Molecular Detection of Human parvovirus B19 in Patients with Thalassemia in Wasit Province, Iraq

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KEYWORDS

Human parvovirus 19, Thalassemia, PCR. Wasit

ABSTRACT

Background :Human B19 (PVB19) isssmall, non-envelope single strand DNA viruses from family of "Parvoviridae" it cause infections in human. In thalassemia are a heterogeneous grouping of genetic disorders that result from a decreased synthesis of alpha or beta chains of hemoglobin (Hb). Numerous factors contribute to functional abnormality found in βeta thalassemia patient like decrease red cells life span, rapidsiron turnover, and tissue deposition of excess iron B19 targets the erythroid progenitors in the bone marrow by binding to the glycosphingolipid.

Materials and Methods: This case cross sectional study and the patients aged between (5-40) years , study has been carried outsin Al-Kut teaching hospitals in Al-Iraq-Wasit in a period between September 2023 and March 2024. This study followed the cross-sectional design. The populations of our study included 120 patients' samples with thalassemia infected by Human Parvovirus B19 in blood transfusion patients 64 male and 56 female. Nested-PCR used to detect of B19V by using primers. PCR reaction was done by using special sets of primers mentioned previously and special components and specific program and procedure according to components of PCR reaction.

Result: Human parvovirus 19 Positive case percentage using polymerase chain reaction indicated that the positive cases was reported according to polymerase chain reaction (PCR) as a percentage of case related to the main characteristic of patients, current study data has been indicated as following: in total of 120 patient sample with thalassemia has been included in this study, PCR positive case was 17 (14.2%), 6 (5%), 2 (1.7%) and 0 (0%) in the age groups (5-15), (16-25), (26-35) and (36-45) respectively. Male was more prevalent 17 (14.2%), than female 8 (6.7%), So as to records of major type in comparision to intermediate 19 (15.8%) and 6 (5%). All positive cases were 25 cases.

Conclusion: Current data showed prevalence of Human parvovirus 19 in Iraqi patients. This prevalence should be alarming public health.

1. Introduction

Human B19 is small, nonenvelope single-strand DNA viruses, it cause infections in human, usually through respiratory tract route (1), blood transfusions, bone marrow transplantations, and verticaly from mothers to fetus during pregnancies (2). Human B19 has a tropism for the erythroid progenitor cell, causing suppressions of an erythropoiesis dueto the cytotoxic effects of this virus (3).

In the infection of this virus, main clinical feature may include dermatologic manifestations, theumatologic finding and hematologic effects. This illness associations with human B19 evolve differently in different individual. Some may-be a symptomatic and others develop prodromal symptom only (4).

Thalassemia are a heterogeneous grouping of genetic disorders that result from a decreased synthesis of alpha or beta chains of hemoglobin (Hb). Hemoglobin serves as the oxygen-carrying component of the red blood cells. It consists of two proteins, an alpha and beta. If the body does not manufacture enough of one or the other of these two proteins, the red blood cells do not form correctly and cannot carry sufficient oxygen, this causes anemia that begins in early childhood and lasts throughout life (5).

Thalassemia (TM) is an autosomal recessive, mean both the parent carrier for this disease or affect with to transferring it to next generations. Its cause by mutation or deletion of the hemoglobin gene, results in absence or underproduction of beta or alpha chain, there were over two hundred mutation has been identified as the culprit for causing thalasemia, α thalassemia cause by deletion of alphaglobin gene, while β thalassemia was cause by point mutations in splicing sites and promoters' region of betaglobin genes on the chromosome eleven (6). Various factor contributes to functional abnormality found in beta thalassemia patients like decrease RBCs life span (7).

Human parvovirus 19 target the erythroid progenitor in bone marrow via binding to glycosphingolipid globosides (Gb4), also recognize as a blood groups P antigena. It was generally harmles in a healthy individual but could have serious clinical effects in susceptible recipient such as patient with shorten red cells survival like Sickle cell diseases and beta thalassemia patient (8).

Immunocompromised patient and pregnant women. Human parvovirus 19 can cause severe diseases, such as transients aplastic crisis in patient suffer from hemolytic disorder (9).

