FREQUENCY OF HEPATITIS 'B' SURFACE ANTIGEN AND HEPATITIS 'C' VIRUS ANTIBODIES IN THALASSEMIC CHILDREN"

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ABSTRACT

OBJECTIVE: To determine the frequency of HBsAg and HCV-antibodies in thalassemic children in tertiary care hospital of Peshawar.

METHODOLOGY: A descriptive study of 100 thalassemic children diagnosed clinically, was done at Department of Pediatrics, PGMI/LRH, Peshawar from 22/03/2007 to 21/12/2007 (completed in 9 months). All the subjects included in the study were investigated for HBsAg and HCV-antibodies using 3rd generation ELISA technique.

RESULTS: Out of 100 thalassemic children, HBsAg(Hepatitis B surface antigen) was positive in 04% cases, HCV(Hepatitis C virus)-antibodies was positive in 13% cases, and 02% cases were positive for both HBsAg and HCV-antibodies. There were 55% male and 45% were female. Majority (69%) cases were in the age group of 1-5 years. Hepatitis B vaccination was done in 17% cases, consanguinity was positive in 56% cases, and thalassemia in family was positive in 42% cases on past history. In 67% cases, blood was transfused from 1-25 times, in 15% cases from 26-50 times, in 13% from 51-100 times, in 03% from 101-150 times, and in 02% from 450-500 times.

CONCLUSIONS: Hepatitis B and C are common in thalassemic children.Hepatitis C is the leading hepatotrophic virus in thalassemic children followed by Hepatitis B.

KEY WORDS: Thalassemia; HBsAg, HCV-antibodies; diagnosis; frequency.

INTRODUCTION

The thalassemias are a group of congenital anemias that have in common deficient synthesis of one or more of the globin sub-units of the normal human hemoglobins (Hb).[1,2]

Thalassemia is considered the most common genetic disorder worldwide. It occurs with a particularly high frequency in a broad belt extending from the Mediterranean basin through the Middle East, Indian subcontinent, Burma, Southeast Asia, Melanesia and islands of the Pacific.[1]

In Pakistan, the disease is seen in almost all parts of the country. The estimated carrier status is around 5-7% meaning thereby that there are about 9.8 million carriers in the total population. Although no documented registry of thalassemic patients exists in Pakistan, the estimate is that over 3,500 to 4,000 thalassemic children are born every year. The average life expectancy in Pakistan is 10 years and at present the disease load is of 90,000 to 100,000 patients throughout the country.[3,4]

Transfusion-dependent patients are more prone to acquiring various transfusion-transmitted infections such as hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV).[5,6]

Hepatitis B is the most common viral infection affecting more than 300 million people worldwide. Over 20 million people are infected annually with this virus globally and there are 350 million chronic carriers of hepatitis B. In Pakistan one out of every ten persons is carrier of hepatitis B virus.[7]

In Pakistan, there are approximately 17-20 million people infected with deadly viruses of hepatitis B and hepatitis C. In general population HBV carrier rate has been reported up to 8-10% and HCV carrier rate of up to 6%.[8]

Hepatitis C virus (HCV) infection is common in transfusion-dependent thalassemia. Multiple transfused patients represent a major risk group for hepatitis C (HCV) acquirement. Blood transfusion is a well-documented route of transmission of hepatitis C virus. In repeatedly transfused patients in Pakistan, the seroprevalence of hepatitis C virus is 60-90%.[9,10,11]

Hepatitis C virus (HCV) infection is highly prevalent in thalassemic patients. This may decrease se-

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rum antibody response to hepatitis B vaccine. The study shows that three standard doses of HBV vaccine are immunogenic and safe in multi-transfused thalassemic patients with or without HCV infection.[12]

The increasing HBsAg positivity rate and high prevalence of anti-HCV antibodies in many local studies indicates that immediate steps should be taken to identify and confirm the clinical status among thalassemic children of our country. It has been recommended that properly screened blood, using a reliable method like Enzyme-linked immunosorbent Assay (ELISA), be only transfused to thalassemic patients in order to avoid/reduce transfusion associated infections.[7,8,9,13]

Thalassemic children pose a serious challenge to the public health services of our country due to their continuous requirement of blood transfusions and thus exposure to hepatitis B and C viruses. That's why this study has been designed to determine not only the frequency of these infections in thalassemic children but also to emphasize the transfusion of safe and properly screened blood.

MATERIAL AND METHODS

STUDY DESIGN: This was a descriptive study.

SETTING: The study was conducted in Department of Paediatrics, Postgraduate Medical Institute, Lady Reading Hospital, Peshawar.

