Human Parvovirus B19 Detection In Thalassemic Patients Associated Serological Responses In Al Najaf Governorate

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Abstract

This study was conducted at from Al zahraa teaching hospital in alnajaf city between August (2022) and January (2023). This study is aimed to detect the prevalence of the Parvovirus B19 on patients with Thalassemia and study the immunological responses of cytokines, IL-6 and C-RP . This study included 105 samples from both genders and different age categories, the samples were divided into two groups: the first group (study group) contained the Thalassemia which included 70 samples and the second group contained 35 control specimens (healthy group). Blood samples for study groups were collected from the patients of the blood diseases center and the Elisa was used to detect the Viral (IgG) and Afias-6 was used to measure the concentrations of IL-6 and C-RP in sera patients. The current study showed that viral infection with thalassemia patients was 58.6% while no viral infections in the control group with a high significance difference of P.v= 0.002. The results also revealed that the viral infection was higher in males than in females with 35.7% and 22.9% respectively with presented high significance difference P.v= 0.005 .The results referred that the highest infection rate in regards to age categories was in age category of 16-26 years old with infection rate of 41.4% and the least was the age category of more than 49 years old with percentage of 0 % with present of significance difference P.v= 0.005 .

The results referred that viral infection rate was a high percentage in the blood transfusion recipients of one blood transfusion routine each 20 days followed by the recipients of one blood transfusion routine every 30 days and lastly the recipients of one blood transfusion routine every 40 days with infection rate of (52.1% , 55% and 50%)

respectively, with presented a high significance difference P. v = 0.006

The results of IL-6 showed presence of high significant difference between patient and control. The mean and standard deviation of IL-6 for patient was 11.78±1.1 pg/ml while for control was 5.29±0.36 pg/ml. and The results of C-RP showed presence of high significant difference between patient and control . The mean and standard deviation of C-RP for patient was 12.7±1.71 mg/dl while for control was 4.61±0.32 mg/dl.

Keywords: Thalassemia, Parvovirus B19 IgG , Interleukin IL-6 , C-reactive protein.

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Introduction

Human parvovirus B19 is a single stranded DNA virus inside the genus erythrovirus of the family Parvoviridae. B19V has a huge range of clinical appearances and diagnosis is fundamentally done by the detection of B19V specific IgM antibodies or B19V DNA[1]. B19V infection generally causes erythema infectiosum, arthralgia, fetal death, transient aplastic crisis in patients with shortened red cell survival, and persistent infection in immunocompromised persons. Less common clinical manifestations include atypical skin rashes, neurological syndromes, cardiac syndromes, and various cytopenia resulting from infection of bone marrow[2,3] . In immunosuppressed patients, B19 infection may persist and lead to pure red cell aplasia, chronic anemia, and less frequently thrombocytopenia, pancytopenia, and neutropenia[4] .

The aim of this study was to detect the prevalence of Human parvovirus B19 antibodies in thalassemia patients in Najaf province, Iraq.

Thalassemia or the Mediterranean anemia is a heredity disorder from parents to offspring through reces from an individual to another from undetectable to lifethreatening. Thalassemia patients are in need of blood transfusion regularly hence, they are at higher risk of viral infection.[5]

Studies have shown that the prevalence of parvovirus B19 infection is higher in individuals with thalassemia compared to the general population. Therefore, individuals with thalassemia need to take precautions to prevent the spread of the virus, such as avoiding close contact with individuals who are known to be infected with parvovirus B19 and practising good hand hygiene, Additionally, individuals with thalassemia who are infected with parvovirus B19 may require close monitoring and supportive care, such as blood transfusions, to manage their anaemia during the acute phase of the infection. [6]

Methods and Materials

The samples were taken in their entirety at Al-Zahra Teaching Hospital in Alnajaf governorate during the period of August (2022) to January (2023). A total of 70 thalassemia patients, with another 35 cases of normal (parentallyhealthy) serving as a control group, with ages ranging from 5 to 64 years. All of the samples were divided into two distinct groups, The initial group contains seventy Samples of thalassemia patients, while the second group contains thirty-five samples and represents as control.

