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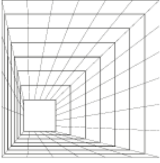

Detection of Human Parvovirus 19 in People with Beta Thalassemia Major (the City of Diwaninah)

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Abstract

study samples were collected at Diwaniyah Teaching Hospital / Hematology Center in Diwaniyah city for the period from 1 November 2023 to 1 February 2024. This study aimed to detect the spread of the Parvovirus B19 virus among patients with Thalassemia beta major type.

The study included 85 samples from both sexes and different age groups. The samples were divided into two groups: the first included patients with Thalassemia beta major type, and included 72 samples, and the second included the control group (healthy people) and included 13 samples. Blood samples were collected for the study groups of patients visiting the Hematology Center, and the linear polymerase chain reaction technique was used to detect viral DNA,

The results of the study showed that the rate of infection with the B19 virus in patients with Thalassemia beta major type was 52.8%, while the control group did not record any infection, with a significant difference of 0.002. Compared with the control group.

The results of the study also showed that the virus spread more among males than females, at a rate of 56.4% and 48.5%, respectively, with a significant difference of = 0.005.

The results of the study indicated that the virus was more prevalent among ages 16-20 years, at a rate of 75%, and less widespread among ages 21-25 years, at a rate of 33.3%, with a significant difference of P.v=0.014.

The results of the study showed that the spread of the virus was greater in urban areas than in rural areas, at a rate of 71.4% (48.3%).

Respectively, with a significant difference of 0.002=v. The results of the study indicated that the virus spread at a high rate among patients who received blood every 20 days, followed by those receiving blood every 30 days, and those receiving every 40 days, at a rate of 55.6%, 52.1%, and 50%, respectively, with a difference of pv.006

الخلاصة

جمعت عينات الدراسة في مستشفى الديوانية التعليمي مركز أمراض الدم في محافظة الديوانية للفترة من 1 تشرين الثاني لعام 2023 إلى 1 شباط لعام 2024 هدفت هذه الدراسة إلى الكشف عن انتشار الفيروس Parvovirus B19 لدى مرضى فقر دم البحر الأبيض المتوسط نوع بيتا الكبرى.

شملت الدراسة 85 عينة من كلا الجنسين وبمختلف الفئات العمرية, قسمت العينات على مجموعتين: الأولى تضمنت مرضى فقر دم البحر الأبيض المتوسط نوع بيتا الكبرى وشملت 72 عينة والثانية مجموعة السيطرة (الاصحاء) وشملت 13 عينة. جمعت عينات الدم للمجاميع الدراسية للمرضى المراجعين لمركز أمراض الدم واستخدمت تقنية تفاعل أنزيم البلمرة المتسلسل الخطي في الكشف عن DNA الفيروس.

أظهرت نتائج الدراسة أن نسبة الإصابة بالفيروس B19 لدى مرضى فقر دم البحر الأبيض المتوسط نوع بيتا الكبرى 52.8% بينما لم تسجل مجموعة السيطرة أي أصابة مع وجود فرقاً معنوياً 0.002 . مقارنة مع مجموعة السيطرة.

كما بينت نتائج الدراسة انتشار الفيروس في الذكور أكثر من الإناث وبمعدل 56.4% 48.5% على التوالي مع وجود فرقاً معنوياً 0.005 .

أشارت نتائج الدراسة انتشار الفيروس أكثر لدى الأعمار 16-20 سنة وبنسبة 75% وأقل انتشاراً لدى الأعمار 21-25 سنة وبنسبة 33.3% مع وجود فرقاً معنوياً 0.014- .P.v

أظهرت نتائج الدراسة انتشار الفيروس في الحضر أكثر من الريف وبنسبة 71.4% 48.3% على التوالي مع وجود فرقاً معنوياً 0.002-v. .

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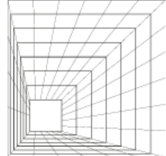

أشارت نتائج الدراسة انتشار الفيروس بنسبة عالية لدى المرضى الذين يتلقون الدم كل 20 يوماً. يليها المتلقون كل 30 يوماً،
والمتلقون كل 40 يوماً وبنسبة 55.6% 52.1%,50% على التوالي مع وجود فرقاً v0.006. .

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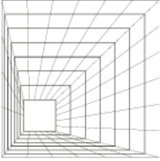

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List of abbreviations

Abbreviation	Word
DN A	Deoxy-ribonucleic acid
Hb H	Hemoglobin
HRP	Horseradish peroxidase
MAF	Macrophage-activiting factor
mRNA	Messenger Ribonucleic acid
B19V	Parvovirus B19
RT-PCR	Real Time Polymerase chain reaction.

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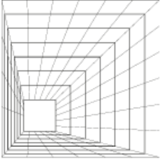
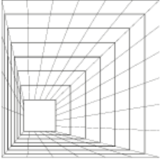


TMB	Tetramethyle -Benzidine
VP1	Viral protein 1
VP2	Viral protein 2



Chapter one
Introduction
1.1 Human parvovirus B19

The B19 virus was discovered in 1974 by the scientist Cossart by searching for the hepatitis B virus in one of the human blood serum samples. The virus was named *(Cossart et al., 1975)* after the number of the sample in which the B19 virus was discovered.

Subsequent electron microscopy studies revealed small animal-sized viral particles with a diameter of 23 nanometers similar to particles of animal parvoviruses. Its relationship to human diseases was determined in 1981 and it was classified as a member of the Parvoviridae family in 1985. It is the only virus within this family that is pathogenic to humans. It is a small virus with a diameter of It is about 22-25 nanometers of DNA type. The virus is single-stranded, linear, 5596 nucleotides long, and is polymorphic (icosahedral). It is located within...

(*Heegaard and Brown, 2002)* Family Parvoviridae

The virus is transmitted in several ways, including respiratory secretions, blood transfusions, and from mother to mother

(*Siritantikorn et al., 2007*) Transplacental fetus.

Epidemiological studies have shown that the spread of infection with the B19 virus varies among countries and societies around the world, and infection is common among different ages, especially children.

