# Detection of Parvovirus B19 in B-thalassemia Major Patients by Serological and Molecular Method

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## Abstract

**Introduction:** Because to the tropism of human parvovirus B19 to erythroid progenitor cells, infection in patients with an underlying hemolytic disorder such as Beta thalassemia major leads to suppression of erythropoiesis, referred to as reticulocytopenia,which could be life threatening, The aim of the study was to determine the rate of occurrence of parvovirus (B19) in beta thalassemia major patient by using real time-PCR, Study the correlation between B19 virus and patient's descriptive data and the correlation between B19 viral load and IgG titer.

**Method:** This case-control study was done to detect the presence of parvovirus B19 DNA in plasma samples and anti-IgG ELISA of patients with beta thalassemia major. The population consisted of 75 patients with beta-thalassemia major who attended the Aban Al-balady hospital in in the City of Baghdad and 75 healthy people as a control.

**Results:** The prevalence of parvovirus B19 in our study population was 28 (37.3%) were positive for B19 DNA by quantitative polymerase chain reaction in which highly significant different P value (< 0.0001), Results of enzyme linked immunosorbent assays showed that IgG antibodies was positive in 16 (21.3%) compared with control group which was 7 (9.3%). According to statistical analysis the difference was in borderline between case and control group in prevalence of B19 virus. There was significant (P= 0.039) association correlation between B19 DNA and anti-IgG positivity, but were significant in WBC (P<0.0001).

**Conclusion:** In study, B19 infections were discovered in patients with beta thalassemia major. Screening of such risky groups will significantly reduce the incidence and prevalence of B19 infection.

***Keywords:*** *Parvovirus B19; Thalassemia; PCR, ELISA, Iraqi patients.*

## Introduction

Human parvovirus B19)HPVB19 (is a tiny single strand DNA virus. It is the only member of Parvoviridae family, genus Erythrovirus, known to be pathogenic to human(1).

After success access to the human host, Parvovirus B19 targets the erythroid progenitors in the bone marrow by bineding to the glycosphingolipid globoside (Gb4), also recognized as blood group P antigen (2). It's generally harmless in healthy individuales but may have a serious clinical effect in susceptible recipients such as patients with shortened red cell survival such as Sickle cell disease and BTM patients, immunocompromised patients and pregnant woman(3). HPVB19 virus may cause more severe disease, like transient aplastic crisis in patients' suffering from chronic hemolytic disorders(4). This virus is resistant to most physicochemical factors and is mainly transmitted through respiratory secretions; however, it can be transmitted through blood and blood products(5).

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The main clinical features of infection with parvovirus B19 include dermatologic manifestation, rheumatologic findings and hematologic effect[6]. The illness association with human parvovirus B19 evolves differently in different individuals. Some may be asymptomatic and other develop only prodromal symptoms. In some, the prodromal illness is followed by a later phase of more definable symptoms. In a few, particularly those who immunosuppressed or suffering from related illnesses which put them at high risk, the disease may become chronic and complicated with long term sequelae. In outbreak, asymptomatic infection occur in ~20% of children and adults expose to the virus[7].

Beta Thalassemia major, owing to chronic hemolytic diseases with shortened half-life of RBC, are at higher risk of acquiring aplastic crisis after exposure to this virus, sudden worsening of anemia, reticulocytopenia and cessation of erythropoiesis of the bone morrow are characteristic feature of transient aplastic crisis [8].

HPV B19 had a strong tropism for hemopoitic stem cell,the virus integrates in a specific site in human genome. The infected cell fail to divide, impairing the production of new RBC, reticulocytecount usually fall to as low as 0.1 to 0.5% from routine values of 6-20% in patient with hemolytic anemia disorder[9].

