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Serodetection of Parvovirus B19 among pregnant women in Riyadh Saudi Arabia

A thesis submitted to Shendi University in Partial fulfillment for the Requirements of M.Sc. in Medical Laboratory Sciences (Microbiology)

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الآية :

((وَلَوْلَا فَضْلُ اللَّهِ عَلَيْكَ وَرَحْمَتُهُ لَهَمَّت طَّائِفَةٌ مِّنْهُمْ أَن يُضِلُّوكَ وَمَا يُضِلُّونَ إِلَّا أَنفُسَهُمْ ۖ وَمَا يَضُرُّونَكَ مِن شَيْءٍ ۚ وَأَنزَلَ اللَّهُ عَلَيْكَ الْكِتَابَ وَالْحِكْمَةَ وَعَلَّمَكَ مَا لَمْ تَكُن تَعْلَمُ ۚ وَكَانَ فَضْلُ اللَّهِ عَلَيْكَ عَظِيمًا)) (113)

سورة النساء

Dedication

In memory of the kind-hearted my beloved father (god bless his soul) whom supported me was proud of me

إلى طيب القلب أبي الحبيب رحمه الله

To my beloved sisters who encouraged me and supported me

Acknowledgement

First of all, thanks to ALMIGHTY ALLAH for blessing me

My supervisors Dr. Hadia & Dr. Fadel who facilitated everything for me until I reached this point

DR Hussain, DR Ali And DR Aabid for their assistance in practical section

ABSTRACT

Background: Parvovirus B19 infection is more prevalent and a major source of clinical manifestation in pregnant women. In fact, after maternal infection during pregnancy, vertical transmission is frequently observed in the 30–50% of non-immune pregnant women. The major cause of outbreaks during the spring season is known to be the occurrence of B19V infection.

Aim: This is a cross- sectional hospital-based study was aimed to detect B19V among apparently healthy pregnant women in King Saud Medical city in Riyadh, Saudi Arabia
Methodology: during the period from October 2022 to May 2023. Blood samples were collected from 86 (n=86) pregnant women their age ranged 17-45 years of age. All samples were tested for the presence of parvovirus B19 IgM & IgG antibodies using ELISA.

Results: Parvovirus B19 IgM antibodies seropositivity was 2(2.3%) of the total samples examined. There was no significant association between IgM positivity and age group or other risk factors. IgG antibodies, indicating past infection or immunity, were present in 45.3% of participants, with no significant associations with risk factors.
Conclusion: The study concluded that Parvovirus B19 IgG antibodies was detected in almost half of the group, while IgM was detected only in 2.3% of participants. There was no association between Parvovirus B19 infection and risk factors (age, miscarriage, history of blood transfusion, hematological disease and gravidity).

المستخلص

الخلفية: تعد الإصابة بفيروس بارفو الصغير(ب19) بين النساء الحوامل أكثر شيوع وسببا للإصابة حيث وجد ما يقرب من 30-50% من النساء الحوامل لم يقمن بالوقاية من الفيروس. ومن المعروف أن الإصابة بعدوى ب19 هي الأكثر شيوعا خلال فترة الربيع.

الهدف:

هدفت هذه الدراسة المقطعية إلى الكشف المصلي عن الأجسام المضادة بارفو(ب19)
في مصل دم النساء الحوامل اللاتي يحضرن إلى مدينة الملك سعود الطبية في الرياض , المملكة العربية السعودية

الطريقة: , خلال الفترة من أكتوبر 2022 إلى مايو 2023. تم جمع عدد 86 عينة من الحوامل الذين تتراوح أعمارهم بين 17-45 سنة.

. igM,igG تم اختبار جميع العينات للكشف عن وجود الأجسام باستخدام اليزا

النتيجة: تم اكتشاف الأجسام المضادة لبارفو فيروس ب19 (م) في 2.3% من العينات التي تم اختبارها (86/2). لم يتم إيجاد أي ارتباط احصائي بين الأجسام المضادة لفيروس بارفو ب19 وعمر المرضى او عوامل لخطر الأخرى.

بالنسبة للجسم المضاد من نوع (ج) , والذي يدل وجوده على مناعة مكتسبة أو إصابة سابقة, تم إيجاده في 45.3% من العينات التي تم اختبارها. أيضا لم يكن هناك علاقة إحصائية بينه وبين عوامل الخطر التي تمت دراستها.

خلصت الدراسة إلى أن نسبة الأجسام المضادة من نوع (ج) كانت ما يقارب النصف من عدد عينات الاختبار بينما الأجسام المضادة من نوع(م) لم يتم إيجاد سوى 2 (2.3%) من مجموع عينات الدراسة.

و ليس هناك علاقة بين مرض البارفو فيروس الصغير ب وعوامل الخطر المدروسة (العمر,الإجهاض,نقل الدم, أمراض الدم وعدد مرات الولادة).

TABLE OF CONTENTS

List of Tables

List of Figures

Abbreviations

B19V	Parvovirus B19
DCM	dilated cardiomyopathy
DNA	Deoxyribonucleic Acid
ELIZA	Enzyme linked immunosorbent assay
EM	Electron microscopy
EPCS	erythroid progenitor
EpoR	erythropoietin receptor
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IUT	Intrauterine erythrocyte
IVIG	Pooled intravenous immunoglobulin G
NS	Nonstructural protein
RNA	Ribonucleic acid
PBMC	peripheral blood mononuclear cells
PCR	Polymerase Chain Reaction
VP	Viral Protein

CHAPTER I

INTRODUCTION

1.1. Introduction:

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. [1] Human parvovirus B19 is a small single-stranded DNA virus (B19V). It is the only erythrovirus in the Parvoviridae family that is known to be hazardous to humans. [2] Every parvovirus has the same icosahedral symmetry in its capsid proteins. B19 measures 20–25 nm in diameter and has a genomic size of 5–6 kb. [3] The structural proteins of the virus control a number of the virus' biological properties, including its capacity to bind to cell receptors, create hemagglutinin, and provoke immunological responses that are neutralizing [4]