This study considered the first one that will includes this issue put little information about the viral causative agents that involved in serious impacts on thalassemia patients, thus this study considered the first that aimed to show the role of human parvovirus in severity of diseases and to understand the factors contributing to the incidence of beta thalassemia major in Wasit province. Thus to accomplish this aim the objectives were done as following.

2. Methodology

2- Materials and methods:

2-1 Sample collection

This case control (cross sectional) aged between (5-40) study has been carry out in AlKut teaching hospital in Al-Iraq-Wasit in a period between September 2023 and March 2024. This study followed the crosssectional design. The populations of our study included 120 patients samples with thalassemia infected by Human Parvovirus B19 in blood transfusion patients 64 male and 56 female. The objectives and methodology were explained to all participants in the current study to gain their consent. Current test included immunological and molecular tests. Clinical data included name, age, etc... were recorded.

2-2 Total Genomic DNA Extraction

The following steps were followed to extract genomic DNA from blood samples using the Genomic DNA Purification Kit, Geneaid, China and following the company's instructions**:**

2-2-1 Virus Nucleic Acid Extraction Kit

Step 1: Cell lysis

It was transfered 200 ul samples to a 1.5 m1 microcentrifuge tubes. It was added 400 ul of Lysis Buffer to samples then mixed by a vortex. It has been incubated at room temperature for 10 minutes.

Step 2: Nucleic Acid Binding

It was added 450 µl of AD Buffer (make sure ethanol was added) to the sample lysate. It was shakeed the tube vigorously to mix. It was placed a VB Column in a 2 ml Collection Tube. It has been transfered 600 ul of lysate mixture with VB Columns. It was centrifuged at 14-16,000 x g for 1 minute. It was discarded the flow-through then place the VB Column back in the 2 ml Collection Tube. It has been transfered the remaining mixture to the VB Column. It has been centrifuged at 14-16,000 x g for 1 minute. Discard the 2 ml Collection Tube containing the flow-through. It has been transfered the VB Column to a new 2 ml Collection Tube.

Step 3: Wash

It was added 400 µl of W1 Buffer to the VB Column then centrifuged at 14-16,000 x g for 30 seconds. It has been discarded the flow-through then place the VB Column back in the 2 ml Collection Tube. It was added 600 µl of Wash Buffer (make sure ethanol was added) to the VB Column. It has been centrifuged at 14-16,000 x g for 30 seconds. The flow-through was discarded and the VB Column was placed back in the 2 ml Collection Tube. The centrifuge at 14-16,000 x g for 3 minutes to dry the column matrix was done.

Step 4: Nucleic Acid Elution

The dried VB Column was placed in a clean 1.5 ml microcentrifuge tube. It has been added 50 µl of RNase-free Water to the CENTER of the VB Column matrix. It was let stand for at least 3 minutes to ensure the RNase-free Water is absorbed by the matrix. Centrifuge at 14-16,000 x g for 1 minute to elute the purified nucleic acid was performed.

Primer after dissolving in Water (according to manufactory instructions) will be 100 µM and storage as stock, after preparation of primers stock it will be diluted with Nuclease-Free Water 1:9 (10 µM) for preparation to work and then stored at -20.

2-3 Polymerase Chain Reaction (PCR)

Detection of *Parvovirus B19* by Nested-PCR

Nested-PCR used to detect of B19V by using primers. PCR reaction was done by using special sets of primers mentioned previously and special components and specific program and procedure according to components of PCR reaction

3. Result and Discussion

The positive cases was reported according to polymerase chain reaction (PCR) as a percentage of case related to the main characteristic of patients, current study data has been indicated as following: in total of 120 patient sample with thalassemia has been included in the study, PCR positive cases were 17 (14.2%), 6 (5%), 2 (1.7%) and 0 (0%) in the age groups (5-15), (16-25), (26-35) and (36-45) respectively. Male was more prevalent 17 (14.2%), than female 8 (6.7%), So as to records of major type in comparision to intermediate 19 (15.8%) and 6 (5%), as showed in table (1). Figures (1) and (2) showed PCR results for first and round respectively.