DURATION OF STUDY: The study was completed in 09 months from 22/03/2007 to 21/12/2007.

SAMPLE SIZE: 100 thalassemic children.

SAMPLING TECHNIQUE: Convenient (Non probability) sampling.

SAMPLE SELECTION

Inclusion criteria

1. Diagnosed thalassemic children (Both trait and major) with at least one or more blood transfusions.

2. Age between 1-15 years.

Exclusion criteria:

1. Thalassemic children with already diagnosed HBsAg or HCV-antibodies before starting blood transfusion.

DATA COLLECTION PROCEDURE

After approval from hospital ethical committee, all thalassemic children, diagnosed clinically (progressive pallor, lethargy, failure to thrive, visceromegaly and thalassemic facies like depressed nasal bridge, prominent forehead and cheeks) and on the basis of hemoglobin electrophoresis, admitted in Paediatric Department of Postgraduate Medical Institute, Lady Reading Hospital, Peshawar, fulfilling the inclusion criteria were included in this study. An informed consent was taken from their parents or relatives for further evaluation.

Detailed history including age, hepatitis B vaccination status, history of consanguinity, family history of thalassemia and number of blood transfusions of every patient was taken from their parents.

All patients were clinically examined for pallor, thalassemic facies and hepatosplenomegaly. All these children were investigated for Hepatitis B surface antigen and HCV-antibodies using 3rd generation Enzyme-linked immunosorbent Assay (ELISA) technique. Hepatitis B Surface Antigen (HBsAg) is the first serological marker to appear following Hepatitis B virus infection.

Two milliliters (ml) of non-oxalated blood was taken from peripheral vein of each patient, properly labeled and sent to the serology section of hospital laboratory. After receiving reports of these patients from hospital laboratory, they were recorded and frequencies of HBsAg and HCV-antibodies among these thalassemic children were determined.

All these informations were entered into a self structured proforma. All the study variables like hepatitis B vaccination status, history of consanguinity, family history of thalassemia, number of blood transfusions, and hepatitis B surface antigen and HCV-antibodies were analyzed for frequencies and percentages. Frequencies of hepatitis B surface antigen and HCV-antibodies was determined among the cases of thalassemic children. Descriptive statistics mean $\pm$ standard deviation was determined for age and number of blood transfusions. Ratio was calculated for sex-distribution. All the data was stored and analyzed on computer using SPSS version 11 and was presented in the form of tables and graphs.

TABLE NO. 1:

FREQUENCY OF HEPATITIS B SURFACE ANTIGEN AND HEPATITIS C VIRUS ANTIBODIES IN CHILDREN WITH THALASSEMIA (n=100)

Frequency	No. of Patients	Percentage
Hepatitis B surface antigen	04	04%
Hepatitis C virus antibodies	13	13%
Both Hepatitis B urface antigen and Hepatitis C virus antibodies	02	02%
TOTAL	19	19%

RESULTS

During the study period a total of 100 cases of thalassemia were collected and among them hepatitis B surface antigen (HBsAg) was positive in 04% cases, hepatitis C virus antibodies (HCV-Ab) was positive in 13% cases of thalassemic children and 02% cases were positive for both HBsAg and HCV-antibodies making a overall frequency of 19% cases among the 100 cases of thalassemic children. (Table No. 1).

In this study there were 55% male children and 45% were female children with male to female ratio of 1.22: 1.

Patients of both sexes were divided into various age groups. Most of the patients, 69% were in the age group of 1-5 years, followed by 23% in the age group of 6-10 years, and 08% in age group of 11-15 years. Minimum age was 01 year and maximum was 14 years with mean age of 4.8121 $\pm$ 3.4537.

On taking history it was revealed that hepatitis B vaccination was done in 17% cases of thalassemia. History of consanguinity was positive in 56% cases, and history of thalassemia in family was positive in 42% cases.

In majority of thalassemic children (67%) , number of blood transfusions were in the range of 1-25 times. In 15% cases blood was transfused from 26-50 times. In 13% cases blood was transfused from 51-100 times. In 03% cases blood was transfused from 101-150 times. In 02% cases blood was transfused from 450-500 times. Minimum blood was transfused for 01 time and maximum was 500 times with mean of 38.0200 $\pm$ 73.2902 (Table No. 2).