(Kelly et al., 2000)

Virus B19 causes many human diseases, including aplasia of red blood cells, Hydrops, Filth disease, Fifth disease, Arthropathy, Myocarditis, Thrombocytopenia, Thrombocytopenia, Fetalis, Virus B19 is associated with neurological diseases and autoimmune diseases, with symptoms similar to those of arthritis, in addition to its relationship. With immune disorders such as chronic fatigue syndrome

(*Adamson-Small et al., 2014*) Fatigue Syndrome

Thalassemia is a hereditary disease that is transmitted through recessive genes from one generation to another. It results from the cessation of the production of one or more globin chains, which leads to a decrease in the production of hemoglobin. The patient suffers from widespread destruction of red blood cells. The severity of the infection varies from one person to another. It ranges from minor and unnoticeable to severe that may lead to death. Patients with Mediterranean anemia require continuous blood transfusions and are therefore vulnerable to infected with the virus (*Surapon, 2011)*

Due to the lack of a study of the B19 virus at the level of Al-Qadisiyah city, this study was designed to aim to :

1.2 The aim of the study

Determine the extent of the spread of parvovirus B19 using polymerase chain reaction technology Linear RT-PCR in patients with Thalassemia, beta major type.

Chapter 2
Literature Review
2-1- Human parvovirus B19V

Human parvovirus 19 Parvovirus B19 is a small, non-enveloped, single-linear, polymorphic DNA virus that belongs to (Family Parvoviridae) Genus Erythrovirsus

The virus replicates with DNA through high-molecular-weight structures linked in a hairpin-like structure due to genomic symmetry (*Ozawa et al., 1986*). Infection with the virus is common and widespread, and the infection causes a wide range of clinical manifestations depending on the degree of anemia and the immune status of patients. Infection with the virus is asymptomatic, even in individuals with weak immunity, and may lead to severe infection and arthropathy.(*Gallinella (2013*)

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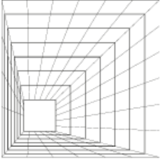

Infection with the B19 virus causes aplasia of red blood cells with a skin rash and joint pain, and the infection leads to a sharp decrease in hemoglobin and anemia, which can threaten Life (*Heegaard and Brown2002*)

Patients with blood disorders are at risk of infection with thشe virus, especially patients with severe anemia and chronic hemolytic anemia, such as Mediterranean anemia, especially patients with the beta-major type. They usually receive frequent blood transfusions and are therefore at high risk.

The risk of infection with the virus is in children with congenital clotting defects and patients with hereditary hemorrhage. The virus has the ability to destroy the progenitor cells of red blood cells (Erythroid progenitor).(*Heegaard and Brown, 2002)* Erythropoiesis inside the bone marrow and causes cells Transmission of infection with the B19 virus occurs by inhaling the virus through droplets as well as from Mother to fetus through the placenta, and through blood transfusion and blood products, bone marrow transplantation, and sold organ transplantation (*Egbuna et al., 2006)*

2.2 Virus classification B19

according to the International Commission for Classification of Viruses, includes small, non-enveloped, single-stranded DNA viruses that infect a wide range of animals. Parvovirinae subfamily, and includes three genera: the first is Dependovirus, which needs helper viruses for replication, and the second is: Parvovirus, and either The third type: Erythrovirus, which is classified as B19V, which self-replicates inside the progenitor cells of red blood cells.

Parvoviridae

Densovirinae
Infects invertebrates

Parvovirinae
Affects vertebrates

Parvovirus
1_Mice minute virus
2-Canine parvovirus
3_Aleutian parvovirus

Dependovirus

Adeno associated virus

Erythrovirus
Human Parvovirus B19
Simian Parvovirus

Figure 2-1- Virus classification. (*King et al., 2011)*

2-3 Structure of the B19 virus

2-3-1 The viral genome

The genome length of the Covid-19 virus is 5596 nucleotides, and a large area of it encodes non-structural proteins and Capsi type (VP2) proteins (1), as in Figure (2-2).

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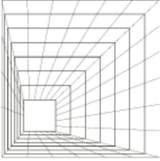
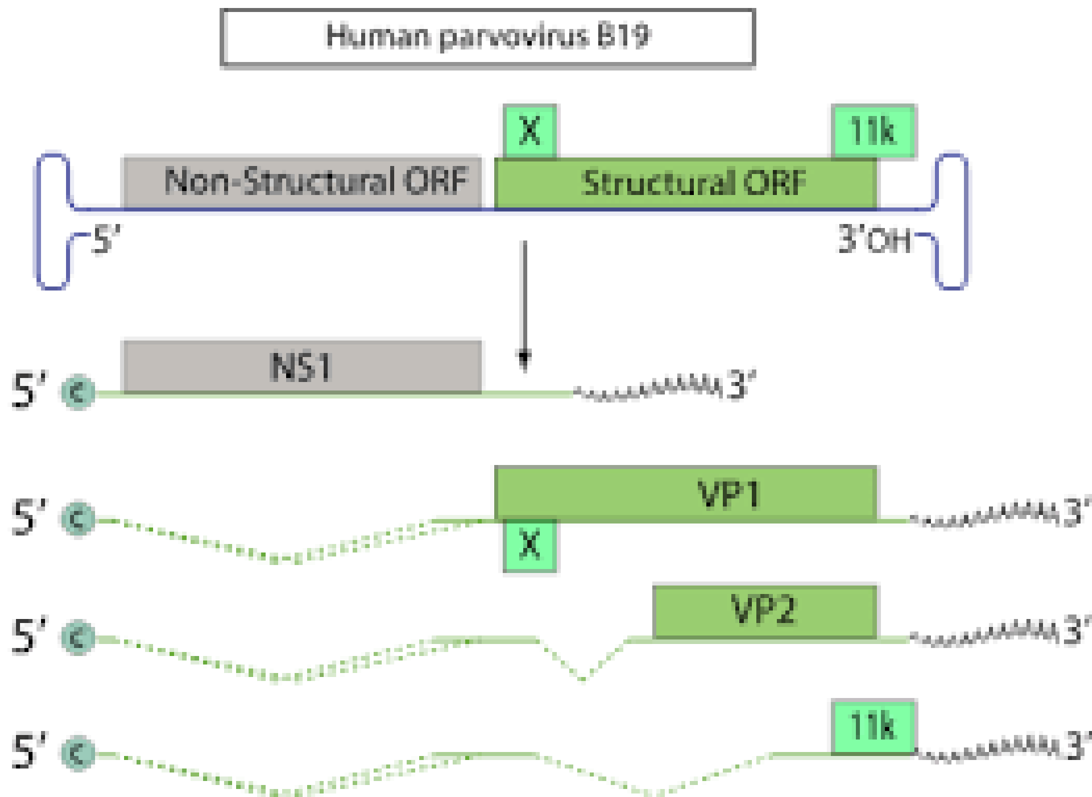


The VPI protein contains a unique region referred to as VP10 and also encodes two types of...The first small proteins are a protein with a molecular weight of 11 kDa and the second is a protein with a molecular weight of 7.5 kilodalton, whose function is not fully known (*Heegaard and Brown 2002*) Gene expression is controlled by a single initiator P6 and is located on the genetic map in the module The sixth part of the viral genome.

