EBV incidence in β-thalassemia Children patients of Najaf Governorate

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ABSTRACT

β-thalassemiais considered a serious inherited disordersand dangerous blood diseases so that the human body cannot produce hemoglobin naturally furthermore unable to perform its function naturally leading to negative effects on the rest of the human body system. It is genetically transmitted from parents to offspring. B-thalassemia involve two main kinds; alpha and beta β-thalassemia. Epstein-Barr virus (EBV) belongs to herpes virus family, which is double stranded DNA, attacks human B-lymphocyte.Transmission of virus infection through the blood and body fluids infects many groups living at low social and economic levels. Multiple blood transfusions of β-β-thalassemia patients play critical role in Epstein-Bar virus spread. Blood samples were collected from 132 β-thalassemia patients who had been admitted to the β-thalassemia Center; undergone for treatment with blood transfusion. This patient's age was ranged between of 2-14 years old and composed of 76 males and 56 females. Samples were divided into two parts: firstly included the serum for detection of viral capsid proteins serologically and secondly involved blood samples in EDTA tubes for investigating of Epstein-Barr nuclear antigen-1(EBNA-1). The results of the present study showed that out of 134 samples, 56% were positive for anti-VCA(viral capsid antigen) antibodies while the control group gave 4% positive. There were no significant differences between males and females. The results showed that 34% was a positive result for EBV DNA while the control group gave 0%. There were significant differences between the patient and control group.

Keyword: EBV, thallssemia,EBNA1 ,VCA

INTRODUCTION

EBV composed of double stranded DNA and it is one of gamma herpes viridae family that has the ability to invade B lymphocyte and undergo latent infection. it's non inheritable throughout time of life. The infection is common in undeveloped countries with low socioeconomic condition[1]. EBV has been related to variety of diseases such as infectious mononucleosis malignant neoplastic disease, Burkett' slymphoma, Hodgkin's lymphoma, variety of complication related to human immunosuppression as well as immunodeficiency diseases(2) and multiple anemia[3] conjointly related to non B cell malignancies like NK cell lymphomas ,extranodal T-cell lymphomas, and malignant neoplastic carcinoma[4].

The hemolytic anemiais due to genetically congenital hemoglobin disorders involved in β-thalassemia. Transfusion dependent hemolytic anemia may differ from mild to a severe depending on heterogeneity of condition in wide spectrum of medical phenotypes[5].

β-thalassemia could be major health problems, inserting an immeasurable emotional, psychological and economic burden on lots of individuals round the world[6]. Recent information indicate that regarding seventh of the world's population revealed that about 3,00,000 -5,00,000 children are born annually with the homozygous diseases states severely and could be a carrier of a hemoglobin disorder[7]. The goal of this study was to study the relationship between virus infection and
β-thalassemia by detection of EBV infection in β-thalassemia patients in Najaf governorate using both serological technique for screening of VCA IgM antibodies in serum and viral DNA in blood samples by using PCR technique.

MATERIALS & METHODS

One hundred thirty two β-thalassemia patients' blood samples were collected from that aged between 2 -14 years old included 76 males and 55 females whom undergoing treatment and blood transfusion. These samples divided into two parts: the first included serum for detection of viral capsid proteins serologically and the second was involved whole blood in the EDTA tubes for detection of Epstein-Barr nuclear antigen -1 (EBNA –1). This research was done at the β-thalassemia Center in Al-Zahraa teaching hospital in Najaf governorate. The study period was from October 2017 to July 2018. The recent study also involved 80 healthy individuals (male and female)as control group.

Viral capsid proteins detection: All plasma or serum samples have been done detection of anti-EBV-CA Immunoglobulin using Euroimmune, Germany ELISA kit.

EBNA-1 detection:: For detection of EBV DNA in blood samples, conventional PCR involving the following primers for EBV nuclear antigen 1 (EBNA-1):

1.5-AGATGGTGAGCCTGACGTG-3as a left ward primer and

2.5-CCAAGTTCCTTCGTCGGTAG-3 as a rightward primer.