Table (1) Percentage of positive HBV19 in Thalassemia patients using PCR

PCR					
Item type	**PCR**			**Total**	P value
	Category	**+ve**	**-ve**		
Age groups					
	5_15	17 (14.2%)	38 (31.6%)	55 (45.8)	0.04
	16_25	6 (5%)	42 (35%)	48 (40%)	
	26_35	2 (1.7%)	12 (10%)	14 (11.7%)	
	36_45	0 (0%)	3 (2.5%)	3 (2.5%)	
Gender	**female**	8 (6.7%)	48 (40%)	56 (46.6%)	0.03
	male	17 (14.2%)	47 (39.1%)	64 (53.3)	
Stage or type disease	**Intermediate**	6 (5%)	29 (24.2%)	35 (29.2%)	
	Major	19 (15.8%)	66 (55%)	85 (70.8%)	0.001
Total		21 (17.5%)	99 (82.5%)	120 (100%)	

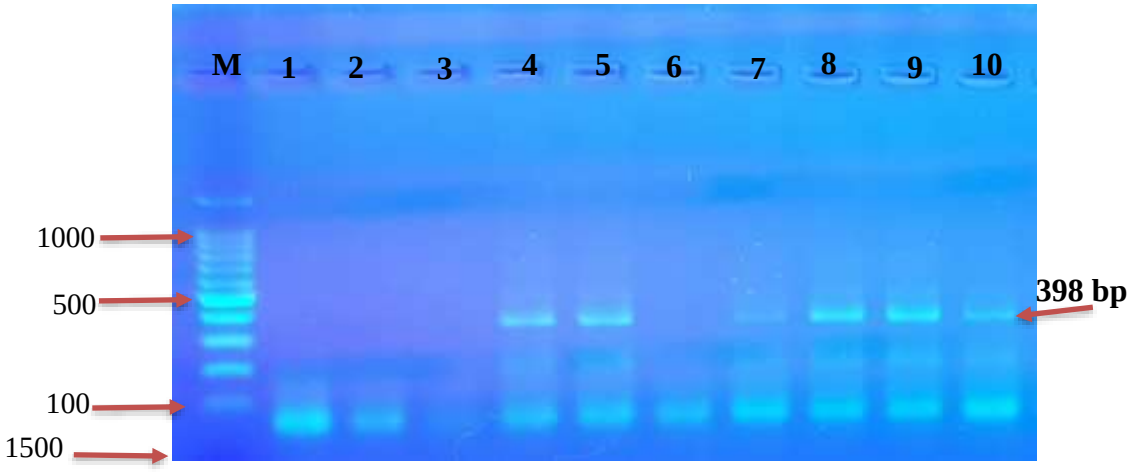

Figure (1) Agarose gel electrophoresis targeting VP1 of B19 virus lane (M) Marker (100-1500 Bp). lane 1-2-3-6 (Negative Samples) . 4-5-7-8-9-10 (398 Bp) positive Samples .