There are three genotypes that differ depending on the strain and can be seen in the Capsid sequence, namely genotypes 2.1 and 3. The first type is the strain that controls the efficiency of the genome. The right end of the genetic material encoding the mRNA is highly encoded in order to allow the formation of the capsid, while the left end of The genetic material encodes mRNA for structural proteins .(*Young and Brown, 2004)*

The short, overlapping open reading frame region encodes the X protein, which is located in the V1 gene, and has been described recently

Functions of the X protein in the B19 virus infection process It is not known because of the difficulty of developing the virus and the infection process carried out by the virus genome will It causes damage to the host cell's DNA and stops vital activities (*Chen and Qiu, 2010*)

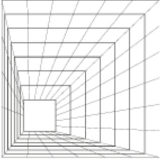


(Zhi et al., 2004) B19 Figure 2-2 Virus genome

2.4.1The life cycle of the B19 virus

The virus replication process occurs in the nuclei of infected cells and includes stages common to most.Types of DNA viruses - docking of the virus with host cell receptors; 2 - Overlap; 3- Genome transport into the nucleus - 4 - DNA replication 5 - mRNA cloning 6 - Capsid assembly: 7 - Genome packaging; 8 The transfer of ferons to other cells, either by destroying cells or Exit without her death as in the figure (2_3) (*Heegaard and Brown 2002)*

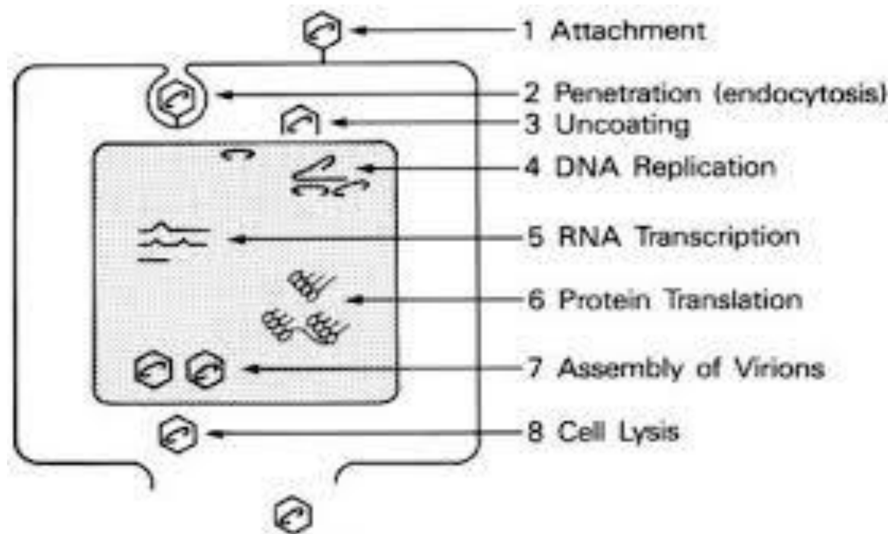
B19 replication is a complex process despite the small size of the genome due to the fact that the DNA is single-stranded and linear. B19V disrupts the host cell's manufacturing machinery for its own replication purposes using the strand replication machinery. As a result, the virus replicates with DNA through high-molecular-weight structures that attach through the terminal spikes of the viral DNA.



The third terminal end of each strand acts as an initiator for the process of initiating DNA synthesis and replication mRNA (*Ozawa et al., 1986)*

The virus has exceptional variations in the progenitor cells of red blood cells, fetal liver cells, and the umbilical cord. The factors responsible for this characteristic include the possession by red blood cells of the group 4-Gb globoside antigen, which is the main receptor for the virus. Some individuals who genetically lack the P antigen have a natural resistance to infection with the virus. It is believed that in the normal course of viral pathogenesis, red blood cells act as effective distributors of the B19 virus in the body (*Bönsch et al., 2008)*

Figure 2-3 The life cycle of the B19 virus in red blood cell progenitor cells (*Heegaard and Brown, 2002)*



