**Sudan University of Science and Technology**

**College of Graduate Studies**

# Seroprevalence of Parvovirus B19 in pregnant women attending antenatal clinics in Military Hospital (2015)

# الانتشار المصلي لفيروس البارفو B19 لدى النساء الحوامل اللائي يترددن علي عيادات ما قبل الولاده في المستشفى العسكري (2015)

A thesis submitted in partial fulfiment for the requirement of M.Sc degree in Medical Laboratory Science (Microbiology)

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**(2015)**

# الآية

## بسم الله الرحمن الرحيم

قَالَ تَعَالَىٰ:

﴿ ٱللَّهُ لَآ إِلَٰهَ إِلَّا هُوَ ٱلۡحَيُّ ٱلۡقَيُّومُۚ لَا تَأۡخُذُهُۥ سِنَةٞ وَلَا نَوۡمٞۚ لَّهُۥ مَا فِي ٱلسَّمَٰوَٰتِ وَمَا فِي ٱلۡأَرۡضِۗ مَن ذَا ٱلَّذِي يَشۡفَعُ عِندَهُۥٓ إِلَّا بِإِذۡنِهِۦۚ يَعۡلَمُ مَا بَيۡنَ أَيۡدِيهِمۡ وَمَا خَلۡفَهُمۡۖ وَلَا يُحِيطُونَ بِشَيۡءٖ مِّنۡ عِلۡمِهِۦٓ إِلَّا بِمَا شَآءَۚ وَسِعَ كُرۡسِيُّهُ ٱلسَّمَٰوَٰتِ وَٱلۡأَرۡضَۖ وَلَا يَـُٔودُهُۥ حِفۡظُهُمَاۚ وَهُوَ ٱلۡعَلِيُّ ٱلۡعَظِيمُ ﴾

سُورَةُ البَقَرَةِ ﴿٢٥٥﴾

**DEDICATION**

*To the fountain of patience and optimism and hope*

*To the big heart* ***my dear father***

*To each of the following in the presence of God and His Messenger,* ***my dear mother***

*To those who have demonstrated to me what is the most beautiful of* ***my brother's life***

*To the people who paved our way of science and knowledge*

*All our* ***a Distinguishedteachers***

*To the taste of the most beautiful moments with* ***my friends***

**ACKNOWLEGEMENT**

*Firstly I would like to thank ALMIGHTY ALLAH for giving patience and health to complete this work.*
*Special thank for my supervisor Prof. YouisfFadlalla , for his patience, giddiness and support.*
*I also thank all members of Microbiology Department for provided good material and equipments.*
*Great thanks to all women who participated in the study and to the doctors and technicians who helped during sample collection in Military Hospital.*

# ABSTRACT

This study was conducted for serological detection of parvovirus B19 antibodies in serum of pregnant women in Khartoum State during the period from April to June 2015. Blood samples were collected from 90 pregnant women less than 45 years of age attending antenatal clinics inOmdurman Military Hospital. All samples were tested for the presence of parvovirus B19 IgG antibodies using ELISA Kits. Parovirus B19 IgG antibodies were detected in 37 (41.1%) of the total samples examined. Most of the positive cases were in pregnant women aged 15-25 years20 (22.2%) followed by 13 (14.2%) in pregnant women between 26-35 years of age. However, the pregnant women between 36-45 years of age had the lowest 4(4.4%) parvovirus B19 seropositivity.Parvovirus B19 seropositivity was 10 (11.1%) among the pregnant women that hadabortion, and this did not reach statistical significance. Most of pregnant women have parvovirus B19 IgG antibodies, were within 3rd trimester 20 (22.2%), followed by 2nd trimester 11 (12.2%), and 1st trimester 6 (6.7%).The high frequency of positive subjects among pregnant women that had number of household children between 0-2 was 30 (33.3%), but the pregnant women that had number of household children between 3-5 was 7 (7.8%). The seropositivty of parvovirus B19 IgG antibodies among pregnant women that had history of blood transfusion was 3 (3.3%).There was ststistical correlation of serological finding of the parvovirus B19 and age of patients, whilenon significantdifference with other risk factors.

**ملخص الاطروحه**

قد أجريت هذه الدراسة للكشف عن الأجسام المضادة المصلية لفيروس البارفو B19 في الدم من النساء الحوامل في ولاية الخرطوم خلال الفترة من ابريل الى يونيو 2015. عينات دم جمعت من 90 امرأة حامل أقل من 45 سنة من العمر يترددن على عيادات ما قبل الولادة في المستشفى العسكري. تم اختبار جميع هذه العينات باستخدام تقنيه الاليزا للكشف عن وجود الاجسام المضاده لفيروس البارفو B19. تم معالجة النتائج التي تم الحصول عليها وتحليلها إحصائيا . تم الكشف عن الأجسام المضادة لفيروس البارفو B19ايجابيه المصل في 37 (41.1%) من مجموع العينات التي تم فحصها. وكانت معظم الحالات الإيجابية لدى النساء الحوامل الذين تتراوح أعمارهم بين 15-25 سنة 20 (22.2% ) يليها 13 (14.2 % ) في النساء الحوامل بين 26-35 سنة من العمر . ومع ذلك ، كان على النساء الحوامل بين 36-45 سنة من العمر أدنى نسبه 4(4.4 % ) لفيروس البارفو B19 إيجابية المصل . كان فيروس البارفو B19 إيجابية المصل 10(11.1 % ) بين النساء الحوامل التي حصلت على الإجهاض ، و هذا لم يصل أهميةاحصائيه.معظم النساء الحوامل لديهم أجسام مضادة لفيروس البارفو B19كانت ضمن الثلث الثالث من الحمل 20 (22.2%) ، تليها الثلث الثاني 11 ( 12.2 %) والثلث الاول6 (6.7 %).كانت وتيرة عالية من الاجسام المضاده لفيروس البارفو بين النساء الحوامل اللاتي لديهن عدد من الأطفال المنزلية بين 0-2 ,30 (33.3% )، ولكن النساء الحوامل اللاتي لديهان عدد من الأطفال المنزلية بين 3-5 ( 7.8 % ) . كانت نسبه الأجسام المضادة لفيروس البارفوB19 بين النساء الحوامل اللاتي لديهن تاريخ من نقل الدم 3 (3.3 %) . كان هناك ارتباط احصائي لتقصي المصلي من B19 البارفو و عمر المرضى ، بينما لا يوجد ارتباط مع عوامل الخطر الأخرى

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# CHAPTER ONE
# INTRODUCTION

# 1. INTRODUCTION

## 1.1 Background

Parvovirus B19 was first discovered by Cossart and colleagues in 1975 in the sera of asymptomatic patients being screened for hepatitis B infection. The name originates from the coding of a serum sample, number 19 in panel B, that gave anomalous results when tested by counterimmunoelectrophoresis and radioimmunoassay (Jin et al., 2003)

Human parvovirus B19 (B19V) is a small single-stranded DNA virus. It is the only member of the Parvoviridae family, genus Erythrovirus, known to be pathogenous to humans (Lukashov and Goudsmit, 2001). Its genome of about 2500 base pairs encodes for three major proteins. Two structural proteins (VP1 and VP2) make up the viral capsid. The viral capsid consists for 95% of VP2; the remaining 5% is made up of VP1. VP1 differs from VP2 only in the N-terminal region with the addition of 227 amino acids that mainly are located outside the virion and accessible to antibody binding (Kaufmann *et al*., 2004). The non-structural protein (NS1) is presumed to be involved in viral replication, activation of viral gene transcription and inducing apoptosis in target cells (Chisaka *et al*., 2003)

It is responsible for erythema infectiosum, fifth disease or slapped cheek syndrome, a common mild self-limiting childhood illness. However, parvovirus B19 infection can cause serious complications, especially in people who have haematological disorders or who are immunocompromised, and in women who are pregnant. It is a cause of nonimmune hydrops and fetal death.

Parvovirus B19 is most commonly spread by respiratory secretions or from hand to mouth contact (clinical practice guideline Royal College of Physicians of Ireland, 2014). Other modes of transmission include blood product infusion and transplacental transfer. As the main mode of transmission is respiratory, epidemics of parvovirus B19 infection can occur. Outbreaks usually occur every three to five years and may last up to six months (Rodis *et al*., 1998a).

Infection with parvovirus B19 is common in developed countries. Studies have indicated that about 15% of preschool children, 50% of adults, and 85% of elderly people have serological evidence of past infection. Seasonal outbreaks of parvovirus B19 infection occur every 3–5 years. During school outbreaks, 10–60% of exposed children develop symptoms consistent with parvovirus B19 infection (clinical practice guideline Royal College of Physicians of Ireland, 2014).

## 1.2 Rationale

Maternal B19V infection can give rise to serious fetal complications during pregnancy. Up to 50% of women are non-immune and susceptible to B19V infection. Infection may result in anemia, spontaneous abortion and/or hydrops fetalis. Pregnancy does not appear to affect the course of the infection, but infection may affect the pregnancy (Alger,1997). The transmission rate of maternal parvovirus B19 infection to the fetus is 17% to 33%( Harger *et al*.,1998). Early diagnosis of B19V infection will identify those at risk and may allow for early intervention therapy, thereby improving fetal survival.

There is only one study done in Sudanese pregnant women infected with parvovirus B19 have been published (Adam *et al*., 2015 ). for these reasons and because of the clinical importance of the disease we found it highly important to study the frequencies of parvovirus B19 in pregnant women attending antenatal clinics in Khartoum State ,Sudan.

