Sudan University of Science and Technology

College of Graduate Studies

Detection of Parvovirus B19 among Pregnant Women in First Trimester in Jeddah -Saudi Arabia

الكشف عن فيروس بارفو B19 بين النساء الحوامل في الفصل الأول بجدة - السعودية

A Dissertation Submitted for Partial Fulfillment for the Requirements of M.Sc. Degree in Medical Laboratory Science (Microbiology)

By:

Fatima Hussein Hassan Mukhtar

(B.Sc. of Medical Laboratory Science, Port Sudan Ahlia College , 2003)

Supervised By:

Dr. Kawthar Abdelgaleil MohammedSalih Ibrahim

February 2022

بسم الله الرحمن الرحيم

الآية

قال الله تعالى :

اللَّهُ لَا إِلَٰهَ إِلَّا هُوَ الْحَيُّ الْقَيُّومُ ۚ لَا تَأْخُذُهُ سِنَةٌ وَلَا نَوْمٌ ۚ لَهُ مَا فِي السَّمَاوَاتِ وَمَا فِي الْأَرْضِ ۗ مَنْ ذَا الَّذِي يَشْفَعُ عِنْدَهُ إِلَّا بِإِذْنِهِ ۚ يَعْلَمُ مَا بَيْنَ أَيْدِيهِمْ وَمَا خَلْفَهُمْ ۖ وَلَا يُحِيطُونَ بِشَيْءٍ مِنْ عِلْمِهِ إِلَّا بِمَا شَاءَ ۚ وَسِعَ كُرْسِيُّهُ السَّمَاوَاتِ وَالْأَرْضَ ۖ وَلَا يَئُودُهُ حِفْظُهُمَا ۚ وَهُوَ الْعَلِيُّ الْعَظِيمُ

صدق الله العظيم
سورة البقرة الآية 255

DEDICATION

To my Parents who always give me great love ,patience , forgiveness, care and
support all my life.

To my lovely sisters & brothers

To my husband for his encouragement and support& to My Son

To those whom I love more, and my Friends and all family members….

To my teachers Who teach me anything.

Acknowledgments

The first and last greatest thanks for Allah who create me and everything in abest form for his greatest blessing that he is Allah the only lord of the words. Iwould like to express my extreme thanks to Dr**. Kawthar Abdelgaleil Mohammed Salih** for her guiding and encouragements, till the study complete. great thanks to khalid yaseen and Eng. Ahmed Gaber who make software to dynex**.** Great thanks to Clinical Center in (AlBadria Tower in Jeddah), for allowing us to collect the samples, and also women from whom we collect the samples. Thanks to Maysa Mahdi for her great effort and help to complete this research. Further thanks to Microbiology staff" teachers' and laboratory assistants" in Sudan University of Science and Technology. Extended thanks to Dr. Inaam for her great help. Thanks to my friends Montaha Mohammad and Dalal Abdullah for help and support.

ABSTRACT

Human parvovirus B19 infection is common during pregnancy. Approximately 30-50% of pregnant women are non-immune to the virus and this is of particular importance during epidemic years, which occur in a 3–6-year cycle. This is cross sectional study was aimed for serological detection of parvovirus B19 antibodies in serum of pregnant women in the first trimester in Albadria Tawer Medical Center in Jeddah, Saudi Arabia, during the period from July 2019 to April 2020. Blood samples were collected from 87 pregnant women their age ranged 17-45 years of age attending antenatal clinic. All samples were tested for the presence of parvovirus B19 IgM antibodies using ELISA (Dynex technology) and analyzed result by using SPSS computer program version 22.0 with T test and P *value* was obtained (P *value* ≤ 0.05 was considered statistically significant. Parvovirus B19 IgM antibodies were detected in 7/87 (8.0%) of the total samples examined. Most of the positive cases were in pregnant women aged 25-35 years 33/87 (37.9%) followed by 28/87 (32.2%) in pregnant women less than 25 years of age. However, the pregnant women less than 35 years of age had the lowest 26 (29.9%) parvovirus B19seropositivity. Parvovirus B19 seropositivity was 2(2.3%) among the pregnant women that had abortion, and this did not reach statistical significance. The seropositivity of parvovirus B19 IgM antibodies among pregnant women that had history of blood transfusion was 1 (1.1%). There was no statistical correlation of serological finding of the parvovirus B19 and age of patients, while non-significant difference with other risk factors. The seropositivity of parvovirus B19 IgM antibodies among pregnant with multigravida in which 7(8.0%) were IgM positive, but there was no significant association. The study concluded that found Lower frequency of IgM (Seropositivity) (8.6%) among Pregnant women .There was insignificant association between B19V infection and others (age, gravidity, history of miscarriage, history of blood transfusion).

مستخلص الدراسة

تعد الإصابة بفيروس بارفو B19 أثناء الحمل أمرًا شائعا حيث وجد ما يقرب من 30-50 ٪ من النساء الحوامل لم يقمن بالوقاية من الفيروس وهذا له أهمية خاصة خلال سنوات الوباء ، والتي تحدث في دورة من 3 إلى 6 سنوات. هدفت هذه الدراسة المقطعية إلى الكشف المصلي عن الأجسام المضادة لفيروس بارفو B19 في مصل دم النساء الحوامل في الشهور الاولي من الحمل في مركز البدرية الطبي في جدة ، المملكة العربية السعودية ، خلال الفترة من يوليو 2019 إلى أبريل 2020. تم جمع عينات الدم من 87 عينة النساء الحوامل الذين تتراوح أعمارهم بين 17-45 سنة في عيادة متابعه ما قبل الولادة. تم اختبار جميع العينات للكشف عن وجود الأجسام المضادة لـ parvovirus B19 IgM باستخدام اليزا وحللت البيانات باستخدام برنامج الكمبيوتر SPSS الإصدار 22.0.وقورنت البيانات باختبار T وقيمة P (قيمة P $\leq$ 0.05 اعتبرت ذات دلالة إحصائية.

تم اكتشاف الأجسام المضادة لـ Parvovirus B19 IgM في 87/7 (8.0٪) من إجمالي العينات التي تم فحصها. كانت معظم الحالات الإيجابية في النساء الحوامل اللواتي تتراوح أعمارهن بين 25 و 35 سنة 87/33 (37.9٪) تليها 87/28 (32.2٪) في النساء الحوامل دون سن 25 سنة. ومع ذلك ، فإن النساء الحوامل أقل من 35 سنة كان لديهن أدنى 26 (29.9 ٪) من فيروس بارفو فيروس B19. كانت الإيجابية لفيروس بارفو 2 B19 (2.3٪) بين النساء الحوامل اللواتي حدث لهن اجهاض من قبل ، وهذا لم يصل إلى دلالة إحصائية. كانت الإيجابية المصلية للأجسام المضادة IgM لفيروس بارفو B19 بين النساء الحوامل اللائي كان لهن تاريخ من نقل الدم 1 (1.1٪). بينما لم يكن هناك ارتباط إحصائي بين الموجودات المصلية للفيروس الصغير B19 وعمر المرضى ، بينما لم يكن هناك فرق واضح مع عوامل الخطر الأخرى. كانت الإيجابية المصلية للأجسام المضادة IgM لفيروس بارفو B19 بين الحوامل المصابات بمرض multigravida حيث كانت 7 (8.0٪) إيجابية IgM ، ولكن لم يكن هناك ارتباط اخر.وخلصت الدراسة الي ان تواتر IgM(الايجابية المصلي) اقل (8.6%) بين النساء الحوامل. ولم يكن هنالك ارتباط معنوي بين عدوى فيروس B19 وغيرها (العمر,تعدد الحمل,تاريخ الاجهاض, تاريخ نقل الدم) .

Table of contents

Title	Page
الآية	I
Dedication	II
Acknowledgments	III
Abstract	IV
مستخلص البحث	V
List of contents	VI
List of tables	X
List of Abbreviations	XI
Chapter One **Introduction, Rationale & Objectives**	
1.0 Introduction	1
1.1 Rationale	3
1.2. Objectives	4
1.2.1 General Objective	4
1.2.2. Specific Objectives	4
Chapter Two **Literature Review**	
2.1 Overview	5
2.1.1 Pregnancy	5

2.1.1.1 First trimester	5
2.1.1.2 Second trimester	5
2.1.1.3 Third trimester	5
2.2 Parvovirus	6
2.2.1 Structure	6
2.2.2 Transmission	6
2.2.3 Replication	6
2.2.4 Attachment and entry	7
2.2.5 Transcription and translation	7
2.3 Epidemology	8
2.4 Pathogenesis	8
2.5 Clinical syndroms commonly associated with parvovirus B19	10
2.5.1 Infection in immunocompetent	10
2.5.1.1 Erythema infection	10
2.5.1.2 Joint disease	10
2.6 other infections	11
2.6.1 Contamination inside the immunodeficient host	11
2.6.1.1 Chronic natural purple mobile aplasia	11
2.7 Immuno response	12
2.8. Parvovirus in pregnant female	13