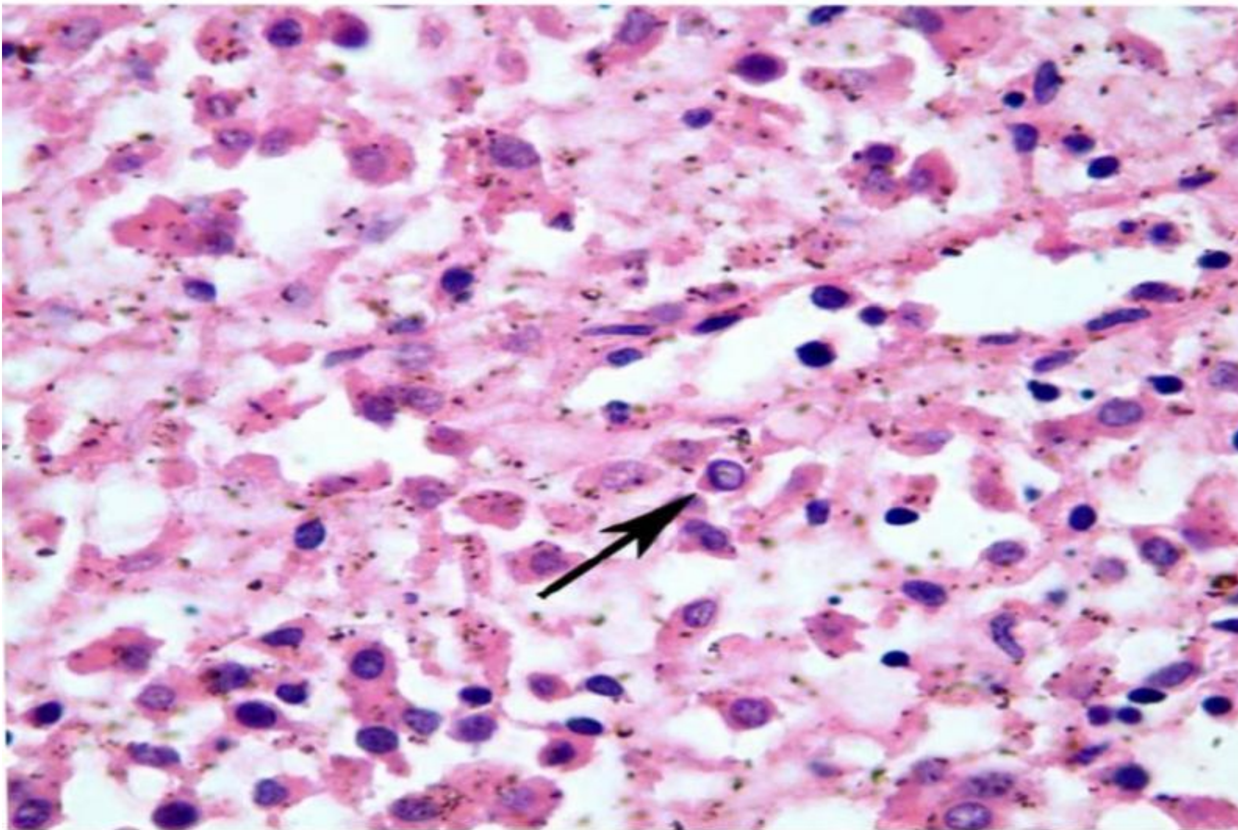


Figure 2-4 shows the shape of a red blood cell infected with the virus (*Bathla et al., 2014)*

2.4.2 Pathogenesis

Infection with the virus without symptoms is common, accounting for 25-32% of infections, with higher rates among individuals with dark skin, as it is difficult to see the rash, especially on the skin.(*Woolf et al., 1989)*

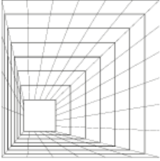
The virus causes many diseases, including the fifth disease (Erythema infection (El), also known as slapped border disease, which is the main manifestation of infection with the B19 virus. The appearance was first described by the scientist Robert Whelan in 1799, and in 1905 it was classified as the fifth disease that affects children, and its symptoms include a cold and a headache. , high body temperature and nausea, and after a period of 2-5 days, a skin rash appears on the cheek and paleness in the first stage of infection, and a skin rash often appears on the trunk. The extremities are in the second stage of infection. In adults, skin symptoms rarely appear ((*Foti et al., 2006*)

Arthritis with symmetrically distributed pain, swelling, and stiffness in adults, especially in women, is a common complication of B19V. Symptoms usually last 1-3 weeks, but in 20% of affected women, the arthropathy persists for a longer period (*Moore, 2000).*

The result of infection with the virus B19 is Transient Plastic Crisis (TAC) in patients with blood disorders such as white-blooded anemia Intermediate thalassemia and hereditary hemolytic anemia

A study in the United States showed that 68% of TAC is associated with infection with the B19 virus in sickle cell anemia patients, including hypoplasia of red blood cells in the bone marrow, shortness of breath, fatigue, confusion, and congestive heart failure, and in some extreme cases, necrosis of the bone marrow and other blood components occurs and some Diseases affected by viral infection to varying degrees, such as Hemophagocytic lymphohistocytosis (*Yates et al., 2009*).

In addition, infection with the virus also causes pure red cell aplasia (aplasia (RCA) in patients with congenital immunodeficiency syndromes such as Nezelof and variant immunodeficiency syndrome) (*Gahr et al., 1991).*



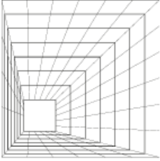
Immunocompromised patients are particularly at risk for complications associated with infection with the virus, and immunosuppressed patients often develop an immune response.
Delayed IgG antibodies and rarely develop immunological symptoms such as rash and Arthropathy. (*Heinz et al.2005*)
The virus infects pregnant women and is transmitted through the placenta to the fetus, causing miscarriage or death of the fetus (Hydrops fetalis). It infects the fetal liver, which is the main site for the production of red blood cells during the early stages of fetal development, and leads to swelling of the fetus due to severe anemia and myositis Cardiovascular disease causing congestive heart failure (*Tolfenstam and Broliden 2009*)

6.2 Epidemiology

The B19 virus is one of the endemic viruses in all parts of the world. IgG antibodies have been confirmed by 50% in children under the age of 15 years and 80% in the elderly.
The rates of infection with the virus vary according to countries, and a higher rate of infection has been found in developing countries Such as Africa, due to its ease of spread in poor areas and crowded populated areas *(Tolfvenstam et .al .,2000*)
Several studies conducted in various countries indicated that IgG specific antibodies were found in patients with Mediterranean anemia in Iraq at a rate of 30.4%, Tunisia at a rate of 39.1%, Egypt at a rate of 18.2%, India at a rate of 80% and Thailand at a rate of 38% (Al *Ghwass et al., 2016*).). A study in (*Slavov et al., 2012*) indicated that the percentage of antibodies in Brazil was 55.3%.
A study conducted in Sudan on sickle cell anemia patients and white blood anemia patients indicated The average, as the rate of infection with the B19 virus was found to be 31 and 26.7, respectively (*Khider, 2005)*. Another study conducted in Iraq in 2016 on people who donated blood found IgM antibody in 97 donors out of a total of 189, representing a percentage of 51.1%. Also in Saudi Arabia, the IgG antibody in donors constituted a percentage of 76.3% (*Nazar 2016)* in a study conducted in India on blood donors 27.97% percentage of IgG antibody form(*Kumar et al ., 2013.)*
A study conducted in Iraq in 2016 on pregnant women who had previously suffered a miscarriage indicated that infection with the virus was identified in 40 women out of a total of 200, which constituted 20% of pregnant women.*(Al-attraqchi et al., 2018*)

2.4.3 Transmission of the B19 virus

The virus is transmitted in several ways, including through the respiratory system, and the virus is also transmitted through blood and its derivatives, as the risk of infection with the virus through the blood increases with the increase in the concentration of viral DNA in the blood of infected people, the presence of the virus in the bone marrow of asymptomatic infected people, and the ability of the virus to multiply after the initial infection occurs with several Years contribute to the spread of Viral infection (*Kooistra et al., 2012)*
A study conducted in the Netherlands in 2010 to detect viral DNA using polymerase chain reaction technology on blood donors who do not show symptoms of infection with the B19 virus showed that they possess the viral DNA without the presence of immune antibodies specific to the virus, and as a result The B19 virus is easily transmitted through blood transfusions (*Kooistra et al., 2012)*
The virus is also transmitted from the mother to the fetus through the placenta, and it constitutes 30%, and 2-5% of infection cases lead to the death of the fetus. The exact mechanism of transmission of the virus to the fetus has not been fully clarified. A study in the United States of America in 2010 indicated that the capillary lining of the fetus In the chorionic villi, they can support the replication of the virus, and inflammation of the chorionic lining leads to structural and functional destruction of blood exchange between the mother and the fetus and facilitates the transmission of the virus to the fetus. The greatest risk of placental transmission is between the first and second trimester of pregnancy (*Lamont et al., 2011)*.