Parvovirus B19 transmission can arise through respiratory fluids and contaminated blood products. In addition, B19V can be transmitted vertically from mother to fetus, which raises the risk of severe fetal anemia, miscarriage, fetal death, or hydrops fetalis. [6]. Vertical transmission of the B19V virus can occur in up to one-third of expectant patients who are severely ill, and additional fetal mortality after maternal infection during the first 20 weeks of pregnancy was found to be 56%. [8]

The virus's capacity to infect fetal erythroid precursor cells and fetal organs makes maternal infection during pregnancy a significant hazards to the fetus [9]. The rapidly increasing red-cell volume and short red-cell life span of the fetus make it especially susceptible to B19 infection, and its potential inability to build a potent immune response makes it even more vulnerable [10].

The possibility of disease and infant mortality decreases as gestation age increases. There will be no chance of virus transmission to the fetus if the mother has immunoglobulin G (IgG) antibodies that are specific to the B19 virus [11].

Enzyme-linked immunosorbent assay is the most popular technique for identifying antibodies that are specific for the B19V virus (ELISA). This test uses B19V antigen to identify B19V immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies in serum. IgM, which is detected 6–10 days after the original infection, is regarded as the first antibody marker for B19V infection. About 12 days after exposure, IgG antibodies start to develop and last a period. IgG antibodies that are particular for B19V are a sign of previous infection. [12]

There are differences between countries in the seroprevalence of B19V in pregnant women. It is well recognized in many developed countries that women of reproductive age have seroprevalence to B19V. [13]

1.2. Rationale:

Serious fetal problems can develop during pregnancy as a result of maternal B19V infection. [14]

For about 3% of pregnant women infected with parvovirus B19, however, these complications can include miscarriage, severe fetal anemia, nonimmune hydrops fetalis, and even fetal death. There are a variety of factors that increase the risk of acute parvovirus B19 infection in pregnant women, including: Women who have one kid are three times more likely to get sick than nulliparous women, and women who have three or more children are 7.5 times more likely. [15] (Avguštin et al., 2018)

A few studies on parvovirus B19-infected pregnant Saudi women have been published. In view of these traits as well as the clinical significance of the condition, the goal of this study was to provide further details regarding the sero-frequency of B19V infection

1.3. Objectives:

1.3.1General objective:

To detect Parvovirus B19 antibodies among Pregnant women in Riyadh Saudi Arabia.

1.3.2. Specific objectives:

1.3.2.1 To detect IgG & IgM antibodies of Parvo virus B19 in serum of pregnant women by employing Enzyme Linked Immunosorbent Assay (ELISA).

1.3.2.2 Measurement of prevalence of anti-parvovirus B19 antibodies in pregnant women.

1.3.2.3 To study the possible major risk factors predisposing to Parvovirus B19 infection among Pregnant women in Riyadh Saudi Arabia.

CHAPTER II

LITRETURE REVIEW

2-1 Parvovirus:

The term parvovirus B19 comes from the Latin word parvus, which means small. It is a single-stranded DNA, non-enveloped virus belonging to the family Parvoviridae and the genus Erythrovirus.[16] Several human diseases and disorders have parvovirus B 19V as their common cause.

Since the label "B19" was given to a serum sample from a healthy blood donor because that serum sample's panel B number 19 was coded [17].

B19V virus is known to spread globally and generate epidemics and pandemics (18) that affect more than 60% of the world's population.[19]

2.1.2 Structure:

The B19 virion has a basic structure made up of the two simplest proteins and a single-stranded, linear DNA molecule. The virus particles have icosahedral symmetry, and both empty and full capsids are typically discernible through poor staining and EM. [20] The coat protein is made up of two structural protein capsomers, which are known as capsid proteins. These two capsomers, VP1 and VP2, are overlaid to create the capsid protein [21].

2.1.3. Transmission:

Human parvovirus B19 may be transmitted by inhaling droplets, transplacental transit, or transfusion of blood or blood products. [22-24]. During the prodromal phase, which lasts 5–10 days after intranasal inoculation, serum and respiratory secretions from healthy individuals show positive findings for B19 DNA.[24,25] Despite nosocomial acquired infections have also been often recorded, patients suffering from a plastic crisis seem to be the main source of B19V infections.[26]

2.1.4 viral life cycle:

Similar to other non-enveloped DNA viruses, B19's life cycle includes the subsequent steps: binding to receptors on host cells; internalization; genome mutations within the host nucleus; RNA transcription; capsid assembly and genome packaging; and finally, cell lysis leading to the release of mature virions. [27]

2..1.4.1. Replication

B19V attachment to host cell via B19V receptors, internalization of B19V within the host cell, transfer of B19V genome to host cell nucleus, those steps are then followed by B19V DNA replication and RNA transcription, and finally assembly of capsid and packaging of genome take place. [28]. A virion adheres to receptors on the host mobile's surface. The virion enters the cell through endocytosis, is discharged from the endosome into the cytoplasm, partners with microtubules, and then moved to a nuclear pore.[29]

2.1.4.2 viral attachment:

The receptors of a host with access abilities are where a virion attaches itself. The B19 virus binds to the blood group P antigen, which functions as the host mobile, and also acts as the receptor. Virion entry into the cell occurs through endocytosis; it then travels to a nuclear pore after leaving the endosome and entering the cytoplasm. Nuclear localization indicators are found in the capsid proteins of various parvoviruses, and the parvovirus virion is small enough to pass through a nuclear pore [29].