2.9 Laboratory diagnosis	14
2.9.1 serological tests	15
2.9.2 viral DNA detection (PCR)	16
2.9.3 viral culture	16
2.9.4 diagnostic cytopathology	17
2.10 Therapy	17
2.11 Management of intrautrerine B19 infection	18
2.12 Prognosis,risk factors and prevention	18
2.12.1 prognosis	18
2.12.2 risk factors	19
2.12.3 prevention	19
2.13 vaccine development	20
2.14 previous studies	20
Chapter Three Material and Method	
3.1 Study design	27
3.2 Study area and duration	27
3.3 Study poplation	27
3.4 Selectin criteria	27
3.4.1 Inclusion Criteria	27
3.4.2 Exclusion Criteria	27

3.5 Ethical Considration	27
3.6 Data collection	27
3.7 sample size	28
3.8 method	28
3.8.1 collection of specimens	28
3.8.2 Enzyme Linked Immunosorbent Assay (ELIZA)	28
3.8.2.1 Principle	28
3.8.2.2 Procedure	28
3.8.2.3 Calculation of the result	29
3.8.2.4 Interpretation of the result	30
3.9 Data Analysis	30
Chapter Four **Results**	
Chapter Five **Discussion, conclusion and recommendation**	
5.1Discussion	34
5.2.Conclusion	36
5.3Recommendation	36
References	
Appendices	

List of Tables

Legend	Page
The Distribution of age group among apparently healthy pregnant women	32
Frequency of parvovirus B19 in pregnant women	32
The association between first trimester and IgM results among apparently healthy pregnant women	32
The association between gravidity and IgM results among apparently healthy pregnant women	33
The association between history of blood transfusion and IgM, results among apparently healthy pregnant women	33
The association between history of miscarriage and IgM results among apparently healthy pregnant women	33

List of Abbreviation

Abbreviate	**Term**
ALL	acute lymphatic leukemia
BM	bone marrow
CIE	counter immune electrophoresis
CMV	Cytomegalovirus
DNA	Deoxyribonucleic Acid
EBV	Epstein Bar virus
ELISA	enzyme-linked immunosorbent assay
EM	Electron microscopy
VZV	varicella zoster virus
RNA	ribonucleic acid
VP	Viral Protein
IgG	Immunoglobulin G
PCR	Polymerase chain reaction
IUT	intrauterine erythrocyte transfusion

CHAPTER I
INTRODUCTION

1.0 Introduction

Pregnancy elicits a reorientation of physiologic prior pities of the woman's body optimal development of the maternal fetus. Specific alterations occur in the hematologic system during pregnancy as the mother provides the nutrients for fetal hematopoiesis and her body prepares for the hemostatic challenge of childbirth (Thomas *et al*., 2014).

Approximately 830 women every day die from preventable cause's related pregnancy and childbirth. 99% of all maternal deaths occur in developing countries. While higher in women living in rural areas and among poorer communities and young adolescent's faces a higher risk of complications and death as a result of pregnancy than other women. (Thomas *et al*., 2014)

In 1974, Cossart *et al* first recognized B19 while evaluating assessments for the hepatitis B virus surface antigen. The name B19 virus originates from the coding of a serum pattern, wide variety 19 in panel B that gave anomalous outcomes while examined through counter immune electrophoresis (CIE) and radioimmunoassay. (Ornoy *et al*., 2017)

Human parvovirus is a non-enveloped, single-stranded DNA virus with icosahedral symmetry (Suliman *et al.*, 2016). B19 was first defined in 1975 (Ornoy *et al.*, 2017) while electron microscopic investigation of sera yielding discrepant hepatitis B surface antigen effects, demonstrated virus-like debris with a diameter of approximately 23nm. in addition, morphological assessment and buoyant density research strongly recommended that this debris had been parvoviruses.

Electron microscopy (EM) revealed the presence of 23-nm-diameter debris resembling animal parvoviruses (Luo *et al*., 2015). B19 become independently defined in Japan 5 years later as the "Nakatani" virus, however, the next testing proved the two viruses to be identical. Extraction of DNA discovered complementary single strands of approximately 5. five kb encapsidated in separate virions indicating that the virus was a member of the genus Parvovirus.

Although originally categorized as “serum parvovirus-like particle” or human parvovirus, it turned into formally identified in 1985 as a member of the Parvoviridae and given the name B19 by way of the global Committee on Taxonomy of Viruses (Avguštin *et al*., 2018).

In view that 1981 when B19 turned into diagnosed because of the motive of aplastic crisis in kids with sickle mobile anemia (Suliman *et al*., 2016), B19 has been related to a variety of different medical syndromes. B19 is effortlessly transmitted through blood transfusion and remedy the usage of plasma-derived products (Luo, 2015).

Importantly, B19V also can be transmitted vertically from mother to fetus where it is able to purpose extreme fetal miscarriage, anemia, fetal demise or hydrops fetalis. The hazard of vertical transmission of B19V is as much as about one-third of acutely inflamed pregnant girls and the excess fetal dying charge after maternal contamination for the duration of the first 20 weeks of gestation became envisioned to be 56% (Luo, 2015).

1.1 Rationale

Parvovirus B19 infection during pregnancy is mostly asymptomatic, but in approximately 3% of infected women in Saudi Arabia it might cause a range of complications, including abortion, severe fetal anemia, nonimmune hydrops fetalis, and even fetal demise. Several factors have been associated with an increased risk of acute parvovirus B19 infection in pregnant women: Women who have only 1 child have a 3-fold greater risk of infection compared with nulliparous women; the risk increases to 7.5-fold in women with 3 or more children. Working in nursery schools or after-school clubs or day care centers also appears to increase the risk. Serious medical conditions and stressful jobs have also been identified as risk factors. There is approximately a 50% risk of infection from close, frequent interaction with an infected child (eg, in the home) (Avguštin *et al*., 2018),

There were a few studies done in Saudi's pregnant women infected with parvovirus B19 have been published for these reasons and because of the clinical importance of the disease, this study is to outline the investigation and management of pregnant women in the first trimester exposed to experiencing symptoms of Parvovirus B19.

The said seroprevalences of B19V in pregnant women fluctuate among nations ranging among ~35% in Spain and 81% in Sweden. In lots of developed international locations, the epidemiology and traits of B19V infection in girls of childbearing age are widely recognized. but, the epidemiological statistics on B19V infection are generally missing in lots of African nations which include Sudan.

1.2 Objectives

1.2.1 General objective

To determine prevalence of Parvovirus B19 among Pregnant Women in First Trimester in Jeddah-Saudi Arabia.

1.2.2 Specific objectives

1. To measure the prevalence of anti -parvovirus B19 IgM antibodies in pregnant women in the First Trimester.
2. To determine the prevalence of B19 in pregnant women in the first trimester
3. To determine the significant effect of the risk factors such as age , gravidity and duration of infection

CHAPTER II

LITERATURE REVIEW

2.1 Overview

2.1.1 Pregnancy

physician’s customarily divide pregnancy into three time intervals called trimesters each of which is slightly longer Than 13 week.by convention (Carel *et al*., 2018).

Pregnancy Trimester

2.1.1.1 First Trimester

The events that lead to pregnancy begin with conception, in which the sperm penetrates the egg produced by an ovary. The zygote (fertilized egg) then travels through the woman's fallopian tube to the uterus, where it implants itself in the uterine wall. The zygote is made up of a cluster of cells formed from the egg and sperm. These cells form the fetus and the placenta. The placenta provides nutrients and oxygen to the fetus (Berk, 2011).

2.1.1.2 Second Trimester

The second trimester is for many women the easiest three month of pregnancy in it feel better and the energy is up to start planning for baby arrival. During the second trimester the baby is growing quickly between 18 and 22 week of pregnancy, mother can see the baby progressing uses ultrasound. Also can see the sex of baby, Vernix and lanugo keep the fetus skin from chapping in the amniotic fluid. Most of the Brain neurons present by 24 weeks and the fetus react to sound; the fetus becomes 30 cm in length and 820gm in weight (Berk, 2011).

2.1.1.3 Third Trimester

The 26 to 40 weeks of third trimester is the period in which fetal organs complete their prenatal maturation, during it, the growth rate decelerates 3200g and is about 50 cm long term is the interval from 37 to 42 weeks normal labor, rhythmic uterine contraction, and birth occurring during this period (Carel *et al*., 2018).

2.2 Parvovirus:

2.2.1 Structure:

The B19 virion has a simple structure composed of the simplest two proteins and a linear, single-strand DNA molecule. The nonenveloped viral particles are approximately 22 to 24 nm in diameter and display icosahedral symmetry, and frequently both empty and complete capsids are visible through bad staining and EM. The limited DNA content material and the absence of a lipid envelope make B19 extraordinarily proof against physical inactivation. (Buller *et al.*, 2016).

The virus is solid at 56°C for 60 min, and lipid solvents haven't any effect. Inactivation of virus can be accomplished by means of formalin, and gamma irradiation (Buller *et al.*, 2016).