2.5 Methods of diagnosing the B19 virus

2.5.1 Cytological diagnosis

Infection with the virus leads to the formation of giant red blood cells in the bone marrow, which are characterized by their absence of cytoplasm. The color of the chromatin is often immature and appears as a thin rim around viral inclusion. The virus can be detected using an electron microscope in plasma and fetal tissues. However, scanning by an electron microscope is a complex method. Technically (*Pasquinelli et al., 2009)*

2.5.2 Serological methods

Accurate diagnosis of acute or old infections with the B19 virus depends on the use of immunofluoresces and radioimmunoassay to detect IgM and IgG antibodies, including enzyme-linked immunosorbent assay (*Bredi et al., 2011)*.

2.5.3 Polymerase Chain Reaction

The first polymerase chain reaction method for detecting B19 virus in serum appeared in early 1989. Polymerase Chain Reaction technology is used to detect the B19 virus. It is one of the sensitive and successful technologies in diagnosing chronic infections and detecting viral DNA present in blood or tissue. DNA can be detected**.** The virus is in the serum four months after acute infection and years later in the tissues (*Söderlund-Venermo et al., 2002)***.**

PCR technology has shown that it has the ability to identify small amounts of viral DNA, and the simple polymerase chain reaction technology is the ideal method in diagnosing B19 virus DNA and identifying DNA sequencing is more accurate, rapid and sensitive than PCR and has advantages including**:**

- Monitor PCR reactions in real time.
- . Increase detection range
- Amplification and detection occur in one tube, minimizing the occurrence of variations or contamination in Results Ishmael and Stellato, 2008

This technique uses fluorescent molecules that participate in the polymerase chain reaction, and the intensity of fluorescence increases with the amount of viral DNA. Fluorochemical reactions involve dyes bound to DNA and primer sequences labeled with special fluorescents (*Ishmael and Stellato, 2008*)

2.6.1 Thalassemia Type Beta

Beta thalassemia is a deficiency or absence of beta globin chains that leads to an increase in alpha globin chains. The manufacturing of beta globin is under the control of a single gene located on chromosome number eleven (11). The beta type occurs as a result of genetic mutations, and most types of beta globin are the result of genetic mutations. (*Galanello and Origa 2010*) Mutations are point mutations and rarely deletion mutations of two genes. A defect in one gene will lead to beta-minor type B-thalassemia, which is a condition that does not show signs of the disease. The patients' red blood cells are characterized by their small size and the patient suffers from moderate anemia. However, in the event of a major defect in both genes or the absence of Their production will lead to beta major B-type disease or what is known as Thalassemia B Major. (*Galanello and Origa (2010*)

Beta major type patients have symptoms from birth due to the presence of hemoglobin, but they become more apparent at six months of age. However, if there is a defect in the beta globin chains in a moderate form, it will lead to beta intermedia type disease, which is a disease that shows symptoms that are less severe than beta major type disease and does not require large blood transfusions (*Campbell, 2009*)

2.6.2 Thalassemia, Beta Major Type

thalassemia, beta major type, or Cooley's anemia, discovered by the scientist Cooley in 1925, is a serious anemia that affects people who inherit two genes. Atypical thalassemia beta anemia is a gene from both parents and occurs when there is a defect in the beta genes. The affected person does not produce enough mature red blood cells or healthy red blood cells, so the skin color is very pale.

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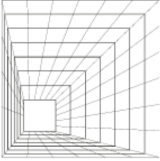


Affected children at three months old are paler, sluggish, and less Appetite and less growth and usually lose their lives at the age of 10-10 years unless
Providing the right medical care (*Muncie and Campbell 2009*)

Chapter 3
Materials and Methods
3.1 Instruments
The devices whose details and origin are shown in Table 3-1 were used to complete the study
Table 3-1 Laboratory equipment used in the study

No	Devices	the manufacture company	Devices
1	Polymerase chain reaction system Real-time RT-PCR	Bioneer	Korea
2	Centrifuge	Hettich	Germany
3	Micro centrifuge	Hettich	Germany
4	Refrigerated centrifuge	Eppeneof	United States of America
5	Scrubber	Biokit	United States of America
6	Reading device	Biokit	United States of America
7	Secondary droplet display For acoustic spectroscopy	Quawell	United States of America
8	Ion-infused water device	Lab. Teh	Korea
9	printer	Epson	Japan

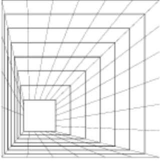
3.2 tools used
Laboratory tools and supplies whose details and origin are shown in Table 3-2 were used
Table 3-2 Laboratory materials and supplies used in the study

Sequence	Materials and supplies	the manufacture company	Origin
1	Ependorf tubes in different sizes	Biobasic	Canada
2	Laboratory tubes	Meheco	China
3	Flasks Flasks	Schott	Germany
4	Stopwatch	Termaks	Germany
5	Syber green dye	Bioneer	Korea
6	Pipette head pieces in sizes Tipe is different	Eppendorf	German
7	Ethanol alcohol 70%	B.D.H	United kingdom
8	Medical syringes	Meheco	China

3-3 -Kit Used In The Study
Table 3-3 shows the tools used in the study, their manufacturer, and their origin

Laboratory diagnostic kit	the manufacture company	Origin	Appendices
DNA extraction kit	Geneoid	Taiwan	Appendix (2
B19 Virus PreMix Kit	Bioneer	Korea	Appendix

3.4 Study totals:



Samples for this study were collected on patients with Mediterranean anemia, Pina major type, for the period from October 1 to December 30, 2018 at Diwaniyah Hospital/The Hematology Center.
Blood samples were taken from 72 patients with thalassemia who had previously been diagnosed with beta major thalassemia by physicians.Hematology specialists took 13 blood samples from healthy people as a control group5 ml of venous blood was drawn from the two study groups and collected in gel and clot tubes, and the blood serum was obtained by using a centrifuge with a speed of 1500 rpm. Minutes and for five minutes, a volume of 0.5 ml of serum was taken and placed in centrifuge tubes The samples were stored at a temperature of 20°C until use.

3.5 Molecular tests

3.5.1 Extraction of genetic material

Genomic DNA was extracted from the serum samples using the Viral Nucleic Acid Extraction kit, and the genetic material was extracted as described in the method attached to the kit, which is
Briefly :
Preparation of solutions
1. Add 20 ml of AD Buffer before use.
2 Add 100 ml of ethanol to the Wash Buffer before use.

