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# Molecular Detection Of Human Parvovirus B19 In B-Thalassemia Patients At Thi-Qar Province/ Iraq

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**Abstract**

**Introduction**

The thalassemia is known as genetic defects that autosomal recessive, characterized by a reduction or absence of production one or two types of globin chains. This condition will have an impact on the quantity and quality of blood produced**.**Human parvovirus B19 has a significant affinity for hemopoitic stem cells and integrates into a particular location in the human genome. The infected cell is unable to divide, resulting in a reduction in the generation of new RBC. Parvovirus is a group of tiny (25nm) non-enveloped viruses. Parvovirus is derived from the Latin word parvus, which means small. A linear single-stranded DNA (ssDNA) genome of 5 to 6 kb is bordered by two terminal hairpin formations**.**

**Method**

Genomic DNA from blood samples were extracted by using (G-spin$^{TM}$ Total DNA Extraction Kit)**.** The extracted blood genomic DNA was checked by using Nanodrop spectrophotometer (THERMO. USA), which measured DNA concentration (ng/µL) and check the DNA purity by reading the absorbance at (260 /280 nm)**.** The Real-Time PCR technique was performed for direct detection of Parvovirus B19 virus based NS1 region in β-Thalassemia patients'blood samples**.**

**Result**

During the current investigation, blood samples from 100 thalassemia patients were utilized to determine the presence of Parvovirus B19 ssDNA in blood samples using the qPCR technique. Parvovirus was found in 54 % of thalassemia patients. Addition, Females were infected with B19v in greater numbers than males (29 vs. 25). The numbers of patients B19v infected with B19v in the countryside is 44, which is higher than the numbers of individuals B19v infected in the city, which is 10.In addition, the numbers of B19v infected people in the first age group is the highest, with 29 patients, followed by the second age group with 20 patients, and groups over 21 with the least number of B19v infected people.The most infected blood type is A +, which has 23 patients, followed by B +, which has 17 patients, and A +, which has nine people. It is the least common blood type among the others. In any event, carriers of type AB + are not infected with B19v.

**Conclusion**

Human parvovirus B19 infections vary depending on an individual's immunity. May be an O+ is the most commonly blood type, thalassemia patients who are often frequent blood transferred to become vulnerable to the risk of contracting the B19 virus.

**Key words: Human parvovirus B19, Viral infection, Real time PCR, β-Thalassemia, Thi-Qar**

## Introduction

Thalassemia is one of the world's most frequent hereditary illnesses. It's a severe health issue that causes a lot of morbidities, early mortality, and a lot of financial and emotional hardship for a family(Al-badry & Al-tamemi, 2019).The thalassemia is known as genetic defects that autosomal recessive, characterized by a reduction or absence of production one or two types of globin chains(Husna et al., 2017). There have been two forms of thalassemia, alpha-thalassemia and beta-thalassemia. When one or more from the four α-globin genes are destroyed or altered, α-thalassemia occurs, while β-thalassemia evolves when both β-globin genes are damaged or mutated. Furthermore, Thalassemia major occurs when a child receives two defective globin genes, one from each parent, whereas thalassemia minor happens when a child receives just one deficient globin gene(Abu-Shaheen et al., 2020).Beta-Thalassemia major people are much more likely to have aplastic crisis after being exposed to B19v as they have chronic hemolytic illnesses with a shorter RBC half-life. Addition, Human parvovirus B19 has a significant affinity for hemopoitic stem cells and integrates into a particular location in the human genome. The infected cell is unable to divide, resulting in a reduction in the generation of new RBC (Rasid et al., 2020).B19V DNA survives in various tissues for the rest of one's life, primarily bone marrow, liver, heart, skin, and synovial with uncertain functional ramifications. Chronic B19V infection is associated with autoimmune symptoms(Arvia et al., 2020).Human parvovirus (B19V) infects patients and replicates in the nuclei of erythroid progenitor cells, causing erythropoiesis problems. As a result, erythema infectiosum, often known as slapped cheek syndrome(Abdelrahman, Al-sadeqet al., 2021).This study was aimed todetects the presence of Parvovirus B19 DNA in β-thalassemia patient's blood by Real-Time PCR.

