Microbiology Research Journal International
18(5): 1-8, 2017; Article no.MRJI.31263
Previously known as British Microbiology Research Journal
ISSN: 2231-0886, NLM ID: 101608140

SCIENCEDOMAIN *international*
www.sciencedomain.org

Molecular and Serological Study of Human Parvovirus B19 among Children with Thalassemia in Mansoura University Children Hospital, Egypt

Nashwa M. Al-Kasaby[1*], Maysaa Elsayed Zaki[2] and Mona Abd El-Latif[3]

[1]Department of Medical Microbiology and Immunology, Faculty of Medicine, Mansoura University, Egypt.
[2]Department of Clinical Pathology, Faculty of Medicine, Mansoura University, Egypt.
[3]Department of Pediatric, Faculty of Medicine, Mansoura University, Egypt.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/MRJI/2017/31263
Editor(s):
(1) Mehdi Razzaghi-Abyaneh, Department of Mycology, Pasteur Institute of Iran, Iran.
(2) Giuseppe Blaiotta, Department of Food Science, Via Università, Italy.
Reviewers:
(1) Otolorin Gbeminiyi Richard, Ahmadu Bello University Zaria, Kaduna State, Nigeria.
(2) Laila Metwally Sherief, Zagazig University, Egypt.
(3) Oğuzhan Avci, University of Selcuk, Konya, Turkey.
Complete Peer review History: http://www.sciencedomain.org/review-history/17834

Original Research Article

Received 28th December 2016
Accepted 8th February 2017
Published 14th February 2017

ABSTRACT

Background: Human parvovirus B19 (HPV-B19) is a member of the *Parvoviridae* family. Children with thalassemia can acquire HPV-B19 through blood transfusions and through contact with other children with infection.
The aim of this study was to detect the prevalence of HPV-B19 infection in children with beta-thalassemia major in Mansoura University Children Hospital, Egypt.
Methods: This study is a cross-sectional case control study that included 63 Egyptian children with beta thalassemia major attending the hospital for blood transfusion and regular blood counts and 60 healthy children as control group.
Blood samples were obtained for determination of specific antibodies IgG and IgM for HPV-B19 by

**Corresponding author: E-mail: nashwakasby2003@yahoo.com;*

enzyme linked immunoassay (ELISA) and for detection of HPV-B19 DNA by nested PCR.
Results: HPV-B19 IgM was positive in 21 beta-thalassemia patient (33.3%), IgG was positive in 27 patient (42.8%) and PCR was positive in 12 patients (19.04%). Comparing these results with the finding in healthy children by Chi-square test, there was statistically significant difference (*P* <0.001). Hematological parameters between beta-thalassemia children with recent HPV-B19 infection and control group, showed statistically significant decrease in total leukocytes counts (4.49±1.3) with reduced neutrophils counts (2.5± 0.73) and increase in lymphocytes (4.1±1.2), *P* value 0.018, ,0.004 and 0.001, respectively. In our study, There was no significant association between blood transfusion and HPV-B19 infection.
Conclusion: HPV-B19 infection is detected in high rates among children with beta thalassemia major (recent infection 19% and prior infection, 42.8%). Direct detection of HPV-B19 DNA by PCR in serum needs to be coupled with serological testing for a more reliable diagnosis of HPV-B19 infections in those children.
HPV-B19 infection in our study, has significant effects on hematological parameters in children with thalassemia with recent infection.
In our study, There was no statistically significant association between number of blood transfusion units and HPV-B19 infection. However the source of HPV-B19 in these patients may be blood transfusion. Whether it is needed for screening of blood units for HPV-B19 or not should be evaluated in larger population studies.

Keywords: Human parvovirus B19; Beta-thalassemia; HPV-B19 DNA.