2.1.4.3 Transcription and translation:

The viral genome encodes for two important nonstructural proteins (NS1 and NS2) and two main structural proteins (VP1 and VP2). Host RNA polymerase II aids in the transcription of the viral genome, producing structural and nonstructural viral proteins. Viral mRNA translation takes place in the host cell's cytoplasm and is crucial to the creation of new viral particles. Due to its ability to release infectious virions that could spread the sickness further, this mechanism is essential to understanding the pathophysiology of parvovirus B19 infection. Developing effective antiviral medicines requires an understanding of the complex mechanisms behind the transcription and translation of parvovirus B19. [30]

2.1.5 Epidemiology:

Parvovirus B19 (B19V) is a serious infection that affects people globally. The B19V infection epidemiology is characterized by a recurrent increase in infections that peaks every three to four years [31]. When an individual comes into direct contact to someone who is infected, the virus can spread through respiratory droplets or contact with contaminated surfaces. It is extremely infectious, particularly among young children [32]. The infection is common through school-age and younger children who attend daycare centers; in young children, the seroprevalence ranges from 5 to 10%, in young adults, it reaches 50%, and in the elderly, it reaches 90%. [32]

Moreover, there is a significant prevalence of recent infection among pregnant women. B19V has been detected in the blood of 1.5% of pregnant women; it is likely that these mothers received the virus from their older children.

Individuals who suffer from hematological disorders including thalassemia and sickle cell anemia are also at greater risk to having B19V infection.[33].

Human parvovirus B19 infections are more common in temperate areas and often arise in late winter or early spring. Small epidemics that happen frequently are also typical. [34]

2.1.6 pathogenesis:

Important pathogen parvovirus B19 (B19V) primarily affects erythroid progenitor cells (EPCs) and is responsible for a number of diseases, including fifth disease, erythema infection, polyarthropathy, and hydrops fetalis.[35,37] The virus is known to infect EPCs through the erythropoietin receptor (EpoR), which is required for B19V replication in these cells after initial virus entry.[35] Erythropoiesis is inhibited by B19V's ability to cytotoxically damage EPCs, potentially leading to severe consequences such myocarditis and hydrops fetalis, as well as anemia. The infection is pathologized in this way. [36,37] Additionally, the virus can persist in several organs, including bone marrow, and the immune system may need several months or even years to completely eradicate it. [37]

2.1.6.1. Erythema infection:

Erythema infection is also known as fifth diease or slapped cheek syndrome. It mostly affects children between the ages of 4 and 6, is the most common clinical manifestation of B19V. Early-stage symptoms, which include a mild fever, headache, and nausea, often appear 14 days after infection. Following the early stages, a recognizable rash resembling a slapped cheek develops, which takes seven to ten days to spread throughout the trunk and limbs.[34]

2.1.6.2. Arthropathy:

B19V infection may only show up as arthritis, especially in middle-aged women, since it has been detected in the synovial fluid and synovium of more than 50% of patients. [38] When developing a differential diagnosis for acute arthritis, a B19 infection must be considered since joint involvement symptoms and signs can also appear with or without rash infection. Arthropathy can resemble the signs and symptoms of rubella, manifesting as asymmetrical arthritis, especially affecting the small joints in the hands.[39] The ability of parvovirus B19 to infect and multiply inside bone marrow cells is considered to contribute to the development of joint inflammation and damage, however the exact mechanisms by which the virus causes arthropathy are not fully understood.[40]

2.1.6.3. Hydrops fetalis:

Parvovirus B19 infection is the main cause of hydrops fetalis, a disorder marked by fluid collection in several bodily cavities. The virus mostly affects the liver of the growing embryo, which may result in fetal anemia and hydrops fetalis. Ascites, pleural effusion, and cardiopathy are a few additional complications that are frequently linked to this disorder.[41] Fetal hydrops appears to be more common in cases of prenatal maternal infection; the reported rate is 4.7% if the infection develops before 25 weeks of gestation, compared to 2.3% after this gestation. [42]

2.1.6.4. Myocardaitis:

One of the cardiac diseases linked to parvovirus B19 (B19V) infection is myocarditis. Viral infections are the most common cause of myocarditis, or inflammation of the heart muscle. The public's health is being seriously threatened by this disease. It is known that B19V-induced acute and fatal myocarditis can result in heart muscle damage and even death. [43]
Numerous studies have shown that people with cardiomyopathy and dilated

cardiomyopathy (DCM) frequently have the cardiotropic virus B19V. In the cardiac arteries, the virus targets endothelial cells instead of cardiomyocytes, resulting in inflammatory responses that damage heart muscle. This condition may be the source of many symptoms, including acute and chronic heart failure. [44]

2.1.7. Immune response to parvovirusB19:

The immune response to B19 parvovirus infection is primarily responsible for the a recovery of disease, and humoral immunity plays a significant role in this response. Certain antibodies produced in reaction to the infection in addition to neutralize the virus's capacity to infect erythroid host cells as well as prevent B19 from establishing a suppressive effect on the formation of erythrocyte colonies in vitro.[46-48] Research indicates that the major capsid protein (58 kD) and minor capsid species (83 kD) of the virus are the targets of antibodies produced during the early and late stages of convalescence, respectively, in the humoral immune response to B19. Alternatively, proliferation assays and peripheral blood mononuclear cells (PBMC) from people with serologic evidence of virus exposure were unable to exhibit a cellular response to purified B19 parvovirus, it appears that cell-mediated immunity is not a major factor in the immune response to B19. [47] Despite the fact that humoral immunity is required for B19 infection recovery, recurrent infections have been reported, and B19 antibodies are extensively distributed across the world's human population. [46-48] The prevalence of antibody response rises to 15%–35% in school-age children, around 50% in adults, and 85% or more in the elderly population over 70 years old, despite the fact that only 10% of children under the age of five have circulating antibodies to B19. [49] Arthralgia and a small maculopapular skin rash appear at the time of B19 IgG detection; these symptoms may be carried on by immune complex deposition, which has been related to the generation of certain neutralizing antibodies. [50] Precisely 50% of the pregnant women with IgM positivity who were exposed to B19 infection cases reported experiencing rash or arthropathy. [51] 8 % of infected children and up to 80% of infected adults-the majority of whom are women-have been reported having joint symptoms linked to a B19 infection. It is possible that individuals who had B19-induced arthropathy had an abnormal immune response to B19 antigens. [49]