3.6 The Method Of Work

First: cell lysis

1. 200 µL of serum sample was transferred to a 1.5 mL centrifuge tube.
2. 400 microliters of lysis solution was added to the sample and mixed using a centrifuge Micro-centralizer at a speed of 1500 r/min for 30 seconds.
3. I left the sample at 25°C for 10 minutes.

Second: DNA binding

1.Add 450 microliters of AD Buffer to the sample.
2 .Mix the sample by vigorous shaking.
3. The sample was transferred to a VB tube attached to a 2 ml collection tube.
4. Transferred 600 microliters of sample to the VB tube.
5. The tubes were centrifuged at 1600 rpm for 1 minute.
6. The filtrate was discarded and the collection tube was replaced with a clean one.
7. Centrifugate the tube again at 1600 r/min for 1 minute.
8. The filtrate was discarded and transferred to another transfer tube) and became DNA after this step linked to the silica column).

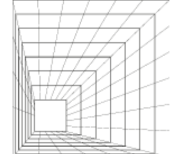
Third: Washing

1. 400 µl of W1 buffer was added to the sample and then centrifuged at 1600 rpm/
Minute for 30 seconds.
2. The filtrate was discarded and the VB column was returned to the collection tube.
3 Add 600 microliters of wash buffer, then centrifuge at 1600 rpm for
30 seconds.
4. The filtrate was discarded and the column was returned to the collection tube and centrifuged at 1600 rpm/ One minute for three minutes in order to dry the VB colum.

Fourth: DNA extraction

1. Transfer the dried VB column to a 1.5 ml microcentrifuge tube.
2 Add 50 µl of RNase free water to the center of the VB column
3. Leave the column for three minutes to ensure that the VB column is saturated with RNase free water.
4. The sample was centrifuged at 1600 rpm for 1 minute to extract DNA
Pure.

3.7 Estimating the purity and concentration of DNA



The purity and concentration of DNA were estimated using a nano drop spectrophotometer. A drop of extracted DNA was added to a device to estimate the concentration in nanograms and microliters. The optical absorption ratio was based on the two positive lengths, 260-280 nanometers, as a criterion for purity. Samples with a result of -1.8 2 were considered to be of adequate purity for testing Subsequent genetic testing.

3.7 Preserving genetic material

The samples were kept in deep freeze until subsequent tests were performed

3.8 Preparation of the primer

The prepared primer was prepared from the Canadian Alpha DNA Company and the primer was used to detect virusParvovirus B19, which was used in a previous study (Nikoozad et al 2015). lyophilized) powder: I dissolve the powder in deionized distilled water, which is free of DNase and Rnase enzymes, for the purpose of preparing the stock solution, which has a concentration of (100/100 poml) and is stored by freezing at a temperature of 20°C until use. The stock solutions were used to prepare the solution. (Working Solution) by adding 10 microliters of stock solution to 90 microliters of deionized distilled water (10 pmol/l) of working solution to obtain the Nuclease concentration.4-3 Explains the name and sequence of the primer used in this study.

Table 3-4 shows the primer used in the study

Initiator	Base sequence	Widget size
3206-33187 front 3271-3290 45 reverse	5 CAAAAGCATGTGGAGTGAGG3 3 CCTTATAATGGTGCTCTGGG5	104 bp

3.9 Amplify The Primer Using RT-PCR

1. The polymerase reaction was carried out in a volume of 20 µl in centrifuge tubes according to the kit used and (Bioneer, Korea AccuPower ® Green Star™ qPCR PreMix) the table (5-3) Explain all the components of the polymerase reaction.

Table 3-5 Components of the RT-PCR reaction medium

PCR Pre mix	the size
5 micro liters	DNA template
0.5 micro liter	Forward primer (10pmol)
0.5 micro liter	Reverse starter (10)
14 micro liters	Deionized water
20 micro liters	Total size

2 The tubes were sealed with optical tape.
3. The tubes were centrifuged at 3000 revolutions for 2 minutes
4 Turn on the RT-PCR machine and put the tubes in it.
5. Adjust the RT-PCR device to the settings shown in Table 3-6 below.
6. After completing the reaction, the results were analyzed

Table (3- 6) RT-PCR program

stage	factors	Number of cycles
Pre-denaturation	95 °C For 5 minutes	1
Denaturation	95 °C For 30 seconds	40
Annealing	60 °C For 30 seconds	
Extension	72 °C for 30 seconds	
Final Extension	72 °C 10 for sec	1

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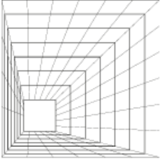
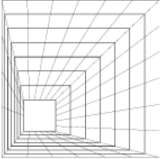


Statistical Analysis

The use of a statistical program Package for Social Science (SPSS Generation 20) The significance value was tested at the level 0.05



Chapter 4

Results and Discussion

4.1 Study Samples

Samples for this study were collected from patients with thalassemia beta major anemia for the period 1 November 2023 to 1 February 2024 at Diwaniyah Hospital.

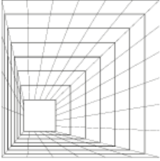
Hematology Center in Diwaniyah. Blood samples were taken from 72 patients with beta major Thalassemia who had previously been diagnosed with beta major Thalassemia by hematologists. 13 blood samples were taken from healthy people as a group control

4.2 Detection Of The B19 Virus Using Linear Polymerase Chain Reaction Technology:

The B19 virus was detected in the study samples using the RT-PCR method. The results of the current study showed that the B19 virus was present in 38 patients with Mediterranean beta major anemia, out of a total of 72 patients, at a rate of 52.8%, and that there was no infection with the virus in 34 patients, at a rate of 47.2%. There is a significant difference compared with the control group, as shown in Table (4-1)

Figure (4-1) shows the positive result for the presence of B19 virus DNA

No	Study samples	the total number	Percentage of people infected with the virus %	Percentage of people uninfected with the virus %	P.v-0.002
1	Patients with Thalassemia beta major type	72	(% 52.8) 38	47.2%)34	
2	Control group	13	nothing	100%)13	
3	Total summation	85	38	47	



