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A Study of the Prevalence of Human Parvovirus B19 in Thalassemia Patients in the Al Najaf Governorate

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Abstract
The purpose of this study was to examine the incidence of human parvovirus B19 in thalassemia patients between August 2022 and January 2023 at the Al-Zahra teaching hospital in the Al-Najaf administration. The ages of the 70 thalassemia patients and the 35 normal (parentally healthy) cases included as a control group range from 5 to 64 years. B19 is a newly discovered agent that can spread through blood transfusions. Patients with thalassemia are at risk for B19 infection since they undergo a high transfusion regimen; B19 is resistant to viral inactivation treatments; and donor units are not checked for B19; nonetheless, there have been very few research conducted on this topic, and none in Asia. Therefore, researchers set out to examine the spread of B19 infection among multi-transfused thalassemia patients.

Keywords: Thalassemia, Parvovirus B19, CBC, Lymphophil, RBCs, WBCs, Hemoglubin

Introduction

The parvoviruses (including B19 and HPV) are a family of icosahedral-capped, single-stranded DNA viruses that range in size from 18 to 26 nm. There are 60 different structural viral proteins (VP) that come together to form the capsid, and they are of two different sizes: a small structural protein called VP1 that accounts for about 5% of the capsid, and a large structural protein called VP2 that accounts for the rest [1].

About 5,596 nucleotides are found in the Parvovirus B19 genome [2]. Infectious erythema, arthropathy, fetal hydrops, and transient aplastic crises are only few of the diseases that can be spread and caused by blood transfusions or through the air. Infectious microorganisms that may infect a wide variety of cells and hosts, from insects to mammals. Human parvovirus B19 and the recently identified human bocavirus are the only two human diseases in the parvovirus family [3].

Bone marrow infections caused by B19V are caused by a member of the Erythroviruses genus, which is considered to be highly harmful for humans. Airborne droplets, blood, blood transfusions, and maternal infection are all potential routes of prenatal parvovirus B19 transmission [4]. Children born with coagulation problems, adults with thalassemia and haemophilia who have received several blood transfusions, and people who have received factor VIII concentrates are all at risk for contracting parvovirus B19 [5].

The inability of the body to manufacture haemoglobin, the protein responsible for transporting oxygen in the blood, lies at the root of thalassemia, a genetic blood illness. Thalassemia patients have trouble getting enough oxygen to their cells because they generate haemoglobin with a defect. Symptoms include lethargy, weakness, pallor, jaundice, and stunted development. Symptoms can be quite minor to extremely debilitating, and in extreme circumstances even fatal. Blood transfusions, iron chelation therapy, and bone marrow transplants are among potential treatments for thalassemia. The lack of haemoglobin protein in RBCs is the pathophysiological basis for thalassemia, which is classified as a hemoglobinopathy. Specific mutations in the genes encoding the alpha and beta globin chains of haemoglobin's quaternary structure are the root cause of alpha and beta thalassemia [6].

Researchers have found that people with thalassemia had a higher incidence of parvovirus B19 infection than the general population. Therefore, people with thalassemia should take measures to limit the transmission of the virus, such as washing their hands often and staying away from people known to be infected with parvovirus B19.

During the acute phase of parvovirus B19 infection, people with thalassemia may need close monitoring and supportive therapy, such as blood transfusions, to manage their anemia [7].

Methods and Materials

All of the samples were collected between August 2022 and January 2023 at Al-Zahra Teaching Hospital in Alnajaf governorate. There were a total of 70 thalassemia patients, ranging in age from 5 to 64, and 35 normal (parentally healthy) cases used as a control group. The samples were split into two groups, with the first consisting of seventy samples from people with thalassemia and the second comprising 35 samples serving as a control.

Collecting Blood Samples

The thalassemia unit of Al Zahra teaching hospital in Al-Najaf City collected the blood samples. Blood was drawn from patients' veins and separated into two groups: one with an anti-coagulant for a total blood count and the other without; the latter group was centrifuged for five minutes at 4,000 revolutions per minute to separate the serum, which was then transferred to sterile tubes with a micropipette fitted with a sterile disposable tip. Each sample was given a unique table number, and until they were needed, they were held in the freezer at a temperature of -20 degrees Celsius.