HPV B19 infect mature erythroid progenitor, preventing further replication and maturation, the more primitive precursor are affected minimally[10]. There are several studies about the seroprevelance of the virus with thalassemia patients which include Kishore study in India by ELISA (IgG) that appeared 73 (81%) from 90[11]. Arabzadehstudy in Iran (Tehran) that resulted 4% from 70 by real-time PCR[12] Mohamed study in Egypt (Fayoum) by ELISA (IgG) that presented 18.2% from 55[13] Same 23 Tarish study in Iraq (Babylon) by ELISA (IgG) that resulted 30.4%from 46[14].

This study was aimed to detect the occurrence of Parvovirus (B19) in Iraqi thalassemia major patients by real-time-PCR and IgG titer and Study the correlation between B19 virus and patient's descriptive data.

## Material And Method

This descriptive cross-sectional study was performed on 150 person, among them 75 patient with beta thalassemia major and 75 apparently healthy persons that served as control group. All patient were attended the Aban AL-balidy hospital (hereditary blood disorders center) Baghdad/Iraqduring period from December 2018 to May 2019. All these individualwere diagnosed as case of thalassemia major by HPLC and all relevant information was obtained from all cases using special questionnaire.

Five millimeters of venous blood were aspirated from all subjects included in the study. Blood sample divided in tow tubes, one of whole blood was collected in EDTA blood tube to get plasma for molecular part and other were allowed to clot and then centrifuged for 15 min at 3000 rpm in order to collect serum. Serum kept deeply in frozen (-20) unless worked immediately for evolution of different parameter.

HPVB19 was detected by immunological and molecular method.

In Immunological Method, Anti-HPV B19IgG was done by using ELISA test kit (MyBioSource/ Germany). The test was done according to manufactures instructions.

In molecular method,HPVB19 DNAwas extracted using a commercial genomic DNA extraction kit (Geneaid, Taiwan).

Virus copy number was for B19 determine by real-time PCR using TaqMan master mix as follows:

Pre-treatment (50°C, 2 min, 1 cycle), Initial denaturation (95°C, 10 min, 1 cycle) and annealing and extension (95 °C, 15 sec, 45 cycles) and using commercial kit (Sacace)/Italy).

SPSS version 21 was used to perform the data analysis and to calculate the descriptive statistics. Chi-Square and Fisher Exact test were used to determine the statistical significance of level of differences between patient (case) control group according to Anti- HPVB19 IgM and IgG. P value<0.05 was considered to be significant.

## Results

The real-time PCR detection of B19 DNA revealed 28 (37.3%) positive and 47 (62.7%) negative samples out of 75 subjects and 3 (4%) positive and 72 (96%) negative samples out of 75 healthy persons as shown in Table (1). there was **significant** different P value (**< 0.0001**), Odds ratio indicate that patients with BTM had 14.3 the ability to get the infection rather than the control.

**Table (1) Results of Real-time-PCR for study group and control.**

| **Chi-sq:P< 0.0001 (highly significant)/Odds ratio= 14.3** | | | |
|---|---|---|---|
| **Case** | | | |
| | **Negative qRT-PCR** | **Positive qRT-PCR** | **Total** |
| Count | 47 | 28 | 75 |
| % within Case | 62.7% | 37.3% | 100.0% |
| % within Real_time_PCR | 39.5% | 90.3% | 50.0% |
| **Control** | | | |
| | **Negative qRT-PCR** | **Positive qRT-PCR** | **Total** |
| Count | 72 | 3 | 75 |
| % within control | 96.0% | 4.0% | 100.0% |
| % within Real_time_PCR | 60.5% | 9.7% | 50.0% |
| **Total** | | | |
| | **Negative qRT-PCR** | **Positive qRT-PCR** | **Total** |
| Count | 119 | 31 | 150 |
| % within Caseand control | 79.3% | 20.7% | 100.0% |
| % within Real_time_PCR | 100.0% | 100.0% | 100.0% |

**Ig Gprevalence of B19 virus among participants:** The overall distribution of B19 IgG antibodies among study and control groups were shown in table (2). The highest seropositivity was noticed among study group (cases) was 16 (21.3%) compared with control group which was 7 (9.3%). According to statistical analysis the difference were in borderline between case and control group in prevalence of B19 virus. The result also indicate that thalassemia patients had 2.63 increasing in the B19 IgG antibodies than the healthy.