The programmed amplification included an activating phase at 95 °C / 15 minutes followed by a denaturation phase at 94 °C / 30 seconds, an annealing phase at 55 °C / 30 seconds, and an extension phase at 72 °C/1 minute. The target PCR product was 241 base pairs [8].

RESULTS

The of this study include the following:

Distribution of cases according to age and gender. Out of all (132) patients (76 males &56 female) showed the most affected age in both gender was (2-6 year) 51% and 50% in male &female respectively .The differences were significant p<0.05.(Table.1).

Table.1:β-thalassemia cases according to age groups and gender

Age groups(yrs)	n	Male	Female
2 - 6	67	39(51%)	28(50%)
6 - 10	44	27(36%)	17(30%)
10 - 14	21	10(13%)	11(20%)
Total	132	76(50%)	56(43%)
X^2 P <0.05			

Anti- EBVCA (IgM)among study group according to Age groups: The current study included an investigation of antiviral IgM antibodies in β-thalassemia patients. The results showed that 24 (31%) had a viral infection, while 90 (68.2%) were negative. The age groups (6-10 and 2-6) were the highest percentage of IgM seropositivity respectively.

In cases of control group, only 4 (5%) gave anti EBV IgM Ab. Positive results, while the negative results were 76 (95%).Table.2 & 3 respectively.

Table 2: EBV detection by IgM antibodies among patients according to age .

Age groups(yrs)	n	EBVCA +	EBVCA -
2 - 6	67	14(33%)	53(59%)
6 - 10	44	19(45%)	24(27%)
10 - 14	21	9(22%)	12(14%)
Total	132	42(31.8%)	90(68.2%)
X^2 P <0.05			

Table.3:EBV detection by IgM antibodies among control group according to age.

Age groups(yrs)	n	EBVCA +	EBVCA -
2 - 6	40	1(2.5%)	39(97.5%)
6 - 10	23	2(8.6%)	21(91.4%)
10 - 14	17	1(5.8%)	16(94.2%)
Total	80	4(5%)	76(95%)
X^2 P <0.05			

PCR technique:
EBV DNA detection by PCR among patients according to age groups: The present study revealed thatEBNA-1 detection positive results were 45(34.1%), while negative results were 87 (65.6%). The highest percentage of was 49% found within age group (6-10)years. while control group samples have no positive results (0%) (table.4&5).

Table.4: EBV detection by PCR among patients group according to age

Age groups(yrs)	n	EBNA-1 (+)	EBNA-1 (-)
2 - 6	67	12(27%)	55(63%)
6 - 10	44	22(49%)	22(25%)
10 - 14	21	11(24%)	10(12%)
Total	132	45(34.1%)	87(65.6%)
X^2 P <0.05			

Table 5: EBV detection by PCR among control group according to age.

Age groups(yrs)	n	EBNA-1 (+)	EBNA-1 (-)
2 - 6	40	0	40
6 - 10	23	0	23
10 - 14	17	0	17
Total	80	0	80
X^2 P <0.05			

PCR results according to IgM antibodies results: The study involved the correlation between anti EBV IgM antibodies and EBNA-1results in the same patients. Out of a total positive samples of anti-viral IgM antibodies, 40 (95%) were positive for viral DNA, while only 5 positive samples of anti-viral IgM antibodies gave negative results. Concerning negative samples of antiviral IgM assay;out of 90 samples, 45 samples showed a positive results to EBNA-1test, while the remaining samples gave negative results.(Table.5).

Table.6: EBV detection by PCR test according to IgM antibodies presence.