## 1.3 Objectives

### 1.3.1 General objective

- To study the prevelance of anti parvovirus B19 IgG antibodies in pregnant women attending antenatal clinic in Military Hospital.

### 1.3.2 Specific objectives

- To measure the prevelance of anti parvo virus B19 IgG antibodies in pregnant women using ELISA.
- To determine the significant effect of the risk factors.

# CHAPTER TWO

# LITERATURE REVIEW

# 2. LITERATURE REVIEW

## 2.1 Taxonomy

The classification of the family *Parvoviridae* relies on morphology and functional characteristics. Parvoviruses are common animal and insect pathogens. Until the recent identification of the circoviruses and the related TT viruses, parvoviruses were among the smallest DNA-containing viruses able to infect mammalian cells; hence, the name "parvum" (Latin), meaning small (Berns, 1996). Based on the ability to infect vertebrate or invertebrate cells the *Parvoviridae* are divided into *Parvovirinae* and *Densovirinae*, respectively (Berns, 1996; International Committee on Taxonomy of Viruses, 2000). *Parvovirinae* are subdivided into three genera according to their transcription maps, the nature of the terminal repeats, and the ability to efficiently replicate either autonomously (genus *Parvovirus*), with helper virus (genus *Dependovirus*), or preferentially in erythroid cells (genus *Erythrovirus*) . Only members of the *Dependovirus* and *Erythrovirus* genera are known to infect humans. The members of genus *Dependovirus*, which includes the adeno-associated viruses 1 to 6, require coinfection of target cells with adenovirus or herpesvirus for efficient replication. B19 is autonomous in the sense that it does not require the presence of a helper virus and was, therefore, until recently classified in the genus *Parvovirus*. Since replication only occurs in erythrocyte precursors, B19 is now classified as a member of the *Erythrovirus* genus, of which it is the only accepted member and type species (International Committee on Taxonomy of Viruses, 2000 ).

## 2.2 Morphology

The B19 virion has a simple structure composed of only two proteins and a linear, single-strand DNA molecule . The nonenveloped viral particles are ~22 to 24 nm in diameter and show icosahedral symmetry, and often both empty and full capsids are visible by negative staining and EM. Mature infectious viral particles have a molecular weight of 5.6 x 106 and a buoyant density in a cesium chloride gradient of 1.41 g/ml. The virion is composed of 60 copies of capsomer, and both negative and positive strands of DNA are packaged. X-ray crystallography has shown that the surface of B19 is significantly different from those of other parvoviruses by lacking prominent spikes on the threefold icosahedral axes involved in host recognition and antigenicity (Heegaard and Brown, 2002). The limited DNA content and the absence of a lipid envelope make B19 extremely resistant to physical inactivation. The virus is stable at 56°C for 60 min, and lipid solvents have no effect. Inactivation of virus may be achieved by formalin, Beta propiolactone, and gamma irradiation (Heegaard, 2002).

## 2.3 Genomic Structure

The single-stranded genome contains 5,596 nucleotides (nt), composed of an internal coding sequence of 4,830 nt flanked by the terminal repeat sequences of 383 nt each. The terminal sequences are palindromic and capable of assuming hairpin duplex configurations, serving as primers for the synthesis of the complementary strand. As in most animal parvoviruses, the B19 genome has two large open reading frames, with the single nonstructural protein (NS1) encoded by genes on the left side of the genome and the two capsid proteins (VP1 and VP2) by genes on the right side. Transcription produces at least nine overlapping mRNA transcripts, all initiating from the single P6 promoter at the extreme left side of the genome. The most important viral proteins include the major nonstructural protein NS1 and the two structural proteins VP1 and VP2 . In addition, while other parvoviruses coterminate RNA species at polyadenylation sites at the far right side, several B19 transcripts terminate in the middle of the genome and use an unusual polyadenylation signal. These

RNAs are derived from the left side of the genome and share an open reading frame with NS1(Heegaard and Brown, 2002).

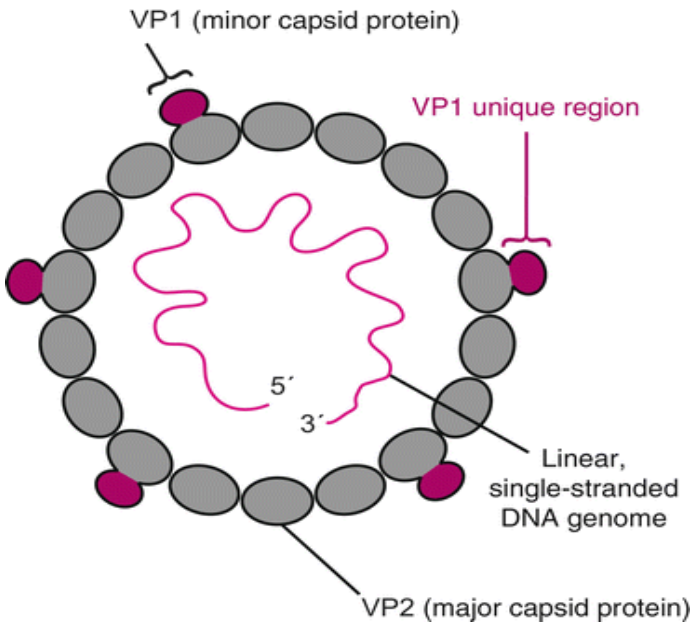



**Fig (2.1) : Structure of parvovirus B19**

### 2.4 Transmission

Parvovirus B19 is transmitted via droplet infection, or via contact with saliva, blood or other body fluids and vertically from mother to fetus. Acutely infected individuals have extremely high viral concentrations in blood and other body fluids such as saliva or urine. Since parvoviruses have no lipid capsule, their infectivity is unaffected by solvents and detergents (Modrow and Gärtner , 2006). Nosocomial transmission has been described infrequently (Miyamoto *et al*., 2000).

### 2.5 Viral Life Cycle and Blood Group P Receptor

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

B19 was initially shown to agglutinate human red cells, and it was hypothesized that the same agglutinin may act as the host cell receptor on erythroid progenitor cells. Thus, the hemagglutinin was identified as the glycolipid globoside, also known as the blood group P antigen, by using hemagglutination as a surrogate marker. B19 and B19 VP2 VLP both bind directly to P antigen, and in tissue culture either excess P antigen or anti-P monoclonal antibody can protect erythroid progenitors from infection with B19, thus demonstrating that P antigen is the B19 receptor. In addition, individuals who genetically lack P antigen (1 in 200,000 individuals) are naturally resistant to B19 infection: none show serologic evidence of past infection, and BM cells in in vitro studies maintain normal erythropoiesis and cannot be infected even in the presence of high concentrations of virus. P antigen is expressed on erythroid progenitors, consistent with the observed tropism of B19. However, the presence of P antigen is almost certainly not sufficient to explain the tropism of B19 to erythroid cells. P antigen is also present on megakaryocytes, endothelial cells, and fetal myocytes, however, none of these cell types have been shown to be permissive for B19 replication. Transfection studies of permissiveand nonpermissive cells with plasmids containing B19 genome suggest that in cells nonpermissive for B19 there may be a block in full-length transcript production, leading to expression of the cytotoxic NS1 but no production of capsid transcripts (Heegaard and Brown ,2002). Alternatively, tropism may be mediated by the presence of a second, as yet unidentified receptor. However, the expression of P antigen on these cell types may mediate transplacental infection, contribute to the rash of erythema infectiosum, or lead to myocarditis. Also, the level of P-antigen expression does not correlate with the efficiency of viral binding, providing further evidence for the existence of a putative cellular coreceptor for efficient entry of B19 into human cells (Weigel-Kelley *et al*., 2001).

## 2.6 Pathogenesis of B19V in pregnancy

B19V is a potent inhibitor of hematopoiesis because it lytically infects erythroid precursor cells. The cellular receptor for B19V is globoside or P-antigen. It is found on erythrocyte progenitor cells (erythroblasts and megakaryocytes), but also on erythrocytes, synovium, placental tissue, fetal myocardium, and endothelial cells (Chisaka *et al*., 2003; Young and Brown, 2004). Viral replication of B19V is restricted to erythroid progenitor cells. Expression of globoside on the cell surface is not sufficient for viral cell entry and therefore for viral replication. Globoside is a binding-site for B19V on the cell surface, but B19V needs α5β1 integrin as a cellular co-receptor and functional activity of β1 integrin for cell entry (Weigel-Kelley *et al*., 2003). Recent data suggest that Ku80 auto antigen may also function as a co-receptor (Munakata *et al.,* 2005). The NS1 protein of B19V is involved in inducing cell death. Both direct toxic cell injury and the induction of apoptosis contribute to this effect. Infection with B19V usually takes place through respiratory droplets, but B19V can also be transmitted by blood and blood derived products and can be transmitted vertically from mother to fetus ((Enders *et al*., 2004). No vertical transmission has been described if the mother is immune at the time of exposure. It is highly unlikely that fetal infection occurs if the mother has IgG antibodies since this is thought to give life-long protection against re-infection with B19V. When maternal infection occurs, maternal viremia reaches its peak approximately 1 week after infection. Symptoms such as erythema infectiosum, mild fever, arthralgia and headache start approximately 10-14 days after infection in about 50% of infected women. At the time of the occurrence of IgM antibodies, presumably during the maternal peak viral load (day 7), the risk of vertical transmission may be maximal (De Haan *et al*., 2005). Until now, the exact route of transmission is unknown.

Jordan *et al*. (Jordan and DeLoia, 1999) showed a decreasing expression of globoside within the villous trophoblast layer of the human placenta with increasing gestational age. This may explain why the incidence of fetal morbidity and mortality related to B19V infection decreases with gestational

age (Enders *et al*., 2004). More likely, passive transfer of maternal antibodies after the age of 25 weeks may reduce fetal morbidity and mortality at late stages of pregnancy.