Parvovirus IgG Elisa kit (SUN LONG, Chain) for Measuring Human Serum Samples

Kit Procedure

1. Fifty microliters each were injected into the control Wells and the experimental Wells.
2. A total of 40 l of sample diluent buffer and 10 l of sample were added.
3. 30 minutes of 37 degrees Celsius incubation after the closure plate membrane was applied.
4. The concentrated washing buffer was diluted with distilled water (30 times for 96T).
5. wash process was repeated for 5 times
6. Each well, besides the blank control well, had fifty microliters of HRP Conjugate working solution injected to it.
7. Each well, besides the blank control well, has fifty microliters of HRP-Conjugate reagent added to it.
8. Incubation in the third stage, as described.
9. Incubation in the third stage, as described.
10. Every well had 50 l of chromogen solution A or B poured to it, and then the whole thing was gently shook before being incubated at 37 °C for 15 minutes to add colour. I used a black and white crayon.
11. The reaction was stopped by adding 50 l of stop solution to each well. There needs to be a transition from blue to yellow in the well.
12. Absorbance optical density reading at 450 nm with a microtiter plate reader. In the blank control well, we've specified an OD value of 0. Within 15 minutes of administering the stop solution, the assay should be completed.

Calculation of Results

Positive controls had an average value of 1.00, whereas negative controls had an average value of 0.10.

1. Using the formula critical value = mean value of negative control + 0.15, the critical value (CUT OFF) was determined.
2. If the optical density (OD) value was less than the cutoff (CUTOFF), the sample was deemed to be Human B19-IgG negative.
3. The positive judgment was calculated if the OD value > CUT OFF the sample of Human B19-IgG positive.

Complete Blood Count (CBC)

DxH500 is widely used to count as well as identify haematological parameters including Hb, HCT, WBCs, RBCs and PLt. The 3 major physical technologies used with the haematological analyzer are electric impedance, cytometry of flow and cytometry of fluorescent flows. They are used in a mixture of chemicals and reagents to lyse or change blood cells to extend the parameters of measurement [14].

Procedure

1. Two ml of whole blood was taken and it was placed in an anticoagulant tube.
2. The blood was mixed well with a shaker for at least 5 minutes.
3. An automated equipment drew blood and displayed the results (WBC differential, Hb, RBC, and PLT) in a flash.
4. The automated equipment included a printer, so the CBC report could be printed out.

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 [15].

Result and Discussion

The Study Group's Distribution According to Infection

Seventy thalassemia patients and thirty-five normal (parentally healthy) cases ranging in age from five to sixty-four provided samples.

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 %).

Fig 1 Distribution results of the control group and the studied groups.

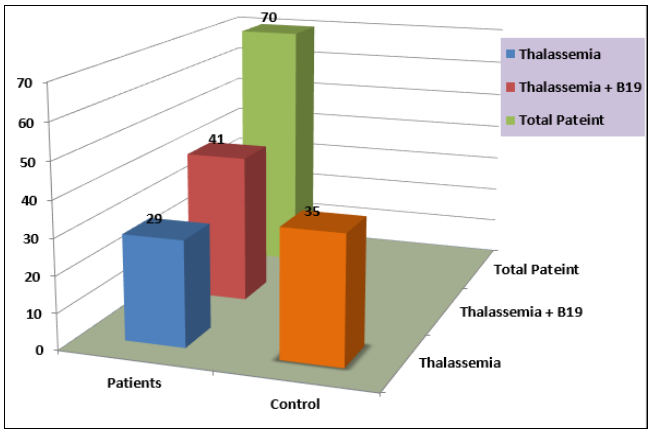

Fig 1: Distribution of the study group according to infection

The Distribution of Age for Study Groups

Fig (2) 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 [16].

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.

Table 1: Distribution of the study group according to age

Age group	Study group No.	Percent %	Control No.	Percent %
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	

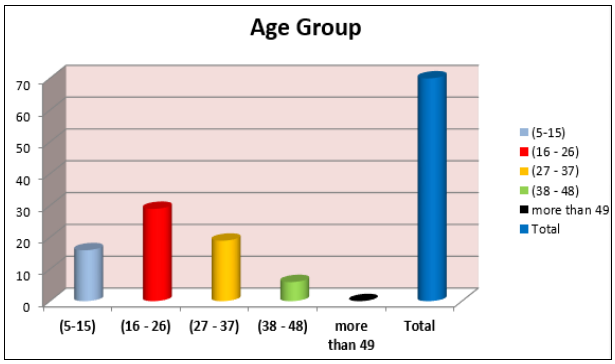

Fig 2: Distribution of the study group according to age

Distribution of Study Groups by Sexes

Table 3 shows the prevalence of the virus in males more than in females, as the virus was found in 25 infected males out of a total of 70 patients, at a percent of 35.7%, and females had 16 infections out of a total of 70 patients, with a percent of 22.9%, with a significant difference of p.v=0.005. Consistent findings were found between the current study and one from Syria in 2015. When the study indicated a greater infection rate in males than females, with 10 of Infected patients out of a total of 101 patients (10% of the total), and 8 infections out of a total of 99 patients (8% of the total) [17].