Collecting blood samples:

Blood samples were obtained in the thalassemia unit of Al Zahra teaching hospital in Al-Najaf City, About five millilitres of blood from each patient via vein puncture and collected into two groups of tubes, one of which included anti-coagulant to assess total blood count and the other group, without an anti-coagulant use for serum preparation, were centrifuged for five minutes at four thousand rotations per minute to obtain serum, which was then transported to sterile tubes using a micropipette with sterile disposable tips. The table number was assigned to the samples, and the full samples were kept in the refrigerator in Deep freeze at (-20°C) until use.

ELISA kit for detecting Human parvovirus B19 antibodies (SUN LONG, Chain).

In Immunological Method, Anti-HPV B19IgG was done by using ELISA test kit (SUN LONG, Chain). The test was done according to manufactures instructions.

Kit Procedure

1. Five microliters were added from negative and positive control to the positive and negative Wells respectively.
2. Forty microliters were added sample diluent buffer & 10 µl sample was added.
3. incubated for 30 min at 37C after sealing with closure plate membrane.
4. The concentrated washing buffer was diluted with distilled water
1. (30 times for 96T).
5. wash process was repeated 5 times
6. fifty microliters was added of HRP Conjugate working solution to each well except the blank control well.
7. fifty microliters HRP-Conjugate reagent has been added to each well except the blank control well.

8. Incubation as described in Step 3.
9. Washing as described in Step 5.
10. Coloring: 50 µl Chromogen Solution A and 50 µl Chromogen Solution B were added to each well, which was mixed with gently shaking and incubated at 37 ℃ for 15 minutes. avoided light during colouring.
11. Termination: 50 µl stop solution was added to each well to terminate the reaction. The colour of the well should change from blue to yellow.
12. Read absorbance O.D. at 450nm using a Microtiter Plate Reader. The OD value of the blank control well is set as zero. The assay should be carried out within 15 minutes after adding the stop solution.

Calculation of results

The average value of the positive control was 1.00, whereas the average value of the negative control was 0.10.

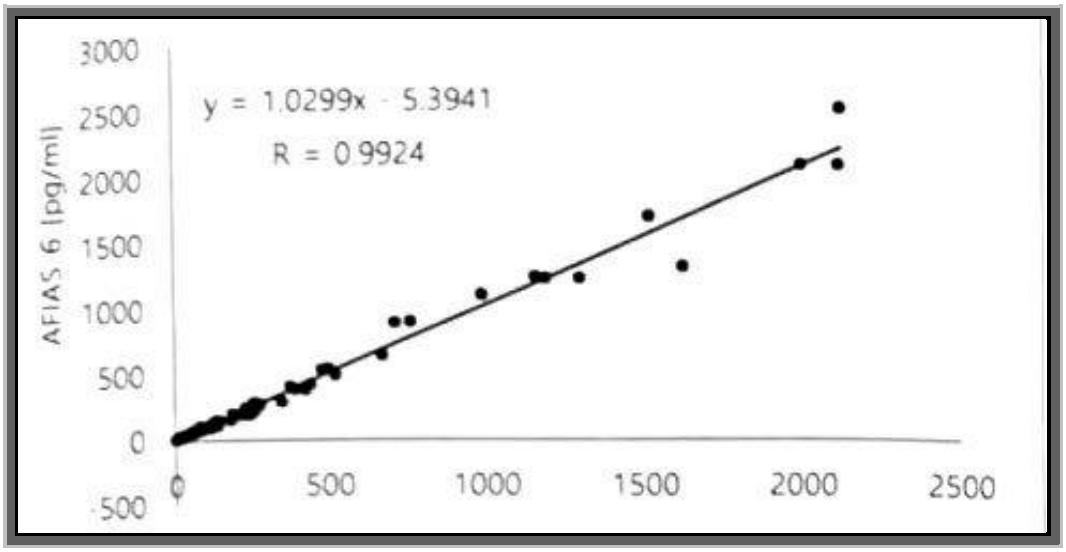
1. The critical value (CUT OFF) was calculated, critical value = average value of negative control + 0.15.
2. The negative judgment was calculated if the OD value < CUT OFF, the sample of Human B19-IgG negative.
3. The positive judgment was calculated if the OD value > CUT OFF the sample of Human B19-IgG positive.