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 and Brown, 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. 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).

**2.7. Fetal Effects of Parvovirus B19 Infection**

**2.7.1 Spontaneous Abortion**

First and second trimester parvovirus infections carry an excess loss risk 10% above baseline (5%) but a low risk of long term sequalae during childhood. The spontaneous loss rate of foetuses affected with parvovirus B19 before 20 weeks' gestation is 14.8% and after 20 weeks' gestation is 2.3% (SOGC, 2002). The reason is uncertain but may be related to multisystem organ damage.

**2.7.2 Congenital Anomalies**

Currently, there does not appear to be any evidence that parvovirus B19 infection increases the risk of congenital anomalies in humans (clinical practice guideline Royal College of Physicians of Ireland,2014).

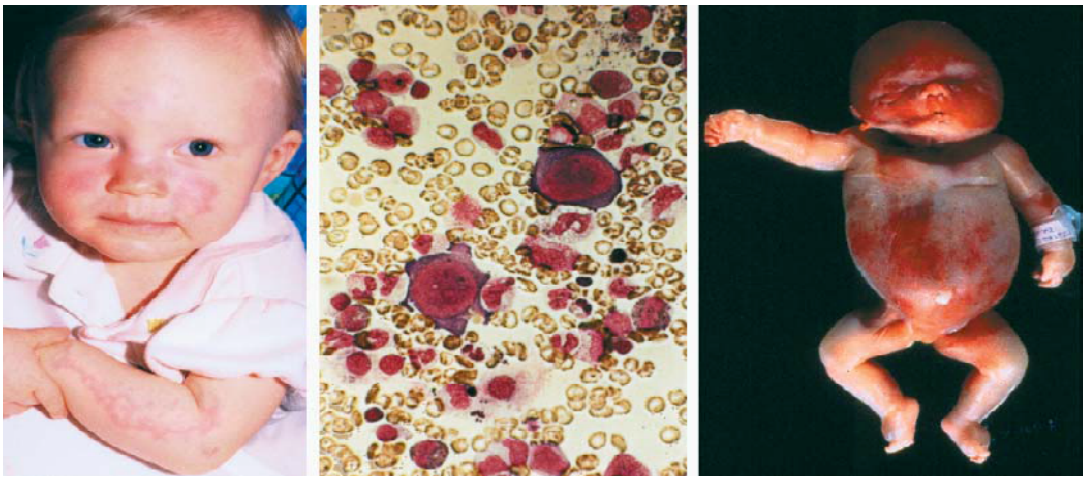
**2.7.3 Hydrops**

Parvovirus B19 has been associated with hydrops fetalis . Possible mechanisms for this include fetal anemia due to the virus crossing the placenta, combined with the shorter half life of fetal red blood cells, leading to the severe anemia, hypoxia, and high output cardiac failure that are associated with fetal hydrops. Other possible causes include fetal viral myocarditis leading to cardiac failure,

and impaired hepatic function caused by direct damage of hepatocytes and indirect damage due to hemosiderin deposits . Another mechanism is the rapidly expanding erythrocyte volume which increases 34-fold during the second trimester. This along with the shorter fetal red cell half life causes increased fetal sensitivity to transient red cell aplasia. There are a number of published studies of the rate of fetal loss and hydrops with parvovirus B19 infection (Harger *et al.,* 1998). Several studies found a higher fetal loss rate when the infection was acquired before 19 to 20 weeks' gestation (14.8%) compared to that after 20 weeks (2.3%) (Rodis *et al*., 1998b). If a fetus develops hydrops, ultrasound signs include ascites, skin edema, pleural and pericardial effusions, as well as placental edema. It is estimated that parvovirus B19 infection accounts for 8% to 10% of non-immune hydrops , although some studies found molecular evidence of parvovirus B19 in 18% to 27% of cases of non immune hydrops (clinical practice guideline Royal College of Physicians of Ireland,2014).

**2.7.4 Long-term Neonatal Outcome**

There have been few studies of the long-term effects on children of maternal parvovirus B19 infection (Rodis, 1999). Case reports of neonatal complications of maternal parvovirus B19 infection have been reported, including hepatic insufficiency, myocarditis, transfusion dependent anemia, and central nervous system abnormalities (SOGC, 2002). However, a case series of 108 children born to women with parvovirus B19 infection during pregnancy and 99 women who had immunological evidence of past infection reported no difference between the groups in the incidence of congenital anomalies, overall learning disabilities, or neurologic morbidity (Rodis *et al*., 1998b). Most children born to mothers who develop parvovirus B19 infection in pregnancy do not appear to suffer long-term sequelae, but further studies are needed (Rodis, 1999). Parvovirus B19 itself does not seem to cause long-term neurologic morbidity, but severe anaemia may be an independent risk factor for long-term neurologic sequelae (Ryan *et al*., 1997).



**A** **B** **C**

**Fig (2.2) : Clinical Manifestations of Parvovirus B19 Infection.**

Panel A shows typical cutaneous eruptions in fifth disease, including "slapped" cheeks in children and a more generalized lacy, reticular pattern of erythema. Panel B shows a bone marrow aspirate with no mature erythroid precursors and with characteristic giant pronormoblasts. In Panel C, hydrops fetalis is evident in an infant who was infected in uterus in midtrimester (Young and Brown, 2004).

## 2.8. Epidemiology of B19V

Infection with parvovirus B19 is common worldwide. The yearly peak incidence of infection occurs during spring and epidemics occur every 4 years (Bosman *et al*., 2002). The prevalence of IgG antibodies directed against B19V in the population ranges from 2 to 15% in children 1–5 years old, 15–60% in children 6–19 years old, 30–60% in adults and more than 85% in the geriatric population (Heegaard and Brown, 2002).

About 35–45% of women of childbearing age do not possess protective IgG antibodies against B19V. The incidence of acute B19V infection in pregnancy is approximately 1–2% in endemic periods, but in epidemic periods infection rate may rise to >10% (Dembinski *et al*., 2003; Trotta *et al*., 2004). In case of maternal infection, vertical transmission occurs in 33–51% of cases, the risk of adverse fetal outcome is approximately 10% (Chisaka *et al* ., 2003). Infections

with B19V are estimated to cause 48 cases of fetal death each year in the Netherlands (Bosman *et al*., 2002).

**2.9 Immune response to B19**

Humoral immune response to B19 seems to be a crucial factor in disease resolution, and cell mediated immunity plays either none or very little role. B19 infection is ordinarily resolved with the production of specific antibodies that neutralise virus infectivity for erythroid host cells, and sera from these persons is also known to neutralise the inhibitory effect of B19 on erythrocyte colony formation in vitro. Nevertheless, persistent infection with B19 is documented and antibodies to B19 are widely found in human populations worldwide. Although only 10% children < 5 yrs have circulating antibodies to B19, the prevalence of antibody response rises upto 15% - 35% in school children, about 50 % in adults (Sharad and Kapur, 2005 ), and it is 85% or more in ageing population > 70 years of age. Seroprevalence in blood donors in developed countries is upto 60%. Based on a study done in 1,000 normal voluntary blood donors in southern India, the seroprevalence of B19 IgG has been reported to be 39.89%. Viral protein 1 (VP1) is considered to be important in the formation of neutralising antibodies to B19 and is also the known target specificity of pooled human immunoglobulin used in therapy of chronic infection. The recombinant empty capsid, containing only VP2, does not elicit a strong neutralising antibody response, but VP2 accounts for haemagglutinating activity of the virus. Antibody titre to NS-1 protein is usually low and has little diagnostic value. However, there is an increasing interest in the role of anti-NS-1 antibodies in chronic persistent B19 infection. At the time of detection of B19 IgG, a fine maculopapular skin rash and arthralgia occur – maybe due to immune complex deposition as it has been shown to be linked with production of specific neutralising antibodies ( Lee *et al*., 2003). Cartter and colleagues found that among the IgM positive pregnant women who had been exposed to cases of B19 infection, only 50% reported symptoms of rash or arthropathy. Joint symptoms associated with B19 infection have been reported to occur in 8% infected children and upto 80% infected adults, the majority of these being

women. Abnormal immune response to B19 antigens has been suspected in patients who developed B19 induced arthropathy.( Sharad and Kapur, 2005 ) reported that B19 DNA can be detected in the synovium of 75% patients but only in 16.7% patients with non-rheumatoid arthritis.

**2.10. Diagnosis of B19**

**2.10.1. Serological tests**

Serologic examination of maternal blood is the first and most useful diagnostic test that should be performed as soon as possible once B19V infection is suspected during pregnancy. B19 IgG or IgM antibody detection now is most often performed by enzyme immune assays, which tend to replace the immunofluorescent technique (Beersma *et al*., 2005). B19V specific IgM antibodies become detectable in maternal serum within 7–10 days after infection, sharply peak at 10–14 days, and than rapidly decrease within 2 or 3 months (De Jong *et al*., 2006). IgG antibodies will rise considerably more slowly and reach a plateau at 4 weeks after infection. As a result, comparison of the IgG and IgM EIA ratio's (or IFA titers) can provide an indication of the actual stage of B19 infection. If IgM titers exceed IgG titers, the B19V infection took place less than month ago, viral load levels will be high, and fetal complications, if absent, may still develop (Beersma *et al*., 2005).