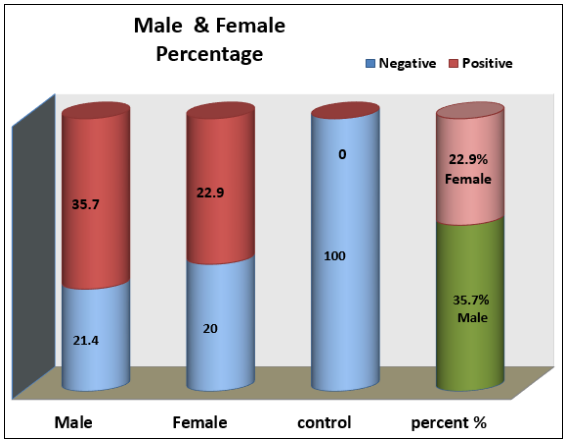

Fig 3: Study Groups Distribution According to Sex

Results of Hematology Parameters

White blood cell (WBC) counts were found to have dropped significantly (6.17 0.97 mg/dl), according to the results of this study. Lymphocyte counts were significantly higher in those with the +ve parvovirus than in the control group (7.160.24 mg/dl vs 37.821.4 mg/dl), and both hemoglobin and red blood cell counts were lower in those with the virus (8.270.26mg/dl vs. 13.850.26mg/dl and 4.610.08 mg/dl, respectively) than in control patients.

Healthcare providers frequently request both the B19 (IgG) and CBC tests to aid make diagnoses. However, the rationale for performing these tests will vary from person to person based on their symptoms and health background, and the results should be interpreted by a medical practitioner. Red blood cells, white blood cells, hemoglobin, and platelets are all part of a complete blood count (cbc). An individual's overall health, such as the existence of infections or blood diseases, can be determined from the results of a CBC.

Measurement of Lymphocyte

The results of lymphocytes showed the presence of a highly significant difference between the patient and control. The mean and standard deviation of lymphocyte for the patient was 42.9±1.6 % while for the control was 37.82±1.4 %. Fig (4)

Lymphocyte show significant differences ($p < 0.05$). Previous research has found a strong correlation between these hematological symptoms and B19V infection [18].

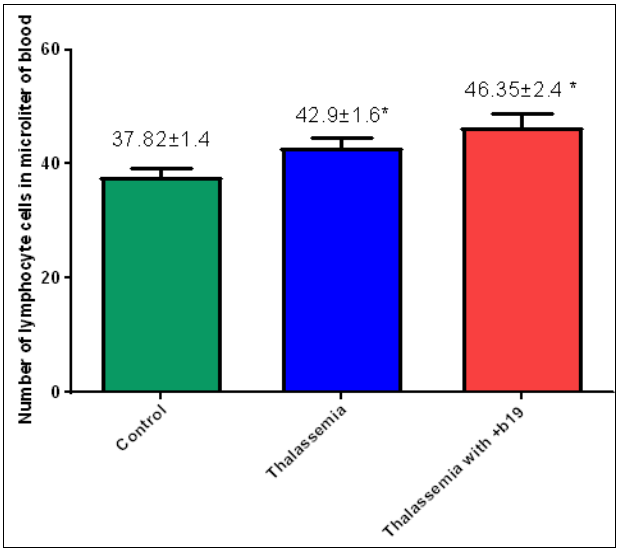

Fig 4: The comparison of Lymphocyte cell numbers in the blood between Thalassemia patients, Thalassemia with +b19 patients compared with the control group. The existence of the star indicates significant ($p < 0.05$)

Table 2: The correlation between lymphocytes with other biomarkers

Correlation Coefficient	R
lymphocyte with the net of	-0.98
lymphocyte with hb	+0.23
lymphocyte with rbc	+0.33
lymphocyte with plate	+0.49

Measurement of Red Blood Cells

The results of RBCs showed the presence of a highly significant difference between the patient and control. The mean and standard deviation of RBCs for the patient was 2.7±0.08 while for control was 4.61±0.08. Fig (5)

Genetic mutations in the hemoglobin gene leads to a decrease in the size of RBC to be smaller (microcytic) and lower dye (hypochromic), then Hb as well as HCT% have been declined. However, the RBC count could be unequally elevated compared to a measure of anemia which can produce very little concentrations of MCV, MCH and MCHC [19].