2.2.2 Transmission:

B19-specific DNA has been detected in breathing secretions on the time of viremia, suggesting that the virus is commonly unfolded within the network via a respiration path. Vertical transmission takes place in a single-1/3 of cases regarding serologically confirmed number one maternal infection. Nosocomial transmission has been described on occasion, and transmission has also been stated amongst a team of workers in laboratories managing native virus (Weiffenbach *et al*., 2012).

2.2.3 DNA Replication:

The small genome of parvovirus can encode just a few proteins, so the virus depends on its host cell (or another virus) to provide critical proteins. Some of those cell proteins (a DNA polymerase and other proteins concerned in DNA replication) are to be had most effective at some stage in the S section of the cellular cycle when DNA synthesis takes vicinity. This restricts the opportunity for parvovirus replication to the S segment. evaluation of this case with that of the huge DNA viruses, together with the herpesviruses, which encode their own

DNA-replicating enzymes, allowing them to reflect in any phase of the cell cycle (Dudleenamjil *et al*., 2010).

2.2.4 Attachment and entry:

A virion attaches to receptors on the floor of an ability host mobile. Inside the case of the B19 virus, the host mobile is a crimson blood mobile precursor and the receptor is the blood group P antigen. The virion enters the mobile via endocytosis and is released from the endosome into the cytoplasm, in which it buddies with microtubules and is transported to a nuclear pore. With a diameter of 18–26 nm, the parvovirus virion is small enough to pass via a nuclear pore, even though there's evidence that the virion must undergo some structural modifications earlier than it could be transported into the nucleus. Nuclear localization indicators were observed within the capsid proteins of a few parvoviruses (Gallinella., 2013).

2.2.5 Transcription and translation:

Inside the nucleus, the unmarried-stranded virus genome is transformed into dsDNA by using a mobile DNA polymerase. The ends of the genome are double-stranded due to base pairing, and on the 3 cease the –OH group acts as a primer to which the enzyme binds (Dudleenamjil *et al*., 2010).

The cell RNA polymerase II transcribes the virus genes and cell transcription elements play key roles. The number one transcript(s) go through diverse splicing events to provide length lessons of mRNA. The bigger mRNAs encode the non-structural proteins and the smaller mRNAs encode the structural proteins. The non-structural proteins are phosphorylated and play roles inside the manager of the gene expression and in DNA replication (Gallinella *et al*., 2013).

After and virion assembly conversion of the ssDNA genome to dsDNA, the DNA is replicated with the aid of a mechanism called rolling-hairpin replication that is a leading strand mechanism and units parvoviruses apart from other DNA viruses, which reflect their genomes through leading and lagging strand

synthesis. Procapsids are produced from the structural proteins and each is filled through a copy of the virus genome, either a (+) DNA or a (−) DNA as appropriate. One of the non-structural proteins capabilities as a helicase to unwind the dsDNA so that an unmarried strand can enter the procapsid (Flint *et al*.,2020).

The two fundamental non-structural proteins and the VP1 and VP2 structural capsid proteins are synthesized within the cytoplasm, and the structural proteins visit the nucleus, wherein the virion is assembled. The VP2 protein is cleaved later to provide VP3. The nuclear and cytoplasmic membrane degenerates, and the virus is released on cell lysis (Xu *et al*., 2019).

2.3 Epidemiology:

Infection with parvovirus B19 is global; infectivity rates, inferred from the presence of anti-parvovirus IgG antibody in serum samples, are similar in the United States, Europe, and Asia. Some isolated Amazonian tribes and populations of remote islands off the coast of Africa have escaped exposure. Parvovirus B19 infection is common in childhood; half of 15-year-old adolescents have specific anti-parvovirus B19 antibodies. B19 infection continues at a lower rate throughout adult life, and by the time, they are elderly, most persons are seropositive. In temperate climates, infections usually occur in the spring, and small epidemics at intervals of a few years are typical. The virus is spread by respiratory droplets, and secondary infection rates among household contacts are very high, Nosocomial infection has been described. Parvovirus B19 has also been transmitted by blood products, specially pooled factor eight and factor nine concentrates. (Osman *et al*., 2018).

2.4 Pathogenesis:

The human B19 virus is a small single-stranded DNA virus classified as a member of precursors. It is the only parvovirus that has been honestly connected with the disease in human beings. B19 virus replicates only in human

cells and belongs to the family Parvoviridae, genus Erythrovirus, whose tropism is broadly speaking for erythroid self-sufficient, this is, not requiring the presence of a helper virus. Specific antiviral antibody production is a concept to symbolize the principal protection against the B19 virus, as human regular immunoglobulin often clears the virus from peripheral blood and effects in clinical improvement in immunosuppressed individuals. (Dijkmans *et al.*, 2012)

The virus replicates in human erythroid progenitor cells of the BM and blood, inhibiting erythropoiesis. Tropism of efficient B19 infection is mainly because of the restrictive mobile distribution of the P blood institution antigen globoside (Gb4), that is located most usually on cells of the erythroid lineage but additionally on platelets; on tissues from the heart, liver, lung, kidney, endothelium and on synovium (Lamont *et al.*, 2011).

Studies performed in volunteers advocate that the B19 virus first replicates inside the nasopharynx or upper respiration tract then spreads with the aid of viremia to the bone marrow and somewhere else, where it replicates and kills erythroid precursor cells (Dijkmans *et al.*, 2012).

People who lack erythrocyte P antigens are very rare (1 in 200000) and seemingly can't be inflamed by using the B19 virus. B19 virus capsids are composed of two structural polypeptides, the minor (4%) VP1 protein (83kDa) and the major (96%) VP2 protein (58 kDa). VP1 immunoglobulin IgM and/or IgG antibodies are always associated with the clearance of PV-B19 virus from serum. The detection of anti-VP1 and anti-VP2 antibodies is the premise for the prognosis of acute or past PV-B19 infections. The dominant humoral immune reaction is to VP2 all through early convalescence and to VP1 during the past due to convalescence. Anti-VP1 and anti-VP2 antibodies play a chief role in limiting B19 contamination in human beings (Kalolekesha *et al*., 2018).

2.5 Clinical syndromes commonly associated with Parvovirus B19:

The development of Parvovirus B19 disease is influenced by the hosts hematological and immunological status. Healthy children usually develop asymptomatic infection, nonspecific illness or benign erythema infectious. But in patients suffering from decreased production or increased loss of erythrocytes, B19 can cause a severe drop in hemoglobin values, leading to aplastic crisis and anemia, which can be fatal. Immunocompromised patients can develop a state of chronic anemia due to their inability to clear the persistent Parvovirus B19 replication (Kerr, 2016).

2.5.1 Infections in immunocompetent:

2.5.1.1 Erythema infection:

Infectious rash occasionally called 'fifth disorder' (The authentic classification of the exanthemata's ailments of formative years comprised six sicknesses: the other five are measles, rubella, scarlet fever, exanthemsubitum, and Duke's ailment, a rash of obscure etiology). greater picturesque name is 'slapped cheek syndrome ', the presenting feature is regularly erythema over the malar areas, observed within the next four days through a maculopapular rash on the trunk and limbs, which may additionally persist for two or 3 weeks (Xu *et al.*, 2019).

Volunteer and field studies show the incubation period to be 13–18 days. There may be some fever and malaise inside the early levels, and mild febrile illness without rash is common that is predominantly an infection of kids however adults may additionally collect it; in them, and in particular, in ladies, the joints are much more likely to be worried (Flint *et al.*,2020).

2.5.1.2 Joint disease:

Signs and symptoms of joint involvement might also occur with or without rash infection, and B19 infection must be taken into consideration within the differential prognosis of acute arthritis. this is greater common in adults, particularly girls, of whom 80% have joint symptoms, in comparison with about 10% informative years. like the rash, arthropathy could be very much like that

visible with rubella, being asymmetrical arthralgia or arthritis concerning especially the small joints of the palms, even though feet, wrists, knees, ankles and other joints may be affected. (Binnicker, 2016). In kids, the arthropathy can be much less symmetrical. Arthropathy usually resolves inside 2–3 weeks, but can also every so often persist or recur for months and very hardly ever for years. some of these patients may be labeled clinically as having early benign rheumatoid arthritis. however, B19 arthropathy is not unfavorable; if the rheumatoid element is detected it does no longer persists, and B19 virus infection isn't causally connected to rheumatoid arthritis (Binnicker, 2016).

2.6 Other infections:

In healthy human beings, case reviews advise that the manifestations of B19 may sometimes be very huge (e.g. meningitis, hepatitis, haemo-phagocytic syndrome or myocarditis). But, as both IgM and DNA might also persist for months, some associations can be informal instead of causal (Lamont *et al.*, 2011).

