J., Servant-Delmas, A., Candotti, D., Mariotti, M., Thomas, I., Brossard ,Y., Lefrere, F., Girot, R., Allain, J.P. and Laperche, S. (2005). Persistent B19 infection in immunocompetent individuals: implications for transfusion safety. *Blood,* 106: 2890–2895.

Lefrere, J.J. and Bourgeois, H. (1986). Human Parvovirus associated with erythroblastopenia in iron deficiency anemia. *Journal of Clinical Pathology,* 39: 1277–1278.

Lefrere, J.J., Courouce, A.M., Bertrand, Y., Girot, R .& Soulier JP (1986a). Human Parvovirus and aplastic crisis in chronic hemolytic anemias: a study of 24 observations. *Journal of Hematology,*23: 271–275.

Lefrere, J.J., Courouce, A.M., Girot, R. and Cornu, P. (1986b). Human Parvovirus and thalassemia. *Journal of Infection,* 13: 45–49.

Lowenthal, E., Wells, A, Emanuel, P., Player, R. and Prchal, J.T. (1996). Sickle cell acute chest syndrome associated with Parvovirus B19 infection. *American Journal of Hematolology,* 51: 207–213.

Lunardi, C., Tinazzi, E., Bason, C., Dolcino, M., Corrocher, R. and Puccetti, A. (2008). Human Parvovirus B19 infection and autoimmunity. *Autoimmunity Reviews,* 8: 116–120.

Lyon, D.J., Chapman, C.S., Martin, C., Brown, K.E., Clewley, J.P., Flower, A.J and Mitchell, V.E. (1989). Symptomatic Parvovirus B19 infection and heat-treated factor IX concentrate. *Lancet,* 1: 1085.

Moffat, S., Yaegashi, N. and Tada, K. (1998). Human Parvovirus nonstructural protein (NS1) induces apoptosis in erythroid lineage cells. *Journal of Virology,* 74: 3018–3028.

Mori, J., Beattie, P., Melton, D.W., Cohen, B. Clewly, J. (1987). Structure and mapping of the DNA of human Parvovirus B19.

Morita E, Tada K, Chisaka H, Asao H, Sato H, Yaegashi N & Sugamura K (2001). Human Parvovirus B19 induces cell cycle arrest at G2 phase with accumulation of mitotic cyclins. of *Journal Virology,* 75: 7555–7563.

Munro, K., Croxson, M.C., Thomas, S. and Wilson, N.J. (2003). Three cases of myocarditis in childhood associated with human Parvovirus (B19 virus). *Pediatric and Cardiology*, 24: 473–475.

Ozawa, K., Ayu, J., Yu-Shu, H. Kurtzman, G., Shimada, T. and Young, N. (1987). Novel transcription map of the B19 (human) pathogenic Parvovirus. *Jornal of Virology,* 61: 2395–2406.

Pallier, C., Greco, A., Le Junter, J., Saib, A., Vassais, I. and Morinet, F. (1997). The 30 untranslated region of B19 Parvovirus capsid protein mRNAs inhibits its own mRNA translation in nonpermissive cells. *Journal of Virology,* 71: 9482–9489.

Pankuweit, S., Ruppert, R., Eckhardt, H., Strache, D. and Maisch, B. (2005). Pathophysiology and aetiological diagnosis of inflammatory myocardial diseases with a special focus on Parvovirus B19. *Journal of Veterinary Medicine* B, 52: 344–347.

Parsyan, A., Szmaragd, C., Allain, J.P. and Candotti, D. (2007). Identification and genetic diversity of two human Parvovirus genotype 3 subtypes. *Journal of General Virology,* 88: 428–431.

Pasquinelli, G., Bonvicini, F., Foroni, L., N. and Galinella, G. (2009). Placental endothelial cells can be productively infected by Parvovirus B19. *Journal of Clinical Virology,* 44: 33–38.

Pattison ,J., Jones, S., Hodgson, J., Davis, L., White, J., Stroud, C. and Murtaza, L. (1981). Parvovirus infections and hypoplastic crisis in sickle-cell anemia. *Lancet,* 1: 664–665.