## Subjects and Methods

**Patients**: One hundred of beta-thalassemia patients were used to detect the presence of Parvovirus B19 ssDNA in blood samples by qPCR technique. 5ml collected from (60 β-thalassemia Patients positive to Parvovirus B19 and negative for HIV, HBV and HCV) and (40 β-thalassemiaPatientsnegative to all viral infections above as a control group.

**Molecular test:**

**Extraction ofviral DNA Genome**

Genomic DNA was extracted from blood samples by using (G-spin$^{TM}$ Total DNA Extraction Kit).

**Estimation the Concentration and Purity of viral DNA**

The estimation of DNA concentration (ng/µl) and its purity by reading the absorbance at (260/280 nm) was done by utilizing Nanodrop spectrophotometer (THERMO/USA).

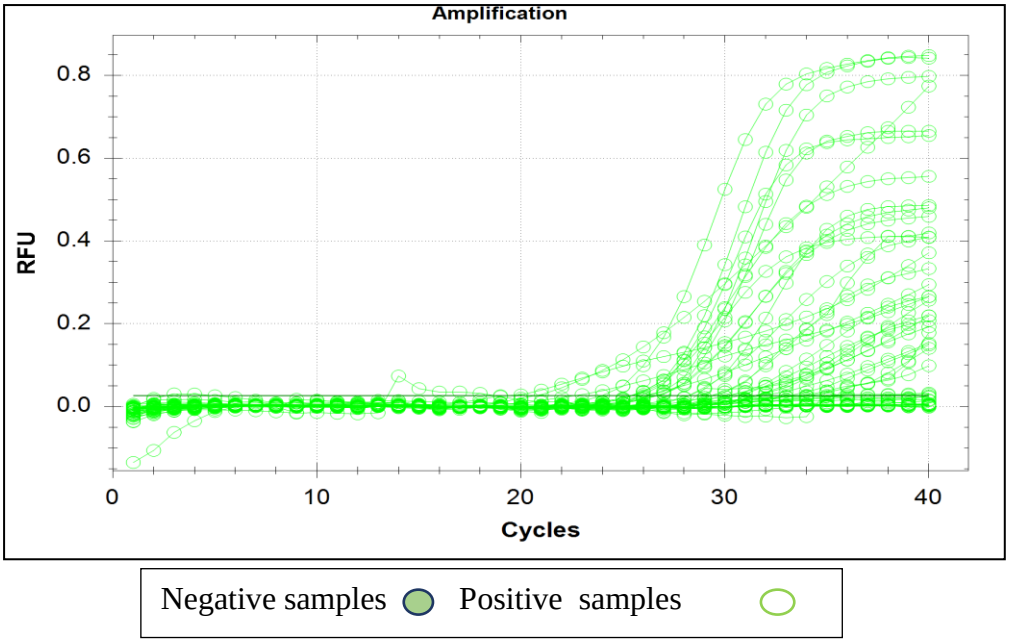
**Detection the Parvovirus B19 virus**

The Real-Time PCR technique was performed for direct detection of Parvovirus B19 virus based NS1 region in β-Thalassemia patients'blood samples and technique was carried out according to method described by (Alves et al., 2019).The Real Time PCR master mixwas prepared by using (**GoTaq® qPCR Master Mix**) as shown in table (1).Real-Time PCRthermocycler conditions was set according to primer annealing temperature and qPCR master mix kit instructions.

**Table(1): The Real-Time PCR primers for detection parvovirus B19 virus.**

| Primer | Sequence (5'-3') | |
|---|---|---|
| Parvovirus B19 NS1 | F | TGCAGATGCCCTCCACCCA |
| | R | GCTGCTTTCACTGAGTTCTTC |

The qPCR data analysis was analysis in BioRad Real Time PCR analysis software by calculation the threshold cycle number (CT value) the positive amplification and negative control sample,as shown in figure(1).



