Inspection of Parvovirus B19 in Patients with Beta Thalassemia Major

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***Abstract*: Parvovirus B19 (B19V) is a small, unwrapped, one strand DNA virus with a 18-26 nm icosahedral capsid. Blood transfusions as one method of treating thalassemia is also the main method of transmitting B19V from infected patients into thalassemia patients. Aim of Study: Inspection of Parvovirus B19 in patients with beta thalassemia by serological and molecular detection.Methods: A case-control study included 120 patients with beta thalassemia major and 50 healthy persons as (control). Whole blood samples were collected and divided into two parts, one for serological diagnosis by detection of anti-B19V IgM and IgG antibodies by ELISA and the other part for detection of *NS1* gene of B19V by conventional Polymerase Chain Reaction (PCR) technique. Results: Serological results revealed that 2 (1.6 %) and 36 (30%) of patients with beta thalassemia major were positive for anti-B19V IgM and IgG antibodies respectively. *NS1* gene of B19V was detected in 15 (12.5%) of patients while the result was negative (0.0%) for control. Statistically there was high significant difference between the patients and control regarding presence of anti-B19V IgG antibody and detection of B19V *NS1* gene. Conclusion: The results of this study suggest increasing of consequences of beta thalassemia major in patients infected with B19V.**

***Keywords*: Beta thalassemia major, ELISA, Parvovirus B19, PCR.**

I. INTRODUCTION

Parvovirus B19 (B19V) is a small, non-enveloped, with genome consist of one strand DNA enclosed by 18-26 nm icosahedral capsid. Capsid structural viral proteins (VP) consists of two types, a small structural protein VP1, which makes up about 5% of the capsid, and a large structural protein VP2, which makes up the bulk of the total capsid composition[1].

B19V transmission occurs predominantly via airborne droplets, transfusion of blood[2,3] or plasma or even during maternal infection [4,5]. There were many documentations involving the role of B19V in different hematological disorders including but not limiting to hemophilia A, congenital blood clotting deficits, and multi-transfusion hemophilia[6,7] .

B19V infections are frequent and widespread, and can lead to a wide variety of clinical findings based on the hematological and immunological condition of the patient[7,8]. Persistent viremia, anemia and fetus's hydrops or intra-uterine death are the main findings in immunocompromised infected fetuses[9,10]. In immunocompetent people, B19V infection can be asymptomatic or mild leading to infectiosum erythema in children and arthritis in adults[11]. The binding of B19V with glycosphingolipid globoside on erythroid progenitor cell lead to death of target cell[12].

Blood disorder patients, particularly those with persistent hemolytic anemia like thalassemia, are at risk of severe clinical diseases[13,14]. In such cases, the level of erythroid progenitor cell production raises to recompense for RBC degranulation[15]. B19V infection can inhibit erythropoiesis as well as lead to acute erythroblastopenia, often called transient aplastic crisis[15,16]. This transitory pause in the development of RBCs happens only in thalassemia patients given the short life-span of the red blood cells, In such patients, the risk of transmission of B19V increases[15]. The aim and objective of the present study is investigating of Parvovirus B19 in patients with beta thalassemia major through serological and molecular detection methods.

II. STUDY GROUPS AND METHODS

A- Study group

A case-control study included 120 patients with beta thalassemia major they referred to the Center of Hereditary Blood Diseases in Al-Nasiriya city, and 50 healthy persons as (control) within period from September 2019 to December 2019. Five milliliter of vein blood had been collected from patients and control then divided into two parts, one part in EDTA tubes and stored in freezer at -20 C^{o} until use for viral DNA extraction and performance of conventional PCR and the second part was collected in gel tube for serum separation and serological detection achieved by ELISA.

B- Hematological Parameters

Different hematological parameters were measured in the present study including Hemoglobin (Hb), Hematocrit (HCT%), Mean corpuscular hemoglobin (MCH), Mean concentration hemoglobin of cell (MCHC) and Mean cell volume (MCV) based on electric impedance and flow

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cytometry of hematological analyzer (Sysmex XP-300, Germany). In addition to measure Adult hemoglobin (HbA2) and Fetal hemoglobin (HbF) through HPLC technique basically on charge for separation of hemoglobin by automated Variant Hemoglobin Testing System (Variant II Beta Thalassemia Short Program, Bio-Rad Laboratories, Japan).

C- Serological Diagnosis

Detection of anti-B19V IgM and IgG antibodies were performed using indirect ELISA and follow kit instructions (Demeditec Diagnostics GmbH, Germany).

D- Detection of B19V *NS1* gene

Viral DNA was extracted from the whole blood samples was performed using the QIAamp Blood Mini nucleic acid extraction kit (Qiagen/Germany), according to the manufacturer's instructions and the quality and purity of extracted DNA were checked by using the Nano drop that reads the absorbance of extracted DNA at (260/280nm). The extracted DNA was stored at -70°C until used for conventional PCR.