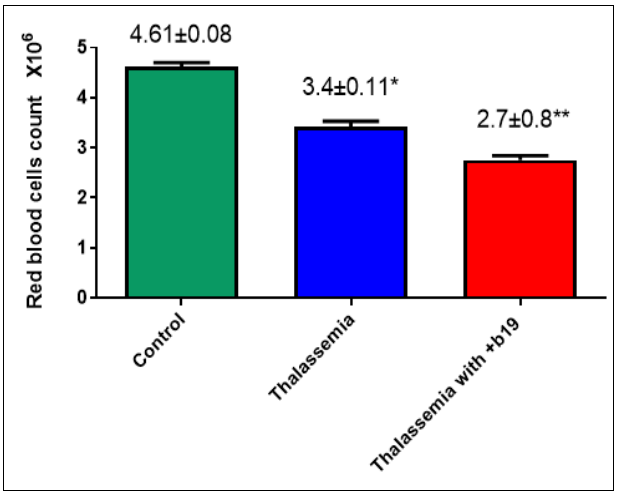

Fig 5: The comparison of R.B.Cs in the blood between Thalassemia patients, Thalassemia with +b19 patients compared with the control group. The existence of the star indicates significant ($p < 0.05$)

Measurement of White Blood Cells

The results of WBCs showed the presence of a highly significant difference between patient and control. The mean and standard deviation of WBCs for the patient was 6.17±0.97 while for control was 7.16±0.24. Fig (6)

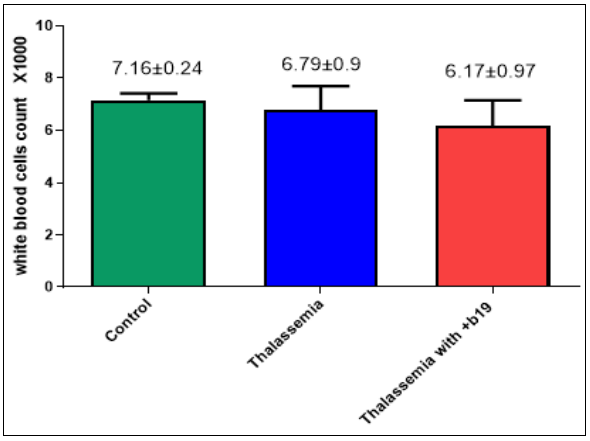

Fig 6: The comparison of WBCs count in the serum between Thalassemia patients, Thalassemia with +b19 patients compared with the control group. The existence of the star indicates significant ($p < 0.05$)

In most cases, leukocytes (white blood cells) are unaffected by a parvovirus infection. However, the body's immune reaction to the infection can sometimes cause a transient drop in white blood cell count. In its fight against the virus, the immune system may temporarily dampen white blood cell production or alter the distribution of these cells throughout the body. The decline in white blood cells seen in parvovirus infection is usually just transitory and goes away when the body heals. The immune system typically eliminates parvovirus infections on its own, so no special treatment is usually necessary [20].

Measurement of Haemoglobin (Hb)

The results of Hb showed the presence of a highly significant difference between the patient and the control. The mean and standard deviation of Hb for the patient was 8.27±0.26 g/dl while for the control was 13.85±0.26 g/dl. Fig (7) and Table (3).

Decreases in Hb level were found to be predictive of B19V, and B19V infections were found to reduce reticulocyte counts Hb level more than any other component. This finding corroborates earlier reports from Papua New Guinea [21].

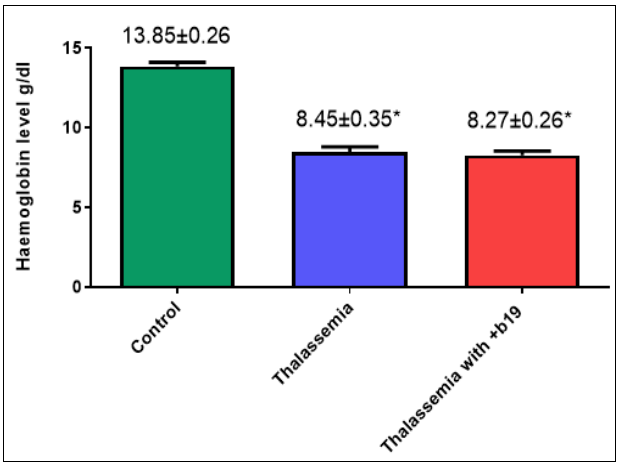

Fig 7: The comparison of Hb level in the blood between Thalassemia patients, Thalassemia with +b19 patients compared with the control group. The existence of the star indicates significant ($p < 0.05$)

Table 3: The correlation between Hb with Other biomarkers

Correlation Coefficient	R
Hb with rbc	+0.91
Hb with plate	-0.23
RBC with plate	-0.005

Conclusion

The detection of parvovirus B19 in the thalassemia serum was higher than in healthy control, and this increases the possibility of this virus as one of the major viral factors causing anaemia. The efficiency of the B19 in thalassemia patients is higher than in normal people because in thalassemia patients are leads to many defects in Hb, RBCs and WBCs.

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