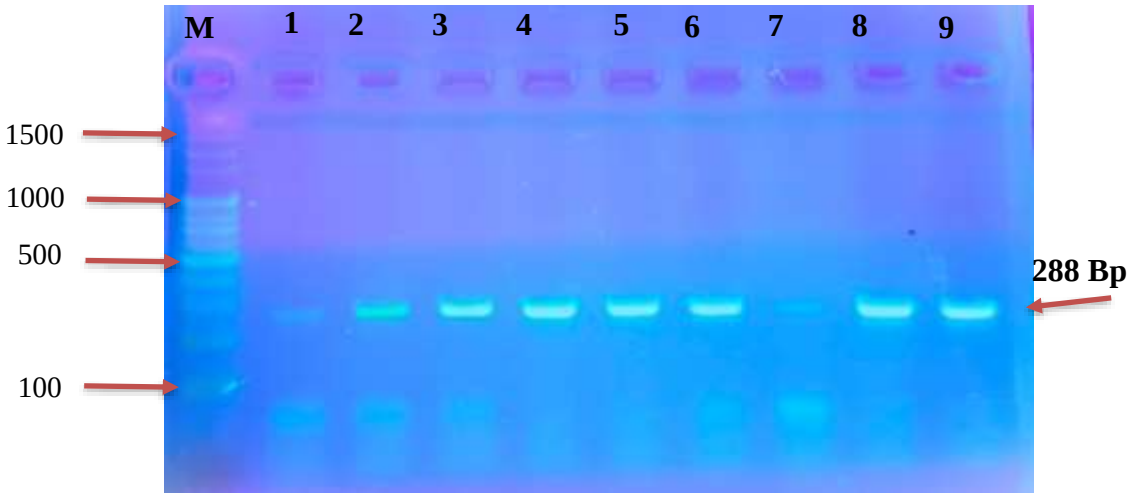

Figure (2) Agarose gel electrophoresis targeting VP2 of B19 virus lane (M) Marker (100-1500 Bp) .lane 1-9 (288 Bp) Positive Samples.

4- Discussion

Only few epidemiological studies on HB19 infections in patient with TM was reported, as the prevalences of its infection has been compare between healthy subject and TM major patient (10). The lacking of sufficient informations about relationships between active human parvovirus19 infections and thalassemia underscore the necesary to determines the rates of B19 infections in TM major patient.

Monitors of high risk group was required for epidemiological diseases prevention and surveillance measurement. Here it was reported a molecular epidemiologic study on humanB19 infection among β-TM major patient in Iranian study. Diagnosis of humanB19 relie on serological tests and viral DNA detection (11).

Polymerase chain reaction (PCR) has been used instead of serological in viral detection because of the highest specificity and sensitivity (12), low cross reactivity (13), and the ability in detection of both

persistent and acute humanB19 infection (14). Human parvovirus B19 was amarked tropism especially to erythroid progenitor cell and could induce temporary infections in bone marrow, eventually leads to the sudden worsen of reticulocytopenia, anemia and transient arresting in erythropoiesis (15). Due to the regular transfusion, multi-transfuse β-TM major patient were most susceptible populations for transfused related infection, includes HBV19 (16). The clinical significant of those genotype was unknown and require further studies. Genotypes I was compose of 3 prototypes B19V isolate and was the prevalent genotypes (17). Genotype III was limited largely to France and Brazil (18).

In This study 25 (20.8%) cases reported positivity in 120 (100%) collected samples, these data were the same of Nikoozad et al., (2015) in Iranian study indicated humanB19 DNA prevalences of 20 percent in 30 TM major patient. However, current result showed reduction in a prevalence (4percent) (10). Slavov et al., (2021) evaluate patient with thalassemia and sickle cells anemia in Brazil. Of 183 patient, 144 and 39 patient was diagnose with BTM and anemia respectively.

The prevalence of human B19 DNA in twenty eight patient with sickle cells anemia and 6 BTM patient was recorded as 19.4 percent and 15.3 percent (19). In Iraqi study detection of human parvovirusB19 genome used conventional PCR. HumanB19 DNA sequence has been detected in 12.5 percent of patient. Concluded that humanB19 genome in TM may be suggests the role for the virus in consequences and pathogenesis of β-TM major patients (20).

But our results were non agreed to the objective of the Iranian study by Arabzadeh et al., (2017) was to evaluating the prevalences of the acuteB19 infection in Iran patient with BTM major via detection of their DNA using realtime PCR, prevalence of humanB19 in a study population was recorded in 4% only. Of 150 patient with TM, six (4percent) was positive for humanB19 DNA (21). Human parvovirus B19 IgM antibody was detected in 15 thalassemia patients, and didnot detected in a control group. While antiHPV B19 IgG antibody were detect in fifty of 130 thalassemia patients (38.5 %). The range of age in thalassemic patients was (2to58 year) and B19 infections was high in the 20-40 years range. And the results showed the high percent to infection to virus in blood transfusion patients from month per months was thirty (in all samples) (22). The small samples size did not providing reliable and precise estimation of prevalence and larger samples size may be need in order to obtaining highest confidence levels.

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