Conventional polymerase chain reaction (PCR) assay was used according to for detection of B19V *NS1* gene. Positive and negative controls were used to verify the absence of PCR inhibition. The materials required for this methods obtained in master mix (Accupower® PCR PreMix kit) from Bioneer/Korea. Each reaction consisting of 20µl total volume include 5µl of extracted DNA, 2µl of forward primer 5'GCTTGGTGGTCTGGGATGAA3' (1715-1734) and 2µl reverse primer 5'CAGGCACAGCTACACTTCCA3' (1834-1815) and 11µl of nuclease free distilled water.

Thirty-five cycles were used in conventional PCR with the following program: 95°C for 1 minute, 55°C for 1.5 minutes, and 72°C for 1 minute, then last extension at 72°C for 7 minutes[17], the PCR product analyzed by gel electrophoresis in 2.0% agarose gel and evaluated on the basis of the position of the DNA marker bands on the gel, the amount of product size was 120 bp.

E- Statistical Analysis

Statistical analysis utilizing SPSS Statistics version 20. Data with numbers were expressed as mean, standard deviation and median. Qualitative data expressed percentage. Chi-square test or Fisher's exact test was used to examine the relation between qualitative variables.

III. RESULTS

The present study comprises 170 individual dividing into 120 thalassemia patients and 50 healthy control. The thalassemia patients included 67 (55.8%) female and 53 (44.2%) male, whereas the control group comprised 27 (54.0%) female and 23 (46.0%) male. Age mean for patients was 11.09 years old while was 11.22 years old for control and statistically there was no significant difference has been approved between patients and control regarding the age and the sex as presented in table 1 and table 2 respectively.

Hematological parameters including Hb, HCT%, MCH, MCHC and MCV in the present study showed significant decreasing compared with control as presented in table 3. The results of other parameters including HbF% (mean ± SD) (60.38 ± 27.76) prove the presence of thalassemia disease in those patients and lack in the control, while HbA2 value (4.55 ± 1.82) appeared within normal value in both groups of this study (table 4). The percentage of Parvovirus B19 infection in the thalassemia patients was 15 (12.5%) while all healthy controls were negative and the Chi-square test showed a significant difference between the thalassemia and control groups (table 5).

Table 1: Study groups distribution according to sex.

		Study groups	
		Patient	Control
Sex	Female	67	27
		55.8%	54.0%
	Male	53	23
		44.2%	46.0%
Total		120	50
		100.0%	100.0%
P value		0.479	

NS: No significant difference

Table 2: Distribution of the study groups according to age

		Study groups	
		Patient	Control
		120	50
Age (year)	Mean	11.09	11.22
	Standard Deviation	7.12	7.85
	Median	10.00	10.00
p value		0.917^{NS}	

NS: No significant difference

Table 3: Hematological parameters of thalassemia patients and control group

Hematological Parameter	Mean ± SD		P Value
	Patients Group	Control Group	
Hb (mg/dl)	7.09 ± 1.19	13.13 ± 1.48	< 0.001
HCT%	23.58 ± 7.65	40.20 ± 3.76	< 0.001

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MCH (pg)	23.56 ± 6.91	27.86 ± 1.74	< 0.001
MCHC (gm)	32.22 ± 3.97	35.18 ± 1.67	< 0.001
MCV (fl)	64.78 ± 13.56	86.68 ± 5.97	< 0.001

Table 4: Fetal Hemoglobin (HbF) and adult hemoglobin (HbA2) in thalassemia patients and control groups

Type of Hemoglobin	Mean ± SD		P value
	Patients group	Control group	
HbF	60.38 ± 27.76	0.26 ± 0.13	<0.001
HbA2	4.55 ± 1.82	3.30 ± 0.38	<0.126

Table 5: Parvovirus B19 infection frequency in thalassemia patients and control groups

		Study groups	
		Patient	Control
PCR	Positive	15	0
		12.5%	0.0%
	Negative	105	50
		87.5%	100.0%
Total		120	50
		100.0%	100.0%
P value		0.004*	

*P< 0.05: (high significant level)

IV. DISCUSSION

Many and different studies indicated to the correlation of different viruses with different genetic and autoimmune diseases and the ability of these viruses to evade the immune response and possess wide spectrum tropism to many critical human cells, for example, studying the role of human Cytomegalovirus in diabetes mellitus[18] , Epstein-Barr virus association with leukopenia and hematological disorders[19] and pathogenesis of Parvovirus B19 in different diseases like arthritis and cardiomyopathies[20] .

Demographic data of the present study showed none of the significant difference was detected between patients with thalassemia major and control regarding the age and the sex also the statistical analysis presented no effect of the age and sex on the infection with B19V. Smaya and Buhtori, 2015 reported that certain age group affected by infection with B19V and there was no effect for sex on such infections[21]. Another study perfomed by Nikoozad *et al*., 2015 presented that the age has no role in the detection of B19V genome in patients with thalassemia major[17].

Hematological parameters had been decreased significantly in the patients with thalassemia major in the present study and this was convenient with of Giardne *et al*., 2007 which showed these tests declined in thalassemia patients and they were used in the primary clinical diagnosis and differentiate between thalassemia patients and control[22.] Genetic mutations in the hemoglobin gene lead to decrease in 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[23] .