Quek, L., Sharpe, C., Dutt, N., Height, S., Allman, M., Awogbade, M., Rees, D., Zuckerman, M. andThein, S. (2010). Acute human Parvovirus B19 infection and nephrotic syndrome in patients with sickle-cell disease. *British Journal of Haematology,* 149: 289–291.

Rajput, R., Sehgal, A., Jain, D., Sen, R. and Gupta, A. (2012). Acute Parvovirus B19 infection leading to severe aplastic anemia ina previously healthy adult female. *Indian Journal of HematologyandBlood Transfusion*,**28**(2): 123-126.

Rayburg, M., Kalinyak, K., Towbin, A., Baker, P. and Joiner, C. (2010). Fatal bone marrow embolism in child with hemoglobin SE disease. *American Journal of Hematology,* 85: 182–184.

Reed, J.L., Kucukcetin, B. and Koering, S. (2000). Mechanisms of Parvovirus B19 persistence in peripheral blood mononuclear cells (PBMC). *Infectious Disease Review,* 2: 169.

Reid, D., Reid, T., Brown, T., Rennie, J. and Eastmond, C. (1985). Human Parvovirus-associated arthritis: a clinical and laboratory description. *Lancet,* 1: 422–425.

Reitblat., T, Drogenikov ,T., Sigalov I, Oren S and London, D. (2000). Transient anticardiolipin antibody syndrome in a patient with Parvovirus B19 infection*Journal of Medicine,*109: 512–513.

Ros, C., Baltzer, C., Mani, B. and Kempf, C. (2006). Parvovirus uncoating in vitro reveals a mechanism of DNA release without capsid disassembly and striking differences in encapsidated DNA stability. *Virology,* 345: 137–147.Saarinen, U.M., Chorba, T.L., Tattersall, P., Young, N.S., Anderson, L.J., Palmer, E. and Coccia, P.F (1986). Human Parvovirus B19-induced epidemic red cell aplasia in patients with hereditary hemolytic anemia. *Blood,* 67: 1411–1417.

Sanphasitvong, W., Poovorawan, K., Boonsuk, P., Assanasen, T., Na Nakorn, T. and Poovorawan, Y. (2005). Parvovirus B19 infection in HIV patient with pure red cell aplasia. *Southeast Asian Journal of Tropical Medicine and Public Health,* 36: 1216–1220.

Serjeant, G.R., Serjeant, B.E., Thomas, P.W., Anderson, M.J., Patou, G, and Pattison, J.R. (1993). Human Parvovirus infection in homozygous sickle-cell disease. *Lancet*,**341**: 1237-1240.

Servant, A., Laperche, S., Lallemand, F., Marinho, V., De Saint Maur , G., Meritet, J. and Garbag-Chenon, A. (2002). Genetic diversity within human erythroviruses: identification of three genotypes.

Servey, J., Reamy, B. and Hodge, J. (2007). Clinical presentations of Parvovirus B19 infection. *American Family Physician,* 75: 373–376.

Simeoni, S., Puccetti, A., Tinazzi, E. and Lunardi, C. (2010). Parvovirus B19. Molecular Detection of Human Viral Pathogens (Liu D, ed), pp. 831–834. *CRC Press, Taylor and Francis Group, Boca Raton, FLorida*

Simmonds, P., Douglas, J., Bestetti, G., Longhi, E., Antinori, S., Paravicini, C. and Corbellino, M. (2008). A third genotype of the human Parvovirus PARV4 in sub-Saharan Africa. *Journal of General Virology,* 89: 2299–2302.

Slavov, S.N., Kashima, S., Pinto, A.C.S. and Covas, D.T. (2011). Human Parvovirus B19: general considerations and impact on patients with sickle-cell disease and thalassemia and on blood transfusions. *FEMS Immunologyand Terminate Medical Microbiology, 62: 247-262.*

Smith-Whitley, K., Zhao, H., Hodinka, R., Kwiatkowski, J., Cecil, R., Cecil, T., Cnaan, A. and Ohene-Frempong, K .(2003). Epidemiology of human Parvovirus B19 in children with sickle-cell disease. *Blood*, 103: 422–427.