TABLE NO. 2:

VARIOUS CHARACTERISTICS OF CHILDREN WITH THALASSEMIA (n=100)

Characteristics	No. of Patients	Mean
Age Ranges (in years)		
01 - 05 years	69 (69%)	Mean age = 4.8121$\pm$ 3.4537
06 - 10 years	23 (23%)	
11 – 15 years	08 (08%)	
Number of blood transfused		
1 - 25 times	67 (67%)	Mean number of blood transfused= 38.0200 $\pm$ 73.2902
26 - 50 times	15 (15%)	
51 - 100 times	13 (13%)	
101 – 150 times	03 (03%)	
450 - 500 times	02 (02%)	
History finding		
Hepatitis B vaccination done	17 (17%)	
Consanguinity	56 (56%)	-
Thalassemia in family	42 (42%)	

DISCUSSION

Thalassemias represent the most common single-gene disorder causing a major public health problem in the world, including India.[14] It is also the most common genetic disorder in Pakistan. It is estimated that over 5000 thalassemic homozygotes are born in Pakistan each year. The mainstay of therapy in thalassemia major is packed red cell transfusion.[15]

Hepatitis B infection is endemic in our region and is associated with significant morbidity and mortality. It is estimated that greater than 350 million people worldwide have hepatitis B infection. In Pakistan, there are estimated to be 4.5 million carriers of HBV with a carrier rate of 3-4%.[16]

Hepatitis C virus infects an estimated 170 million persons worldwide, approximately 3% of the world's population and thus represents a viral pandemic.[17]

Hepatitis C virus primarily spreads through contact with infected blood. High-risk groups include recipients of multiple or repeated blood transfusions or blood products, intravenous drug abusers, prisoners, hemodialysis patients, healthcare workers exposed to needle stick and sharps injuries. In about 50% of infected patients (so-called 'sporadic' cases) have no obvious risk factor.[18]

According to a review approximately 10 million people have been infected with HCV in Pakistan. The majority of patients have acquired their infection through unsafe injections, reuse of syringes and needles and community barber shops used for face and armpit shaving. More than two-thirds of HCV patients were 40 to 50 years old.[19]

Blood transfusion is an important and established source of transmitting viral disease to the recipients. Transfusion of un-screened blood products from professional blood donors has a 30% chance of transmitting these viruses to the recipients. Increased and improved donor selection and screening along with increased vaccination of the donor and recipient population has markedly reduced the incidence of hepatitis B virus transfusion associated infections. Less than 10% of all post-transfusion hepatitis is now attributable to the HBV. The risk for contracting hepatitis B is 1: 200,000 transfusion. [5,6,7,20]

The seroconversion rate for HCV in recipients is 40-93% and is major cause of post-transfusion hepatitis. Recipients of blood are at risk of acquiring HCV

through transfusion as HCV is prevalent in 0.5 to 8% of the blood donors in different parts of the world. A study of infectivity of blood seropositivity for HCV antibodies revealed that it was 20 times more likely to have posttransfusion HCV.[21,22]

Testing donated blood for the presence of antibodies to HCV, using the first generation assay, reduced the incidence of posttransfusion HCV infection to 3 in 10,000 transfusion episodes. Since the implementation of an improved screening assay for HCV antibodies, this risk has been lowered further (1 in 103,000).[23,24]

Transfusion-dependent patients are more prone to acquiring various transfusion-transmitted infections such as hepatitis B (HBV), hepatitis C (HCV) and human immunodeficiency virus (HIV). In a study the aim of the study was to investigate the prevalence of these infections in patients with thalassemia and with sickle cell anemia (SCA) receiving multiple blood transfusions. The subjects of the study were 399 multi-transfused patients with beta-thalassemia major or intermedia and SCA who have been registered at the two regional hemoglobinopathy centers in Turkey since 1996. Hepatitis B surface antigen (HBsAg), hepatitis C virus antibodies (anti-HCV) and human immunodeficiency virus antibodies (anti-HIV) tests were assayed by a second-generation enzyme-linked immunosorbent assay method. Results of the study showed that of the 399 patients, 3 were HBsAg positive (0.75%), 18 were anti-HCV positive (4.5%), and none was anti-HIV positive. It was concluded that after introduction of more sensitive screening tests and stringent donor selection procedures, incidence of HCV infection was significantly reduced, but there was still a serious risk for HCV infection, and there was a minor risk for HBV infection in patients with thalassemia and SCA.[5]

Major thalassemia (MT) is the most common form of anemia requiring blood transfusion in Iran. HCV infection is found in more than 60% of MT patients throughout the world. The probability of transmission of the virus has been reduced significantly due to recent vigilant screening of blood donors; however, similar to the other part of the world, more than 60% of multitransfused patients with major thalassemia are infected by the virus in Iran.[25]

In our study we have found that there were overall 19% cases positive for HCV-antibodies and HBsAg with the frequency of 13% HCV-antibodies, 4% HBsAg, and 2% both HBsAg and HCV-antibody cases among the thalassemic children. This high frequency of HBsAg and HCV in our study could be due to the small sample size. Due to cost constraints, many blood transfusion services could not carry out screening tests in blood donors which could be the major cause of HBV and HCV transmission in the thalassemic patients. However, it is not only screening of blood but a number of other factors which are involved in the transmission of these diseases especially to thalassemics. One important factor is the use of re-use of disposable syringes by unscrupulous elements in the society when these patients seek chelation therapy.

