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Research Article

Molecular detection of human Parvovirus B19 in patients with hemoglobinopathies in Basrah province-Iraq

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ABSTRACT

Background: Human Parvovirus B19 infects human and cause no or mild disease, but more severe disease may occur in some people especially those with thalassemia and sickle cell anemia. In HPV-B19 infections lead to suppression of the erythrocytes formation and acute erythroblastopenia often called transient aplasia crisis which may be life-threatening. The aim of this study was to detect the prevalence of Human parvovirus B19 in thalassemia and sickle cell patients in Basrah provance, Iraq molecularly.

Patients and Methods: A 208 serum samples of both sexes with the age range 1 to 47 years' old were collected from patients with haemoglobinopathies. The nested PCR protocol was used to amplify two DNA fragment of B19. The PCR products of the second round (288 bp) and a 926 bp DNA fragment spanning the NS1-VP1u junction were sent for sequencing.

Results: Human parvovirus B19 was detected in 42/208(20.2%) and 15/100(15%) of patients and control groups respectively. The studied patients were divided into 10 age groups, the age periods (1-5) and (10-15) were significantly prone to the infection (P< 0.05). In contrast, the age period (46-50) years had significantly lower infection (P< 0.05). Also, the patients were divided into 5 study groups. Sequencing of DNA for confirming virus identification showed that of 57 PCR products, 26 isolates were successfully sequenced.

Conclusions: we concluded that low prevalence of HPV-B19 in thalassemia and sickle cell patients.

Keywords: Human Parvovirus B19, Thalassemia, sickle cell, Polymerase Chain Reaction.

INTRODUCTION

Beta-thalassemia refers to group of genetic blood disorders in which there is quantitative imperfection in beta chain creation of ordinary hemoglobin (Hb) molecules, bringing about a range of anemias varying in severity]1,2[. It is considered one of the most genuine medical issues around the world, representing a significant number of child passing every year]3,4[.

Sickle cell disease (SCD) is a hereditary blood disease which is because of the presence of an irregular type of hemoglobin, hemoglobin S, which precipitates under low oxygen tension and results in the cell expecting the shape of a sickle. SCD is at times used interchangeably with sickle cell anemia (SCA) which is a recessive hereditary disorder due to a point mutation in globin gene chain on chromosome 11 that leads to substitution of glutamic amino acid by valine amino acid at the sixth position of the beta globin chain]5[SCA is associated with high morbidity and mortality among sickle cell suffers in developing countries] 6[.

Human Parvovirus B19 (HPV-B19), the virus answerable for the erythema infectiosum, was discovered within the U.K. in 1975 by means of COSSART et al., who recognized the virus in the serum of a healthful blood donor [7]. It is the only pathogenic human virus belonging to the Parvoviridae family and having, worldwide distribution [8]. Human parvovirus B19 is a single stranded DNA virus inside the genus erythrovirus of the family Parvoviridae. HPV-B19 has a huge range of clinical appearances and diagnosis is fundamentally done by the detection of HPV-B19 specific IgM antibodies or HPV-B19 DNA [9].

Human parvovirus B19 is a single-stranded DNA virus which replicates primarily within the erythroblasts in the bone marrow and it's been shown to persist life-long in many different cell types throughout the body following acute infection [10]. HPV-B19 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 [10,11].

HPV-B19 transmission may be by via respiratory droplets, vertical transmission of mom to fetus or via the receipt of HPV-B19-contaminated blood or blood products (e.g. packed red cells, plasma, or platelets) [12].Upon infection with HPV-B19, high levels of virus appear in the circulatory system within days to weeks and may exceed 10^{13} viral particles/mL, followed by the appearance of virus-specific IgM and ultimately high-titer IgG [13]. HPV-B19 is composed of two distinct viral proteins, namely VP1 (83 kDa) and VP2 (58 kDa), which form the 20 nm diameter viral capsid [14].

