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Serological Detection of Human Parvovirus B19 in Thalassemia Patients of Wasit Province

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ABSTRACT: Human ParvovirusB19 is nonenvelope single-strand DNA virus in family of Parvoviridae that cause infections in human, it is related strongly to thalassemia. This case control (cross sectional) aged between (5-40) study was carried out in Al-Kut teaching hospital in Al-Iraq. 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 and ELISA test was done. The positive cases were recorded according to enzyme linked immunosorbent assay test (ELISA) as a percentage of causes related to the main characteristics of patients, current study data has been indicated as following: in a total of 120 patients' sample with thalassemia were included in this study, ELISA positive cases were 13 (10.8%), 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 (14 (11.7%), than female (7 (5.8%), so as to records of major type in comparison to intermediate 15 (12.5%) and 6 (5%).

Keywords: ELISA, Human B19, Thalassemia. Wasit

1. INTRODUCTION

Human Parvovirus B19 is nonenvelope single-strand DNA virus from a family of Parvoviridae that cause infections in human, frequently enter through a respiratory tract (1), through bone marrow transplantations or by blood transfusions (2). Human ParvovirusB19 has a tropism for erythroid progenitor cell, cause suppression of erythropoiesis due to the cytotoxic effects (3).

Thalassemia is a heterogeneous group of blood disorders affecting the hemoglobin genes and resulting in ineffective erythropoiesis, the decreased production of hemoglobin results in anemia in early age and frequent blood transfusions are required to keep up the hemoglobin levels(4).

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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 (5).

Human ParvovirusB19 infections usually related to the spectrum of clinical manifestation, depending on immunology competence and age of patient, from subclinical and asymptomatic infection to erythema infection, transient aplastic, chronic anemia and arthropathy (6). In child, human B19 infections are most frequently present as febrile diseases with the characteristics of skin rashes, while during adulthoods, human B19 most commonly present as a unspecific fever (1).

2. MATERIALS AND METHODS

2-1 Sample collection

This Cross-Sectional study conducted to patients with thalassemia, aged between (5-40) years old. current study was carried out in Al-Kut teaching hospital in Al-Iraq-Wasit. The populations of this study included 120 patient's samples with thalassemia 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.

2-2 Blood collection

Four milliliters of venous blood were drawn from each participant into EDTA tube, two milliliters for total genomic DNA extraction, plasma drown using a clean pipette into sterile plastic tubes and kept frozen at -20C till the serological examination to detect of human B19 Ab by ELISA Kit.

2.3 ELISA test

2.3.1 Principle of ELISA test used in current study

The ELISA kit uses Sandwich ELISA as a main method. Micro Elisa stripplates provides in Kit was precoated with antigens specific to the HB19-IgG. Standard or sample were added to an appropriate Microelisa stripplates well and combine to a specific antigen. Then the Horseradish Peroxidase (HRP)-conjugate antigens specific to HB19 was add to all Micro Elisa stripplate wells and were incubate. Free component was washing away. TMB substrates solutions was add to all wells. Only well containing HRP conjugate B19 antigens and B19-IgG were appeared blue color and turned yellow after an addition of stop solution. Optical density was measured spectrophotometrically at wave length of 450 nanometer (7).

Samples preparation

Serum preparation

After the collections of a blood samples, it was allowed a blood to be clotted by leave it at the RT, usually for ten to twenty minutes. It has been removed the clot after centrifuged at 2000-3000 round per minute for twenty minutes. If was precipitate appear during reservations, again the sample were centrifuged.

3. RESULTS AND DISCUSSION

The positive cases were recorded according to enzyme linked immunosorbent assay test (ELISA) as a percentage of causes related to the main characteristics of patients, current study data has been indicated as following: in a total of 120 patients sample with thalassemia were included in this study, ELISA positive cases were 13 (10.8%), 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 (14 (11.7%), than female (7 (5.8%), so as to records of major type in comparison to intermediate 15 (12.5%) and 6 (5%), as showed in table (1) and figures (1) and (2).