Soliman, Oel-S, Abd El-Aal, M., El-Ashry, L,, Zaghloul, M. and Kora, B. (2009). Parvovirus B19 infection in pediatric oncology patients: diagnostic value of clinical and serologic parameters compared with nested PCR. *Journal of Pediatric Hematology and Oncology*, 31: 173–176.

St Amand, J., Beard ,C., Humphries, K. and Astell, C. (1991). Analysis of splice junctions and in vitro and in vivo translation potential of the small, abundant B19 Parvovirus RNAs. *Virology*, 183: 133–142.

Sukanya, T., Pilaiwan, K., Rattana, K., Pakaphan, K., Junya, J. and Thawlwong, R. (2006). Hydrops fetalis caused by Parvovirus B19 infection case report and literature. *Journal Medical Association Thailand, 89: 1277-1268.*

Summers, J., Jones, S .and Anderson, M.(1983). Characterization of the genome of the agent of erythrocyte aplasia permits its classification as a human Parvovirus. *Journal of General Virology*, 64: 2527–2532.

Toan, N.L., Duechting, A., Kremsner, P.G., et al. (2006). Phylogenetic analysis of human Parvovirus B19, indicating two subgroups of genotype 1 in Vietnamese patients. *Journal of General Virology*,87: 2941–2949.

Tolaymat, A., AlMousily, F., MacWilliam, K., Lammert,. N. and Freeman, B. (1999). Parvovirus glomerulonephritis in a patient with sickle cell disease. *Pediatric Nephrology*, 13: 340–342.

Tolfvenstam, T. and Broliden ,K. (2009). Parvovirus B19 infection. *Seminars in Fetal Neonatal Medicine*,14: 218–221.

Toyokawa ,Y., Kingetsu, I., Yasuda, C., Yasuda, J., Yoshida, K., Kurosaka, D. and Yamada, A. (2007). A case of pure red cell aplasia complicated by Evans syndrome. *Modern Rheumatology*,17: 333–337.

Van Elsacker-Niele, A,M., Salimans, M.M., Weiland, H.T, Vermey- Keers, C., Anderson, M.J. and Versteeg, J. (1989). Fetal pathology in human Parvovirus B19 infection. *British Journal of Obstetric and Gynaecology*,96: 768–775.

Von Landenberg, P., Lehmann, H.W., Knoll, A., Dorsch, S. and Modrow, S. (2003). Antiphospholipid antibodies in pediatric and adult patients with rheumatic disease are associated with Parvovirus B19 infection. *Arthritis Rheumatoid,* 48: 1939–1947.

Wan, C., Soderlund-Venermo, M., Pintel, D. and Riley, K. (2002). Molecular characterization of three newly recognized rat Parvoviruses. *Journal of General Virology,* 83: 2075–2083.

Watanabe, D., Taniguchi, T., Otani, N., Tominari ,S., Nishida, Y., Uehira T. and Shirasaka, T. (2010). Immune reconstitution to Parvovirus B19 and resolution of anemia in a patient treated with highly active retroviral therapy. *Journal of Infections and Chemotherapy,* 17: 283–287.

Weigel-Kelley, K., Yoder, M. Srivastava, A. (2003). Alpha-5 beta-1 integrin as a cellular co-receptor for human Parvovirus B19: requirement of functional activation of beta1 integrin for viral entry. *Blood,* 102: 3927–3933.

Weiland ,H.L., Salimans, M.M., Fibbew, N.E., Kluin, P.M and Cohen, B.J. (1989). Prolonged Parvovirus B19 infection with severe anemia in a bone marrow transplant patient. *British Journal of Haematology,*71: 300.