Kit for IL-6 estimation (Boditech, Korea).

Principle

This test used a sandwich immune detection method ; the detector antibodies in buffer bind to the antigen in the sample , forming antigen-antibody complexes and migrated onto nitrocellulose matrix to be captured by the other immobilized-antibodies on test strip .

More antigen in the sample will form the more antigen-antibody complexes which lead to stronger fluorescence signal by detector antibodies , which is processed by instrument for AFIAS tests to show IL-6 concentration in the sample. Figure (1)



Figure(1) Concentration of IL-6

INTERPRETATION OF TEST RESULT

The instrument for AFIAS tests calculates the test result automatically and displays IL - 6 concentration of the test sample in terms of pg / mL

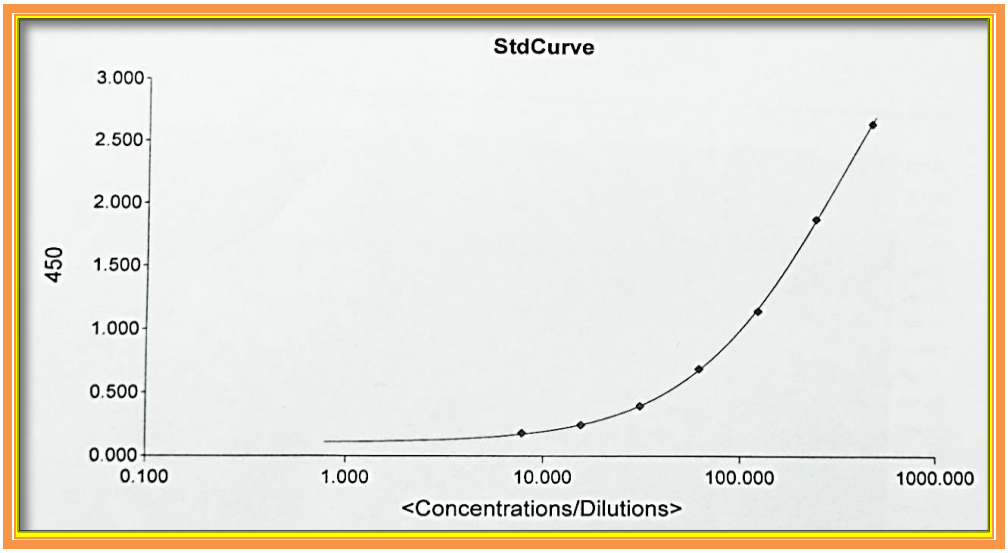
* Reference value : 7 pg / mL
* Working range : 2-2,500 pg / mL .

Kit for C-RP estimation (Boditech, Korea).

Principle

This test used a sandwich immune detection method ; the detector antibodies in buffer bind to the antigen in the sample , forming antigen-antibody complexes and migrated onto nitrocellulose matrix to be captured by the other immobilized-antibodies on test strip .

More antigen in the sample will form the more antigen-antibody complexes which lead to stronger fluorescence signal by detector antibodies , which is processed by instrument for AFIAS tests to show C-RP concentration in the sample. Figure(2)



Figure(2) Concentration of C-RP pg/ml

INTERPRETATION OF TEST RESULT

The instrument for AFIAS tests calculates the test result automatically and displays C-RP concentration of the test sample in terms of pg / mL .

* Reference value : < 6 pg / mL .
* Working range : 0.6 , 200 pg / mL .

Statistical Analysis

This means SE was used to express all values. The data were analyzed using the computer programs SPSS (T-test) version 17 and Microsoft Excel, with a P value of less than 0.05 (p 0.05) being used as the lowest level of significance [7].