**Table (2): Frequency of B19 virus among participants.**

| **Chi-sq:P= 0.06 (borderline significant)**<br>**Odds ratio=2.63, P=0.04** | | | |
|---|---|---|---|
| **Case** | | | |
| | **Negative B19 IgG** | **Positive B19 IgG** | **Total** |
| Count | 59 | 16 | 75 |
| % within case | 78.7% | 21.3% | 100.0% |
| % within Parvo_B19_IgG | 46.5% | 69.6% | 50.0% |
| **Control** | | | |
| | **Negative B19 IgG** | **Positive B19 IgG** | **Total** |
| Count | 68 | 7 | 75 |
| % within control | 90.7% | 9.3% | 100.0% |
| % within Parvo_B19_IgG | 53.5% | 30.4% | 50.0% |
| **Total** | | | |
| | **Negative B19 IgG** | **Positive B19 IgG** | **Total** |
| Count | 127 | 23 | 150 |
| % within Case-control | 84.7% | 15.3% | 100.0% |
| % within Parvo_B19_IgG | 100.0% | 100.0% | 100.0% |

**Association between RBC count and B19 virus by PCR) in thalassemia patients:** The result showed that in BTM patients 26(40%) where found to be positive by qRT-PCR PV19 and had low RBC count and 2 (20%) had normal RBC count. 15 (23.1%%) patients were found to be positive by ELISA with low RBC count and 1 (10.0%) patients were found to be normal count. there were **no significant** P value as shown in figure (1) and figure (2).