2.1.9.laboratory diagnosis:

Parvovirus B19 may lead to a wide range of scientific symptoms, depending on the degree of infection and the host's immune condition. The diagnosis procedure is special for the exclusive scientific symptoms. Since the virus cannot yet be grown in standard diagnostic virology laboratories, the diagnosis is achieved only on the basis of parvovirus B19-specific antibodies found by serologic tests and nucleic acid amplification testing. [52]

2.1.9.1 serology:

The first and most effective diagnostic test that has to be carried out as soon as a B19V infection is detected during pregnancy is a serologic analysis of the mother's blood. Serological tests are considered a reliable diagnostic tool for detecting B19V infection. These tests involve the detection of anti-B19V (IgM-IgG) antibodies using techniques such as ELISA. [53] Anti-B19V-IgM antibodies are detected in the plasma of B19V patients and in the bloodstream of B19V-infected individuals 7-10 days after the infection initially appeared, even 6 months after the infection has cleared up.[54].

2.1.9.2. Detection of B19 viral DNA:

Nucleic acid amplification is the most sensitive method for detecting B19V DNA in a sample. Most of the reported PCR tests are sensitive to viral DNA at concentrations of 1 to 100 copies/mL, making them especially useful for detecting the virus in fetuses, immunocompromised or immunosuppressed adults, and individuals with insufficient antibody-mediated immune response. In these cases, serological testing for B19V is unreliable. [55,56] While PCR has a sensitivity of almost 100%, B19V-specific IgM detection in fetal blood has a sensitivity of just 29%. However, because low B19V DNA levels might last for years after acute infection, low-positive B19V PCR results do not always indicate recent infection. [55]

2.1.10. Treatment:

Although there is presently no antiviral medicine available to treat B19V infection, pooled immunoglobulin G is an effective therapy for recurrent infection (27). The majority of infection cases are reported to fully resolve. Pooled intravenous immunoglobulin G (IVIG) has been shown to successfully treat B19V persistent infections in 66% of immunocompetent patients. [57]

Intrauterine erythrocyte transfusion (IUT) is a crucial technique for controlling B19V infection since it can greatly lower death rates and treat fetal anemia. The chance of fetal mortality can be decreased by promptly performing IUT on fetuses with severe hydrops.[58]

2.1.11. Vaccine: Effective vaccinations are available against animal parvoviruses, and parvovirus B19 infection is most likely avoidable as well. Normal individuals responded with neutralizing antibodies to a single dosing of 2.5 μg of empty capsids. Since it lacks DNA, the recombinant immunogen-which is being developed as a vaccine for the human virus-is not virulent. It has been possible to overexpress the very immunogenic VP1 in empty capsids. [1] Like many previous vaccine developments, the development of a parvovirus B19 vaccine has been limited more by commercial concerns than by safety or efficacy issues. In certain immunocompromised persons, pure red-cell aplasia, and transient aplastic crises in sickle cell disease or other hemolytic anemia patients might all be avoided if seronegative mothers had this vaccination early in pregnancy. [1]

2.2. Pregnancy:

The process of becoming pregnant is complicated and causes a woman's body to undergo major physiological changes. Pregnant women may become more susceptible to infectious infections as a result of these changes. Pregnancy problems are an extreme risk, around 15% of pregnancies end poorly, including stillbirth and spontaneous pregnancy loss. [59]

2.2.1. viral infection during pregnancy:

Pregnant women are particularly at risk of viral infection due to physiological and hormonal changes that occur during pregnancy, which in turn cause immunological changes that occur during the pregnancy period. [34] In considering the serious health risks associated with such crises, viral infection during pregnancy has always been regarded as a global health concern. [27] Pregnancy causes major immune system changes that may alter a person's susceptibility to viral infections. One such alteration that might support viral infection is the suppression of immunity mediated by T cells. The inhibition of this process is believed to be mediated by variations in hormone levels, such as those of progesterone and estrogen. These changes in hormone levels might affect immune system regulation, which could lead to increased virus replication and worsening of disease outcomes. [60]

2.3 Previous studies:

In study done by Ayman in year 2016, the study included 364 pregnant women, mean of age 27. Result show that parvovirus was positive in 50% of women. igG was 50% positive. [62]

In a second study done by Ghazi in 2007, 1200 blood samples were examined for parvovirus B19 antibodies using the enzyme-linked immunosorbent assay (ELISA) on pregnant Saudi women in Makkah. IgG antibodies against parvovirus B19 were discovered in 46.6% of the various age groups, whereas IgM antibodies were reported in 2.25%. [64]

An investigation by Adam and collegeas, in 2014, found the B19V seroprevalence in Sudanese pregnant women. ELIZA was used to screen 500 pregnant women for B19V IgG and IgM antibodies. There was one positive IgM test, and the study revealed that B19V had a 61·4% IgG seroprevalence. [61]

In 2009 Elnifro and other in Tripoli, Libya found that out of 150 pregnant women, 61% of pregnant women were positive for anti-B19V-IgG antibodies and 5% were positive for anti-B19V-IgM Of women under study. [65]

CHAPTER III

MATERIALS AND METHODS

3.1 Study design:

This is a descriptive cross –sectional and hospital-based study

3.2 Study area and duration:

The study was conducted in reference laboratory in King Saud Medical City (KSMC), Riyadh, Saudi Arabia. during the period from October 2022 to May 2023.

3.3 Study population:

The study subjects included in this study were pregnant women who attended the reference laboratory in KSMC.