Although measurement or maternal IgM is highly sensitive and specific, one should be aware of two classic pitfalls. First, after a recent contact, there will be a serologic window of 7 days, during which both IgG and IgM remain negative. Secondly, at the time of clinically overt hydrops fetalis, IgM levels may already have become low or (rarely) even undetectable. It is even conceivable that continued antigenic shedding resulting from the fetal infection may contribute to the decline of maternal IgM levels. In such cases, PCR analysis of the same blood sample will be highly informative.

In contrast to the reliability of B19V serology in maternal blood, serologic examination of fetal and neonatal blood samples is highly unreliable since most unborn children will not produce IgG or IgM responses to B19V. Therefore examination of fetal serum or neonatal (cord blood) for B19V infection should

be confined to B19 DNA detection by PCR, which effectively will confirm or exclude fetal B19V infection (De Jong *et al*., 2006).

### 2.10.2. Viral DNA detection (PCR)

Nucleic acid amplification to detect B19V DNA is an extremely sensitive means (most published PCR assays are able to detect viral DNA at 1–100 copies/mL) to detect viral DNA in a sample. This method is especially useful in patients lacking an adequate antibody-mediated immune response, immunocompromised or immunosuppressed individuals, and fetuses. In such cases serological testing for B19V is unreliable (Jordan, 2001). Using standard procedures, detection of B19V specific IgM in fetal blood has a sensitivity of 29% compared to almost 100% for PCR (Beersma *et al*., 2005; Enders *et al*., 2004). However, low B19V DNA levels may persist for years after acute infection and therefore low-positive PCR results for B19V do not prove recent infection (Lindblom *et al*., 2005).

### 2.10.3 . Ultrasound measurements

If a recent parvovirus infection is suspected during pregnancy, ultrasound examination should be performed to exclude the presence of fetal anemia and hydrops. Blood flow in anemic fetuses has a hyperdynamic pattern, which can be detected non-invasively with Doppler ultrasonography in various fetal blood vessels. These changes in blood flow are thought to result from increased cardiac output and decreased viscosity of fetal blood. Blood flow in the middle cerebral artery is the first to respond to fetal anemia due to the early response of brain tissue to anemia. An increase in the middle cerebral artery peak systolic velocity (MCA-PSV) (Fig. 2.3) is a very sensitive measure to identify fetal anemia caused by B19V infection (Cosmi *et al*., 2002). Timing of intrauterine transfusion for treatment of fetal anemia and prevention of fetal hydrops can be based on these MCA-PSV measurements. B19V-associated fetal hydrops, an accumulation of excess fluid in at least two body compartments of the fetus, can be easily seen on fetal ultrasound (Fig.2.4) (De Jong *et al*., 2006). Hydrops caused by anemia usually manifests itself first by ascites, with enlargement and thickening of the fetal heart. Untreated, fluid

accumulation progresses with skin edema, pericardial effusion and placental edema. Pleural effusions are late and minimal in anemic hydrops. Amniotic fluid volume may be normal or even decreased; polyhydramnios is rare (Van Kamp *et al*., 2001).

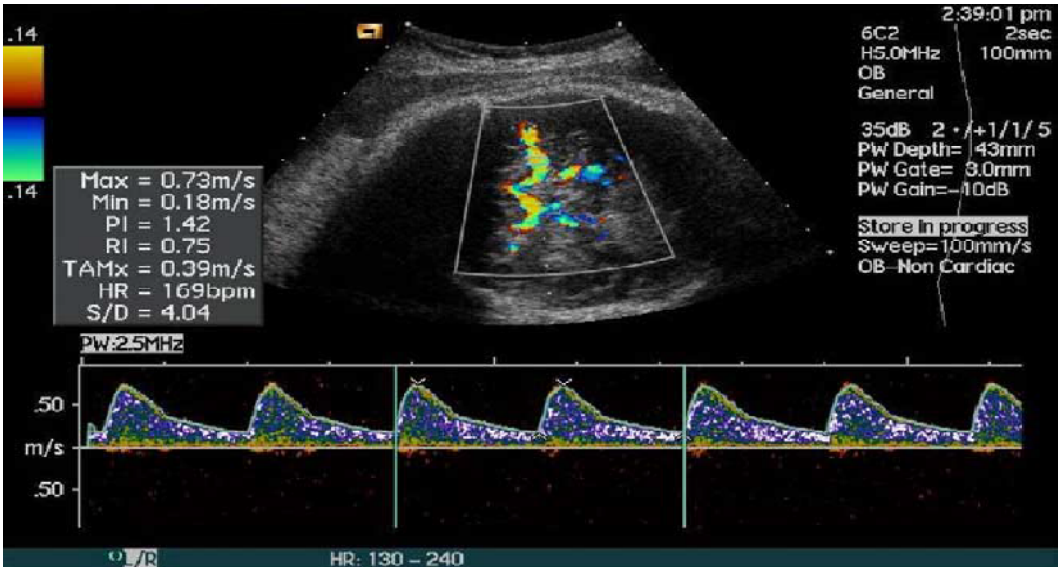



**Fig (2.3) : Measurement of peak systolic velocity in the middle cerebral artery (MCA-PSV).**

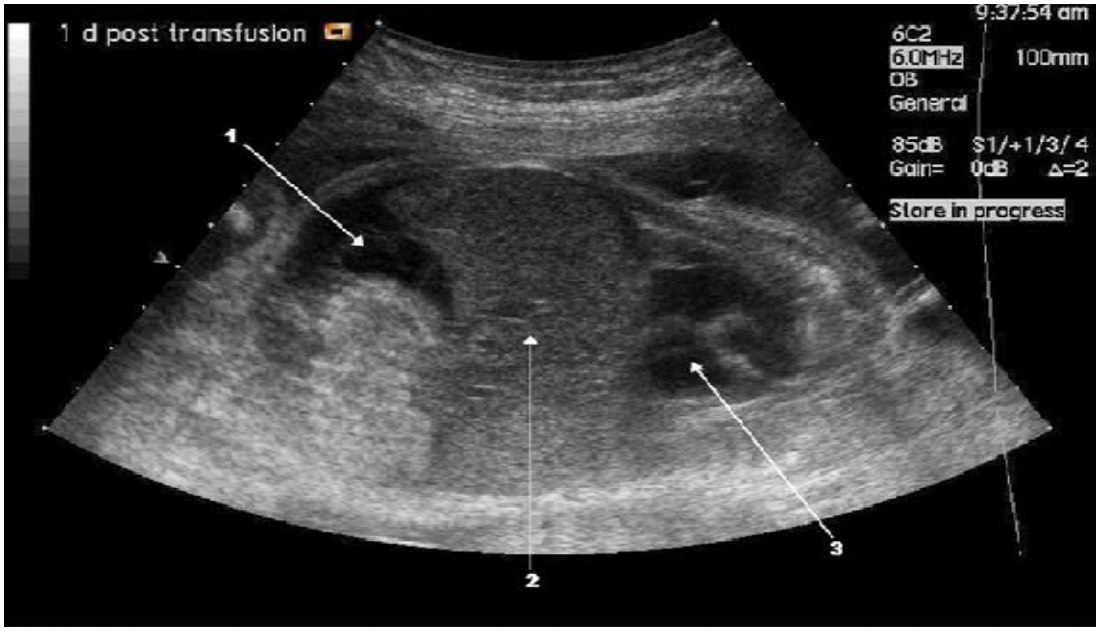



**Fig (2.4) : Fetal ascites (1) and enlargement of fetal liver (2) and fetal heart (3).**

### 2.10.4 Culture

There is no animal model for B19, and virus can only be grown in culture with difficulty. In vitro studies of B19 in explanted human BM cultures have confirmed the erythroid specificity of this virus, with B19 inhibition of the colony formation of late erythroid progenitors and the more primitive burst-forming erythroid progenitors and sparing of the myeloid precursors. While the pluripotent stem cell is sheltered, the susceptibility of erythroid progenitors to B19 infection increases with differentiation. B19 can be cultured in erythroid progenitor cells from a variety of sources, including human BM , fetal liver, umbilical blood, and peripheral blood. In all culture systems erythropoietin is required to maintain viral replication, probably by supporting the rapid division of erythroid progenitors. All systems are culture explants only and are not suitable for long-term culture.

However, B19 can also be propagated in a few specialized cell lines: two megakaryoblastoid cell lines, MB-02 and UT-7/Epo, and two human erythroid leukemia cell lines, JK-1 and KU812Ep6 (Heegaard and Brown ,2002). These lines have been used to study mechanisms of replication and to develop neutralization (Bostic *et al*., 1999) and infectivity assays (Miyagawa *et al*., 1999). However, the yield of virus from all these cultures is poor, and they cannot be used as a source of antigen for diagnostic tests.

### 2.10.5. Diagnostic Cytopathology

The cytopathic effect of infection of erythroid progenitor cells with B19, both in vivo and in vitro, is manifested as giant pronormoblasts (alternately referred to as lantern cells), first recognized in 1948 in the BM of patients with transient aplastic crisis. Giant pronormoblasts are early erythroid cells with a diameter of 25 to 32 _m, large eosinophilic nuclear inclusion bodies, and cytoplasmic vacuolization, and occasionally, “dogear” projections may be observed (as indicated in Fig. 2.5). EM of cells reveals cytopathic ultrastructural changes that include pseudopod formation, marginated chromatin, and virus particles in the nucleus (Heegaard and Brown ,2002).