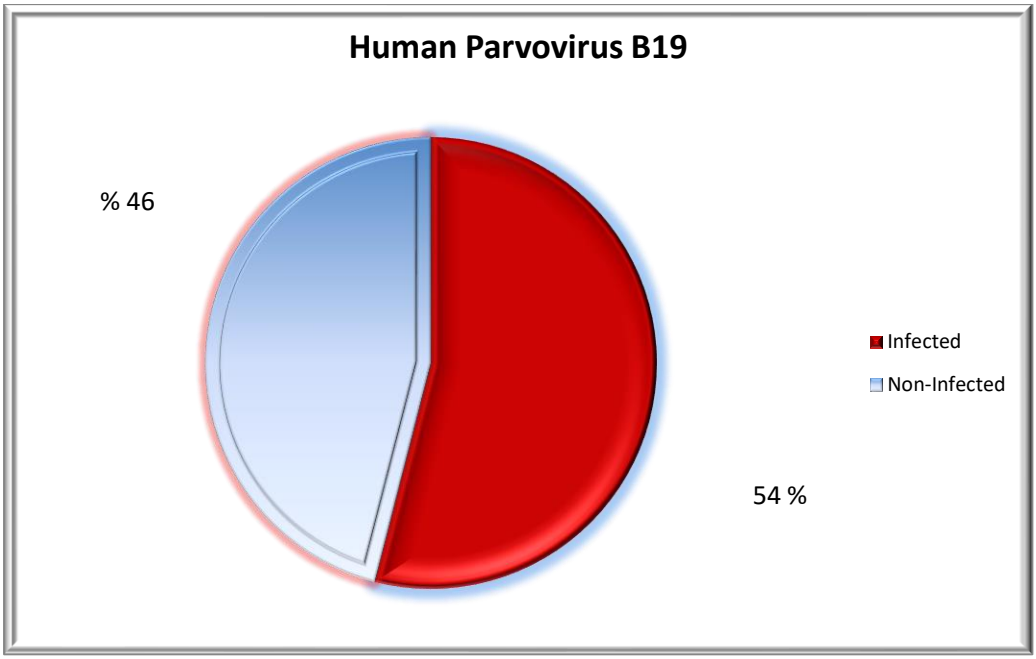

**Figure(1): The Graph of positive Human parvovirus B19 obtained by RT-qPCR Thermocycler.**

**Statistical Analysis**

The data of the current study is analysis by using SPSS (Statistical Package of Sociot Science) based on descriptive Chi-Square and Nonparametric Chi-Square.

**Results**

During the current investigation, blood samples from 100 thalassemia patients were utilized to determine the presence of Parvovirus B19 ssDNA in blood samples using the qPCR technique. Parvovirus was found in 54% of thalassemia patients, whereas it was not found in 46% percentas shown in figure (2).




**Figure(2):The Finding of Parvovirus B19 infections in The-Qar province.**

According to the current study,females have a higher rate of parvovirus infection than males,as shown in table (2).However there were no statistically significant differences between the incidence of infection and the patient's gender at a significance threshold of < 0.05.

**Table(2): Percentage of Parvovirus B19 infections, according to gender.**

| Infectious Status / Gender | Infected No. | % | Total | |
|---|---|---|---|---|
| | | | No. | % |
| **Male** | 25 | 46.3 | 52 | 52 |
| **Female** | 29 | 53.7 | 48 | 48 |
| **Total** | 54 | 100 | 100 | 100 |
| **CalX$^2$= 0.296** | **TabX$^2$= 3.84** | **DF=1** | **P. value > 0.05** | |

The current study found that the incidence of parvovirus infection is higher in the countryside than in the city, so there are statistically significant variations in the infection rates and the patient's residence at a significant level of< 0.05, as shown in table (3).