1. INTRODUCTION

Human Parvovirus B19 (HPV-B19) is a member of the *Parvoviridae* family. It is a single strand DNA virus and it is the only member of its family that can cause human infection [1-3]. The virus targets rapidly growing erythroid progenitor cells, which are found in human bone marrow, fetal liver, human umbilical cord and peripheral blood [4].

HPV-B19 is transmitted via respiratory secretions, contaminated blood and blood products, organ transplantation and vertical transmission from mother to fetus [5-7]. Children with thalassemia can acquire HPV-B19 through blood transfusions and through contact with other children with infection [8,9].

Clinical manifestations of HPV-B19 infection varies and is influenced by both the hematological and the immunological status of the infected individual. Healthy children usually develop asymptomatic infection, nonspecific illness or benign erythema infectiosum [10].

But in patients suffering from decreased production or increased loss of erythrocytes, HPV-B19 can cause a severe drop in hemoglobin values, leading to aplastic crisis and anemia, which can be fatal. Immunocompromised patients can develop a state of chronic anemia due to their inability to clear the persistent HPV-B19 replication [11,12].

Patients with thalassemia, like other types of chronic hemolytic anemia there is enhanced proliferation of erythroid progenitor cell to compensate for the shortened life span of red blood cells. HPV-B19 infection can affect the erythropoiesis process leading to acute erythroblastopenia, which is often referred to as transient aplastic crisis [5,13,14].

In Egypt, thalassemia is a common disorder estimated to affect around 1.000/1.5 million per year live births [15].

Laboratory diagnosis of HPV-B19 includes serological detection of specific antibodies IgM and IgG beside molecular detection of HPV-B19 DNA [16].

There are several data concerning the implication of HPV-B19 and chronic anemia associated with malignant disorders in children [17]. In case of children with thalassemia there are several reports about the seroprevalence of antibodies to parvovirus in those patients [17] and in Egypt [18]. However, there are no studies in Egypt dealing with active HPV-B19 infection and thalassemia in children.

The aim of this study was to detect the prevalence of HPV-B19 infection in beta-

thalassemia major patients at Mansoura University Children Hospital, Egypt.

2. MATERIALS AND METHODS

The study is a cross-sectional case control study that was carried out in Mansoura University Children hospital, Egypt from January 2015 till January 2016.

This study included 63 Children with beta thalassemia major attending the hospital for regular blood counts and blood transfusion when indicated were included in the study. Exclusion criteria were patients above 18 years, patients with other types of hemolytic anemia and patients with non-hemolytic anemia were excluded from the study.

Sixty healthy control children (age- and sex-matched), without any history of transfusion of blood or blood products and without any hematological disorders.

The study was approved by Mansoura Faculty of Medicine ethical committee and informed consent was obtained from the parent of each child.

The study included full medical record of each child, data of complete clinical examination, laboratory findings regarding the number and characteristics of red and white blood cells and history of blood transfusion.

2.1 Sample Collection and Processing

Three militre blood samples was obtained from each patient and control and divided into three aliquots. One was with EDTA for complete blood counts and differential counts. The second aliquot was for serological study of specific HPV-B19 IgG and IgM after sera separation by enzyme linked immunoassay (ELISA) (*RIDASCREEN, Darmstadt, Germany*).

The third aliquot, sera was separated and kept frozen at -20℃ for further determination of HPV-B19 by nested PCR.

2.2 Detection of HPV-B19 IgM and IgG by ELISA

The ELISA kit uses microtiter plated coated with VP1 and VP2 for detection of specific HPV-B19 IgG, for HPV-B19 IgM the plate is coated with VP2. After incubation of patients' sera with the antigen wash was performed and enzyme-labeled anti-human antibodies were added. This converts (H2O2/TMB) to a blue end product. The reaction was stopped by adding sulphuric acid. The final measurement is carried out at 450 nm on a photometer using a reference wavelength ≥ 620 nm.

Value of more than 12 U/ml and 5 U/ml is considered positive for HPV-B19 Ig M and HPV-B19 Ig G, respectively [19].