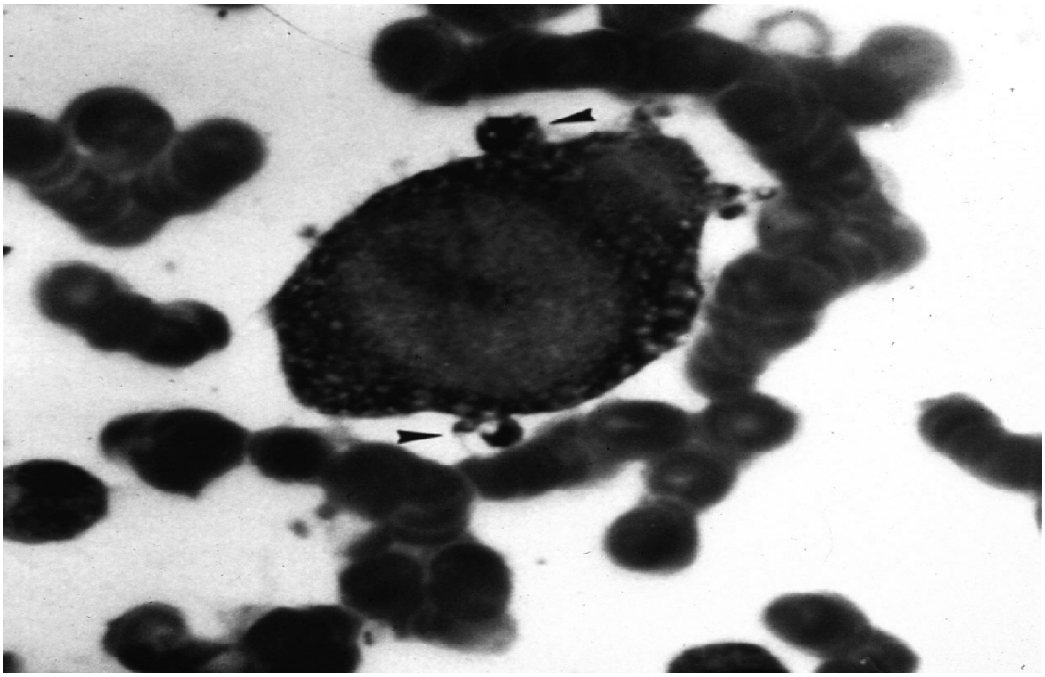


**Fig (2.5) : Giant pronormoblast. Arrowheads indicate dog-ear projections.**

## 2.11. Therapy

Management of B19V infection with IUT can correct fetal anemia and may reduce the mortality of B19V infection significantly (Fig. 2.6). Timely IUT of fetuses with severe hydrops reduces the risk of fetal death (Enders *et al*., 2004; Rodis *et al*., 1998b). In most cases, one transfusion is sufficient for fetal recovery. Following successful transfusion, it may take weeks for all hydropic signs to disappear. A few cases of spontaneous resolution of hydrops due to parvovirus infection have been described. This has led to discussion on the best time to intervene or whether to intervene at all. Only fetal blood sampling can provide information on fetal hemoglobin and reticulocyte count, and thus on whether the fetus may be in a spontaneous recovery phase or not. Most clinicians choose to proceed with transfusion when the fetal blood sample shows anemia, even if there is already evidence of recovery of erythropoiesis by a high reticulocyte count. Due to the rarity of the disease, a randomized trial to find the best policy is unlikely ever to be performed.

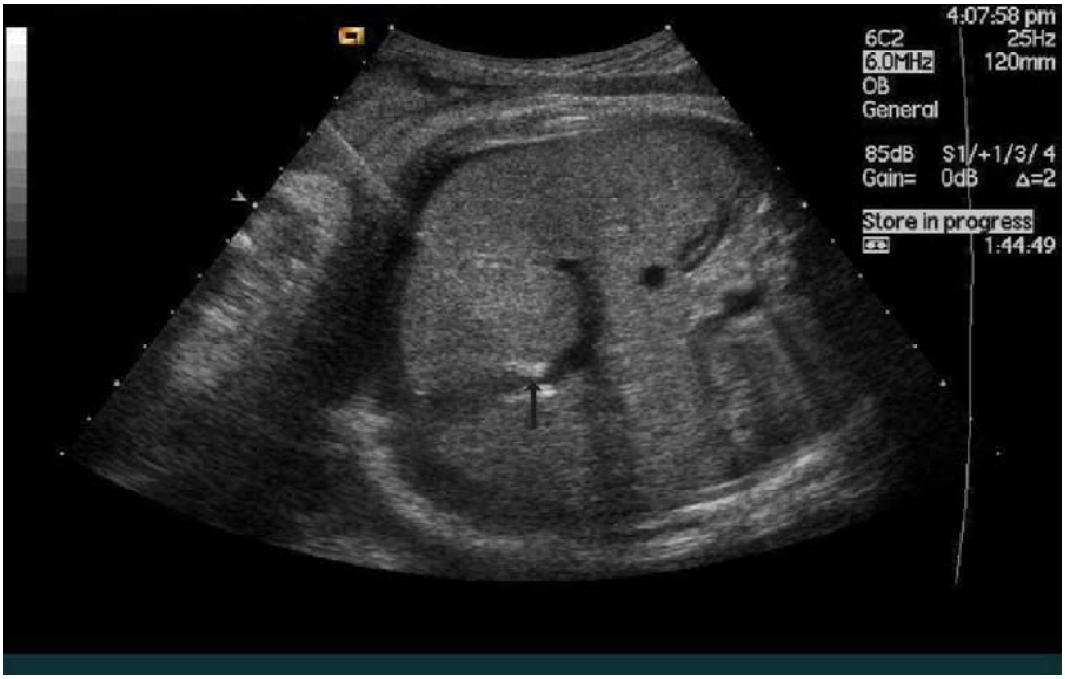



**Fig (2.6) : Insertion of the transfusion needle in the umbilical vein (arrow).**

## 2.12. Management of intrauterine B19V infection

Pregnant women who have been exposed to B19V, or those developing symptoms compatible with B19V infection, should be assessed for susceptibility or the presence of infection, by determining their B19V IgG and IgM status (Fig. 2.7). If the woman is immune to B19V (IgG positive, IgM negative) she can be reassured that recent exposure will not result in adverse consequences in her pregnancy. If there is no immunity to the virus and no seroconversion has taken place after 1–2 weeks, the woman is not infected with the virus. She should be counseled about the risk of B19V infection. If the woman has been infected with B19V (IgM positive), the fetus should be monitored for the development of hydrops fetalis by ultrasound examination including assessment of MCA-PSV, preferably weekly until 10–12 weeks post-exposure. If the fetus subsequently develops hydrops and/or anemia (increase in MCA-PSV), an IUT should be considered. A fetal blood sample should be taken during IUT to perform a measurement of B19V DNA, hemoglobin and reticulocyte counts. If the fetus is near term or at term, delivery should be considered (Cohen and Kumar, 2005). Intrauterine transfusion may be preferable to delivering a severely hydropic fetus, even close to term. Hydropic

neonates usually are prone to severe respiratory problems, which can be prevented by allowing intrauterine recovery.

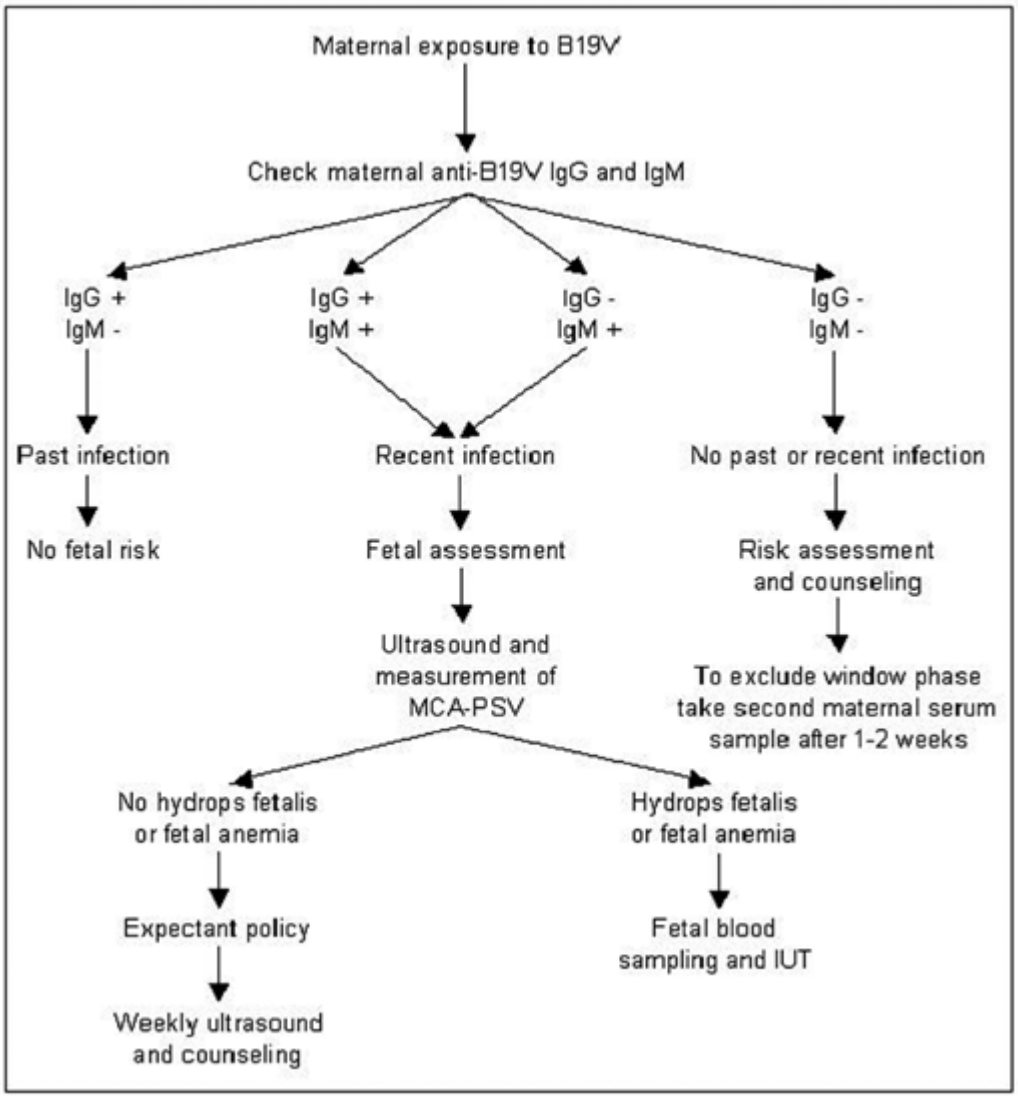



**Fig (2.7) : Clinical and laboratory protocol for the first weeks after maternal exposure to B19V. This does not apply to longer intervals after exposure** (De Jong *et al*., 2006).