In immunosuppressed patients, B19 infection may persist and lead to pure red cell aplasia, chronic anemia, and less frequently thrombocytopenia, pancytopenia, and neutropenia [15]. Parvovirus B19 has been shown to affect the erythroid as well as myeloid and platelet production in normal volunteers [16]. HPV-B19V is genetically variable, such that it has three genotypes; 1, 2, and 3, with genotype 1 being the most prevalent worldwide [17].

MATERIALS AND METHODS

Patients and samples

A 208 serum samples of both sexes with the age range 1 to 47 years' old were collected from patients with haemoglobinopathies that obtained from Basrah Center for Hereditary Blood Diseases (BCHBD) and premarital clinic in Al-Sadr teaching hospital during the period between March to December, 2018. Furthermore, 100 individuals as control group, 49 of them are males and 51 females with age range 1 to 47 years. All the samples were stored at -40 ^{0}C until DNA extraction.

MOLECULAR DETECTION

DNA extraction

The viral DNA was extracted using ReliaPrep™ Blood gDNA Miniprep System kit (Promega / USA). according to the manufacturers' instructions.

Nested PCR protocol

The nested PCR protocol was used to amplify two DNA fragment of B19 in the overlapping region common to the minor (VP1) and major (VP2) capsid protein genes according to Yamakawa et al., 1995[18].

The first round, involved usage of outer primers sequences5'-CAAAAGCATGTGGAGTGAGG-3' (nt 3187–3206) and5'-CTACTAACATGCATAGGCGC-3' (nt 3584–3565) to amplify 398 bp of target region. The reaction mixture (25µl), was composed of 5 µl of DNA templet, 2 pmol of primers, 12.5 µl of master mix (Promega, USA) and the volume completed to 25µl with D-Water. While in the second round, inner primers (sequence 5'-CCCAGAGCACCATTATAAGG-3' (nt 3271–3290) and 5'-GTGCTGTCAGTAACCTGTAC-3' (nt 3558–3539) were used to amplify 288 bp of first product. The reaction mixture (25µl), was composed of 5 µl of the first PCR product as a templet, 2 pmol of primers, 12.5 µl of master mix and the volume completed to 25µl with D-Water. The reaction conditions were 94^{0}C for 5 min., 30 cycles of 55^{0}C for 2 min. (for first round) or 57^{0}C for 2 min. (for second round), and 72^{0}C 3 min., with a final extension at 72^{0}C for 5 min. The Amplified products were visualized on 2 % agarose gel.

Human B19 sequencing

The PCR products of the second round (288 bp) and a 926 bp DNA fragment spanning the NS1-VP1u junction (from nucleotide 1,765–2,691) was obtained by semi-nested PCR according to Candotti et al., 2004[19] were sent to Macrogen Inc. (Macrogen Korea: 10F, 254 Beotkkot-ro, Geumcheon-qu, Seoul, 08511, Rep. of Korea) for sequencing. A 926 bp DNA fragment spanning the NS1-VP1u junction was obtained by semi-nested PCR outer primers PVB-1(5-CACTATGAAAACTGGGCAATAAAC-3) (nt 1747-1770) and B19SR (5-CCAGGCTTGTGTAAGTCTTC-3) (nt 2691- 2672) were used to in the first round. While in the second round, semi inner primer PVB-3 (5-ATAAACTACACTTTTGATTTCCCTG-3) (nt 1765-1789) and the outer primer B19SR were used to amplify 926 bp of first product. The reaction conditions were 94^{0}C for 30s, 50 cycles (for first round) or 38 cycles (for second round) of 55^{0}C for 1 min., 72^{0}C 1.5 min., with a final extension at 72^{0}C for 10 min. The Amplified products were visualized on 1 % agarose gel.

Statistical analysis

Analysis of the data obtained was made by using SSPS software version SPSS 24. P values <0.05 were considered statistically significant.