2.6.1 Contamination inside the Immunodeficient Host

2.6.1.1 Chronic natural purple mobile aplasia

In immunocompromised patients unable to mount a neutralizing antibody reaction because of chronic bone marrow (BM) insufficiency, B19 infection may additionally motive chronic anemia. Predisposing situations include Nezelof's syndrome, acute lymphatic leukemia (ALL), acute myeloid leukemia, chronic myeloid leukemia, myelodysplastic syndrome, Burkitt's lymphoma, lymphoblastic lymphoma, astrocytoma, Wilms's tumor, HIV contamination, SCID, BM transplantation, organ transplantation, class switch illness, steroid and cancer chemotherapy remedy (Dijkmans*et al.*, 2012)

Medical hallmarks include fatigue and pallor, even as immune-mediated symptoms (rash and arthralgia) are typically no longer present, contamination may additionally serve as a prodromal of an underlying sickness, and anemia

might also right away remit after immunoglobulin or antiviral chemotherapy (in HIV sufferers) (Abiodun *et al.*, 2013).

2.7 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 neutralize virus infectivity for erythroid host cells, and sera from these persons is also known to neutralize 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 up to 15% -35% in school children, about 50 % in adults (Verdonschot, 2015), and it is 85% or more in ageing population > 70 years of age. Seroprevalence in blood donors in developed countries is up to 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 neutralizing 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 neutralizing antibody response, but VP2 accounts for hemagglutinating activity of the virus. Antibody titer 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 neutralizing antibodies (Dudleenamjil *et al.*, 2013). Bua 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 up to 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. (Verdonschot, 2015) reported that B19 DNA can be detected in the synovium of 75% patients but only in 16.7% patients with non-rheumatoid arthritis.

2.8 Parvovirus in pregnant Female:

Despite the fact that B19 infection can purpose fetal loss, most pregnancies bring about the start of a normal toddler. There's no proof of birth defects or developmental abnormalities in kids exposed to B19 in the uterus. Transplacental infection can arise at some point in acute maternal contamination, whether or not signs of B19 infection occur inside the mom, earlier than maternal antibody has advanced and crossed the placenta to guard the fetus. infection within the first 20 weeks can lead to intra-uterine dying (increased hazard 9%) and non-immunological fetal hydrops (danger 3%), of which approximately 1/2 die and are blanketed within the 9%. this is in assessment to hydrops which takes place following Rhesus incompatibility and is immune-mediated (Binnicker, 2016).

Considerably, the chance of fetal demise is maximum after B19V infection in early gestation. The prevalence of fetal anemia and hydrops fetalis is especially high at some stage in the second one trimester while the erythrocyte mass expands unexpectedly, mixed with the quick lifespan of fetal erythrocytes. Timely transfusion of packed erythrocytes of fetuses is the treatment of choice in excessive fetal anemia and hydrops ensuing in a massive discount of fetal mortality. The threat of acquiring B19V contamination all through being pregnant is set 1–2% in endemic durations, but it is able to upward push to >10% at some point in epidemic intervals. (Abiodun *et al.*, 2013).

2.9 Laboratory Diagnosis:

Parvovirus B19 produces numerous scientific manifestations, relying on the level of infection and the immune popularity of the host. The diagnostic approach is exclusive for the exclusive scientific manifestations. The virus can not presently be cultured in recurring diagnostic virology laboratories and, therefore, diagnosis is based totally on serologic exams to hit upon parvovirus B19-particular antibodies and on nucleic acid amplification trying out (Shafik *et al*., 2019).

Acute parvovirus B19 contamination reasons the fifth sickness, additionally called exanthemsubitum. Because patients with 5th ailment are typically viremic, the diagnosis can be installed by means of the use of PCR to locate parvovirus B19- unique DNA in serum. An opportunity approach is to check for parvovirus B19-precise IgM antibodies, which are gift inside several days of the onset of signs. A drawback of this approach is that tests for parvovirus B19 IgM may additionally pass-react with other viruses, in particular rubella, measles, EBV, and CMV. Serologic diagnosis of acute parvovirus B19 contamination can also be carried out by means of evaluating parvovirus B19-unique antibody titers in acute and convalescent specimens, with seroconversion or a fourfold upward thrust taken as diagnostic of latest infection. Parvovirus B19 is also the principal cause of hemolytic disaster in individuals with chronic hemolytic anemia, particularly sickle cell disease (Shafik *et al*., 2019).

Hemolytic disaster takes place earlier after contamination than the fifth sickness, and the position of parvovirus B19 is greatly confirmed the use of nucleic acid amplification because antibodies might not be detectable for approximately 1 week after the onset of the crisis. Parvovirus B19 contamination can purpose persistent hypoplastic anemia in immunocompromised patients. these patients are chronically viremic and, therefore, PCR is the exceptional method for making a virologic diagnosis. Serologic checking out is generally not helpful due to the fact sufferers with

chronic parvovirus B19 infection may fail to expand parvovirus B19-precise antibodies. The opportunity of the use of parvovirus nucleic acid amplification assays to screen blood donors is underneath dialogue, specifically for pooled plasma products. In the end, parvovirus B19-precise immunity can be tested by using trying out for parvovirus B19-specific IgG antibodies (Puccetti *et al*., 2012).

2.9.1 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 (Jordan *et al.,* 2017). B19V specific IgM antibodies become detectable in maternal serum within 7–10 days after infection, sharply peak at 10–14 days, and then rapidly decrease within 2 or 3 months (De Jong *et al.,* 2016). 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 (Jordan *et al.,* 2017).

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. (Jordan *et al.,* 2017)

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 (Xu *et al.,* 2019).

2.8.2 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,*et al* 2017). Using standard procedures, detection of B19V specific IgM in fetal blood has a sensitivity of 29% compared to almost 100% for PCR (Jordan *et al*., 2017). 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 (Jordan *et al*., 2017).

2.9.3 Viral Culture:

In all culture systems erythropoietin is require to maintain viral replication probably by supporting the rapid division of erthyroid progenitors. All systems are culture explants only and are not suitable for long turn cultured.HoweverB19V can also be propagated in a few specialized cell line: two magakaryoblastoid cell line, MB-02 and UT-7/EPO, and two human erythroid leukemia cell line, JK-1 and KU812EP6 (Bua 2016). These lines have been used to study mechanisms of replication and to develop neutralization and infectivity assays (Bua 2016).

2.9.4 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 μm, large eosinophilic nuclear inclusion bodies, and cytoplasmic vacuolization, and occasionally, "dogear" projections may be observed. EM of cells reveals cytopathic ultrastructural changes that include pseudopod formation, marginated chromatin, and virus particles in the nucleus (Jordan *et al*., 2017).

2.10 Therapy

Management of B19V infection with intrauterine erythrocyte transfusion (IUT) can correct fetal anemia and may reduce the mortality of B19V infection significantly. Timely IUT of fetuses with severe hydrops reduces the risk of fetal death (Wang *et al*.,2020). 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. (Wang *et al*.,2020)

2.11 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. 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. (Mukhtar, 2015) 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. 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.(Mukhtar, 2015)

2.12 Prognosis, risk factors and prevention

2.12.1. Prognosis

Children who survived a successful IUT forB19V-induced fetal anemia and hydrops fetalis have a good neurodevelopmental prognosis (Xu *et al.*, 2018).

2.12.2. Risk factors

A prospective evaluation of 618 pregnant women exposed to B19V in an endemic period was performed by Bonvicini. 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 (Bonvicini *et al*., 2017).

2.12.3. 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 (Bonvicini *et al*., 2017). This would require an active policy of serological testing of these categories early in pregnancy. Recently (Xu *et al*., 2018) 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. (Xu *et al*., 2018)

2.13 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. (Ornoy *et al*., 2017) 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 (Ornoy *et al*., 2017)

2.14 Previous studies:

A study to determine the seroprevalence of immunoglobulin G (IgG) to Erythrovirus B19 in Saudi pregnant women in the cities of Makkah and Jeddah in Saudi Arabia. A total of 364 blood (serum) samples were tested for Erythrovirus B19-specific-IgG antibody in Saudi pregnant women in the cities of Makkah and Jeddah in Saudi Arabia. Erythrovirus B19-specific-IgG antibodies were detected in 182/364 (50%) of Saudi pregnant women of different age groups. Conclusion: Their study indicated that B19V is clearly circulating in the community in a way

that is similar to what is found in most nontemperate countries. (Johargy *et al.*, 2016)

Another to determine the seroprevalence rate of immunoglobulin G (IgG) and immunoglobulin M (IgM) to parvovirus B19 in pregnant Saudi women in Makkah. Using enzyme-linked immunosorbent assay (ELISA), a total of 1200 serum samples were tested for antibodies to parvovirus B19 known to cause a variety of clinical syndromes in women and newborn infants. Parvovirus B19 IgG antibodies detected in 46.6% and IgM antibodies were found in 2.25% of different age groups. they Concluded, The previous exposure to parvovirus B19 was determined, and 560 (46.6%) of 1200 pregnant Saudi women tested at their first antenatal visit were seropositive for specific IgG. The rate of maternal infection in susceptible pregnancies was 2.25%. These results were in accordance with previous studies performed in other countries.(Ghazi., 2007)

A study aimed to 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). Our data showed that B19V infection is prevalent in Sudan and recommend further studies in Sudanese women, particularly in those with complications and adverse outcomes of pregnancy (Adam *et al.*, 2015)

Another study to assess the seroprevalence of human parvovirus B19 among the pregnant women in Damascus, Syria and to compare this with that in other regions. A total number of 273 participants were included in the study, consisting of women of child-bearing age ranging from 21 to 42 years, and divided into age groups as follows: ≤ 21 years, 22-27, 28-32, 33-36, and ≥ 37 years. Specific IgM and IgG antibodies were measured using a commercial ELISA kit. The overall

prevalence of IgG and IgM among women of child-bearing age was 61.2% and 4.8% respectively. It was observed that pregnant women in the age group between 37 and 42 had no detectable IgM. The presence of IgG and absence of IgM indicate immunity to primary infection. Our data showed that B19V infection is prevalent in Syria, and over one-third of the studied populations are at risk of primary infection with human parvovirus B19 which could adversely affect their pregnancy (Buhtori *et al.*,2015).