## 2.13. Prognosis, risk factors and prevention

### 2.13.1. Prognosis

Children who survived a successful IUT forB19V-induced fetal anemia and hydrops fetalis have a good neurodevelopmental prognosis (Dembinski *et al*., 2002).

### 2.13.2. Risk factors

A prospective evaluation of 618 pregnant women exposed to B19V in an endemic period was performed by Harger *et al* (1998). In this study the single statistically significant risk factor that was found for B19V infections in pregnant women was exposure to B19V by their own children. Other studies have found an increased risk for B19V infections in elementary school teachers and day-care workers (De Jong *et al*., 2006).

### 2.13.3. Prevention

Because maternal exposure to B19V occurs before her child or any other contact has a rash or is otherwise symptomatic, and considering that around 20% of children are asymptomatic, no reasonable strategy to avoid B19V exposure to pregnant women is apparent. Also, it would not be justified excluding pregnant women from the workplace during endemic periods, since the risk of occupational infection may be similar to or less than in the community or at home. Individual counseling of pregnant women, identified to be seronegative, with a high-risk profile (school teachers, daycare workers) should be done to prevent unnecessary fetal death (Enders *et al*., 2004; Harger *et al*., 1998). This would require an active policy of serological testing of these categories early in pregnancy. Recently ( Ballou *et al*., 2003) described a recombinant parvovirus B19 vaccine composed of VP1 and VP2 capsid proteins, which proved to be immunogenic and safe to use in human volunteers. Vaccination of non-immune pregnant women could be a highly effective method to prevent fetal infection with B19V, but doubt exists about the cost-effectiveness of this strategy in the general population.

## 2.14. vaccine development

Effective vaccines are available for animal parvoviruses, and it is likely that parvovirus B19 infection can also be prevented. The recombinant immunogen that is being developed as a vaccine for the human virus lacks DNA and is therefore noninfectious, empty capsids have been engineered to overexpress the highly immunogenic VP1, and a single dose of 2.5 μg of empty capsids elicited neutralizing antibody responses in normal volunteers. ( Ballou *et al*., 2003) As with many other vaccines, commercial interest rather than lack of efficacy or safety has limited the development of a parvovirus B19 vaccine. Such a vaccine could prevent transient aplastic crisis in patients with sickle cell disease or other hemolytic anemias and pure red-cell aplasia in some immunodeficient persons, as well as hydrops fetalis, if seronegative women were inoculated early in pregnancy. Chimeric viral capsids have been proposed as more general vehicles for the delivery of antigens, and parvovirus B19 is especially attractive for this purpose, because the VP1 unique region can be entirely replaced with other protein sequences, allowing, for example, the presentation of a conformationally and functionally intact enzyme on the surface of the empty viral capsid. This method is now being adapted for protection against an agent of bioterrorism: a domain of protective antigen of anthrax is being incorporated on a parvovirus B19 particle (Young and Brown, 2004)

## 2.15 Parvovirus B19 antibodies and correlates of infection in pregnant women attending an antenatal clinic in central Nigeria

The purpose of this study was to establish the prevalence of B19 IgG and IgM antibodies, including correlates of infection, among pregnant women attending an antenatal clinic in Nigeria. Subsequent to clearance from an ethical committee, blood samples were collected between August-November 2008 from 273 pregnant women between the ages of 15-40 years who have given their informed consent and completed self-administered questionnaires. Recombinant IgG and IgM enzyme linked immunosorbent assay kits (Demeditec Diagnostics, Germany) were used for the assays. Out of the 273

participants, 111 (40.7%) had either IgG or IgM antibodies. Out of these, 75 (27.5%) had IgG antibodies whereas 36 (13.2%) had IgM antibodies, and those aged 36-40 years had the highest prevalence of IgG antibodies. Significant determinants of infection ($p < 0.05$) included the receipt of a blood transfusion, occupation and the presence of a large number of children in the household. (Emiasegen *et al.,* 2011).

**2.16 Seroprevalence of parvovirus B19 among pregnant women in Tripoli, Libya**

This study was conducted to assess the seroprevalence of human parvovirus B19 among the pregnant women in Tripoli, Libya. A total number of 150 participants were included in the study, consisting of women of child-bearing age ranging from 18 to 41 years, and divided into age groups as follows: ≤ 21 years, 22-27, 28-32, 33-37, and ≥ 38 years. Specific IgM and IgG antibodies were measured using a commercial ELISA kit. IgG was observed to be prevalent (61%) among the women of child-bearing age. The sero-prevalence of IgM was found to be 5% overall and there was no detectable IgM in the age group between 33 and 37 ( Elnifro *et al.*, 2009).

**2.17 The seroprevalence of parvovirus B19 infection in pregnant women in Sudan**

Parvovirus B19 (B19V) infection during pregnancy may have serious consequences like fetal anaemia, hydrops fetalis, and fetal loss. Since epidemiological data on B19V infection are generally lacking in Sudan, this study was the only one study done in Sudan determine the seroprevalence of B19V in Sudanese pregnant women. Five hundred women, attending antenatal clinics in Khartoum State between November 2008 and March 2009, were enrolled and screened for B19V IgG and IgM antibodies by enzyme immunoassays. The study revealed a B19V IgG seroprevalence of 61·4%, with one subject positive for IgM. B19V DNA was not detected by PCR in any of the tested individuals. B19V IgG seroprevalence was significantly correlated with multigravidity P=0·046 (Adam *et al*., 2015 ).

# CHAPTER THREE

# MATERIALS AND METHOD

# 3.MATERIALS AND METHODS

## 3.1 Study design

This was prospective, cross –sectional and hospital baswd study , to detect anti parvovirus B19 IgG antibodies in serum of pregnant woman attending antenatal clinic in Khartoum State, Sudan.

## 3.2 study area

The study was carried out in Military Hospital in Omdurman, Khartoum State, Sudan.

## 3.3 Study population

The study subjects included in this study were pregnant women who attended the antenatal clinic in the above mentioned hospital.

## 3.4 Study duration

This study was conducted from April 2015 to June 2015

## 3.5 Data collection

Data were collected from each subject using interviewed questionnaire, which includes: age, resident, Gestational age, abortion, Number of household children , history of blood transfusion and diabetes.

## 3.6 Ethical consideration

Ethical approval for this study was obtained from Research and Ethical Committee at College of Graduate Studies, Sudan University of Science and Technology. All subjects were informed about the study and consented before enrolment.

## 3.7 Inclusion and exclusion criteria

The study included women who were pregnant, attended the antenatal clinic and provided signed consent forms and interviewed questionnaire. The exclusion criteria were non pregnant women, an age below 15 or above 40 years and failure to sign a consent form.

### 3.8 Sample size

A total of 90 pregnant women serum sample (n= 90) were collected in plain containers, serum separated and stored at -20 ºC.

### 3.9 Method

#### 3.9.1 Collection of specimens

Three milliliters of blood sample was collected in plain containers from each woman and left for 30 minuets to clot. Serum samples were separated by centrifugation at 3000 rpm for 3 minuets and then stored at -20 °C until tested.

#### 3.9.2 Enzyme linked immunosorbent assay (ELISA)

##### 3.9.2.1 Principle

The ELISA test kit (EUROIMMUN, Germany) provides a semi quantitative in vitro assay for human antibodies of the IgG class against parvovirus B19 in serum or plasma . The test kit contains microtiter strips each with 8 break –off reagent wells coated with parvovirus antigens. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgG antibodies will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalyzing a colour reaction.

##### 3.9.2.2 Procedure

The following techniques were used according to the instructions of the manufactures.

**Sample dilution**

patient samples were diluted 1:101 in sample buffer and mixed well by vortexing .

**Numbering wells**

the strip needed was set in strip-holder and number sufficient number of wells including two calibrator 3,one positive control, one negative control and one blank (neither samples nor conjugate should be added into the blank well).

**Sample incubation**

100µl of calibrator3,positive control, negative control, and diluted patient sample were added into their respective wells and mixed by tapping the plate gently, covered the reagent wells with the protective foil and incubated at 37°C for 60 minutes.

**Washing**

After the incubation each well was washed 3 times with diluted wash buffer. After the final washing cycle, the strip plate was turned onto blotting paper or clean towel, and tapped to remove any remainders.

**Conjugate incubation**

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

**Washing**

Each well was washed 3 times with diluted wash buffer .

**Substrate incubation**

100µl of chromogen/substrate solution was added to each well including the blank and incubated for 15 minutes at room temperature avoiding direct sunlight.