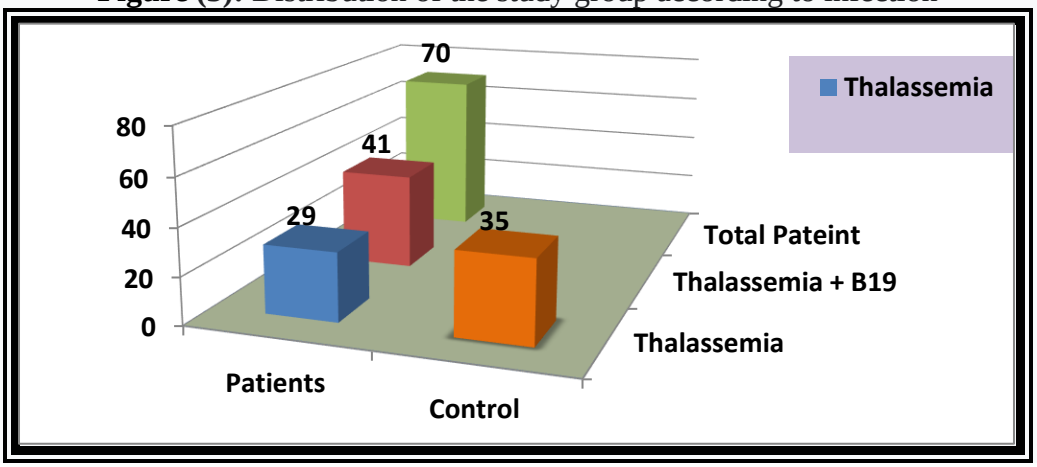
Result and discussion

The study group's distribution according to infection

The samples were taken total of 70 thalassemia patients, with another 35 cases of normal (parentallyhealthy) serving as a control group, with ages ranging from 5 to 64 years.

The results showed positive sera B19 of 41 (58.6%) and negative sera 29(41.4%) in the studied groups and the control 35 patient all negative (0 %) .

Figure (3): Distribution of the study group according to infection



The Distribution of age for study groups

Figure(4) showed the distribution of age for study groups, which shows an increase in infection of parvovirus in Young age between (16-26) years , Three of twelve patients in an Iranian study conducted in 2015 tested positive for the virus, for a prevalence rate of 25%; this age range was found to be particularly at risk.1 out of 8 individuals (12.5%) tested positive for the virus, suggesting that infection begins before the age of 20 [8] .

Parvovirus infection in adults can be more serious than in children, especially for individuals with weakened immune systems or underlying medical conditions, as the virus can cause fetal anaemia and hydrops fetalis, a potentially life-threatening condition in the fetus.

Figure (4): Distribution of the study group according to age.