3.4 Selection criteria:

3.4.1 Inclusion criteria:

The study included women who were pregnant with different age provided

3.4.2 Exclusion criteria:

The exclusion criteria were non pregnant women.

3.5 Ethical consideration

Ethical approval for this study was obtained from Institutional Research Board committee at King Saud Medical City. (Appendix 5)

3.6 Data collection

A structured data collection sheet was designed to collect information regarding sociodemographics and risk related data. Data includes: age, hematological diseases, abortion, and history of blood transfusion.

3.7 Sample size: A total of 86 pregnant women serum sample (n= 86) were collected in duration from October 2022 to march 2023.

3.8 Method

3.8.1 Collection of specimens

Three-ml blood sample was collected from 86 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) (appendix 1), and the serum (the supernatant) was transferred and stored at -20° C until required for use (appendix 2).

3.8.2 Enzyme linked immunosorbent assay (ELISA)

3.8.2.1 Principle

The ELISA test kit(Vircell) provides a semiquantitative in vitro assay for human antibodies of the immunoglobulin class IgM and igG 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 IgG) 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 29 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 oC ± 1 oC 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 min at room temperature (+18 oC to + 25 oC).

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 oC to + 25 oC. 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 Vircell. appendix 6 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 30

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.

A negative serological result does not exclude an infection. (appendix3) 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:

4.1. Demographic Results

The study categorized 86 pregnant participants into three age groups: 17-28 years, 29-35 years, and 36-44 years. The distribution was 33.7% (n = 29) in the 17–28-year group, 38.4% (n = 33) in the 29–35-year group, and 27.9% (n = 24) in the 36–44-year group. (Figure 4.1) Regarding the history of miscarriage, 43.0% (n = 37) of the participants reported having had a miscarriage (figure 4.2), while 57.0% (n = 49) did not. In terms of blood transfusion history, 33.7% (n = 29) had received a transfusion, and 66.3% (n = 57) had not (figure 4.3). Over half of the participants (54.7%, n = 47) reported having a hematological disease, with iron deficiency being the most common condition (78.7%, n = 37), followed by sickle cell anemia (6.4%, n = 3) and microcytic hypochromic anemia (6.4%, n = 3) (figure 4.4). Participants were also classified based on gravidity, with 15.1% (n = 13) being primigravida and 84.9% (n = 73) being multigravida. (Figure 4.5)

4.2. Parvovirus Results:

The presence of IgM antibodies, indicating recent parvovirus B19 infection, was found to be very low among the participants, with only 2.3% (n = 2) testing positive (table 1). The analysis revealed no significant association between IgM positivity and age group (p = .669), history of miscarriage (p = .214) (table 2), history of blood transfusion (p = .307) (table 3), hematological disease (p = .116) (table 4), or gravidity (p = .163) (table 5). IgG antibodies, indicating past infection or immunity, were present in 45.3% (n = 39) of participants. Similar to IgM, no significant associations were found between IgG positivity and age group (p = .849) (table 2), history of miscarriage (p = .224) (table), history of blood transfusion (p = .598) (table 4), hematological disease (p = .568) (table 5), or gravidity (p = .950) (table 6).

These findings suggest that while past exposure to parvovirus B19 is relatively common among pregnant women, recent infections are rare, and the examined demographic and medical history factors do not significantly influence the presence of parvovirus antibodies