RESULTS

Patient's characteristics

A total of 208 patients with the age range 1 to 47 years old and 92 (44.2%) males and 116 (55.8%) females were included; 127 (61%) Sickle cell patients, 51 (24.5%) Thalassemia patients and 30 (14.5%) Sickle -Thalassemia patients. Also, the patients were divided into 5 studied groups (Table, 1) gives up the follows percentages [No, %]: Sickle cell trait SA [88, 42.3%; 52(1.9%)

females and 36(9٠٩٠%) males], Sickle cell anemia SS [39, 18.8%; 24(5591%) females and 15(7.21%) males], Thalassemia Trait TT [45, 21%;25(1195%) females and 20(44.4%) males], Sickle-Thalassemia ST [30 , 14.42%; 14(46.7%) females and 16(53.3%) males] and Thalassemia Major TM [6, 2.88%;1(16.7%) females and 5(83.3%) males], In addition to control group [100; 51(51%) females and 49(49%) males].
The studied patients were divided into 10 age groups (Table, 2), the age periods (6-10) and (11-15) were more numerous (P< 0.05) than others periods. In contrast, the age period (46-50) years was less numerous.

Table 1:Studied groups and sex distribution of subject

Studied groups	Female	Male	Total
SA	52 (59.1%)	36 (40.9%)	88 (42.3%)
SS	24 (61.5%)	15 (38.5%)	39 (18.8%)
ST	14 (46.7%)	16 (53.3%)	30 (14.4%)
TM	1 (16.7%)	5 (83.3%)	6 (2.9%)
TT	25 (55.6%)	20 (44.4%)	45 (21.6%)
Total	116 (55.8%)	92 (44.2%)	208 (100%)

Table 2 :Age and sex distribution of subjects

Age groups	Patients No. (%)	Control No. (%)	Total No. (%)
1-5	35(16.8%)	17(17%)	52(16.9%)
6-10	64(30.8%)	31(31%)	95(30.9%)
11-15	40(19.2%)	20(20%)	60(19.5%)
16 -20	25(12%)	12(12%)	37(12%)
21-25	20(9.6%)	9(9%)	29(9.4%)
26-30	7(3.4%)	3(3%)	10(3.2%)
31-35	7(3.4%)	3(3%)	10(3.2%)
36-40	5(2.4%)	2(2%)	7(2.3%)
41-45	4(1.9%)	2(2%)	6(2%)
46-50	1(0.5%)	1(1%)	2(0.6%)
Total	208 (100%)	100(100%)	308(100%)
Sex			
Male	92(44.2%)	49(49%)	141(45.8%)
Female	116(55.8%)	51(51%)	167(54.2%)
Total	208(100%)	100(100%)	308(100%)

Molecular detection
PCR and sequencing
The extracting DNA was amplified by polymerase chain reaction (PCR) technique, the PCR products was then subjected to gel electrophoresis. PCR products showed a sharp band on agarose gel (Figure, 1).

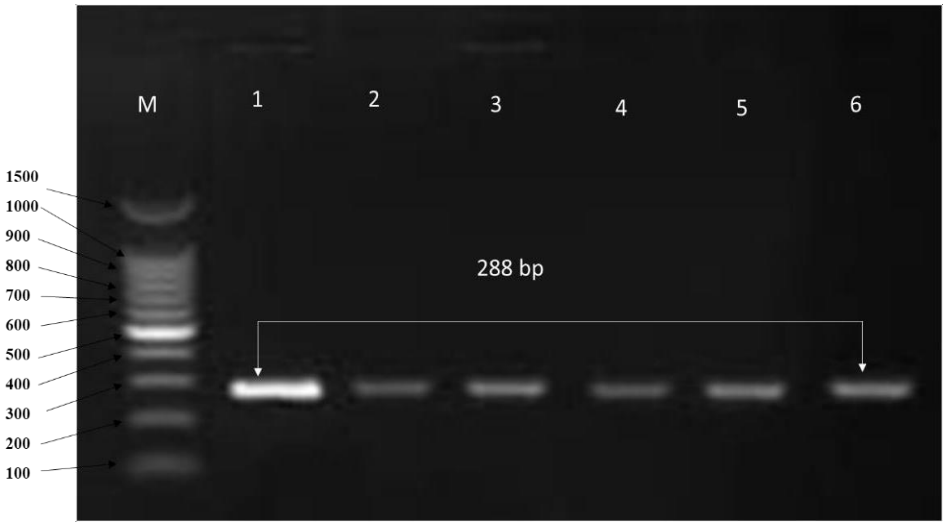

Fig.1:Gel electrophoresis of PCR products of human parvoviruses B19
Lane M: DNA marker (100-1500 bp DNA marker), lanes 1-6: positive results for amplification (288bp).