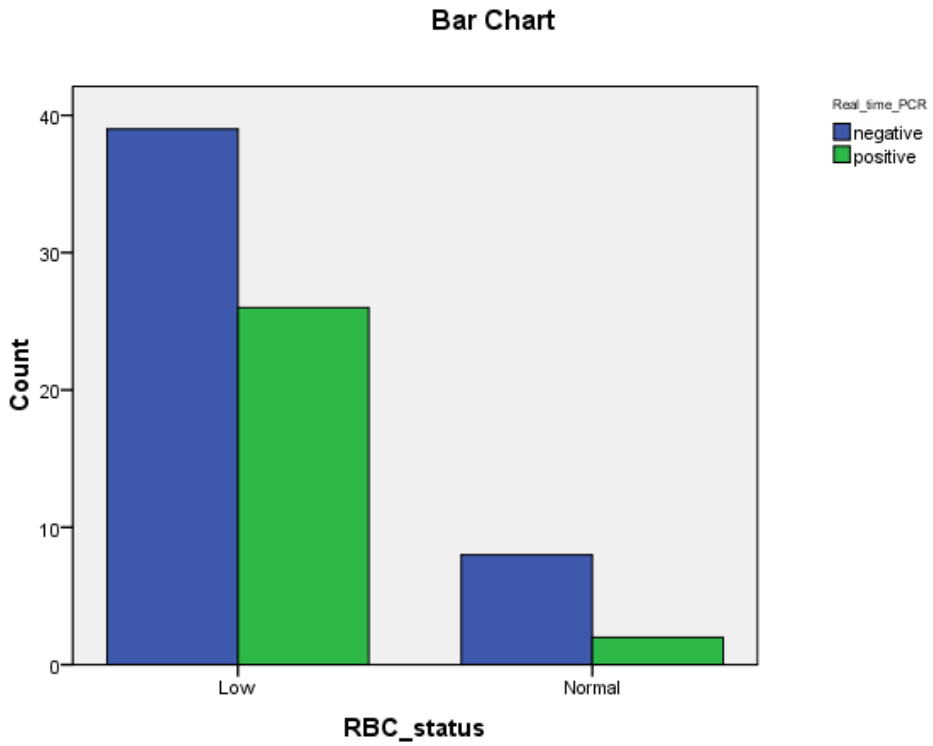
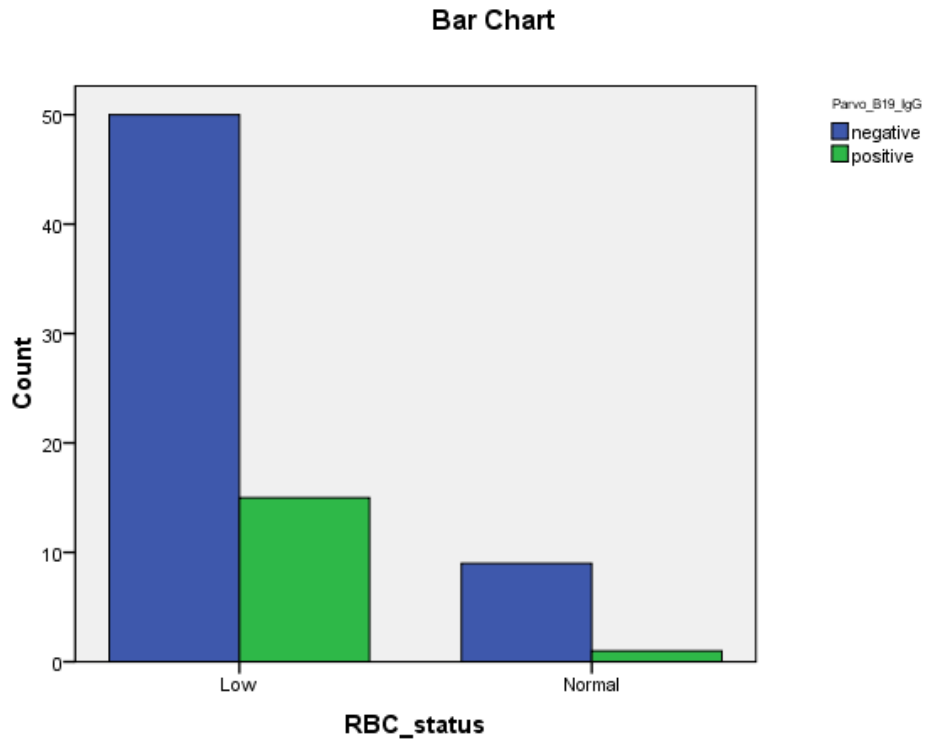



**Figure (1) Association between RBC count and B19 virus (by PCR) in thalassemia patients.**




**Figuer (2) Association between RBC count and B19 virus (by ELISA) in thalassemia patients.**

**Detection of WBC level within Thalassemia -B19 positive byqRT-PCR:** Table (3) show the effect of viral infection detected by qRT-PCR on the WBC level in thalassemia patients, the result indicted the following: No patient had leukopenia, 26(92.9%) had leukocytosis and finally 2(7.1%) with Normal leukocyte number.

In Thalassemia -B19 negative the results were 36 (76.6%) had normal leukocyte number, 9(19.1%) with leukocytosis and 2(4.3%) had leukopenia. these difference were significantly important.