Another study conducted to evaluate seroprevalence of rubella virus (RV), cytomegalovirus (CMV), varicella zoster virus (VZV), and parvovirus B19 (PB19) in 404 Tunisian pregnant women, and to determine reliability of maternal past history of eruption. Sociodemographic characteristics, risk factors, and past history of eruption were collected through a questionnaire. Serologic tests were performed using enzyme immunoassays. Risk factors were analyzed using univariate and multivariate logistic regression models. Seroprevalences were 79.7% for rubella, 96.3% for CMV, 80.9% for VZV, and 76.2% for PB19. In multivariate analysis, the number of persons per room (> 2) in the house during childhood was associated with CMV infection (P = 0.004), irregular professional husband's activity was correlated with VZV infection (P = 0.04), and an age of more than 30 years was associated with PB19 infection (P = 0.02). History of rubella, varicella, and PB19 infection was unknown for, respectively, 55.8%, 20%, and 100% of women. False history of rubella and varicella were found for 7.4% and 15% of women, respectively. The positive and negative predictive values (PPV and NPV) of rubella history were, respectively, 92.6% and 17.2%, and were, respectively, 84.9% and 20.9% for varicella history. Susceptibility to RV, VZV, and PB19 infection remains high in pregnancy in our population. Preventive strategies against congenital rubella must be reinforced. Vaccination against VZV should be considered in seronegative women. Systemic CMV screening is not warranted in our country where high immunity is acquired probably in childhood.

Since maternal history of eruption is not reliable, we recommend serologic testing to determine immune status of women (Hannachi *et al.*, 2011).

In a study done to detect parvovirus B19 IgG and IgM. Between 2007-2010, 55 women in the age between 21 and 40 years were tested for both B19V IgG and IgM antibodies and sociodemographic information was collected. Among the study group, the mean age was 30 years, 43.6% of women were positive only for B19V IgG antibodies, 9% were positive for both B19V IgG and IgM antibodies and 11% were positive only for B19V IgM antibodies. Women negative for B19 IgG antibodies (47.3%) were considered as a high-risk group of B19V viremia. The serological profile indicating infection with Toxoplasma gondii was considered as a risk factor for fetal distress. The T. gondii IgG antibodies were detected in 51% cases, in 32.7% antibodies were positive for both IgG and IgM, while in 16.3% cases both IgG and IgM were negative. Their conclusions was, B19V infection and overlapping of other independent risk factors during pregnancy pose a significant hazard to fetus during development. Therefore, we recommend further broadening the epidemiological database of B19V infection prevalence among women. B19V infection should be taken into account during differential diagnosis as a cause of miscarriage (Zajkowska, *et al.*, 2015)

In published literature retrieved through searches of PubMed and The Cochrane Library. Results were restricted to systematic reviews, randomized control trials/controlled clinical trials, and observational studies. There were no date restrictions but results were limited to English or French language materials. The quality of evidence in their document was rated using the criteria described in the Report of the Canadian Task Force on Preventive Health Care. The outcomes evaluated were maternal outcomes including erythema infectiosum, arthropathy, anemia, and myocarditis, and fetal outcomes including spontaneous abortion, congenital anomalies, hydrops fetalis, stillbirth, and long-term effects.

Recommendations 1. Investigation for parvovirus B19 infection is recommended apart of the standard workup for fetal hydrops or intrauterine fetal death. (II-2A) 2. Routine screening for parvovirus immunity in low-risk pregnancies is not recommended. (II-2E) 3. Pregnant women who are exposed to, or who develop symptoms of, parvovirus B19 infection should be assessed to determine whether they are susceptible to infection (non-immune) or have a current infection by determining their parvovirus B19 immunoglobulin G and immunoglobulin M status. (II-2A) 4. If parvovirus B19 immunoglobulin G is present and immunoglobulin M is negative, the woman is immune and should be reassured that she will not develop infection and that the virus will not adversely affect her pregnancy. (II-2A) 5. If both parvovirus B19 immunoglobulin G and immunoglobulin M are negative (and the incubation period has passed), the woman is not immune and has not developed the infection. She should be advised to minimize exposure at work and at home. Absence from work should be considered on a case-by-case basis. (II-2C) Further studies are recommended to address ways to lessen exposure including the risk of occupational exposure. (III-A) 6. If a recent parvovirus B19 infection has been diagnosed in the woman, referral to an obstetrician or a maternal-fetal medicine specialist should be considered. (III-B) The woman should be counselled regarding risks of fetal transmission, fetal loss, and hydrops and serial ultrasounds should be performed every 1 to 2 weeks, up to 12 weeks after infection, to detect the development of anemia (using Doppler measurement of the middle cerebral artery peak systolic velocity) and hydrops. (III-B) If hydrops or evidence of fetal anemia develops, referral should be made to a specialist capable of fetal blood sampling and intravascular transfusion. (II-2B) (Crane *et al*., 2014).

In a study by Di Domenico *et al*. (2012), anti-parvovirus B19 IgG levels were present in the cord serum of 24% of babies born in Rome, Italy (indicating

maternal immunity), while Barros De Freitas *et al*. found an 84% rate of seropositivity among mothers from Brazil (Barros De Freitas *et al*., 2013). Valeur-Jensen *et al*. found that the rate of seropositivity in pregnant women from Denmark was 65%, and the risk of acute infection in pregnancy correlated directly with the number of children at home. During pregnancy, the risk of acquiring parvovirus B19 infection is quite low, ranging from 0 to 16.5%, typically with normal outcome (Di Domenico *et al*., 2012).

A cohort of 113 228 children born to women tested for parvovirus B19 infection during pregnancy in a major diagnostic laboratory in Denmark, from 1994 to 2009. Information on 20 selected morbidity diagnoses and on mortality was obtained from the Danish National Patient Register, the Danish Cancer Register and the Danish Civil Registration System. Incidence rate ratios (IRR) were estimated by log-linear Poisson regression with adjustment for age and sex of the child, maternal age and year of maternal parvovirus B19 test. A total of 1095 (1.0%) children were born to mothers who were infected with parvovirus B19 during pregnancy. During 1 million person-years of follow-up, 10 856 children experienced morbidity and 590 children died. Overall, maternal infection status was neither associated with morbidity during infancy (IRR 0.64; 95% CI: 0.40 to 1.02) or childhood (IRR 0.93; 95% CI: 0.77 to 1.14), nor with infant mortality (IRR 0.98; 95% CI: 0.44 to 2.20). Specifically, there was no association with 19 of 20 morbidities. An excess risk of cancer in the central nervous system was observed (IRR 5.88; 95% CI: 1.41 to 24.6); however, the number of exposed cases was very small (n = 2). Conclusions Parvovirus B19 infection during pregnancy was not associated with overall morbidity or mortality in infancy and childhood (Lassen *et al*.,2014)

A case of parvovirus B19 infection related to hemophagocytic lymphohistiocytosis during pregnancy. The patient experienced fever and pancytopenia. A bone marrow biopsy demonstrated hemophagocytosis and a giant proerythroblasts,

which is characteristic of a parvovirus B19 infection. Viral serology for parvovirus B19 was positive. Prompt treatment was started because of the high level of certainty of viral-associated hemophagocytic lymphohistiocytosis, and the patient was successfully treated with prednisolone administration. She delivered a healthy newborn without any complications. Hemophagocytic lymphohistiocytosis should be considered when encountering unexplained cytopenia and fever. Prednisolone was an effective treatment (Mayama *et al*., 2014) In a study done to investigate the presence of Cytomegalovirus (CMV), herpes virus simplex (HSV), and parvovirus B19 (PVB19) in the placental tissue of patients who underwent abortions without an otherwise-defined aetiology. a cross-sectional study was conducted in a high-risk obstetric maternity facility at a University Hospital in Belo Horizonte, Brazil, from January 2013 to December 2015. Immunohistochemistry used monoclonal anti-CMV antibody, anti-PVB19 antibody, and anti-HSV1/HSV2 antibodies. Viral agents were detected in five patients (7.14%) in the villitis group. Three patients were positive for CMV, one for PVB19, and one for HSV type 2. Foetal and maternal complications were significantly higher in the group with villitis compared with those in the group without villitis ($p = .002$). their Conclusions was, The prevalence of transplacental viral infections as a cause of spontaneous abortion should be considered high in the placenta with villitis. Thus, this study highlights the need for developing diagnostic tests to clarify the aetiology of abortion and foetal loss. (Oliveira *et al.*, 2019).