Regarding the results, Human parvovirus B19 was detected in 42/208(20.2%) and 15/100(15%) in patients and control groups, respectively (Table, 3). The present results of PCR show (Table, 4) that 25(59.5%) are females with significantly higher rate (P<0.05) than 17(40.5%) males.

The studied patients were divided into 10 age groups (Table, 5), the age periods (1-5) and (10-15) are significantly more prone to the infection (P< 0.05). In contrast, the age period (46-50) years is significantly less prone to the infection (P< 0.05).

Also, the positive results of PCR for Human parvovirus B19 are distributed among the study groups of patients as follow (Table, 3): SA group (15, 35.71%), SS (11, 26.2%), ST (6, 14.3%), TM (2, 4.8%) and TT (8, 19.04%).

Sequencing of DNA for confirming virus identification showed out of 57 PCR products, 26 isolates were successfully sequenced. The alignments of the virus isolates were identified and the molecular identity of the samples performed by multiple alignment of each sample sequence with NCBI database using basic local alignment search tool (BLAST) software. The best references were selected to show the results (Table, 6). The sequences have been deposited in the GenBank: accession numbers LC548641, LC548642, LC548643, LC548644 LC548645, LC548646, LC548647, LC548648, LC548649, LC548650, LC548651, LC548652.

Table 3:PCR results of the studied groups

Studied groups	PCR		Total No. (%)
	Positive No. (%)	Negative No. (%)	
SA	15 (17%)	73 (83%)	88 (100%)
SS	11 (28.2%)	28 (71.8%)	39 (100%)
ST	6 (20%)	24 (80%)	30 (100%)
TM	2 (33.3%)	4 (66.7%)	6 (100%)
TT	8 (17.8%)	37 (82.2%)	45 (100%)
Total	42(20.2%)	166(79.8%)	208(100%)

Table 4 :Distribution of sex and age groups according to PCR

Age groups	PCR		Total No. (%)
	Negative No. (%)	Positive No. (%)	
1-5	24(14.5%)	10(23.8%)	34(16.3%)

6-10	51(30.7%)	13(31%)	64(30.8%)
11-15	30(18%)	9(21.4%)	39(18.8%)
16 -20	21(12.7%)	6(14.2%)	27(13.0%)
21-25	18(10.9%)	2(4.8%)	20(9.7%)
26-30	7(4.2%)	0 (0.0%)	7(3.3%)
31-35	6(3.6%)	1(2.4%)	7(3.3%)
36-40	5(3%)	0(0.0%)	5(2.4%)
41-45	3(1.8%)	1(2.4%)	4(1.9%)
46-50	1(0.6%)	0(0.0%)	1(0.5%)
Total	166(100%)	42(100%)	208(100%)
Sex			
Male	75(45.2%)	17(40.5%)	92(44.24%)
Female	91(55.8%)	25(59.5%)	116(55.76%)
Total	166(100%)	42(100%)	208 (100%)