Figure 4.1 Frequency of age group among pregnant women

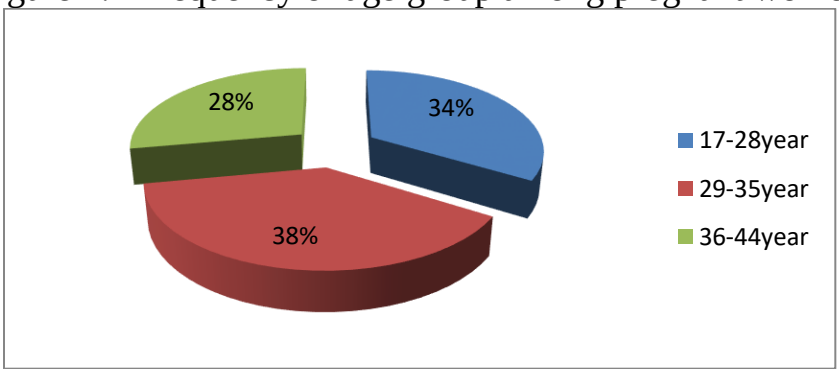

Figure 4.2 Frequency of miscarriage among pregnant women

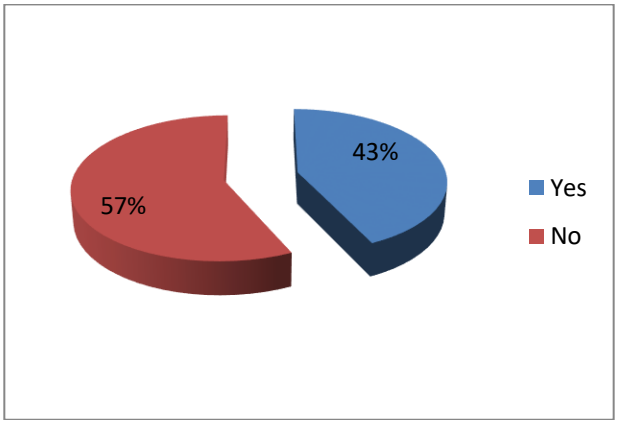

Figure 4.3 Frequency of blood transfusion history among pregnant women

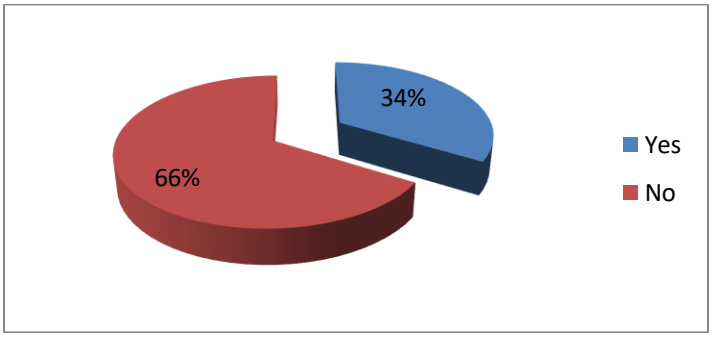

Figure 4.4 Frequency of hematological diseases among pregnant women

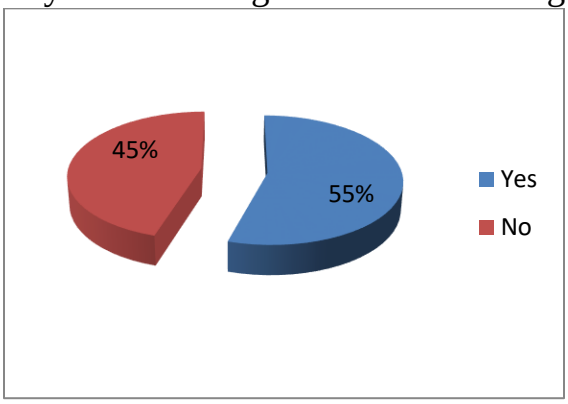

Figure 4.5. Frequency of gravidity among pregnant women

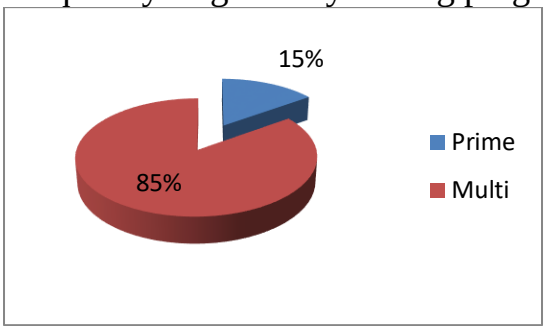

Figure 4.6 Distribution of study population according to type of hematological disease

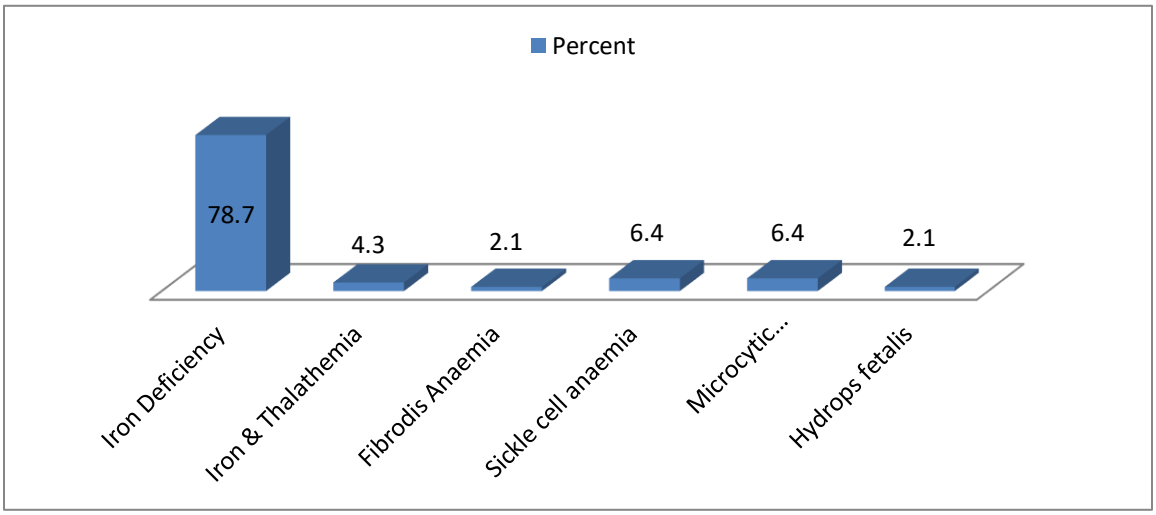

Table 1 Distribution of study population according to IgG and IgM results

igM			igG		
Result	**Frequency**	**Percent**	**Result**	**Frequency**	**Percent**
Positive	2	2.3%	**Positive**	39%	45.3
Negative	84	97.7%	**Negative**	47%	54.7
Total	86	100%	**Total**	86%	100

Table 2. Association between \age groups and B19V IgM & IgG antibodies among pregnant women

Age	**IgM**		**Total**	**P value**	**IgG**		**Total**	**P value**
	Positive	Negative			Positive	Negative		
17-28	1(3.4%)	28(96.5%)	29	0.669	13(44.8%)	16(55.1%)	29	0.849
29-35	1(3%)	32(97%)	33		14(42.4%)	19(57.5%)	33	
36-44	0	24(100%)	24		12(50%)	12(50%)	24	
Total	2(2.3%)	84(98%)			39	47		

Table 3 Association between history of miscarriage and B19V IgM & IgG antibodies among pregnant women

IgM			Total	P value	IgG		Total	P value
History of miscarriage	Positive	Negative			Positive	Negative		
yes	0	37	37	0.214	14(37.8%)	23(62%)	37	0.224
No	2(4%)	47(96%)	49		25(51%)	24(49%)	49	
Total	2	84			39	47		

Table 4 Association between history of blood transfusion and B19V IgM & IgG antibodies among pregnant women

History of blood transfusion	IgM		Total	P value	IgG		Total	P value
	Positive	Negative			Positive	Negative		
YES	0	29	29	0.307	12(41%)	17(58.6%)	29	0.598
NO	2(4%)	55(96%)	57		27(47%)	30(52%)	57	
	2	84			39	47		