CHAPTER III

MATERIALS AND METHODS

3.1 Study design:

This is descriptive cross –sectional and hospital-based study

3.2 Study area and duration:

The study was conducted in antenatal clinic in Albadria Tower Medical Center in Jeddah, Saudi Arabia. during the period from October 2019 to April 2020.

3.3 Study population:

The study subjects included in this study were pregnant women in first trimester who attended the antenatal clinic in Albadria Tawer Medical Center.

3.4 Selection criteria:

3.4.1 Inclusion criteria:

The study included women who were pregnant with different age, in the first trimester, attended the antenatal clinic and provided signed consent forms and interviewed questionnaire. Written and virbal consents used.

3.4.2 Exclusion criteria:

The exclusion criteria were non pregnant women, pregnant women in the second and third trimester and age below 15 or above 45 years and failure to sign a consent form.

3.5 Ethical consideration

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

3.6 Data collection

A structured questionnaire was designed to collect information regarding socio-demographics and risk related data. Most the questions questionnaire was the yes/no questions which includes: age, Gestational age, abortion, and history of blood transfusion.

3.7 Sample size

A total of 87 pregnant women serum sample (n= 87) were collected in plain containers aged from 17 years, serum separated and stored at -20 ºC.

3.8 Method

3.8.1 Collection of specimens

Five-ml blood sample was collected from 87 pregnant women by venipuncture, the suitable vain was selected and the skin cleaned by alcohol swab, sterile syringe was used to collect the blood, then transferred into sterile anticoagulant-free bottle, and allowed to clot. The clotted blood sample was centrifuged (3700 rpm, 10 min), and the serum (the supernatant) was transferred and stored at -20° C until required for use.

3.8.2 Enzyme linked immunosorbent assay (ELISA)

3.8.2.1 Principle

The ELISA test kit (EUROIMMUN, Germany) provides a semiquantitative in vitro assay for human antibodies of the immunoglobulin class IgM against parvovirus B19 in serum or plasma. The test kit contains microtiter strips 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 IgM antibodies (also IgA) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled antihuman IgM (enzyme conjugate) catalyzing a colour reaction.

3.8.2.2 Procedure:

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

Sample dilution patient samples were diluted 1:10 (10 ul from sample and 1.0 ml from sample buffer) in sample buffer and mixed well by vortex.

The reagents and samples were allowed to reach room temperature (+ 18o C to +25 o C) and the samples were diluted 10 ul and 1.0 ml from sample buffer. Then

was transferred 100 µl of the calibrator, positive control, negative controls , 1.0 ml from sample buffer and 10 ul from sample into the individual respective wells of the 96-well microtiterplate. The finished test plate was incubated for 60 minutes at 37 ^{o}C ± 1 ^{o}C automatically in the incubator of dynex technologies instrument .and the plate was washed 3 times using 450 µl of working strength diluted wash buffer .The washing buffer was left in each well for dispose of 30 to 60 seconds per washing cycle.

100 ul of enzyme conjugate (peroxidase-labelled anti human IgM) was added into each of the microplate wells and the plate was incubated for 30 minutes at room temperature (+18 ^{o}C to + 25 ^{o}C). Then washed as method above.

100 ul of chromogen\substrate solution was added into each of microplate wells and the plate was incubated for 15 minutes at room temperature (+18 ^{o}C to + 25 ^{o}C. protects from direct sunlight).

100 ul of stop solution was added into each well to stopping the reaction in the same order and same speed as 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

3.8.2.3 Calculation of the result

The extinction value of the calibrator defines the upper limit of the reference range non-infected persons (cut-off) recommended by EUROIMMUN. Values above the indicated cut-off are to be considered as positive, those below as negative.

Semiquantitative: Result can be evaluated semi quantitatively by calculating a ratio of the extinction of the control or patient sample over the extinction value of calibrator. Use the following formula to calculate the ratio:

Extinction of the control or patient sample ÷ Extinction of calibrator = Ratio

3.8.2.4 Interpretation of the results

Ratio <0.8: negative

Ratio <1.1: borderline

Ratio ≥1.1: positive

For duplicate determinations. The mean of the tow values should be taken. If the tow values deviate substantially from one another. EUROIMMUN recommended resulted the samples.

A negative serological result does not exclude an infection. Particularly in the early phase of an infection, antibodies may not yet be present or are only present in such small quantities that they are not detectable. Significant IgM titer increases seroconversion in a follow-up sample taken at least after 7 to 10 days can indicate an acute infection.

For diagnosis, the clinical picture of the patient always needs to be taken into account along with the serological finding.

3.9 Data analysis

The data was analyzed using SPSS computer program version 22.0. T test and P *value* was obtained (P *value* ≤ 0.05 was considered statistically significant).

CHAPTER IV

RESULTS

4. RESULTS

A total of 87 apparently healthy pregnant women who attended Albadria Tawer Medical Center in Jeddah Saudi Arabia during period from July 2019 to April 2020 were enrolled in this study to determine the frequency of B19V. Their age range between17-45 years and the majority of them 33/87 (37.9%) were at age between 25-35 years **(Table 1).**

Antibody detection of parvovirus B19 7/87 (8.0%) were positive for IgM and 80/87 / (92.0%) were negative for IgM as showed in **(Table 2).**

Most of pregnant women in first trimester had anti-parvovirus B19 IgM antibodies were within second month 5/87 (5.7%) and no association between gestational age and seropositivity of anti-parvovirus IgM (*p value*= 0.144) **(Table 3).**

Concerning gravidity pregnant women with Primigravida had anti-parvovirus B19 IgM antibodies negative 19/89 (21.8%) when 61/87 (70.1%) of women with multigravida had negative IgM and Concerning gravidity pregnant women with multigravida had anti-parvovirus B19 IgM antibodies positive 7/87 (8.0%) and there was no association between gravidity age and seropositivity anti- parvovirus IgM (*P.value*= 0.145) **(Table 4).**

Most of pregnant women had history of blood transfusion 1 (1.1%) were positive for anti- parvovirus B19 IgM antibodies and there was no association between history of blood transfusion and seropositivity of anti-parvovirus IgM (*p value*=0.959) **(Table 5).**

Parvovirus sero-positivity among pregnant women with history of miscarriage was 2(2.3%) anti-parvovirus B19 IgM antibodies and there was no association between history of miscarriage and sero-positivity of anti-parvovirus IgM (*p value* = 0.591) **(Table 6).**

Table (1): The Distribution of age group among apparently healthy pregnant women:

Age groups	**Frequency**	**Percentage**
Less than 25 Years	28	32.2%
25-35Years	33	37.9%
More than 35 Years	26	29.9%
Total	87	100

Table (2): Frequency of parvovirus B19 in pregnant women.

Result	**Frequency**	**Percent**
Positive	7	8.0%
Negative	80	92.0%
Total	87	100.0%

Table (3): The association between first trimester and IgM results among apparently healthy pregnant women:

Serological tests		First Trimester				*p-value*
		First month	Second month	Third month	Total	
IgM Result	Positive	1 (1.1%)	5 (5.7%)	1 (1.1%)	7 (8.0%)	0.144
	Negative	10(11.5%)	29 (33.3%)	41 (47.1%)	80(92.0%)	
Total		11(12.6%)	34 (39.1%)	42 (48.3%)	87 (100%)	

Table (4): The association between gravidity and IgM results among apparently healthy pregnant women:

Serological tests		**IgM result**		***p-value***
		Positive	**Negative**	
Gravidity	**primigravida**	**0(0.0)**	**19(100%)**	**0.145**
	Multigravida	**7(10.3%)**	**61(89.7%)**	
Total		**7(8.0%)**	**80(92%)**	

Table (5): The association between history of blood transfusion and IgM, results among apparently healthy pregnant women:

Serological tests		**History of blood transfusi**		**Total**	***p-value***
		No	**Yes**	**No.**	
IgM Result	**Positive**	**6(6.9%)**	**1(1.1%)**	**7(8.0%)**	**0.959**
	Negative	**68(78.2%**	**12(13.8%)**	**80(92.0%)**	
Total		**74(85.1%**	**13(14.9%)**	**87(100%)**	

Table (6): The association between history of miscarriage and IgM results among apparently healthy pregnant women:

Serological tests		History of miscarriage		Total	*p-value*
		Yes	No	No.	
IgM Result	Positive	2(2.3%)	5(5.7%)	7(8.0%)	0.591
	Negative	16(18.4%)	64(73.6%)	80(92.0%)	
Total		18(20.7%)	69(79.3)	87(100%)	

CHAPTER V

DISSCUSSION, CONCLUSION &RECOMMENDATIONS

5.1Discussion

Parvovirus B19 is a widespread infection that may affects 1-5% of pregnant women, mainly with normal pregnancy outcome (Feldman *et al.*,2010). Infection during pregnancy can cause a variety of other signs of fetal damage. The risk of adverse fetal outcome is increased if maternal infection occurs during the first two trimesters of pregnancy but may also happen during the third trimester (Ergaz *et al.*,2006)

This study was conducted to detect parvovirus B19 IgM antibodies in pregnant women attending Albadria Tower Medical Center in Jeddah Saudi Arabia during period from October 2019 to April 2020.