2.3 Detection of HPV-B19 DNA by Nested-PCR

2.3.1 DNA extraction

HPV-B19 DNA was extracted from serum samples by the use of QIAamp, (Qiagen, Hilden, Germany) according to the instructions of the manufacturer.

2.3.2 Nested PCR

The primers used for the amplifications procedures were listed in Table 1 [20].

The first round of amplification was performed in total volume of 90 µl of the amplification mixtures containing 100 pmol of each of the first and second oligonucleotide primers and 10 µl of the extracted DNA. The amplification program was denaturation for 10 min at 94C followed by 36 cycles (94C for 45 seconds, 55C for 60 seconds and 72C for 90 seconds).

At the second stage of nested-PCR, 3 µl of the first amplification round added to 100 µl of PCR amplification mixture with 100 pmol of each of the third and the fourth primers. The amplification procedure was the same as the first.

Negative controls were used in PCR as sterile distilled water and a positive serum sample containing the HPV-B19 genome was used as the positive control [20].

Analysis of nested PCR product was performed by electrophoresis using 1.5%gel stained with ethidium bromide and visualized by UV illumination.

Positive samples yielded product of 104 bp as shown in Fig. 1.

2.4 Statistical Analysis

Data was analyzed using Statistical Package for Social Science software computer program version 17 (SPSS, Inc., Chicago, IL, USA). Quantitative data was presented in mean and standard deviation, while qualitative data was presented in number and percentage. Analysis of variance (ANOVA) and turkey were used for comparing quantitative means of the three groups. Chi-square "χ2" or Fischer's exact tests, as indicated, were used to compare the qualitative data. *P* value less than 0.05 was considered statistically significant.

3. RESULTS

This study included 63 beta-thalassemia major children and 60 healthy children with matched age and gender as a control group.

Thalassemia patients mean age was 7.9 ± 3.8, they were 45 (71.4%) males and 18 females (28.6%). The majority of patients had hepatosplenomegaly (58.7%), fever (57.1%) and some reported presence of nonspecific rash (33.3%). Patients had anemia with mean hemoglobin 7.9± 2.7gm/dl, (Table 2).

In the study of HPV-B19 markers among beta-thalassemic major children IgM was positive in 21 children (33.3%), PCR was positive in 12 patients (19.04%) and IgG was positive in 27 children (42.8%). Comparing these results with the finding in healthy children by Chi-square test there was significant difference (*P* value <0.00) Table 3.

On comparison between demographic data, clinical findings and hematological parameters between beta-thalassemia children with recent infection, prior infection and those without infection with HPV-B19, there was statistically significant decrease in total leukocytic counts (4.49±1.3) with reduced neutrophils counts (2.5± 0.73) and increase in lymphocytes (4.1±1.2) among thalassemia patients with recent infection (*P* value 0.018, 0.004 and 0.001, respectively) Table 4.

There was no statistically significant association between blood transfusion and HPV-B19 infection (*P* value 0.7).

4. DISCUSSION

The presence of Human parvovirus B19 (HPV-B19) in children with different hematological malignancies has been studied previously in many reports. However, lesser reports studied the association of HPV-B19 in children with beta thalassemia major [18].

Table 1. Sequences of the primers used in nested PCR for detection of HPV-B19

First Round	5′-AATACACTGTGGTTTTATGGGCCG-′3 5′-CCATTGCTGGTTATAACCACAGGT-′3
Second Round	5′-AATGAAAACTTTCCATTTAATGATGTAG-′3 5′-CTAAAATGGCTTTTGCAGCTTCTAC-′3

Table 2. Demographic, clinical and hematological parameters among beta-thalassemia major children (n=63)