**Table(3): Percentage of Parvovirus B19 infections, according to Habitation.**

| Infectious Status / Habitation | Infected | % | Total | |
|---|---|---|---|---|
| | No. | | No. | % |
| **Urban** | 10 | 18.5 | 18 | 18 |
| **Rural** | 44 | 81.5 | 82 | 82 |
| **Total** | 54 | 100 | 100 | 100 |
| **CalX$^2$= 21.407** | **TabX$^2$= 3.84** | **DF= 1** | **P. value < 0.05** | |

According to the current study, the first age group (1-10) had the highest rate of parvovirus infection, followed by the second age group (11-20), and the last age group had the lowest rate of infection (above 20 years), as shown in table (4). There were also statistically significant variations between the incidence of infection and the patient's age at a significant level of < 0.05, according to the study.

**Table(4): Percentage of Parvovirus B19 infections, according to Age Groups.**

| Infectious Status / Age Groups | Infected | % | Total | |
|---|---|---|---|---|
| | No. | | No. | % |
| **1 - 10 years** | 29 | 53.7 | 51 | 51 |
| **11 - 20 years** | 20 | 37.0 | 42 | 42 |
| **21 – 30 years** | 3 | 5.6 | 4 | 4 |
| **Above 30 years** | 2 | 3.7 | 3 | 3 |
| **Total** | 54 | 100 | 100 | 100 |
| **CalX$^2$= 38.889** | **TabX$^2$= 7.81** | **DF= 3** | **P. value < 0.05** | |

Based on the current study, individuals with type of blood O+ had the highest incidence of parvovirus infection, followed by those who have type of blood B+. Individuals with blood groups B-, AB-, and O- had the lowest incidence of infection, while people who are carriers had no infection. AB+ blood type,as shown in table (5). At a significant threshold of < 0.05, the study found statistically significant differences between the incidence of infection and the patient's blood group.

**Table(5): Percentage of Parvovirus B19 infections according toBlood Groups.**

| Infectious Status / Blood Groups | Infected | % | Total | |
|---|---|---|---|---|
| | No. | | No. | % |
| **A +** | 9 | 16.67 | 24 | 24 |
| **A -** | 2 | 3.7 | 3 | 3 |
| **B +** | 17 | 31.48 | 32 | 32 |
| **B -** | 1 | 1.85 | 2 | 2 |
| **AB +** | 0 | 0.0 | 2 | 2 |
| **AB -** | 1 | 1.85 | 1 | 1 |
| **O +** | 23 | 42.6 | 34 | 34 |
| **O -** | 1 | 1.85 | 2 | 2 |
| **Total** | 54 | 100 | 100 | 100 |
| **CalX$^2$= 63.444** | **TabX$^2$= 12.59** | **DF= 6** | **P. value < 0.05** | |

**Discussion**

**Detection of Human Parvovirus B19 by real time PCR**

Human Parvovirus B19 infection is very common and spreads all over the world (Tomás-Velázquez et al., 2020).

The result of present study revealed the prevalence of B19v was estimated as 54% and this results is higher than those in previous studies conducted in Iraq by Fanos & Mohammed,(2021) and Rasid et al., (2020) were they reported the prevalence in Thi-Qar province (12.5%) and in Baghdad (37.3%) respectively, and more than results by Ibrahem et al., (2014) in Basra (47.5%), Majeed, (2018) of B19v children infection in Thi-Qar (20%), Fisal & Agha, (2020) seroprevalence in Erbil (39.8%), and Sadeq K. Al-Salait, (2021)in Basra (15%).

In comparison with results of studies in other countries, the results are in agreement with those in China, Singapore, and Turkeybelow 30%,in Iran reaching (65.63%), while extremely high prevalence in Eritrea about (56-91%) in different age groups (Mor et al., 2021).B19V DNA positivity among blood donors in the Southern part of Brazil we found

53.9% B19V IgG seroprevalence and 1.9% positivity of B19V DNA (Slavov et al., 2019), in Washington 22% had positive PCR (Majumdar et al., 2020).