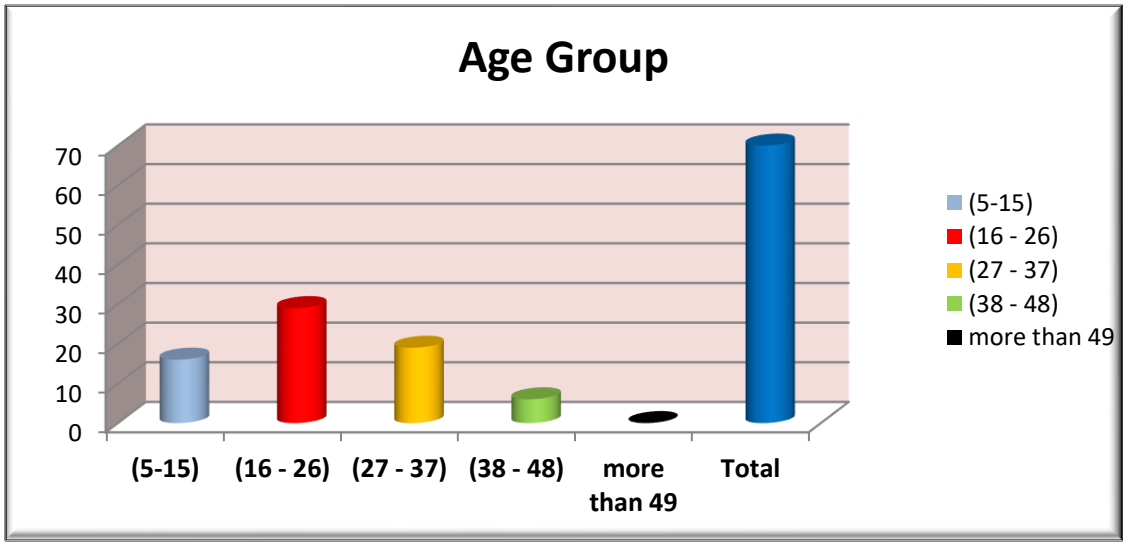


Table (1): Distribution of the study group according to age

Age group	Study group No.	Per cent %	Control No.	Per cent %
5-15	16	22.9 %	1	2.9 %
16-26	29	41.4 %	5	14.3 %
27-37	19	27.1 %	12	34.3%
38-48	6	8.6 %	11	31.4 %
More than 49	0	0%	6	17.1 %
Total	70		35	

Cytokines concentrations

Interleukin 6 (IL-6) measurement level

The results of IL-6 showed the presence of a highly significant difference between the patient and control. The mean and standard deviation of IL-6 for the patient was 11.78±1.1 % while for the control was 5.29±0.36 %. Figure (5) IL-6 show significant differences ($p < 0.05$) .These hematological findings were significantly associated with-B19V infection in previous studies this agrees with Kerr [9], another study in Sweden found that IL-6 concentration increase in acute infections and normal levels in chronic infections. concentration of IL-6 is affected by several factors including inflammations, iron levels, Lombardi referred that IL-6 concentration is low or undetectable in thalassemia patients because they suffer from low CD4+ activity which stimulates IL-6 secretion through IL-2, or because IL-6 is active but with low concentrations.[10]

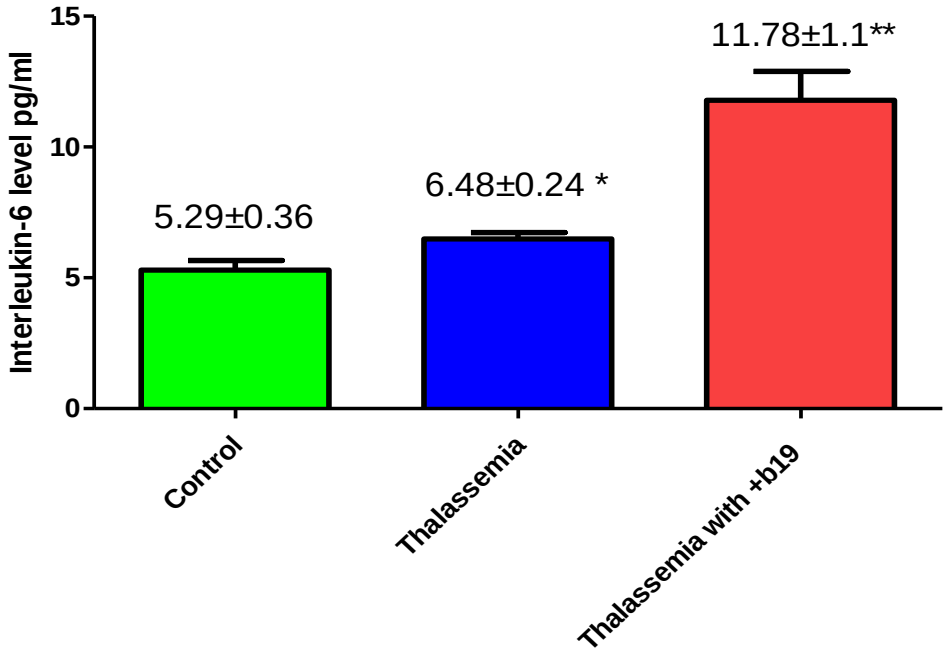


Figure (5): The comparison of IL6 level in the serum between Thalassemia patient , Thalassemia with +b19 patient compared with control group.
The existence of the star indicates significant **(p < 0.05)**