**Table (3) Detection of WBC level within Thalassemia by qRT-PCR**

| **P<0.0001 (Significant)** | | | |
|---|---|---|---|
| **Leukopenia** | | | |
| | **Negative B19 DNA** | **Positive B19 DNA** | **Total** |
| Count | 2 | 0 | 2 |
| % within WBC_status | 100.0% | .0% | 100.0% |
| % within Real_time_PCR | 4.3% | .0% | 2.7% |
| **Normal leukocyte number** | | | |
| | **Negative B19 DNA** | **Positive B19 DNA** | **Total** |
| Count | 36 | 2 | 38 |
| % within WBC_status | 94.7% | 5.3% | 100.0% |
| % within Real_time_PCR | 76.7% | 7.1% | 50.7% |
| **Leukocytosis** | | | |
| | **Negative B19 DNA** | **Positive B19 DNA** | **Total** |
| Count | 9 | 26 | 35 |
| % within WBC_status | 25.7% | 74.3% | 100.0% |
| % within Real_time_PCR | 19.1% | 92.9% | 46.7% |
| **Total** | | | |
| | **Negative B19 DNA** | **Positive B19 DNA** | **Total** |
| Count | 47 | 28 | 75 |
| % within WBC_status | 62.7% | 37.3% | 100.0% |
| % within Real_time_PCR | 100.0% | 100.0% | 100.0% |

**Frequency of WBC level within Thalassemia -B19 IgG positive patients:** From the sixteen thalassemia patients positive by ELISA, 11(68.6%) had leukocytosis, 4(25%) normal leukocyte number and only one (6.2%) with leukopenia .while in negative Thalassemia patients, 34(57.6%) with normal leukocyte number, 24(40.7%) with leukocytosis and only one (1.7%) with leukopenia.

## Discussion

Patients with BTM are at higher risk of acquiring aplastic crisis, sudden worsening of anemia, reticulocytopenia and cessation of erythropoiesis of the bone morrow (transient aplastic crisis) after exposure to parvovirus B19 infection[15].

The present study revealed a high prevalence of the viruses in the thalassemia major patients 37% (28/75) when compared with study done in Iran by Nikoozad Et al[16], in this study the rate was 20% (6/30) .the difference may be due to the sample size of the. A small sample size will not provide a precise and reliable estimation of the prevalence and a larger sample size is needed to obtain a higher confidence level.

Also, our results differ from study done in Hong

Kong[17] a Thailand (5) but agree with Heegaard ET.al.[18].

Seroepidemiologic studies of several countries showed that prevalence of parvovirus B19 infection varied among countries and populations[19]. Our results was lower than a study done in Babylon city/Iraq, Their result was 30.4% positive for Anti-B19 IgG[14], But result is in same rang with that reported by Fayoum study/Egypt[13] of 55 beta thalassemia major patient (Anti-B19 IgG is 18.2%).

Results obtained by Siritantkorn *et al*;[5] from Thailand, of 60 thalassemic major patient (Anti-B19 IgG is 38%).More or less similar rates were found by a study done in USA on patients with sickle cell disease; it showed that 30% had evidence of old PB19 infection at first testing[20].

The difference perhaps related to geographical variation in prevalence of HPVB19 infection[19].

Chronic parvovirus B19 infection was demonstrated by the presence of parvovirus B19 DNA and anti-parvovirus B19 IgG in patients' plasma, while lacking of anti-parvovirus B19 IgM. Thalassemic patients who had parvovirus B19 DNA in their blood plasma but had no anti-parvovirus B19 IgG might be in an early phase of acute parvovirus B19 infection [21].In This search, a total of ((28)) patients were tested positive for B19 infection by using PCR and ((16)) by using ELISA techniques. The discrepancies between DNA and IgG findings refer that searching for specific IgG is a cheap and easy diagnostic tool for basic screening; but the sensitivity of the test may be very low in selected groups of objected. It is advisable that if IgG turns out to be negative, to continue searching for possible B19 infection by employing. About RBC count in this study revealed to 26 from 28 of BTM infected with B19 DNA had low RBC count and 2 were normal count this due to beta thalassemia major anemia that result from the absent synthesis of beta globin chains, leading to excess alpha chains. B-thalassemia generally presents as severe form of the disease because it produces severe anemia in their homozygous and compound heterozygous states[22]. In present study apperaed result Leukocytosis in BTM infected with PB19 DNA (74.3%) and anti PB19 IgG (31.4%), properly due mix infection with unknown bacteria that may be encourage PB19 infection also the Leukocytosis can use as a sign of infection with PB19.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest:** The authors declare that they have no conflict of interest.

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