Patient Data	Parameter
Age	7.9± 3.8
Gender	
• Male	45 (71.4%)
• Female	18 (28.6%)
Fever	36 (57.1%)
Rash	21(33.3%)
Hepatosplenomegaly	54 (58.7%)
Lymphadenopathy	21(33.3%)
HB gm/dl	7.9± 2.7
RBCs $X10^6$/µl	3.4± .63
WBCS$X10^3$/µl	7.04± 1.4
Platelets$X10^3$/µl	159.9± 22.3

Data are presented as no (%) or mean ± standard deviation

Table 3. Laboratory markers of thalassemia children and control group

Laboratory markers of HPV-B19	Patients (n=63)	Control (n=60)	P value
IgM	21 (33.3%)	1 (1.7%)	<0.001
IgG	27 (42.8%)	3 (5.0%)	<0.001
PCR	12 (19.04%)	0 (0%)	0.001

Test used: Fisher exact test significance when P <0.05

Table 4. Comparison between thalassemia patients with recent, prior and without HPV-B19 infection

Parameter	Patients with recent parvovirus B19 (IgM +ve/PCR +ve) (n=12)	Patients with prior parvovirus B19 (IgG +ve) (n=27)	Patient without infection (n=24)	P value
Gender				
• Male	8 (66.7%)	14(51.9%)	12 (50%)	0.6
• Female	4 (33.3%)	13(48.1%)	12 (50%)	
Age	8.2± 1.6	7.6± 2.7	7.2 ± 2.5	0.5
Fever	10 (83.3%)	25 (92.6%)	1(4.2%)	0.001*
Rash	6 (50%)	15 (55.6%)	5(20.8%)	0.2
HSM	8 (66.7%)	25 (92.6%)	17(70.8%)	0.74
Blood transfusion units (mean± SD)	7.5 ± 1.2	6.5 ± 2.5	6.5 ± 1.5	0.7
RBCsX10^6/µl	2.8± 0 .8	3.0± 0.47	2.9± 0.89	0.7
WBCsX10^3/µl	4.49± 1.3	6.53± 2.2	5.5± 1.7	0.018*
NeutrophilsX10^3/µl	2.5± 0.73	3.5± 0.86	2.7± 0.82	0.004*
LymphocytesX10^3/µl	4.1± 1.2	2.1± 0.7	2.9± 0.93	<0.001*
PlateletsX10^3/µl	160.5± 23.5	159.8± 22.3	157.5± 20.3	0.9
Absolute Reticulocytics X 10^6/µl	0.12± .02	0.09± 0.01	0.025± .0.01	0.4

Data expressed either as mean±SD or frequency(No-%)
** P value <0.05 is considered as statistically significant*
Test used: One way ANOVA followed by post-hoctukey for data expressed as mean±SD and chi-square for data expressed as frequency

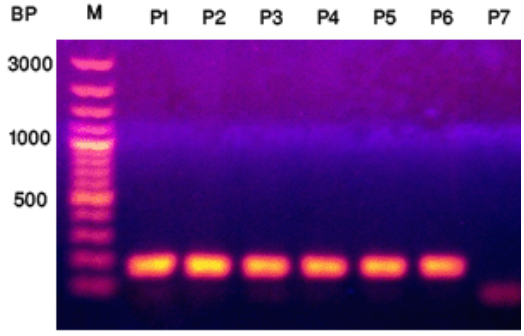

Fig. 1. Agarose gel electrophoresis pattern of HPV-B19 DNA amplicons in thalassemia patients
M: DNA marker, lanes P1-P6 positive samples, P7 negative sample

In the present study, 63 children with beta thalassemia major and 60 healthy control were included in the study. There was no significant clinical finding in infected patients with HPV-B19 that can be used as a clue for such infection. These findings were also common in other studies for parvovirus infection as it is known as a silent infection detected by laboratory methods [16,21].

In the current study, HPV-B19 infection was classified as recent infection in patients with IgM positivity and/or in those positive for B19 DNA by PCR, prior HPV-B19 infection in patients with only IgG positivity and no infection by absence of any of the HPV-B19 markers.