Table 5 Association between hematological status and B19V IgM & IgG antibodies among pregnant women

History of Hematological diseases	IgM		Total	P value	IgG		Total	P value
	Positive	Negative			Positive	Negative		
Yes	0	47	47	0.116	20(51%)	19(49%)	39	0.568
No	2(5%)	37(94.8)	39		27(57%)	20(42%)	47	
Total	2	84			47	39		

Table .6 Association between hematological status and B19V IgM & IgG antibodies among pregnant women

Gravidity	IgM		Total	P value	IgG		Total	P value
	Positive	Negative			Positive	Negative		
Prime	1(7%)	12(93%)	13	0.163	6(46%)	7(54%)	13	0.950
Multi	1(1.3%)	72(98.6%)	73		33(45%)	40(54%)	73	
Total	2	84			39	47		

CHAPTER V

DISSCUSSION, CONCLUSIONS AND RECOMMENDATION

5.1. Discussion

The results of this study show that among 86 pregnant women, the prevalence of IgM antibodies, indicating recent parvovirus B19 infection, was 2.3%, and the prevalence of IgG antibodies, indicating past infection or immunity, was 45.3%. These findings can be compared with several previous studies conducted in different regions and on larger cohorts.

In a study conducted by Ayman in 2016, which included 364 pregnant women with a mean age of 27 years, 50% of the women tested positive for parvovirus B19 IgG antibodies, indicating a past infection rate of 50%. This is slightly higher than the 45.3% IgG positivity found in our study. However, the IgM positivity rate in Ayman's study was not specified, so a direct comparison of recent infection rates is not possible.

Elnifro's 2009 study in Tripoli, Libya, examined 150 pregnant women and found that 61% were positive for anti-B19V IgG antibodies, and 5% were positive for anti-B19V IgM antibodies. The IgG seroprevalence in Elnifro's study is significantly higher than our finding of 45.3%. The IgM positivity rate of 5% in Elnifro's study is also higher than our 2.3% IgM positivity, suggesting a higher recent infection rate in the Libyan cohort.

In Makkah, Ghazi's 2007 study used ELISA to analyze 1200 blood samples from Saudi pregnant women. showed that seroprevalence increases with age, indicating a larger possibility of previous parvovirus B19 infection in older women. This contrasted our results, which showed no significant correlation with age groups (p =.669).

Adam and colleagues' 2014 investigation in Sudan screened 500 pregnant women for parvovirus B19 antibodies using ELISA. Parvovirus B19 seropositivity was low amongst the pregnant women that had abortion, and there was no significant association between history of miscarriage and seropositivity for IgM antibodies(P value 0.834),this is in agreement with present study which found p value 0.214.

Samuel E Emiasegen study found A history of blood transfusion (in the last 5 years) and occupation were both significant determinants of recent cases of B19 infection in this population were positive23% (p value 0.05). Pregnant women that have been transfused with blood in the last five years demonstrated a significantly higher rate of IgM antibodies. Its not consistent with our study which reported no association between history of blood transfusion and seropositivity of anti parvovirus (p value=0.307).

Furthermore, Adam's study, reported a significant association between the number of gestations and seroprevalence with multigravida shows higher rates of IgG positivity (64.3%) compared to primigravidae (54.3%). This is contrast to present study which found no correlation between gravidity and the presence of IgM (p = .163) or IgG antibodies (p = .950).

Buraa et al recorded a considerable association between maternal anemia and B19V IgM positivity, with a p-value of less than 0.001. This result proposes that maternal anemia may raise the risk of infection with parvovirus B19. In addition, Aldawmy et al. reported a directly proportional relationship between anemia and parvovirus B19 infection. Their study demonstrated that pregnant women with anemia had a higher incidence of B19V infections. In contrast, my research did not reveal a significant association between parvovirus B19 seropositivity and hematological disease, with a p-value of 0.568.

These comparisons indicate that the seroprevalence of parvovirus B19 among pregnant women varies across different regions and studies but generally falls within a comparable range for both IgG and IgM antibodies. The differences observed could be due to variations in sample size, geographical location, population characteristics, and methodologies used in the studies.

5.2. Conclusion:

Parvovirus B19 infection is a major global concern, especially in pregnant women. B19V virus can transmit vertically in up to one-third severely ill expectant patients, leading to 56% additional fetal mortality after maternal infection during the first 20 weeks of pregnancy. The study concluded that Parvovirus B19 IgG antibodies was detected in almost half of the group, while IgM was detected only in 2.3% of participants. There was no association between Parvovirus B19 infection and risk factors (age, miscarriage, history of blood transfusion, hematological disease and gravidity). For pregnant women, we recommend parvovirus B19 detection to be a routine investigative procedure such as TORCH screen including parvovirus B19.

5.3. .Recommendations:

Further studies including larger sample size should be used to accurately establish the rate of B19V infection.

For pregnant women, parvovirus B19 detection need to be a routine investigative procedure such as TORCH screen including parvovirus B19.

It should be standard procedure for pregnant women to have their parvovirus B19 status checked. All women of reproductive age should undergo routine screening for B19 IgM and IgG antibodies. Positive patients should then be treated clinically

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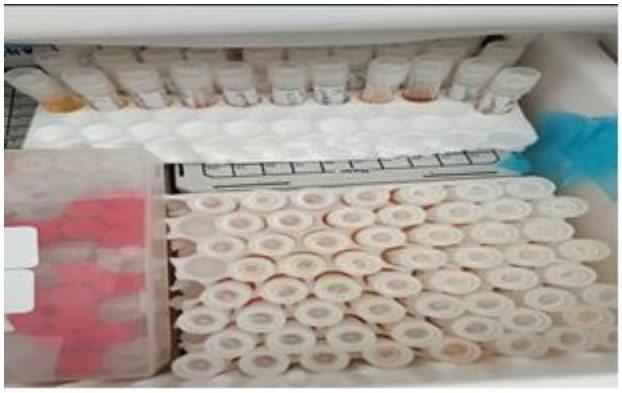
Appendix