Table (2) The correlation between il6 with Other biomarkers.

correlation coefficient	R
il6 with wbc	-0.24
il6 with lymphocyte	-0.39
il6 with netrof	+0.4
il6 with hb	+0.14
il6 with rbc	-0.42
il6 with plate	-0.25

C-reactive protein (C-RP) measurement level

The results of C-RP showed the presence of a highly significant difference between the patient and control. The mean and standard deviation of C-RP for the patient was 12.7±1.71 % while for the control was 4.61±0.32%. Figure (6) C-RP show significant differences ($p < 0.05$) .These hematological findings were significantly associated with-B19V infection in previous studies

In normal individuals infected, either naturally or experimentally, with B19 parvovirus antibodies to virus form rapidly, usually within a week of inoculation [11]. Fifth disease is itself an antibody-mediated, probably immune complex disorder, occurring at the time that antibodies are present in the circulation and seldom associated with the presence of virus in serum [12]. We have observed classic symptoms of fifth disease in immunosuppressed, persistently infected patients following immune plasma [E.Fr.] or immunoglobulin [E.Fo.]

These findings corroborate previous findings that increase C-RP levels [13]. While disagreeing with[14], who suggested that low levels of C-RP in the serum of thalassemia patient could be linked to a lack of inducing effects for T-cell and B-cell growth.

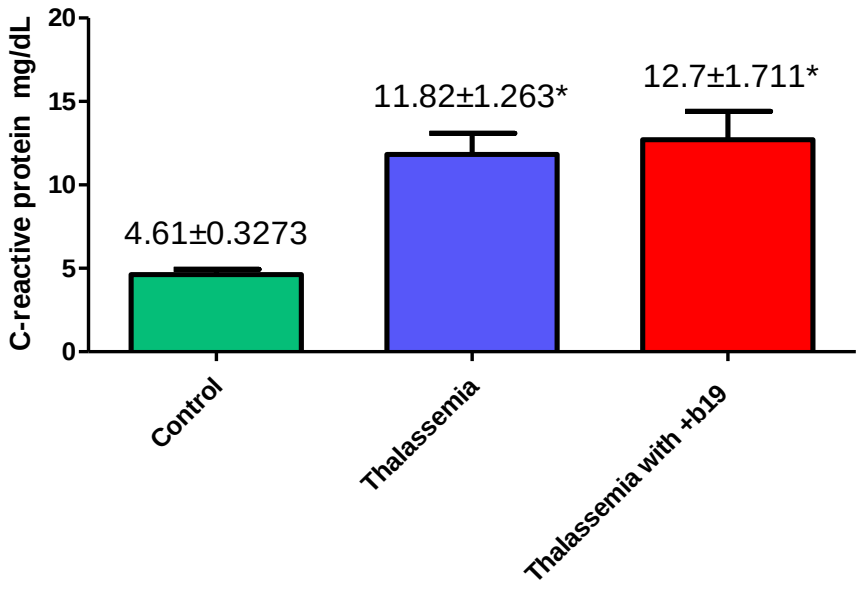


Figure (6): The comparison of c-reactive protein level in the serum between Thalassemia patient , Thalassemia with +b19 patient compared with control group.
The existence of the star indicates significant **($p < 0.05$)**

Table (3) The correlation between C-reactive protein with Other biomarkers.

correlation coefficient	R
C-reactive protein with CXCL10	-0.13
C-reactive protein with TNF	-0.31
C-reactive protein with il6	+0.38
C-reactive protein with wbc	-0.54
C-reactive protein with lymphocyte	+0.53
C-reactive protein with netrof	-0.54
C-reactive protein with hb	+0.51
C-reactive protein with rbc	-0.489
C-reactive protein with plate	+0.32

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