The prevalence of recent HPV-B19 infection as diagnosed by positive IgM and HPV-B19 DNA by nested PCR was found in 19% of thalaseemia children. This finding comes in agreement with

previous study reported the presence of parvovirus DNA in 20% of Iranian children with thalassemia [22].

The seroprevalence of antibodies among the studied patients for HPV-B19, were 33.3% for HPV-B19 IgM and 42.8% for HPV-B19 IgG. This is consistent with previous studies which showed prevalence of HPV-B19 IgM and IgG in children with hematological disorders with a range from 13% to 40% for and IgM and 15 to 80% for Ig G, respectively [22,23].

In Tunisia, higher prevalence of HPV-B19 IgG was reported among patients with hematological disorders (56.5%) while lower rate was reported for viremia (8.7%) [5].

In previous study from Egypt, Al Ghwass et al. [18] reported lower rates of HPV-B19 specific IgM and IgG (14.5% and 18.2%; respectively) in patients with beta-thalassemia major.

Regarding control subjects the prevalence of IgM and IgG was (1.7%, 5%) respectively. This is less than previous study that reported IgG 21% in the control [24]. DNA was not detected in any of the control group, This also in agreement with other studies [21, 25].

The variation in the seroprevalence of HPV-B19 in different studies can be attributed to various factors such as the difference in the geographical distribution, the difference in the hematological disorders of the affected patients in the study and the difference in the immunological status of the studied groups.

In our study HPV-B19 IgM found in 33.3% of thalassemic patient, DNA of HPV-B19 was detected in 19%.

The discrepancies between HPV-B19 DNA and HPV-B19 IgM findings indicate that searching for specific IgM may be a cheap and easy diagnostic tool for basic screening but the sensitivity may be very low.

This disparity between PCR and ELISA results was also reported in similar studies [2,24,25] but with different prevalence rates of HPV-B19 markers.

Serological detection of HPV-B19 IgM and detection of HPV-B19 DNA by PCR are informative and the evaluation of both is necessary to achieve a high level of diagnostic accuracy.

In the study of hematological parameters among children with thalassemia and with evidence of laboratory recent parvovirus infection (IgM positive) there was significant decrease in total leukocytes count (4.49±1.3) with reduced neutrophils counts (2.5± 0.73) and increase in lymphocytes (4.1±1.2) among thalassemia patients with recent infection, (*P* value 0.018, , 0.004, 0.001 respectively). These hematological findings were significantly associated with HPV-B19 infection in previous studies [18,21,26,27].

Though the level of hemoglobin in the present study was not significantly lower in patients with recent HPV-B19 infection than those with no infection, this may be attributed to the repeat of blood transfusion that make their hemoglobin levels at a rather steady value.

In our study, There was no significant association between number of blood transfusion and HPV-B19 infection. This is in accordance with other studies [18,28].

The possibility of acquiring parvovirus through blood transfusion depends mainly upon the prevalence of asymptomatic infected blood donors among general population that is estimated to range from 1 in 20,000 to 1 in 500,000 donors [9,23].

However, the exact prediction of the danger of parvovirus transmission through blood transfusion is an arbitrary figure as there is no screening strategy available for detection of HPV-B19 in blood donors.

The current study is to the best of our knowledge, the first report that described the presence of active viremia in patients with beta-thalassemia major in Egypt.

5. CONCLUSION

In the present study, HPV-B19 infection was detected in high rates among children with beta thalassemia major. It has effects on hematological parameters with no specific clinical manifestation in recent infection therefore, HPV-B19 should be suspected and screened in these patients.

Direct detection of HPV-B19 DNA by PCR in sera needs to be coupled with serological testing for accurate diagnosis of HPV-B19 infections.

In our study, There was no significant association between number of blood transfusion and HPV-B19 infection. However the source of this infection may be blood transfusion. Whether it is needed for screening of blood units for HPV-B19 or not should be evaluated in larger population studies.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/17834