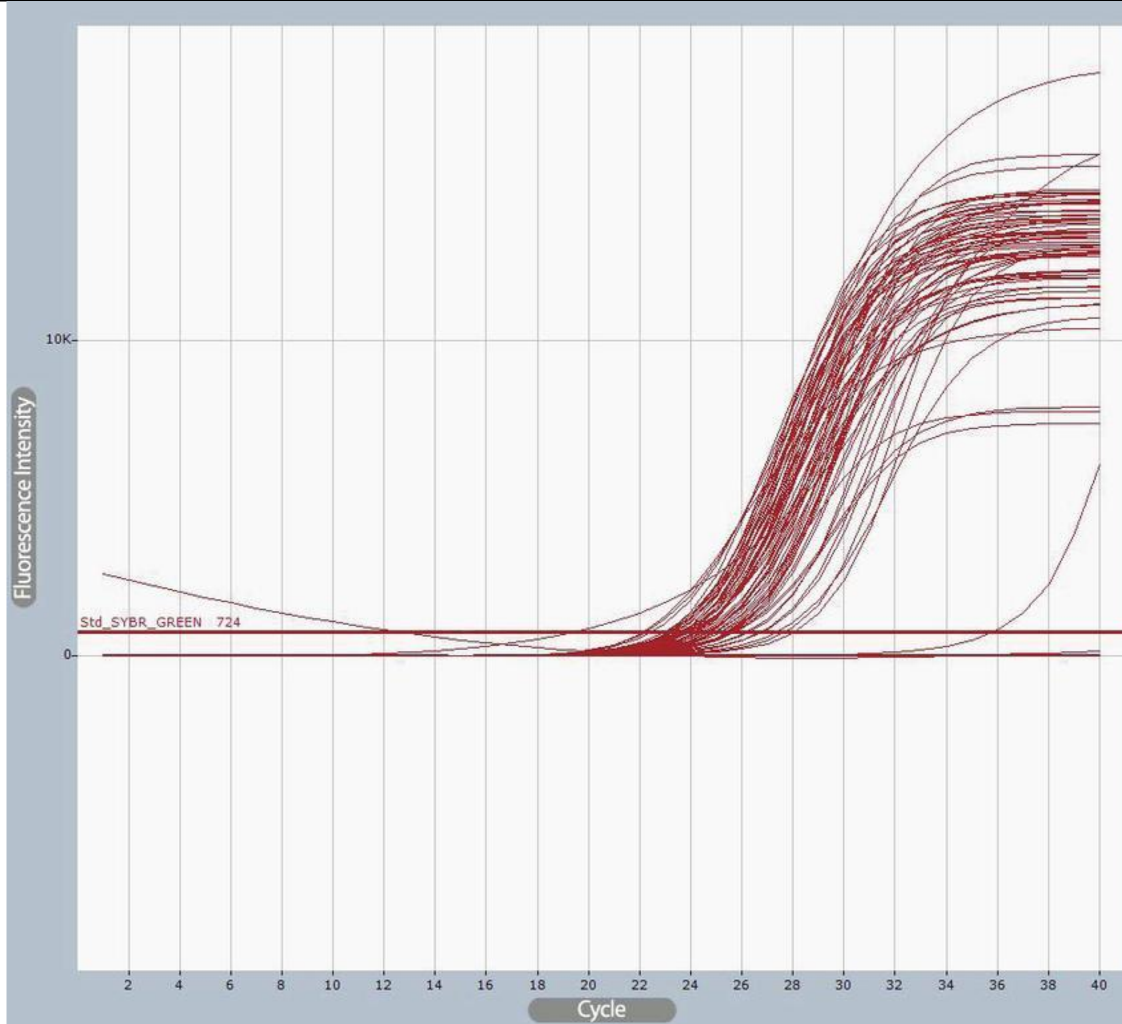


Figure (4-1) Positive result for screening for the B19 virus using an RT-PCR device.

One study in Iran in 2015, using PCR technology, indicated the spread of the virus in 6 infected people out of a total of 30 patients, at a rate of 20% (*Nikoozad et al., 2015*). In a study

Another study conducted in Iran in 2016, using RT-PCR technology, indicated that the virus had spread to 6 infected people out of a total of 150 patients, at a rate of 4%. The researcher explained the reason for the decrease in infection among patients to the period of sample collection, as the samples were collected in the summer; The spread of the virus decreases in the winter

Summer due to high temperatures, and virus activity increases in the winter and hot seasons(*Bokharaei-salim et al., 2017)*

study in Turkey in 2007, using PCR technology, showed the spread of the virus among 23 with a percentage of 29.15% of infected people out of a total of 79, (Us et al., 2007)

A study conducted in Egypt in 2017, using PCR technology, showed that the virus was found in 6 infected children out of a total of 39 patients, with a percentage of 15.4 (*Salama, 2017)*. Likewise, a study conducted in Brazil in 2011, using PCR technology, showed the spread of the virus in 2 of those infected out of a total of 10 patients and a percentage (*Slavov et al.* , *2011*)

A study conducted in India in 2012 using PCR technology indicated that the virus had spread to 13 people of 238 patients were infected, with a percentage of 5.5% (*Jain et al., 2014)*

Also, a study in France in 2014 using PCR technology showed the spread of the virus in 6 people A total of 76 patients were infected with a percentage%8(*Lefrère et al., 2014)*

The difference in proportions between societies is due to several factors, including climatic and demographic differences, the period of sample collection, sample size, type of sample, sensitivity of

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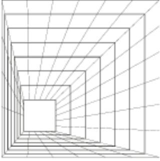


the technique used, and the percentage of contamination, in addition to differences in health care and awareness

4.3 Spread Of Infection With The Virus According To Gender

Table (4-2) Beta major type patients infected with the virus, by gender

No	Sex	Total number of patients with beta major type	Percentage of people infected with the virus (%)	
1	Females	33	48.5%)16	P.v=-0.005
2	Male	39	56.4%)22	
3	Total summation	72	38	

Table 4-2 shows the prevalence of the virus among males more than females. The virus was found in males, 22 infections out of a total of 39 patients, at a rate of 56.4, and in females, 16 infections out of a total 33 patients, representing 48.5%, with a significant difference P. V =0.005.

The results of the current study did not agree with a study conducted in Iran in 2015 using PCR technology. The researcher pointed out that the virus spread more among females than males. The virus was found in females with four infections out of a total of 15 patients, at a rate of 26.6%, and in males with two infections out of a total of 15. patients and a percentage of13.3%(*Nikoozad et al., 2015*)

The results of the current study agreed with a study conducted in Syria in 2015 using PCR technology the study showed a higher incidence of infection among males than females, and the virus was found in 10 of Males were infected out of a total of 101 patients, at a rate of 10%, and females had 8 infections out of a total of 99 patients and a percentage of 8% (Smaya and Buhtori,2015).