Appendix 1

Appendix 2



Appendix 3

Siemens Healthcare Diagnostics Products GmbH
Postfach 11 49
D-35001 Marburg

12/7/23 10:08:36

Plate report: Raw values

PlateID:	0031	Strips:	1 - 12
BES Version:	V5.1.1	Validation Formula:	
Analyzer:	322255	Unit:	OD
Test Name:	B19.IgG	Validation ranges	
Cut Off:	0.642	POS	0.900 - 9.999
Cut Off Formula:	Mw(COF)	COF	0.550 - 1.500
Repetition Range:	0.000 - 0.000	NEG	0.000 - 0.500
Blank correction:	-		
Meas. correction:	-		
Wavelengths M/R:	450 nm / 620 nm		
Combipack no.:	-		
Plate defined at:	10/7/23 14:04		

!	Well suspicious	*	Result negative
#	Result calculation not possible		Result positive
∞	Result suspective	?	Result inside "retest" range

Pos	SampleID	Value	Flag	Pos	SampleID	Value	Flag	Pos	SampleID	Value	Flag
										1.686	*

Appendix 4

Appendix 5

Kingdom of Saudi Arabia
Ministry of Health
King Saud Medical City

مدينة الملك سعود الطبية
KING SAUD MEDICAL CITY

المـملكة العـربية السـعوديـة
وزارة الـصحة
مدينة الملك سـعود الطبية

IRB Registration Number with KACST, KSA: **H-01-R-053**
IRB Registration Number U.S. Department of HHS IORG #: **IORG0010374**

- Memorandum -

Date: February 5, 2023

Proposal Reference No.	: **H1RE-31-Jan23-02**
Proposal Title	: "Seroprevalence of Parvovirus B19 among pregnant women in Riyadh Saudi Arabia"
PI	: Ms. Neda Mohamed Ibrahim Sayed
Co-Investigators	: Dr. Fadhel H. Hababi; Hadia Abbas Eltaib Ahmed
Type of Review	: Modification
Category of Approval	: Exempt
Date of IRB Approval-Expiration (Validity)	: 05/02/2023 04/02/2024 (12 months)

Dear Ms. Neda Mohamed Ibrahim Sayed:

We are pleased to inform you that the above-referenced research proposal has been reviewed and was approved. The Institutional Review Board (IRB) committee found that the research met the applicability criteria and was eligible for exempt review. However, to commence the collection of data a permission letter must be issued from the Director of the Research Center first.
This approval is valid for **12 months** from the date of IRB review when approval is granted. The approval will no longer be in effect on the date listed above as the IRB expiration date. Please note that you are obligated to submit the following to IRB committee:

1. progress/final report on the **12 months (04-Feb-2024)** (or earlier in the case the study has completed)
2. any manuscript resulting from this research for approval by IRB before submission to journals for publication.

The approval of the conduct of this proposal will be automatically suspended after 12 months, in the case the Progress Report (or Final Report, if relevant) is pending acceptance. You also need to notify the Research Centre as soon as possible in case of:

1. any amendments to the proposal;
2. termination of the study;
3. any serious or unexpected adverse events;
4. any event or new information that may affect the benefit/risk ratio of the proposal.

All records relating to the research including consent form must be retained and available for audit for at least 3 years after the research has ended.

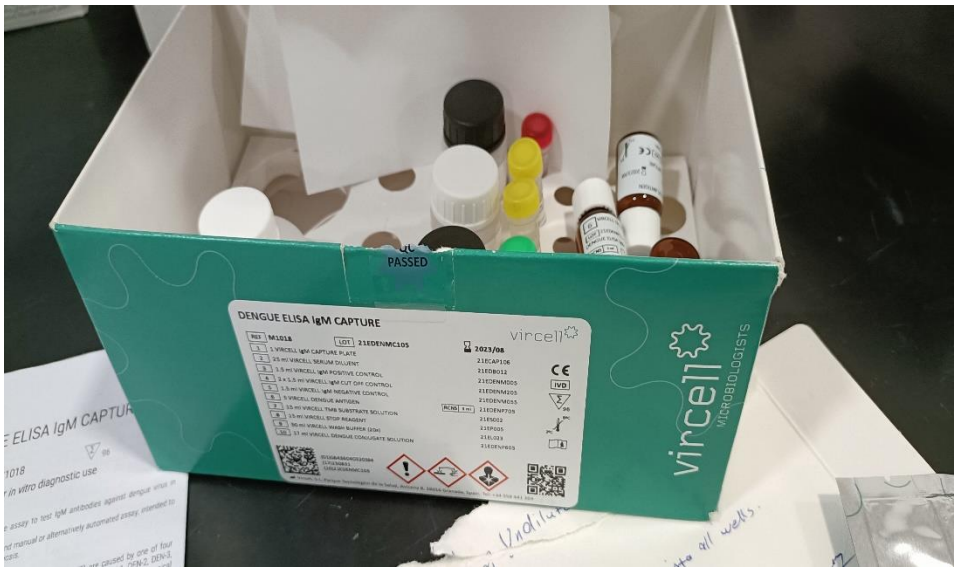
We wish you every success in your research endeavors.

Dr. Faisal Almazroua
Chairman, Institutional Review Board (IRB)
King Saud Medical City Riyadh, KSA

وزارة الصحة المملكة العربية السعودية
مدينة الملك سعود الطبية
King Saud Medical City
لجنة أخلاقيات البحوث (IRB)

☎ +966118371777 Ext. 78733-78734 irb@ksmc.med.sa

Appendix 6



Demographic data sheet :

No	age	History of miscarriage	History of blood trtansfusion	Hematological disease	gravidity