Molecular technique	IgM +	IgM -	Total
EBNA-1 (+)	40(95%)	5(6%)	45
EBNA-1 (-)	2(5%)	85(94%)	87
Total	42	90	132
T p < 0.05			

Figure 1: EBNA-1 primer detection by PCR technique.
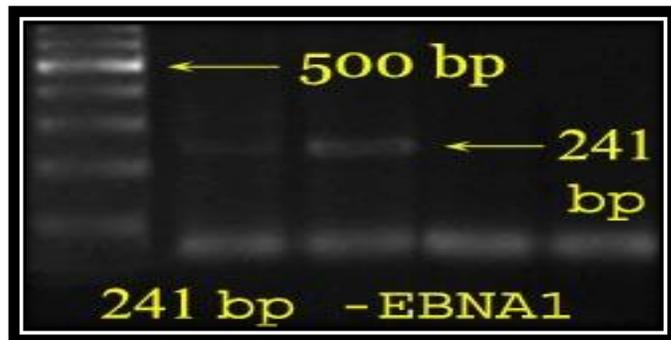
DISCUSSION

One of the most important factors that rendersβ-thalassemia patients most susceptible to viral infections is the frequent transfusion of blood, making them vulnerable to EBV infection and the bar as well as their immune system does not work with normal efficiency.

Table.2 showed that the results obtained through the diagnosis of EBVCA IgM antibodies in serum samples of β-thalassemia patients by use of serological tests; out of a total of 132 samples, There were 42 (31.8%) positive results while the remaining 90 (68.2) gave a negative result.

while 5% of the control have seropositive of EBV infection (Table.3) .This indicate that the Serologic responses are more suggestive of high viral replication within patients though serologic investigation determine exact reactivation time point (8). Moreover, there were significant statistical differences when comparing the

results of healthy people who were used as control group, indicating that β-thalassemia patients are more susceptible to virus than healthy.

Age group (6-10year) while the highest percentage of positive result in control group was 8.6% found within age group (6-10 years) with significant differences. Many further studies indicated that some patients are risky & also revealed that the reactivation of EBV in some patients with immunosuppression by using molecular techniques[9,10]

Jasim B.S. exibited that autoimmune thyroiditis in Patients showed increased titers of anti-EBV antibodies their sera as a compared with healthy individuals[11]

Table.5 showing the presence of anti EBV CA antibodies related with presence of viral gene to indicate the presence of active infection of the virus and viral replication in blood, this resembling the results of another study who studied the sequential infection of the virus[12]

As the results of the examination of ELISA, only the presence of an immune response or not, whereas the presence of DNA in the blood of the patient indicates the presence of an active and effective virus.

The results showed that most of the positive samples for ELISA test yielded positive results for the presence of viral DNA. Our data displayed that 95% of IgM positive results showed EBNA-1 positive. A very small percentage gave negative result. This means that the antibodies were formed at the acute infection but there is no active replication of virus. And this explanation in agree with *Morissette & Flamand* who explained the chromosomal integration during latent infection[13].

The same table shows that the number of negative samples for viral antibody examination Gave positive results for the examination of viral DNA and this indicates the presence of the viral genome with the absence of antibodies, and this always happens with viruses that have the ability to injury inactive and then regain activity again. In addition, the study results indicated there was a consequence of EBV in β-thalassemia patients. And this finding is in agree with[14] who indicated that EBV play role in infection and pathogenesis in autoimmune and immune compromised patients.

The finding of EBV DNA does not indicate active viral infection because virus was associated with latent infections. In case immunosuppression, latent viral infection may arise due to red blood cells transfusions and lead to reactivation of endogenous viruses.

Because this member of herpes virus family undergoes latent infection in individuals[15] refers to the specificity between B cells and virus portions and the specificity of infection and reactivation.

The PCR has been measuring in imitation of keep a sensitive, certain or precise method along pair big advantages when relevant because of the detection about viral genomes.

CONCLUSIONS

We conclude from the current study that there was significant differences between β-thalassemia patients and healthy individuals which indicated that patients with β-thalassemia are more susceptible for EBV infection and reactivation than healthy people.

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