The frequency of B19V IgM antibodies detected in this study was seven cases which interestingly close to other studies carried out in Sudan by (Marwa.2019), which reported that out of 93 participants ,8(8.6%) was positive for B19-IgM, in Libya by (Elnifro *et al.*,2009) which reported that among 150 pregnant women tested ,8(5%) was positive for IgM to parvovirus B19, also agreement in study by (Kelishadi *et al.*, 2016) in Iran.

In the present study found 7(8%) were positive for IgM, this study was higher than the previous reported study done in Sudan by (Adam *et al.*,2015) which found that the seropositive was positive in one subject that may be due the large sample size, long duration between the two studies, different type of ELISA Kits and B19V infection is difficult to prevent since the infection is frequently asymptomatic and exposure is common during epidemics.

Another agreement In Saudi Arabia, the prevalence of IgM was also low in study done by (Ghazi .,2007) who estimate B19 among pregnant women, who reported that majority of the study population were presented at age range 25-35 years and the frequency of IgM antibodies were increased with age, which matched with previous study in Saudi Arabia done by (Ghazi .,2007).

in this study Parvovirus B19 seropositivity was low among the pregnant women that had abortion, and there was no association between history of miscarriage

and seropositivity of anti-parvovirus IgM *p value* = 0.591 that mean there was in significant association, it similar to previous reported study done in Sudan by (Adam *et al.*,2015) which found P.value was 0.834. 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 few numbers of participants Mirzaie *et al.*, 2008), and a study by Nyman with *et al.*,*(*2002) revealed that DNA of B19 Parvovirus was observed in negative of abortions in the first trimester and positive in second trimester in fetal tissues, this difference may be related to specimen population and the diagnostic techniques used.

In the present study there was no association between IgM result and abortion (P.value was 0.591) that not agree with study done by (Jensen *et al.*,2012) in Denmark, which done in first-trimester serum samples were tested for parvovirus B19 IgM positivity, Parvovirus B19 IgM positivity was associated high-rate increased risk of fetal loss, a strong statistical association was observed between the presence of B19 IgM antibody during pregnancy and spontaneous abortion.

The seropositivity of anti-parvovirus B19 IgM antibodies among pregnant women that had history of blood transfusion was presented as low number so there was no association between history of blood transfusion and seropositivity of anti-parvovirus IgM (*p value*= 0.959), it is similar to the previous reported study done in Nigeria by (Emiasegen e*t al.*,2011) which found that no association between history of blood transfusion and seropositivity of anti-parvovirus IgM (*p value*= 0.62)

Women in their second month of first trimester of pregnancy were had the highest frequency of B19-IgM antibodies in compare to those in their first month and third month and no association between gestational age and seropositivity of anti-parvovirus IgM (*p value*= 0.144), The frequency of B19V antibodies was higher in pregnant women with multigravida in which IgM positive, but there was no significant association which disagree with study

done by Adam and his colleagues (2015) in Sudan, which found there was significant association between gravidity and seropositivity (*P.value* = 0.046).

5.2 Conclusion

-Lower frequency of B19V-IgM (Seropositivity) (8.6%) in pregnant women.

-There was insignificant association between B19V infection and others (age, gravidity, history of miscarriage, history of blood transfusion)

5.3 Recommendations

Based on this study we recommended that:

-Detection of parvovirus B19 should be routine investigation for pregnant women.

-Routine screening for B19 IgM and IgG antibodies for all women of childbearing age and subsequent clinical management of positive cases.

-Further studies in pregnant 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.

-Public awareness of B19V must be increased.

REFERENCES

AAK, A.E. and KSE, A.W.,(2018). Egyptian Mother-to-fetus transmission of Parvovirus B-19 in the first trimester abortions. *The Egyptian Journal of Hospital Medicine,* **39**(1):288-294.

Abiodun, I., Opaleye, O.O., Ojurongbe, O. and Fagbami, A.H., (2013). Seroprevalence of parvovirus B19 IgG and IgM antibodies among pregnant women in Oyo State, Nigeria. *The Journal of Infection in Developing Countries*; **7**(12):946-950.

Adam, O., Makkawi, T., Reber, U., Kirberg, H. and Eis-Hübinger, A.M., (2015). The seroprevalence of parvovirus B19 infection in pregnant women in Sudan. *Epidemiology & Infection*; **143**(2):242-248.

Avguštin, N.O., Sršen, T.P., Stritar, B.Š. and Mujezinović, F., (2018). Slovenian recommendations for parvovirus B19 infection in pregnancy. *Zdravniski Vestnik*, **87**(1-2).

Berk L.E. (2011) .Infants and children, 7 th editions. USA Published by Pearson.

Binnicker, M.J. and Razonable, R.R., (2016). Molecular diagnostics for viral infections in transplant recipients. Molecular Microbiology: *Diagnostic Principles and Practice*,476-486.

Bonvicini, **F**., Bua, G. and Gallinella, G.**,** (2017). Parvovirus B19 infection in pregnancy—awareness and opportunities. Current Opinion in Virology, 27, pp.8-14.

Bua, G., (2016). Parvovirus B19: Virus-Cell Interactions, Pathogenetic Mechanisms and Development of Compounds with Antiviral Activity.

Buhtori, M.M., (2015). Seroprevalence of parvovirus B19 among pregnant women in Damascus Syria. *Int J Pharm Sci Rev Res*, 30, pp.227-9.

Buller, R.S.,(2016)**.** Parvoviruses. Clinical Virology Manual, pp.443-455.

Carel A. B., Eduard R. A., David E. B and Barbara G. (2018). Fundamentals of Clinical chemistry, 6th edition, Saunders an imprint of Elsevier Inc.

Crane, J., Mundle, W., Boucoiran, I., Gagnon, R., Bujold, E., Basso, M., Bos, H., Brown, R., Cooper, S., Gouin, K. and McLeod, N.L., (2014). Parvovirus B19 infection in pregnancy. *Journal of Obstetrics and Gynaecology Canada*, **36**(12):1107-1116.

Dijkmans, A.C., de Jong, E.P., Dijkmans, B.A., Lopriore, E., Vossen, A., Walther, F.J. and Oepkes, D., (2012). Parvovirus B19 in pregnancy: prenatal diagnosis and management of fetal complications. Current Opinion in Obstetrics and Gynecology, 24(2)95-101.

Di Domenico C, Moschese V, Chini L, *et al*. 2012. [Perinatal infections of B19 Parvoviruses]. Ig Sanita Pubbl LVIII: 155– 162.

Dudleenamjil, E., Lin, C.Y., Dredge, D., Murray, B.K., Robison, R.A. and Johnson, F.B**.(**2010). Bovine parvovirus uses clathrin-mediated endocytosis for cell entry. *Journal of general virology*, **91**(12):3032-3041.

Emiasegen, S.E., Nimzing, L., Adoga, M.P., Ohagenyi, A.Y. and Lekan, R., (2011). Parvovirus B19 antibodies and correlates of infection in pregnant women attending an antenatal clinic in central Nigeria. *Memorias do Instituto Oswaldo Cruz*, **106**(2):227-231.

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. *The Journal of Infection in Developing Countries*, **3**(03):218-220.

Ergaz, Z., & Ornoy, A. (2006). Parvovirus B19 in pregnancy. *Reproductive Toxicology*, **21**(4): 421-435.

Feldman, D.M.,Timms, D. and Borgida, A.F., (2010). Toxoplasmosis, parvovirus, and cytomegalovirus in pregnancy. *Clinics in laboratory medicine*, **30**(3):709-720.

Flint, S.J., Racaniello, V.R., Rall, G.F., Hatziioannou, T. and Skalka, A.M., (2020)**.** Principles of virology, Volume 2: pathogenesis and control. John Wiley & Sons.

Gallinella, G., (2013)**.** Parvovirus B19 achievements and challenges. International Scholarly Research Notices, 2013.

Ghazi, H.O., (2007). Prevalence of antibodies to human parvovirus B19 in Saudi women of childbearing age in Makkah. *Journal of family & community medicine*, **14**(1):15.

Hannachi, N., Marzouk, M., Harrabi, I., Ferjani, A., Ksouri, Z., Ghannem, H., Khairi, H., Hidar, S. and Boukadida, J.,(2011). Seroprevalence of rubella virus, varicella zoster virus, cytomegalovirus and parvovirus B19 among pregnant women in the Sousse region, Tunisia. *Bulletin de la Societe de pathologie exotique (1990)*, **104**(1):62-67.

Johargy, A.K., **(**2016). Seroprevalence of erythrovirus B19 in Saudi pregnant women. *Journal of family & community medicine***, 23**(2):105.