**Stopping the reaction**

100µl of stop solution was added to each well in the same order and at the same speed as the chromogen/substrate solution was introduced.

**Measurement**

photometric measurement of the colour intensity was made at a wavelength of 450 nm and reference wavelength 630 nm within 30 minutes of adding the stop solution.

### 3.9.2.3 Calculation of the result

Result can be evaluated semiquantitatively by calculating a ratio of the extinction value of the control or patient sample over the extinction value of calibrator 3. Calculate the ratio according to the following formula **:**

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

### 3.9.2.4 Interpretation of the results

Ratio <0.8 : negative

Ratio <1.1 : borderline

Ratio ≥1.1: positive

## 3.10 Data analysis

The data was analyzed using SPSS computer program (software version 11.5). Frequencies , Chi-square test values were calculated.

# CHAPTER FOUR

# RESULT

# 4. RESULTS

## 4.1 Detection of anti parvovirus B19 IgG antibody in serum sample tested by indirect ELISA.

Table( 1 ) demonstrates that out of the 90 serum specimens examined 37 were show positive (41.1 %) for anti parvovirus B19 IgG antibody, while 53 subject were found negative (58.9 %)

**Table 1 : Frequency of parvovirus B19 in pregnant women.**

| Result | Frequency | Percent |
|---|---|---|
| **Positive** | 37 | 41.1% |
| **Negative** | 53 | 58.9% |
| **Total** | 90 | 100.0% |

## 4.2 Age distribution of anti parvovirus B19 IgG antibody among pregnant women.

Table (2) revealed that Pregnant women aged 15-25 years had the highest prevalence of IgG antibodies of 20 (22.2%), but pregnant women aged 36-45 years had the lowest prevalence of IgG antibodies 4 (4.4%). There was significant between age and seropositive of anti parvovirus IgG antibodies .

**Table 2: Age distribution of anti parvovirus B19 IgG antibody among pregnant women.**

| Age group | Positive | Negative | Total |
|---|---|---|---|
| **15-25** | 20<br>22.2% | 16<br>17.8% | 36<br>40.0% |
| **26-35** | 13<br>14.4% | 34<br>37.8% | 47<br>52.2% |
| **36-45** | 4 | 3 | 7 |

| | | | |
|---|---|---|---|
| | 4.4% | 3.3% | 7.8% |
| **Total** | 37<br>41.1% | 53<br>58.9% | 90<br>100.0% |

Pearson Chi-Square = 7.358

P value = 0.025

**4.3 Distribution of anti parvovirus B19 IgG antibodies in pregnant women according to trimester.**

Table (3) most of pregnant women have anti parvovirus B19 IgG antibodies, were within 3rd trimester 20 (22.2%), followed by 2nd trimester 11 (12.2%), and 1st trimester 6 (6.7%).

**Table 3: Distribution of anti parvovirus B19 IgG antibodies in pregnant women according to trimester**

| Trimester | Positive | Negative | Total |
|---|---|---|---|
| **1st** | 6<br>6.7% | 7<br>7.8% | 13<br>14.4% |
| **2nd** | 11<br>12.2% | 12<br>13.3% | 23<br>25.6% |
| **3rd** | 2o<br>22.2% | 34<br>37.8% | 54<br>60% |
| **Total** | 37<br>41.1% | 53<br>58.9% | 90<br>100% |

Pearson Chi-Square =0.935

P value =0.627

**4.4 Distribution of anti parvovirus B19 IgG antibodies in pregnant women according to the abortion:**

Table (4) Parvovirus seropositivity was 10 (11.1%) among pregnant women that obtained abortion. These findings showed no significant difference.

**Table 4: Distribution of anti parvovirus B19 IgG antibodies in pregnant women according to the abortion**

| Abortion | Positive | Negative | Total |
|---|---|---|---|
| **Yes** | 10<br>11.1% | 18<br>20% | 28<br>31.1% |
| **No** | 27<br>30% | 35<br>38.9% | 62<br>68.9% |
| **Total** | 37<br>41.1% | 53<br>58.9% | 90<br>100% |

Pearson Chi-Square = 0.489

P value = 0.489

**4.5 Distribution of anti parvovirus B19 IgG antibodies in pregnant women according to the blood transfusion :**

Table (5) the seropositivty of anti parvovirus B19 IgG antibodies among pregnant women that had history of blood transfusion was 3 (3.3%). These findings showed no significant difference.

**Table 5: Distribution of anti parvovirus B19 IgG antibodies in pregnant women according to the blood transfusion**

| Blood transfusion | positive | Negative | Total |
|---|---|---|---|
| **yes** | 3<br>3.3% | 6<br>6.7% | 9<br>10% |
| **no** | 34<br>37.8% | 47<br>52.2% | 81<br>90% |
| **Total** | 37<br>41.1% | 53<br>58.9% | 90<br>100% |

Pearson Chi-Square = 0.250

P value =0.617

**4.6 Distribution of anti parvovirus B19 IgG antibodies in pregnant women according to the diabetes :**

Table (6) the result illustrated that (1) of the diabetic pregnant women (1.1%) was positive for the anti parvovirus B19 IgG antibodies.

**Table 6: Distribution of anti parvovirus B19 IgG antibodies in pregnant women according to the diabetes**

| Diabetes | Positive | Negative | Total |
|---|---|---|---|
| **yes** | 1<br>1.1% | 3<br>3.3% | 4<br>4.4% |
| **no** | 36<br>40% | 50<br>55.6% | 86<br>95.6% |
| **Total** | 37<br>41.1% | 53<br>58.9% | 90<br>100% |

Pearson Chi-Square = 0.449

P value =0.503

**4.7 Distribution of anti parvovirus B19 IgG antibodies in pregnant women according to the number of household children:**

Table (7) revealed that there was high frequency of positive subjects among pregnant women that had number of household children between 0-2, 30 (33.3%). These results showed no significant difference.

**Table 7: Distribution of anti parvovirus B19 IgG antibodies in pregnant women according to the number of household children**

| Children group | Positive | Negative | Total |
|---|---|---|---|
| **0-2 (primigravidae)** | 30<br>33.3% | 43<br>47.8% | 73<br>81.1% |
| **3-5** | 7 | 10 | 17 |

| | | | |
|---|---|---|---|
| **(multigravidae)** | 7.8% | 11.1 | 18.9% |
| **Total** | 37<br>41.1% | 53<br>58.9% | 90<br>100% |

Pearson Chi-Square = 0.00

P value = 0.995

## 4.8 Summary for distribution of anti parvovirus B19 IgG antibodies in pregnant women according to the risk factors.

| **Risk factors** | **Positive** | **Negative** | **P. value** |
|---|---|---|---|
| **Abortion:** | | | |
| yes | 10 (11.1%) | 18 (20%) | 0.489 |
| no | 27 (30%) | 35 (38.9%) | |
| **Blood transfusion:** | | | |
| yes | 3 (3.3%) | 6 (6.7%) | 0.617 |
| no | 34 (37.8%) | 47 (52.2%) | |
| **Diabetes:** | | | |
| yes | 1 (1.1%) | 3 (3.3%) | 0.503 |
| no | 36 (40%) | 50 (55.6%) | |
| **No. of household children** | | | |
| 0-2 (primigravidae) | 30 (33.3%) | 43 (47.8%) | 0.995 |
| 3-5 (multigravidae) | 7 (7.8%) | 10 (11.1%) | |

# CHAPTER FIVE

# DISCUSSION

## 5.1 DISCUSSION

Parvovirus B19 (B19V) infection during pregnancy may have serious consequences like fetal anaemia, hydrops fetalis, and fetal loss**.** Since Data on B19V epidemiology in Sudan are very limited. This study was conducted on parovirus B19 IgG antibodies in pregnant women attending antenatal clinici in Military Hospital in Omdurman. The frequency of parovirus B19 IgG antibodies detected in this study was (41.1 %) it is lower than the previous reported study done in Sudan by (Adam *et al*., 2015 ) which found that the seropositive was (61.4%) and may be due to sample size (500). The seroprevalence rate of immunoglobulin G (IgG) and immunoglobulin M (IgM) to parvovirus B19 in pregnant Saudi women in Makkah was 46.6% and 2.25% respectively (Ghazi, 2007), this result is higher than that found in present study and may be due to sample size (1200), demographic and geographical variations season.

In a study of urban and remote rural populations in northern Brazil, the B19 parvovirus seroprevalence in the urban population of Belem was found to be similar to that of developed countries (42.6%), these results are in agreement to the present result, while it was considerably lower among the remote rural tribes 4.7 – 10.7% (Public Health Laboratory Service Working Parry on Fifth Disease, 1990). In an African study, prevalence of 58.4% and 55.0% were found in Malawi and Mauritius respectively, compared with a very low prevalence of 2.2% on remote Rodriguez Island (Public Health Laboratory Service Working Parry on Fifth Disease, 1990). More recently, Elnifro *et al.* (2009) reported a prevalence of IgG and IgM antibodies of 61% and 5%, respectively, among pregnant women in Libya. In some studies conducted in Iran, the prevalence of infection is estimated to be 45% and 60% of

reproductive age and pregnant women respectively, these results are higher than that found in present study. Emiasegen *et al*. (2011) reported a prevalence of IgG and IgM antibodies of 27.5% and 13.2%, respectively, among pregnant women in Nigeria, these results are lower than that found in present study. The low or high frequency of parvovirus B19 antibodies observed by different investigators could be attributed to several factors including, the study population, the diagnostic techniques used , the incidence rate of the virus in different environment, the living condition and standards of the study group and the season during which the study was conducted .