Table 1: Percentage of positive HBV19 in Thalassemia patients using ELISA

ELISA					
Item type				**Total**	**P value**
	Category	**+ve**	**-ve**		
Age groups					
	5_15	13 (10.8%)	42 (35%)	55 (45.8)	**0.03**
	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%)	
SEX	**female**	7 (5.8%)	49 (40.8%)	56 (46.6%)	**0.02**
	male	14 (11.7%)	50 (41.6%)	64 (53.3)	
Stage or type disease	**Intermediate**	6 (5%)	29 (24.2%)	35 (29.2%)	

	Major	15 (12.5%)	70 (58.3%)	85 (70.8%)	**0.1**
Total		21 (17.5%)	99 (82.5%)	**120 (100%)**	

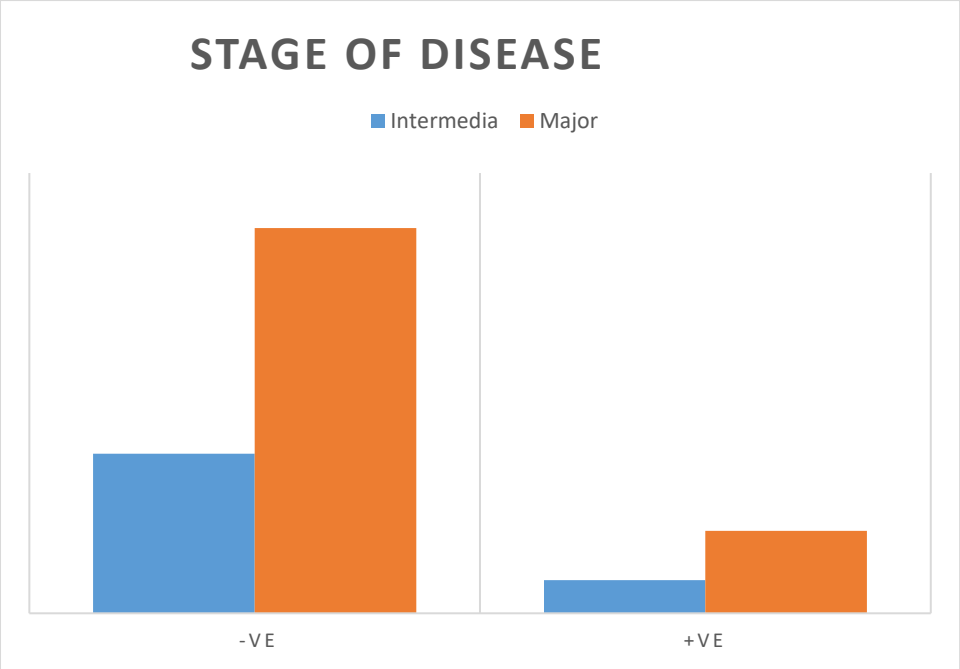

Figure 1: Representive of positive HBV19 using ELISA test according to stage

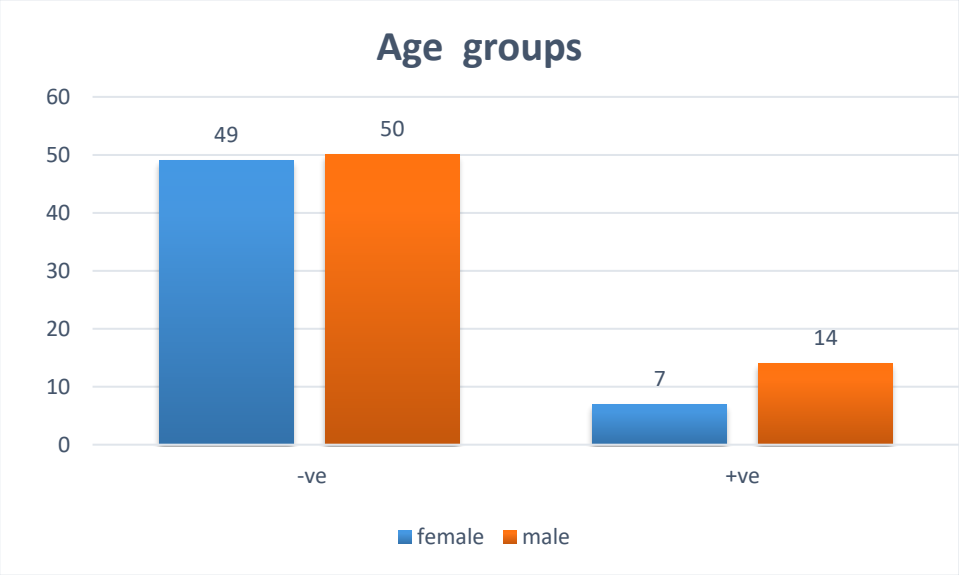

Figure 2: Represented of positive HBV19 using ELISA test according to age groups

The human parvovirus B19 (B19V) is a non-enveloped, single-stranded DNA (ssDNA) virus that is tiny and belongs to the Parvoviridae family's Erythroparvovirus genus (1). It might prevent

lysosomal fusion and guarantee the virions' nuclear entrance. A better-characterized 11 kDa protein that is implicated in viral pathogenesis is one of the extra non-structural proteins that are encoded by other minor open reading frames (ORFs) (2).

HB19 infection was prevalent and frequent; depending on the patient's immunological and hematological condition, the infection may induce a wide range of clinical manifestations (3). In immune-compromised individuals, human B19 infections may be benign or asymptomatic can cause infectiosum and erythema arthropathy. Chronic infections can occur in immunocompromised patients (4).

The main ways that HB19 is spread are by respiratory droplets and blood products. Patients with hematological disorders are typically at a higher risk of being seriously clinically unwell, especially those who have thalassemia. Furthermore, patients with β-thalassemia major are typically on hyper-transfusion regimens; as such, they frequently have a significant risk of contracting diseases linked to viruses that are transmitted during transfusions. Patients receiving Factor VIII concentrate and those with multitransfusion hemophilia have also been reported to have contracted HB19 infection (5).