Jordan, J.A., 2017. Clinical manifestations and diagnosis of parvovirus B19 infection. UpToDate.[Updated Dec 15, 2014].

Kalolekesha, M.C., (2018)**.** *Seroprevalence of parvovirus B19 in blood donors at the Kitwe Central Hospital, Blood Bank, Kitwe, Zambia* (Doctoral dissertation, The University of Zambia).

Kelishadi, R., Heshmat, R., Shahsanai, A., Djalalinia, S., Motlagh, M. E., Keikha, M., ... & Qorbani, M. (2016). Determinants of tobacco and hookah smoking in a nationally representative sample of Iranian children and adolescents: The Caspian-IV Study. *Iranian Red Crescent Medical Journal*, **18**(8).

Kerr, J.R., (2016). The role of parvovirus B19 in the pathogenesis of autoimmunity and autoimmune disease. Journal of clinical pathology, 69(4):279-291.

Lamont, R.F., Sobel, J.D., Vaisbuch, E., Kusanovic, J.P., Mazaki-Tovi, S., Kim, S.K., Uldbjerg, N. and Romero, R., (2011). Parvovirus B19 infection in human pregnancy. *BJOG: An International Journal of Obstetrics & Gynaecology*, **118**(2):175-186.

Luo, Y. and Qiu, J., (2015). Human parvovirus B19: a mechanistic overview of infection and DNA replication. *Future virology*, **10**(2):155-167.

Lassen, J., Bager, P., Wohlfahrt, J., Böttiger, B. and Melbye, M., (2013). Parvovirus B19 infection in pregnancy and subsequent morbidity and mortality in offspring. International journal of epidemiology, **42**(4):1070-1076

Lassen, J., Jensen, A.K., Bager, P., Pedersen, C.B., Panum, I., Nørgaard-Pedersen, B., Aaby, P., Wohlfahrt, J. and Melbye, M., (2012). Parvovirus B19 infection in the first trimester of pregnancy and risk of fetal loss: a population-based case-control study. *American journal of epidemiology*, **176**(9):803-807.

Mirzaei, F.A.T.E.M.E.H., Arabzadeh, S.A.M., Jeihoni, S.H. and Molaei, H.R., (2008). Comparison of the frequency of CMV and Parvo B19 infections in intrauterin fetal death and normal pregnancy. *Journal of Kerman University of Medical Sciences*, **15**(4):273-281.

Mukhtar, E.S.E., (2015). Seroprevalence of Parvovirus B19 in pregnant women attending antenatal clinics in Military Hospital 2015 (Doctoral dissertation, Sudan University of Science and Technology).

Nyman, M., Tolfvenstam, T., Petersson, K., Krassny, C., Skjöldebrand-Sparre, L. and Broliden, K., (2002). Detection of human parvovirus B19 infection in first-trimester fetal loss. *Obstetrics & Gynecology*, **99**(5):795-798

Oliveira, G.M.D., Pascoal-Xavier, M.A., Moreira, D.R., Guimarães, V.S., Aguiar, R.A.L.P.D., Miranda, D.M.D. and Romanelli, R.M.D.C., (2019). Detection of cytomegalovirus, herpes virus simplex, and parvovirus b19 in spontaneous abortion placentas. *The Journal of Maternal-Fetal & Neonatal Medicine*, **32**(5):768-775.

Ornoy, A. and Ergaz, Z., (2017). Parvovirus B19 infection during pregnancy and risks to the fetus. *Birth defects research*, **109**(5):311-323.

Osman, S.A.M., (2018). Serodetection of Parvovirus B19 among Blood Donors Attending Haj Al Safi Teaching Hospital–Bahri (Khartoum State) (Doctoral dissertation, Sudan University of Science & Technology).

Puccetti, C., Contoli, M., Bonvicini, F., Cervi, F., Simonazzi, G., Gallinella, G., Murano, P., Farina, A., Guerra, B., Zerbini, M. and Rizzo, N., (2012).

Parvovirus B19 in pregnancy: possible consequences of vertical transmission. *Prenatal Diagnosis*, **32**(9):897-902.

Shabani, Z., Esghaei, M., Keyvani, H., Shabani, F., Sarmadi, F., Mollaie, H. and Monavari, S.H., 2015. Relation between parvovirus B19 infection and fetal mortality and spontaneous abortion. *Medical journal of the Islamic Republic of Iran*, 29, p.197..

Shafik, A.F., Hussein, M.Z., Elfiky, A.A., Elshafiey, R.M.G. and El-Naghy, W.S., (2019). Comparative study between Immunochromatography, Enzyme immunoassay and Real-time PCR for diagnosis of Rotavirus infection in infants and young children with acute winter diarrhea. *Life Science Journal*, **16**(10).

Suliman, M.A.A.,(2016). *Seroprevalence of Parvovirus B19 among Children with Acute Lymphocytic Leukemia in Al-Gazira State* (Doctoral dissertation, Sudan University of Science and Technology).

Verdonschot, J., Hazebroek, M., Merken, J., Debing, Y., Dennert, R., Brunner- La Rocca, H.P. and Heymans, S., (2016). Relevance of cardiac parvovirus B19 in myocarditis and dilated cardiomyopathy: review of the literature. European journal of heart failure, 18(12), pp.1430-1441.

Wang, J., Ganaie, S.S., Cheng, F., Xu, P., Ning, K., Wang, X., Kleiboeker, S., Cheng, S. and Qiu, J., (2020). RNA binding motif protein RBM45 regulates expression of the 11-kilodalton protein of parvovirus B19 through binding to novel intron splicing enhancers. Mbio, 11(2), pp.e00192-20.

Weiffenbach, J., Bald, R., Gloning, K.P., Minderer, S., Gärtner, B.C., Weidner, A., Hanke, M. and Enders, M., (2012). Serological and virological analysis of maternal and fetal blood samples in prenatal human parvovirus b19 infection. *Journal of Infectious Diseases*, **205**(5):782-788.

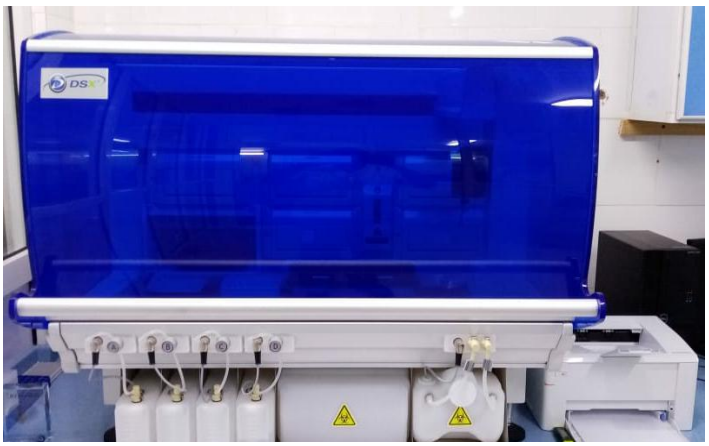
Xu, P., Chen, A.Y., Ganaie, S.S., Cheng, F., Shen, W., Wang, X., Kleiboeker, S., Li, Y. and Qiu, J., (2019). The 11-kilodalton nonstructural protein of Human

parvovirus B19 facilitates viral DNA replication by interacting with Grb2 through its Proline-rich motifs. *Journal of virology*, **93**(1).

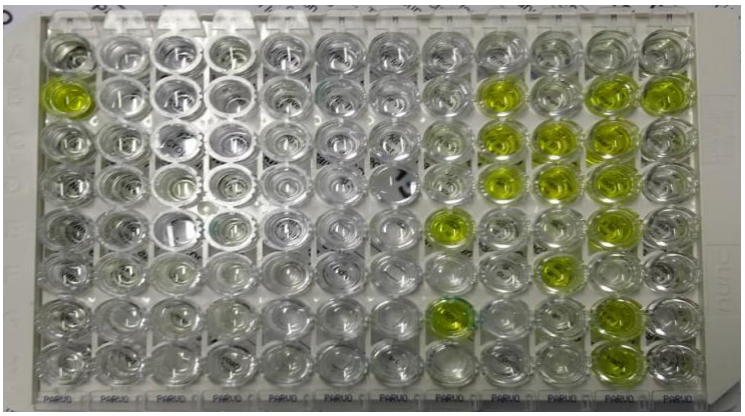
Xu, P., Ganaie, S.S., Wang, X., Wang, Z., Kleiboeker, S., Horton, N.C., Heier, R.F., Meyers, M.J., Tavis, J.E. and Qiu, J., (2018). Endonuclease activity inhibition of the NS1 protein of parvovirus B19 as a novel target for antiviral drug development. Antimicrobial agents and chemotherapy, 63(3), pp.e01879-18.

Zajkowska, A., Garkowski, A., Czupryna, P., Moniuszko, A., Król, M.E., Szamatowicz, J. and Pancewicz, S., (2015). Seroprevalence of parvovirus B19 antibodies among young pregnant women or planning pregnancy, tested for toxoplasmosis. *Przegl Epidemiol*, **69**(3):479-82

Appendix 1



Appendix 2



Appendix 3