White,and Fenner, F. (1994). Parvoviridae. Medical Virology, 4th edn (White D & Fenner F, eds), pp. 285–293. *Academic Press, San Diego.*

White, D., Woolf, A., Mortimer, P., Cohen, B., Blake, D. and Bacon, P. (1985). Human Parvovirus arthropathy. *Lancet,* i: 419–421.

Wierenga, K., Pattison, J., Brink, N., Griffiths ,M., Miller ,M., Shah ,D,J., Williams,W.,Serjeant, B.E. and Serjeant, G.R. (1995). Glomerulonephritis after human Parvovihomozygousickle-cell disease. *Lancet,* 346: 475–476

Wierenga, K.J., Serjeant, B.E. and Serjeant. (2001). Cerebrovascular complications and Parvovirus infection in homozygous sickle cell disease. *Journal of Pediatric,* 13: 438–442.

Williams, M.D., Cohen, B.J., Beddall, A.C., Pasi, K.J.,Mortimer, P.P. and Hill, F.G. (1990). Transmission of human Parvovirus B19 by coagulation factor concentrates. *Vox Sang,* 58: 177–181..

Yaegashi, N., Niinuma, T., Chisaka, H., Watanabe, T., Uehara, S., Okamura, K., Moffat, S., Sugamura, K.and Yajima, A. (1998). The incidence of, and factors leading to, Parvovirus B19-related hydrops fetalis following maternal infection; report of 10 cases and meta-analysis. *Journal of Infection,* 37: 28–35.

Yates, A., Hankins, J., Mortier, N., Aygun, B. and Ware, R. (2009). Simultaneous acute splenic sequestration and transient aplastic crisis in children with sickle cell disease. *Pediatric Blood Cancer*,53: 479–481.

Zaki, M. Ashray, R .(2010). Clinical and hematological study for Parvovirus B19 infection in children with acute leukemia. *International Journal of Laboratory Hematology,* 32: 159–166.

Zaki, M., Hassan, S., Seleim, T. and Lateef, R. (2006). Parvovirus B19 infection in a children with a variety of hematological disorders. *Hematology*, 11: 261–266.

Zhi, N., Mills, I., Lu, J., Wong ,S., Filippone, C. and Brown, K. (2006). Molecular and functional analyses of a human Parvovirus B19 infectious clone demonstrates essential roles for NS1, VP1, and the 11-kilodalton protein in virus replication and infectivity. *Journal of Virology*,80: 5941–5950.

APPENDICES

APPENDIX I

ETHICAL APPROVAL

YOBE STATE HOSPITALS MANAGEMENT BOARD
Yobe State Specialist Hospital, Damaturu.

P. M. B.:- 1012
Tel:- 08033374973
e-mail:- gsashdtr2013@gmail.com

Our Ref:- YSSH/DTR/GEN/302
Your Ref:
Date: 25th April, 2018.

Kaltume Bulama Yusuf,
Department of Medical Laboratory Science,
Bayero University Kano,
Kano State.

Ma,

RE: REQUEST FOR ETHICAL CLEARANCE

After careful scrutiny of your request for the above mentioned subject matter in respect of your proposed research work titled **"PERVOVIRUS B19 ANTIGENEMIA AMONG PREGNANT WOMEN AND SICKLE CELL PATIENTS" (A study of Yobe State Specialist Hospital Damaturu)**, I have the honour of conveying the approval of the Ethnic Review Committee for you to proceed with the study.

Best wishes.

Yours faithfully
25 APR 2018
Dauda Abdullahi
(Secretary Ethics Review Committee)

All Correspondences to be addressed to the Medical Director

APPENDIX II

RESPONDENT INFORMED WRITTEN CONCEPT

I agreed to participate in this titled "**PARVOVIRUS B19 ANTIGENEMIA AMONG PREGNANT WOMEN AND SICKLE CELL PATIENTS ATTENDING ANTENATAL CLINIC AND SICKLE CELL CLINIC,YOBE STATE**. The detail procedure and probable benefits of this test method were fully explained to me.