Infection with B19V is particularly dangerous in high-risk people who are often transfused. Immunocompromised individuals, particularly those with hematological diseases, and pregnant women are among them. Because of childhood infection, it is estimated that 30–75 percent of the world's population is B19V seropositive (Abdelrahman, Al-Sadeq, Smatti, Taleb, Abuodeh, Al-Absi, Al-Thani, Coyle, Al-Dewik, Al Qahtani, Yassine, Nasrallah, et al., 2021).

Infection with B19V is particularly dangerous in high-risk people who are often transfused. Immunocompromised patients, particularly those with hematological illnesses, and pregnant women, especially those in the first and second trimesters, are among them. Because of childhood infection, it is estimated that 30–75% of the world's population is B19V seropositive(Abdelrahmanet al., 2021).

**The positive percentage of B19v relation to gender**

The results by real-time PCR technique were corresponding to detect the prevalence of B19V infection among males and females. This results was in agreement with other studies by Al-Ghanimi et al., (2019), IsabeL de oLIVeIra et al., (2016), (Ashaka et al., 2018) and (Kosaryan et al., 2020).

Also these results were in agreement with a previous studies in other countries: in Taiwan and Sweden Arabzadeh et al., (2017), and in Iran Sabahi et al., (2015) who found that infection rate in females higher than in males.

There was no significant difference in infections between males and females, perhaps to equal the chance of thalassemia patients in the number of blood transfusions that could be the cause of infection with the virus.

**The positive percentage of B19v relation to habitation**

The prevalence of B19V according to the current study appears to be high in rural areas of the Thi-Qar province, this result was in agreement with Zyoud et al., (2016), whereas disagreement with the results given by Salimi et al., (2008) who is found that B19V infections in urban areas more than of that in rural areas. While, Sabahi et al., (2015)and Arabzadeh et al., (2017) found it somewhat equal between urban and rural.

The fact that the rural population is a more consanguineous marriage rate than the urban population may contribute to the transfer of genetic features such as thalassemia

mutations, which required frequent blood transfusions, as well as a lack of health awareness.

**The positive percentage of B19v relation to age group**

The results of this study showed that B19V prevalence is higher within age groups (1-20) years than older persons according to real time PCR technique, and this result was in agreement with Abdelrahman et al., 2021) who studied B19V prevalence at the general population in Qatar. Exposure of this group to multiple transfusion and weak immune system which hemoglobinopathy lead to infection.

In Iraq, Hussein et al., (2021) have recorded that the highest infection rate B19v of patients belong to the age group (1-20) years old.

The reason for decrease of infections in age groups older than (above 30) years was the death due to bone marrow failure and infection with other etiology, and may be that the baby is born with B19v infected, which is vertically transmitted from the mother.

**The positive percentage of B19v relation to blood group**

The result of this study demonstrated a correlation between B19V infections and blood groups. The prevalence of B19V was found to be higher in patients with blood group O, this finding is not unexpected as there is usually a high demand for blood group O which is the most common blood group in the general population, and lower in patients with blood group AB, this result is in agreement with Mohammed, (2018).

In 2008, a study of Iranian blood donors found that no samples were positive for parvovirus DNA, suggesting the low risk of parvovirus transmission through blood cells, (which can put patients with hematological disorders at risk) (Bokharaei-salim et al., 2017).

## Conclusion

Human parvovirus infections differ depending on a person's immunity, as well as their habits and traditions. In the villages, there is a lack of health awareness and culture, which leads to the spread of human B19v. Furthermore, the rate of infection in children increases as a result of a weakening immune system and a lack of a very well diet. Also because the O+ blood type is perhaps the most prevalent, thalassemia children who have frequent blood transfusions are at risk of catching the B19v.

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