The results of the current study also agreed with a study conducted in Iran in 2016 using PCR-RT technology. The researcher found the spread of the virus among females, one infection out of a total of 75 patients, with a rate of 1.3%There are 5 infected males out of a total of 75 patients And in percentage 6.6 % (*Bokharaei-salim et al., 2017*)

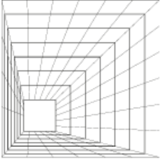
The results of the current study are compatible with a study conducted in Taiwan in 2003 using PCR technology. The researcher indicated that the infection was higher in males than in females, and he found infection in 7 infected males out of a total of 179 patients, at a rate of 4%, and 2 infections in females out of a total of 99. patients And in percentage 2% (*Lee et al., 2003*)

4.4 The Spread Of Infection With The Virus And According To Age Groups

Table (4-3) Those infected with the virus and according to age groups

No	Age groups of patients in years	Total number of patients with beta major type	Number of infected people	
1	1-5	15	7(46.7%)	P.v=0.014
2	6-10	15	10(66.7%)	
3	11-15	21	8(38.1%)	
4	16-20	12	9(75%)	
5	21-25	6	2(33.3%)	
6	26 and more	3	2(66.7%)	
7	Total summation	72	38(52.8%)	

Table (4-3) shows the results of the current study, where the study showed that the most infected ages with the virus are within the age group of (20 -16) years, at a rate of 75%, and the least ages infected with the virus are within the age group from (21-25) years, at a rate of 33.3%, with a significant difference of P.v=0.014.



A study conducted in Iran in 2016 using RT-PCR technology indicated that the virus spread more among ages 31-35 years, and the virus was found in 3 infected people out of a total of 36 patients with percentage%8.3 (Bokharaei-salim et al., 2017)

A study conducted in Iran in 2015, using PCR technology, showed that the virus spread more among ages 20-40 years, as the virus was found in 3 infected people out of a total of 12 patients, with a percentage of 25% It is less prevalent among people under 20 years of age, as the virus was found in one infected person out of a total 8 patients and a percentage of 12,5% (*Nikoozad et al., 2015)*
A study conducted in Syria in 2015 using PCR technology showed that the most common age infected with the virus was 168 years, where the virus was found in 12 infected people out of a total of 76 patients, with a percentage of %15.7.(*Smaya and Buhtori, 2015)*
A study conducted in Taiwan in 2003 using PCR technology, where the researcher indicated that the age most infected with the virus was 30-39 years, where the virus was found in one infected person out of a total of 21 patients, at a rate of 4.7%, and the least infected ages were 40-49 years, where one infection was found in a total of 4.7%
35 patients with the percentage of %2.8)(*Lee et al., 2003)*
Researcher Zaki indicated in a study conducted in Egypt in 2006 that the increase in infection with the virus B19 in children due to a lack of neutrophils and lymphocytes
(*Zaki et al., 2006)*

4.5 The Spread Of Infection With The Virus According To Residence

Table(4-4) People infected with the virus by residence

No	Accommodation type	Total number of patients with beta major type	Number of people infected with the virus %	
1	Urban	14	10(71.4%)	P.v-0.002
2	The countryside	58	28(48.3%)	
3	Total summation	72	38(52.8%)	

Table 4-4 shows that infection with the virus was higher in cities than in the countryside, and the infection was in 10 infected people out of a total of 14 patients, at a rate of 71.4%, and in the countryside in 28 infected people out of a total of 58 patients And in percentage 48.3% Although there is a significant difference P.V =0.002
The difference between the spread of infection between the countryside and the city may be due to the difference in the number of samples in the countryside more than in the city, population density and environmental pollution in the city more than in the countryside, and demographic changes after 2003. As a study in Sweden in 2000 indicated, the virus spread more.In crowded populated areas (Tolfvenstam et al. 2000).

4.6 The Spread Of Infection With The Virus And Its Relationship With The Number Of Blood Transfusions

Table(4-5)People infected with the virus, according to the number of blood transfusions

No	Number of blood transfusions	the total numberFor patients with beta major type	Number of infected people With virus (%)	
1	Once every 20 days	18	10(55.6%)	
2	Once every 30 days	48	25(52.1%)	
3	Once every 40 days	6	3(50%)	P.v-0.006
4	Total summation	72	38(52.8%)	

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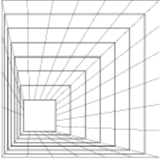

Table 4-5 shows the highest incidence among patients who receive blood every 20 days, followed by the every 30 days group, followed by the every 40 days group, at a rate of 52.1.55.6%, respectively, with the presence of
Significant difference P. V=006
The results of the current study are consistent with a study conducted in Iran in 2016, which indicated that patients who receive blood every 20 days are more infected with the virus, and the virus was found in 3 infected patients out of a total of 62 patients and in percentage 4.8% (*Bokharaei-salim et al., 2017)*
Study was conducted in Iran in 2015 using PCR technology, and the researcher indicated the spread of the virus For blood recipients, the virus was found in 25 infected blood recipients, at a rate of 20%.(*Nikoozad et al., 2015)*
A study conducted in Egypt in 2012 indicated the spread of infection among blood recipients, as the virus was found in 16 infected people out of a total of 41 patients, at a rate of 39 (Zaki, 2012). Likewise, in a study conducted in Egypt in 2017, the researcher indicated that there is a consistent relationship between the spread of infection with the virus and the number Blood transfusion times(*Salama 2017)*
It Is clear from the results of the current study that infection with the virus increases with the number of blood transfusions
It is possible that the virus is present in donors. A study conducted in South Africa indicated the presence of the virus in blood donors. The virus was found in 40 infected people out of a total of 1,500 donors, at a rate of 2.6%. Likewise, a study in Sudan found the virus in 8 infected people out of a total of 110 donors, at a rate of 7.3% (*Osman et al., 2017; Francois et al., 2019)*

Conclusions and Recommendations

Conclusions

The current study concluded that:
1. The spread of the virus was high using the linear enzyme polymerase chain reaction technique Patients with thalassemia, beta major type, in Diwaniyah city .
2. The highest rate of infection with the virus was in the age group of 16-20 years
3 .The incidence is higher in the city than in the countryside.
4.The infection with the virus increases with the number of blood transfusions.

Recommendations

1. Conducting immunological tests in conjunction with viral DNA tests to determine the type of viral infection
2. Conduct a study on the field marshal's blood to detect viral infection.
3. Conducting multiple studies to reveal the effect of viral toxicity in pregnant women and its effect on the fetus
4. To conduct studies that clarify the relationship between the virus and the rest of the cellular dynamics
5. Screening for the B19 virus in patients with other types of anemia, such as hemophilia and sickle cell anemia

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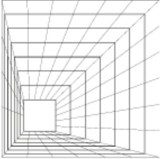

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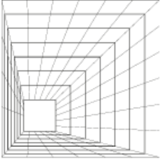

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