I Understand that my sample that will be collected for the test is willingly and the test result will be communicated to me in confidential manner.

I made this consent willingly without being forced or pressure.

Participant phone no……

Name of researcher……**Signature**……..……

APPENDIX III

Questionnaire for the study of parvovirus B19 Antigenemia among pregnant women and sickle cell patient attending antenatal clinic and sickle cell clinic in Yobe state.

My name is Kaltume Bulama Yusuf,a postgraduate student of Bayero University Kano. I am carryingout this research as part of the requirement for the award of masters in the department of medical laboratory science.

Supervisors: Dr Lawal Dahiru Rogo

Proff. M .Y. Gwarzo

Subject Number:_____

1.Name:_____

2.Age (years):_____

3.Religion; Islam ( ) Christian ( ) Others ( )

4. Marital Status; Married ( ) ,Single ( ) Divorce ( )

5.Education ;Primary ( ) , Secondary ( ) ,Tertiary ( ) Others ( )

6.Employment;School Age Employee ( ) ,Government Employee( )

7.Type Of Crisis; Aplastic Crisis ( ) , Fetal Hydrops( )

8.Previous History Of Abortion;;Yes ( ) ,No( )

9.Previous History Of Anemia ;Yes ( ) ,No( )

10.General Improvement Since The Onset Of Treatment;Yes ( ) ,No( )

11.Symtomatic? Yes ( ) ,No( )

12.Asymtomatic? Yes ( ) ,No( )

13.Previous History Of Fetal Death;Yes ( ) ,No( )

14.Previous History Of Preterm Labour;Yes ( ) ,No( )

15.Previous History Of Joint Pain;Yes ( ) ,No( )

Result;Elisa……..

Sign And Date:_____

APPENDIX IV

Descriptive Statistics

	Mean	Std. Deviation	N
Age	18.25	10.417	365
MaritalStatus	1.45	.498	365
Levelof Education	3.64	.696	365
PreviousHistory of Abortion	1.79	.407	365
History of Anemia	1.55	.499	365
Symtomatic	1.81	.394	365
History ofFatalDeath	1.92	.279	365
History of PretermLabour	1.96	.199	365
HistoryofJoinPain	1.52	.500	365
Result Status	1.55	.498	365

Correlations

		Age	MaritalStatus	LevelofEdu	PreviousHisAbort	HistoryofAnemia	Symtomatic	HistoryofFatalD	HistoryofPretermL	HistoryofJoinPain	ResulStatus
Pearson Correlation	Age	1.000	-.722	.085	-.329	.553	.487	-.264	-.183	.587	.060
	MaritalStatus	-.722	1.000	-.354	.463	-.845	-.511	.255	.187	-.903	-.097
	LevelofEdu	.085	-.354	1.000	-.179	.427	.027	-.102	-.048	.405	.056
	PreviousHisAbort	-.329	.463	-.179	1.000	-.401	-.216	.376	.132	-.441	.422
	HistoryofAnemia	.553	-.845	.427	-.401	1.000	.449	-.239	-.106	.913	.153
	Symtomatic	.487	-.511	.027	-.216	.449	1.000	-.148	-.066	.463	.444
	HistoryofFatalD	-.264	.255	-.102	.376	-.239	-.148	1.000	.283	-.235	.260
	HistoryofPretermL	-.183	.187	-.048	.132	-.106	-.066	.283	1.000	-.117	.119
	HistoryofJoinPain	.587	-.903	.405	-.441	.913	.463	-.235	-.117	1.000	.093
	ResulStatus	.060	-.097	.056	.422	.153	.444	.260	.119	.093	1.000
Sig. (1-tailed)	Age		.000	.052	.000	.000	.000	.000	.000	.000	.125
	MaritalStatus	.000		.000	.000	.000	.000	.000	.000	.000	.032
	LevelofEdu	.052	.000		.000	.000	.304	.026	.179	.000	.143
	PreviousHisAbort	.000	.000	.000		.000	.000	.000	.006	.000	.000
	HistoryofAnemia	.000	.000	.000	.000		.000	.000	.022	.000	.002
	Symtomatic	.000	.000	.304	.000	.000		.002	.105	.000	.000
	HistoryofFatalD	.000	.000	.026	.000	.000	.002		.000	.000	.000
	HistoryofPretermL	.000	.000	.179	.006	.022	.105	.000		.013	.011
	HistoryofJoinPain	.000	.000	.000	.000	.000	.000	.000	.013		.039
	ResulStatus	.125	.032	.143	.000	.002	.000	.000	.011	.039	
N	Age	365	365	365	365	365	365	365	365	365	365
	MaritalStatus	365	365	365	365	365	365	365	365	365	365
	LevelofEdu	365	365	365	365	365	365	365	365	365	365
	PreviousHisAbort	365	365	365	365	365	365	365	365	365	365
	HistoryofAnemia	365	365	365	365	365	365	365	365	365	365
	Symtomatic	365	365	365	365	365	365	365	365	365	365
	HistoryofFatalD	365	365	365	365	365	365	365	365	365	365
	HistoryofPretermL	365	365	365	365	365	365	365	365	365	365
	HistoryofJoinPain	365	365	365	365	365	365	365	365	365	365
	ResulStatus	365	365	365	365	365	365	365	365	365	365