The current study tested two of the most important types of hemoglobin which used in the diagnosis of thalassemia, one of them was HbF and the results showed highly significant difference (P < 0.001) between patients and control groups. HbF is a confirmatory and differential test to distinguish beta thalassemia major. During the first 6 months after birth, the fetal hemoglobin HbF ($\alpha 2\gamma 2$), γ chain was produced and gradually the production decreased and is replaced by β chain synthesis. A complex mechanism involved in γ to β switching, mutations in the gene that codes for either factor results in elevated HbF[24]. The another hemoglobin was HbA2 and the results showed that the percentage for the patients were close to that of control and there was no statistical difference between them (P < 0.126). Khan and Shaikh, 2020 showed in their study that HbA2 could be within normal to mildly elevated in Beta-thalassemia major[25], and this was convenient with the result of the present study.

Regarding serological diagnosis of B19V, the presented study founded that the percentage of positive anti-B19V IgM antibody in the serum of thalassemia patients was low (1.7%) and was negative in control samples (0.0 %). Statistically there was no significant difference between the patients and control (P < 0.497).

A study conducted in Kurdistan of Iraq showed percentage of anti-B19V IgM antibody 3.7% in thalassemia patients and this was very close to the result of the present study[26]. On the other hand, there was a study performed in Al-Hilla city at the middle of Iraq showed relatively high percentage (13%) of anti-B19V IgM antibody in patients with thalassemia major[27]. The cause of low percentage of anti-B19V IgM antibody in the present study may be due to the short life span of IgM antibody. The anti-B19V IgM antibody present in the fifth day of infection and reach to the optimal titer at day 15 then the antibody starts to decline until vanished after 40 days of infection. Detection of anti-B19V IgM antibody indicated to a recent or acute infection. Acute B19V infection is commonly asymptomatic or may induce an influenza-like disease[28] . In related to anti-B19V IgG antibody, the results founded that the percentage of positive anti-B19V IgG antibody in the serum of thalassemia patients was high (30.8%) while the control result was (0.0%). Statistically there was highly significant difference between the patients and control (P<0.001).

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This study was agreed with the study conducted in Al-Hilla city at the middle of Iraq which gave 30% positive for anti-B19V IgG antibody in thalassemia major patients[27]. The percentage of anti-B19V IgG antibody in other countries presented in between, a study conducted in Tunisia showed 39% positive[29] while in India the percentage was 81%[30].

The majority of infections occur during childhood and adolescence, seroprevalence based on the presence of VP1/VP2-specific IgG antibody is approximately 2–20% in children, increases with age and may reach maximal levels of more than 80% in the elderly population (>70 years of age), although these values may vary slightly by country[31]. Detection of anti-B19V IgG antibody is a double edged sword, as on the one hand it indicates to the time and nature of the infection which could be past or persistent and the later type of infection resulting in pure red cell aplasia and sever chronic anemia. On the other hand, this type of antibody indicates to the strength of the immune response against B19V infection as the presence of this antibody denote the protective status of the patients against the virus[28].

PCR results presented that *NS1* gene of B19V was founded in 12.5% of 120 thalassemia patients selected from Thi-Qar Province in Iraq, which is coordinated with the results of previous studies performed in other countries. The results of present study indicate to presence of high significant difference between the patients and control regarding infection with B19V ($P < 0.004$) and this ratio was very close to a study conducted in Thailand [22]. Other studies presented different percentages of B19V infection in thalassemia patients, one was conducted in the Iranian capital, Tehran, showed low (4%) infectious ratio[32], and another study conducted in Isfahan, Iran showed high (20%) infectious ratio[17]. However, The presence of B19V in thalassemia patients considered as a risk factor for sever consequences, one explanation of that was alteration occur in erythropoietic process due to implying genetic defects or exhausting physiological conditions affecting the cellular turnover, block in erythropoiesis as a result of infection, which usually appears in the form of an acute episode of profound anemia, also deficiency of the immune system and resultant inability to neutralize, and clear the virus, infection may become persistent and manifest with chronic anemia of different grades[33,34]. Other factors which contribute in the spreading of B19V infection also increase the incidence of these consequences, such as the climate, so the infection index is high during the winter and spring, yet infections occur in the other seasons of the year[35], Socio-economic conditions, overpopulation and geographic density also have an important influence on B19V infectious ratio[36]. On the other hand, B19V infection is worsening patient's baseline anemia lead to more complex manifestations because this virus directly affects production of red blood cells by invading the red cell precursors and proliferate in and lysing them[37]. B19V infection almost prevent red blood cell production for two to three days and for long consequences may lead to shorten red cell life result in an abrupt, life-threatening situation[38].

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CONFLET OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTION

AM designed the manuscript, figures and tables and supervised and reviewed the manuscript. HF drafted the manuscript and compiled information from the literature. SM managed references and data resources.

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DATA AVAILIABILITY

The data that support this study will be shared upon reasonable request to the corresponding author.

ETHICAL STATMENT

This study was carried out in accordance with the recommendations of Committee of Ethical Standards in the College of Science, University of Thi-Qar, which affiliated to the Ministry of Higher Education and Scientific Research, Iraq.

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