Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 10, Issue, 04, pp.68663-68667, April, 2018

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

FREQUENCY OF VIRAL HEPATITIS B AND C INFECTIONS IN THALSSEMIC CHILDRENS AT TERTIARY CARE HOSPITAL

***Ameer Ali Jamali, Anwar Ali Jamali, Ghulam Mustafa Jamali, Bella Shaikh, Iqrar Ali Kanhar and Bhojo Mal Tanwani**

Department of Pediatrics Medicine, Peoples University of Medical and Health Sciences for Women, Nawabshah, Sindh, Pakistan

ARTICLE INFO

Article History:
Received 10th January, 2018
Received in revised form
24th February, 2018
Accepted 09th March, 2018
Published online 30th April, 2018

Key words:

Thalassemia,
Hepatitis B,
Hepatitis C.

ABSTRACT

Introduction: Thalassemia is the most well known inherited issue of public health in Pakistan. It had evaluated that more than 4000 thalassemic youngsters conceived in Pakistan every year. Thalassemics are among the most commonly transfused children and in this way presented to transfusion related infections. Among these diseases, Hepatitis B and C are the most widely recognized.

Objective: The aim of current study is to conclude frequency of Hepatitis B and C viral infection in thalassaemia major patients presenting to tertiary care hospital.

Material and methods:

Study design: Cross sectional study.

Setting: Paediatric ward, PMCH Nawabshah

Duration of study: From October 2016 to November 2017.

Subjects and methods: One hundred and twenty nine subjects after fulfilling inclusion criteria were enrolled.

Results: From 129 subjects, 82 (63.6%) male and 47 (36.4%) were female. Only 02 (1.6%) patients belong to high socioeconomic class, 52 (40.3%) to middle and 75 (58.1%) to low socioeconomic class. 126 (97.7%) muslim while only 03 (2.3%) were non- muslim patients. 5 (3.9%) subjects received blood transfusion every week, whereas greater part [124 (96.1%)] of thalasaemic subjects received blood transfusions once a month. Blood transfusion from hospital blood bank was observed in 25 (19.4%), from private blood banks in 17 (13.2%) and 87 (67.4%) had source of blood transfusion both from hospital as well as private blood banks. Majority of patients had exchanged blood transfusion [119 (92.2%)], while only 10 (7.8%) had blood transfusion from family donors. On analysis of frequency of viral hepatitis in study population it was observed that 05 (3.9%) [2 male, 3 female] had hepatitis B, 24 (18.6%) [19 male, 5 female] had Hepatitis C, 03 (2.3%) [1 male, 2 female] had both Hepatitis B & C (p=0.170).

Conclusions: Children with thalassemia are at high risk to acquire hepatitis "B" and "C" virus infections due to repeated blood transfusions. These infections could be prevented if proper preventive measures taken before transfusions.

Citation: Ameer Ali Jamali , Anwar Ali Jamali, Ghulam Mustafa Jamali, Bella Shaikh, Iqrar Ali Kanhar and Bhojo Mal Tanwani, 2018. "Frequency of viral hepatitis b and c infections in thalssemic childrens at tertiary care hospital", *International Journal of Current Research*, 10, (04), 68663-68667.

INTRODUCTION

The thalassaemia is derivative of "thalassa" a Greek word, which means "the sea" submitting to Mediterranean plus "emia", denoting "related to Blood". Thus, thalassaemia is defined as a heterogeneous cluster of genetic disorders related with of synthesis hemoglobin. The globin chains are synthesized abnormally that leads to anaemia. Erythropoiesis is ineffective; erythroblasts are destroyed in the bone marrow as well as erythrocytes are destroyed in peripheral blood (Vento *et al.,* 2006).

Corresponding author:* **Ameer Ali Jamali,
Department of Pediatrics Medicine, Peoples University of Medical and Health Sciences for Women, Nawabshah, Sindh, Pakistan.