Our results are higher than a study in which HBsAg was positive in 0.75%, and anti-HCV was positive in 4.5%,[5] while it was less than a local study in which a high frequency of HCV was found in 30% and HBV in 14% cases of thalassemia.[26] While in few other studies hepatitis C virus was detected in 86 to 100% patients.[27,28]

In Pakistan, few local studies reported HBV and HCV frequencies with different ranges in thalassemia patients. A study conducted by Mohammad J and colleagues[13] reported that among 80 thalassemic children, 7.59% were positive for HBsAg and 36.25% for anti-HCV antibodies. In another local study by Shah MA et al[7] conducted at Khyber Medical College and Fatimid Foundation, Peshawar reported that out of 250 multitransfused thalassemia major patients, 8.4% and 56.8 patients have been screened positive for HBsAg and anit-HCV antibodies respectively. Burki MFK et al[9] reported that out of 180 multitransfused children, 75 (41.7%) children were positive for anti-HCV antibodies. Another local study by Younas M et al[10] reported in their study that among 75 patients of thalassemia major who had received at least 10 transfusions were tested for anti-HCV antibodies and found that 42% were seropositive for anti-HCV antibodies. The variation in incidence of HBV and HCV in thalassemic patients may be due to small and large number of sample size selected in these various studies.

It will be noticed in our study that the number of older patients was less while the number of younger patients was increasing. This could be due to increasing disease load and shortened life expectancy. The oldest patient in this study was 14 years old, while the mean age was 4.8121 +/- 3.4537. In contrast to our study many studies reported older age groups. In one local study age was ranged from 2-17 years,[3] other local study by Shah MA et al[7] has reported the patient's ages ranged from 1 ½ year to 19 years. Burki MFK et al[9] included in their study the children of 3 months to 12 years age.

Hepatitis C virus infection is highly prevalent in thalassemic patients. This may decrease serum antibody response to hepatitis B virus vaccine. There is also some alteration in the immune system of multitransfused thalassemic patients as a consequence of iron overload. In a study it was deduced that HCV infection may reduces the effectiveness of HBV vaccine in multi-transfused thalassemic patients. Results showed that during the vaccination periods, patients in all 3 groups did not show any significant adverse reactions. So they concluded that three standard doses

of HBV vaccine are immunogenic and safe in multi-transfused thalassemic patients with or without HCV infection.[12] In our study, results showed that in only 17% cases of thalassemia, history of HBV vaccination was positive. This may the reason of only 05% cases positive for HBV in our study. While in a local study it was found that majority of their patients was vaccinated and they found only one patient (1.7%), who was hepatitis B positive.[3]

The possibility of hepatitis transmission through blood and blood products were known since 1950s.[29] Thalassemic patients receiving multiple blood transfusions often acquired Hepatitis B and hepatitis C infections.[30]

In a study conducted by Lee WS et al[11] in 72 children there were 0.88 transfusion episode/patient per month, 1.41 units of blood transfused/ patient/per month. While in our study minimum blood transfused was 01 time to maximum of 500 times with mean of 38.02 +/- 73.29 times, which could be the reason of acquiring hepatitis C virus and hepatitis B virus in our patients with high frequencies.

CONCLUSIONS

From the results of this study it is concluded that:

There were 4% cases positive for HBV and 13% patients were positive for HCV antibodies and 2% cases having both HBV and HCV, making an overall frequency of 19% among the thalassemic children.

Majority of patients 69% were in the age group of 1-5 years, followed by 23% in the age group of 6-10 years.

Blood transfusions were in the range of 1-25 times in majority of cases that is 67% cases. Minimum blood transfused was 1 time to maximum of 500 times with mean of 38.02 +/- 73.2902.

RECOMMENDATIONS

1. Multi transfused patients specially thalassemic children are at risk of acquiring hepatitis B & C viruses, emphasizing the need of safe blood transfusion.

2. Screening of blood products should be strengthened with more expert vigilance.

3. Mass vaccination against hepatitis B virus (even who suffer from hepatitis C virus) in Pakistan must be undertaken.

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