Coefficient Correlations[a]

Model			ResulStatus	LevelofEdu	HistoryofPretermL	HistoryofFatalD	MaritalStatus	PreviousHisAbort	Symtomatic	HistoryofAnemia	HistoryofJoinPain
1	Correlations	ResulStatus	1.000	-.137	-.076	-.212	.013	-.544	-.554	-.177	.106
		LevelofEdu	-.137	1.000	.013	.034	-.001	.077	.247	-.142	-.070
		HistoryofPretermL	-.076	.013	1.000	-.226	-.179	.058	.009	-.023	-.080
		HistoryofFatalD	-.212	.034	-.226	1.000	.005	-.129	.149	.100	-.047
		MaritalStatus	.013	-.001	-.179	.005	1.000	-.135	.190	.102	.571
		PreviousHisAbort	-.544	.077	.058	-.129	-.135	1.000	.278	.062	.000
		Symtomatic	-.554	.247	.009	.149	.190	.278	1.000	.035	-.046
		HistoryofAnemia	-.177	-.142	-.023	.100	.102	.062	.035	1.000	-.636
		HistoryofJoinPain	.106	-.070	-.080	-.047	.571	.000	-.046	-.636	1.000
	Covariances	ResulStatus	1.104	-.084	-.151	-.325	.023	-.705	-.748	-.339	.246
		LevelofEdu	-.084	.339	.014	.029	-.001	.055	.185	-.150	-.090
		HistoryofPretermL	-.151	.014	3.595	-.623	-.602	.136	.022	-.081	-.336
		HistoryofFatalD	-.325	.029	-.623	2.121	.012	-.232	.278	.264	-.151
		MaritalStatus	.023	-.001	-.602	.012	3.136	-.296	.432	.327	2.229
		PreviousHisAbort	-.705	.055	.136	-.232	-.296	1.518	.440	.139	.001
		Symtomatic	-.748	.185	.022	.278	.432	.440	1.653	.083	-.130
		HistoryofAnemia	-.339	-.150	-.081	.264	.327	.139	.083	3.305	-2.549
		HistoryofJoinPain	.246	-.090	-.336	-.151	2.229	.001	-.130	-2.549	4.865

a. Dependent Variable: Age

ANOVA[a]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	23304.652	9	2589.406	56.771	.000[b]
	Residual	16192.159	355	45.612		
	Total	39496.811	364			

a. Dependent Variable: Age

b. Predictors: (Constant), Result Status, LevelofEducation, HistoryofPretermLabour, HistoryofFatalDeath, MaritalStatus, Previous History of Abortion, Symtomatic, HistoryofAnemia, HistoryofJoinPain

APPENDIX V

YOBE STATE MAP SHOWING THREE ZONES