To determine how many abortions are due to infection with B19 virus in this study, it was necessary to show the virus DNA in fetal tissues by PCR method which was not the aim of current study, however, present findings may be a background for future studies in this field. In this study Parvovirus B19 seropositivity was (11.1%) among the pregnant women that had abortion,it lower than previous reported study done in Sudan by (Adam et al., 2015) which found that the seropositive was (29.8%) and may be due to large sample size, large number of pregnant women that had abortion in the study population . Also in a study performed by Mirzaei *et al*., to determine the prevalence of Parvovirus B19 in IUFD (intra uterine fetal death), virus DNA was observed in 10 percent of participants (Mirzaie *et al*.,2008), and a study by Nymon *et al*., revealed that DNA of B19 Parvovirus was observed in 3% of abortions in the first trimester and 12% in second trimester in fetal tissues (Nyman *et al*., 2002), this differences may be related to specimen population and the diagnostic techniques used. In a study by Jensen *et al*., in Denmark, a strong statistical association was observed between the presence of B19 IgM antibody during pregnancy and spontaneous abortion. It was reported that the percentage of spontaneous abortion in pregnant women with positive IgM antibody is 12.9% (Jensen *et al*., 2000). The limitation of this study was lack of the detection of seroprevelance of anti parvovirus B19 IgM antibodies.

Women in their third trimester of pregnancy were found to demonstrate the highest rate of IgG antibodies 20 (22.2%) followed by those in their second 11

(12.2%) and first 6 (6.7%) trimesters, compared with other previous study done in Nigeria by (Emisaegen *et al*., 2011) which found that the highest rate of IgG antibodies among pregnant women in their second trimester 42 (31.1%) followed by those in their first trimester 30 (26.3%) and third trimester 3 (12.5%), and may be due to in this study most of the pregnant women present in third trimester (54).

The most important risk factors for exposure to B19V are having contact with young children and also the number of children at home (Rohrer *et al*.,2008). In this study The seroprevelance of anti parvovirus B19 IgG antibodies was higher in primigravidae 30 (33.3%) compared with multigravid 7 (7.8%) women. In other study The incidence of Parvovirus specific antibody was positive in 19(20.9%) of participants with non children, 42(46.1%) of women with 1-3 children ,and 7 (7.7%) of women with 4-5 children (Hasan *et al*., 2013**)**, these results are not in agreement to the present result, and may be due to the living condition and standards of study group and the incidence rate of the virus in different environment.

the seropositivty of anti parvovirus B19 IgG antibodies among pregnant women that had history of blood transfusion was 3 (3.3%), it is lower than the previous reported study done in Nigeria by (Emisaegen *et al.*, 2011) which found that the seropositive was 12 (30.8%) , and may be due to sample size.

The seroprevalence of anti parvovirus B19 IgG antibodies among pregnant women that had diabetes was (1.1%). These findings showed no significant difference.

## 5.2 CONCLUSION

The seroprevalence rate of B19V in Sudanese pregnant women was 37 (41.1%).). There was ststistical correlation of serological finding of virus and age of patients, and non significant difference with other risk factors.

## 5.3 RECOMMENDATIONS

1. Detection of parvovirus B19 should be routine in pregnant women.
2. further studies in pregnant Sudanese women, mainly in those with complications and adverse outcomes of pregnancy, as well as in other high-risk groups including patients with haemoglobinopathies and immunological disorders.

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# APPENDIX

## Appendix 1

**Questionnaire**

**Sudan University of Science and Technology**

**College of Graduate Studies**

# Seroprevelance of Parvovirus B19 among pregnant women attending antenatal clinics in Khartoum State, Sudan

1.date ……

2.Number……

3. Age ……

4. Residence……

5.Trimester……

6. Abortion……..

7. History of blood transfusion……..

8. Diabetes …….

9. the Number of household children…….

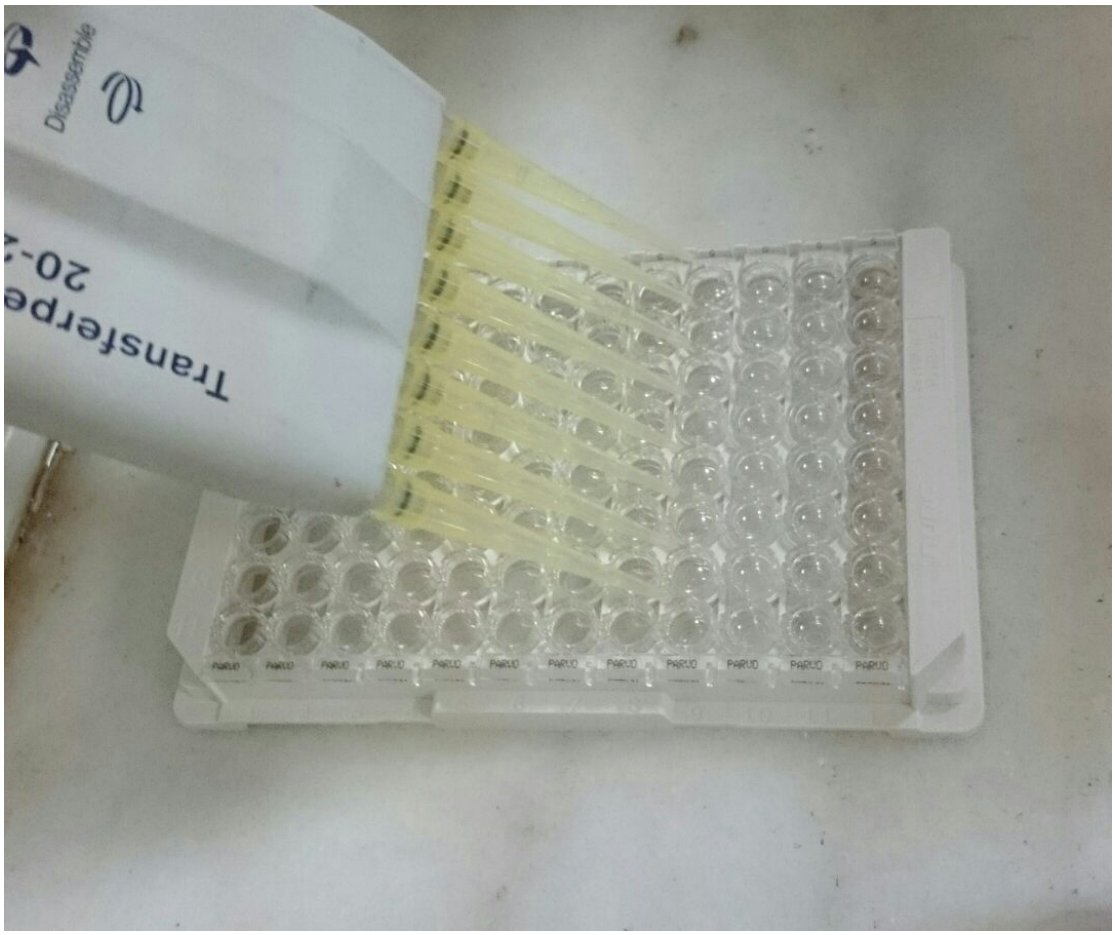
**Signature :……..**

# Appendix 2

## ELISA reader

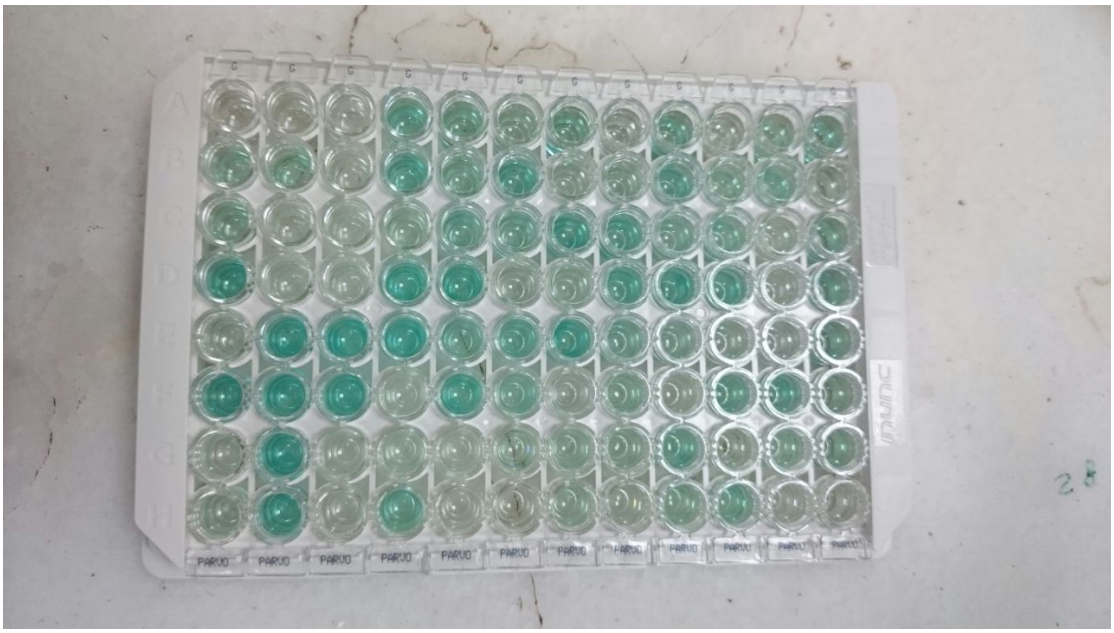
# Appendix 3

Manual wash



# Appendix 4

Result after addition of substrate



# Appendix 5

Result