Table (4-6): Nucleotides Sequencing Data for Isolates for gene 288 bp

No.	Isolate No.	Compatible with	E value	Identity %	Query Name of the new strain in cover ge e bank (Accession number)	
1	M1-F1	EU478568.1	1e-111	96.43%	95%	LC548641
	M1-R1	EU478568.1	5e-120	99.59%	90%	-
2	M2-R1	KM659027.1	4e-52	90.74%	74%	-
3	M3-F1	JN211126.1	1e-138	99.27%	91%	LC548642
4	M3-R1	JN211146.1	2e-170	99.08%	92%	-
5	M4-F1	JN211146.1	4e-75	97.77%	71%	LC548643
	M4-R1	EU478568.1	2e-154	99.66%	97%	-
6	M5-F1	MH534950.1	2e-28	95.18%	46%	LC548644
7	M6-F1	KY940273.1	7e-114	99.15%	94%	LC548645
	M6-R1	KY940273.1	9e-128	100%	97%	-
8	M7-F1	KM065415.1	3e-46	87.29%	73%	LC548646
	M7-R1	LN211182.1	2e-43	90.60%	66%	-
9	M9-F1	EU478584.1	5e-48	85.99%	91%	LC548647
	M9-R1	MN105993.1	2e-22	94.67%	34%	-
10	M10-F1	JN211146.1	8e-121	97.22%	86%	LC548648
	M10-R1	EU478568.1	3e-123	99.17%	97%	-
11	M11-F1	JN211146.1	6e-129	98.44%	90%	LC548649
	M11-R1	EU478568.1	6e-125	99.59%	92%	-
12	M12-F1	JN211146.1	3e-120	96.85%	90%	LC548650

	M12-R1	**EU478568.1**	**2e-106**	**97.74%**	**81%**	-
13	**M13-F1**	**JN211146.1**	**1e-127**	**98.81%**	**92%**	**LC548651**
	M13-R1	**EU478568.1**	**2e-128**	**96.35**	**97%**	-
14	**M15-F1**	**JN211146.1**	**9e-98**	**96.77%**	**90%**	**LC548652**
	M15-R1	**EU478568.1**	**7e-121**	**99.16%**	**90%**	-

DISCUSSION

Human Parvovirus B19 (HPV-B19) is a pathogenic virus that is occasionally considered as life-threatening specifically for those individuals who have sickle cell and Thalassemia due to which a risk of transient aplastic crisis increases [20].

Our study of prevalence of HPV-B19 DNA was found in 42/208(20.2%) of thalassemia and sickle cell. This finding comes in agreement with the study reported the presence of parvovirus DNA in 20% of Iranian children with thalassemia [21]. Our findings were higher than that reported in Damascus-Syria (9%), Saudi Arabia (2.89%), Thailand (4%), Tunisia (4.4%), and Brazil (17.2%). However, it was lower than 37% reported in Jamaica [22, 23, 24, 25].

This our result is compatible with all studies conducted in Syria, Saudi Arabia, Tunisia, Brazil, Jamaica and New York that indicating no difference in DNA positive rates between sexes [22,23,24,25,26]. Slavov et al., 2012 found an evaluated patient with sickle cell anemia and thalassemia in a similar survey in Brazil, of 183 patients, 144 and 39 patients were diagnosed with sickle cell anemia and beta-thalassemia, respectively. The prevalence of parvovirus B19 DNA in 28 patients with sickle cell anemia and six beta-thalassemia patients were 19.4% and 15.3%, respectively [24], In other countries like Vietnam with similar prevalence of Human Parvovirus B19 DNA (21.4%) (27).

Higher prevalence rates of (56.5%) HPV-B19V infection were observed in study Regaya et al., 2007conducted in Tunisian patients with sickle cell disease or other hemoglobinopathies]28[. In Brazil, a study conducted by Slavov et al., 2012, found a prevalence of 55.3% of Human Parvovirus B19 infection among 47 patients with sickle cell disease and beta thalassemia]29[.

The differences in Human Parvovirus B19 prevalence rates observed in these studies may be mainly related to the average age of participants, ranging from 8 to 12 years, since the prevalence of virus increases with age, as demonstrated in the present report. In addition to age, the diagnostic methods sensitivity (because nested PCR is more sensitive than other methods) for virus detection and the geographic differences among the analyzed populations may have contributed to the variation observed among the studies. The difference might also belong to the difference in age groups and number of samples in this study compared to previous studies.

CONCLUSION

This study was the first time that detected prevalence of Human Parvovirus B19 infection at Basrah -Iraq and proved that no significant effect of sex. However, there was a relationship between infection and age as the infection increases up to (9%), years.

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