The study included 120 patients with thalassemia, of which the majority fell within the age range of 5 to 15. Of these, 13 (10.8%) were positive cases using ELISA as a percentage of causes related to the main characteristics of patients; males were more common than females, at 14 (11.7%) compared to 7 (5.8%).
According to current data, which is consistent with several research, 28 (37.3%) of the study populations tested positive for HB19, and there was a positive connection between the cases and IgG (6). The current results (AntiB19 IgG was 18.2%) were comparable to those of the Fayoum 55 beta thalassemia major patients. (7).

. Outcomes of Siritantkorn *et al*., (2007) in Thailand, of sixty thalassemia major patients (AntiB19 IgG was 38%) (8). Chronic human parvovirus B19 infections were demonstrated by presence of its DNA and antiparvovirus HB19 IgG in the plasma of its patients, while the lack of antiparvovirus B19 Immunoglobulin M (9).

But our results were opposite to Soltani, Zakeri *et al*., (2020) results indicated 4 and 7 studies for the prevalence of the parvovirus B19 in β-thalassemia and SCD, respectively. Among the β-thalassemia patients, the B19V seroprevalence for IgG and IgM were ranged from 18.2–81% and 14.5–41.1%, respectively; meanwhile, B19V DNA positively results was 4–15.3% (10). Epidemiological researches were showed that prevalence of human B19 infections varied in many countries around our world (11). The high seroprevalence of Immunoglobulin G ant parvovirus B19 has been recorded in Iranian patients (12). Moreover, 88 percent of seropositivity participant were

from urban and rural areas (13). In other studies, the human B19 Immunoglobulin G prevalence in patient with hemophilia has been reported in 74% of patients.

A study conducted in Iran on blood donor revealed that there were no positive sample for Human B19 DNA, suggested low risks of parvovirus transmissions through blood cell (14). The difference among results in all mentioned studies could be attributed to that current outcomes were based on only small study populations.

4. CONCLUSION

Current information indicated the incidence of *Human B19* in thalassemia patients at Wasit province with ratio considered somewhat serious.

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