Thalassaemia a single gene disorder is the commonest syndromes corresponding as a most important genetic health issue in the globe with a carrier rate of 7% and 300,000 to 400,000 annual births of thalassaemia major children (Zaidi *et al.,* 2004). Thalassaemia is the most frequent inherited haematological disease in Pakistan. In Pakistan, the birth rate of children with β thalassaemia is 1-2 per 1,000 live births and nearly 5,000 affected children are born annually (Zaidi *et al.,* 2004). In Pakistan, carrier rates are estimated up to 5.3% for β thalassaemia major (Shah *et al.,* 2005). All subjects with thalassaemia major develop anaemia, so blood transfusions are required early at the age of two to three years as to anticipate extreme anaemia and its physical outcomes (Kapoor *et al.,* 2007). Standard blood transfusion regimens maintaining

haemoglobin level above 10 gram/ dl expose thalassaemia major patient to receive monthly packed red cells (Kapoor *et al.,* 2007). Youngsters with β thalassaemia major who had multiple blood transfusions are in danger of securing transfusion associated viral diseases. The most significant transfusion associated viral diseases in these subjects are hepatitis B, C and HIV (Lee *et al.,* 2005). Viral Hepatitis B is the most renowned disease, influencing in excess of 300 million individuals around the world. Increased incidence of viral Hepatitis B had been accounted from different areas of Pakistan. The frequency of viral hepatitis B in children probably parallels that in adults. Asian subcontinent bears nearly 80% of all viral hepatitis B carriers and occurrence of HBsAg (Hepatitis B surface antigen) ranges from 8-10% in this region (Mohammad *et al.,* 2003).

In Pakistan the carrier rate for viral hepatis B is intermediate and rages 3-4% also the Chronic Hepatitis B is a major issue of health in Pakistan. In a community based local study prevalence of Hepatitis B core antibodies was 31% and that of HBsAg was 4.3% (Jafri *et al.,* 2006). Approximately 160 millions of people are estimated to be infected with Hepatitis C Virus as reported by the WHO, the large majority of whom resides in developing countries of the world (Amin *et al.,* 2004). HCV (Hepatitis C virus) infection emerges as endemic in nearly all areas worldwide with frequency of about 3%., Incidence of sero positivity in Pakistani population had usually been accounted as of blood donors. In southern Pakistan, the prevalence of HCV is 1.18% and 6.21% in Northern areas in professional donors and in voluntary donors it ranges between 1-3% (Khokhar *et al.,* 2004). In a study conducted at Iran, reported incidence of HCV was 33% and HBV was found to be 7% in transfusion dependent thalassaemic children [10]. However, a local study at Bolan Medical Complex Hospital Quetta has showed prevalence of HBV to be 14% and HCV 30% in thalassaemic patients (Kapoor *et al.,* 2007). Infections with Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV) can lead to CLD (chronic liver disease) and HCC (hepato-cellular carcinoma) can occur due to HBV and HCV infection. As HBV and HCV are commonly transmitted by means of blood transfusion therefore thalassaemia major patients who require repeated blood transfusions are more at risk. Our study would indicate the burden of diseases in these patients so that adequate measures could be made in order to prevent such conditions.

Operational definition

Hepatitis C virus: Hepatitis C was diagnosed by Enzyme Linked Immunosorbent Assay (ELISA), which detects antibodies for HCV.

Hepatitis B virus: Hepatitis B was diagnosed by ELISA by detecting HBsAg (Hepatitis B surface antigen) in blood tests.

Elisa: ELISA is a biochemical procedure utilized most importantly in immunology for detection of antibodies or antigens in sample.

Thalassaemia major: People with β thalassaemia major (also called Cooley's anemia) have severe thalassaemia. Presence of any one or more of following signs and symptoms which could occur at the age of 1-2 years and may be characterized by, pale look, reduced desire for food, passage of dark urine, sluggish growth and puberty may be delayed compared with normal. On examination; jaundice, hepato spleenomegaly, and cadiomegaly may be observed. It is confirmed by Haemoglobin electrophoresis.

MATERIAL AND METHODS

Study design: Cross sectional study

Setting: Paediatric ward PMCH Nawabshah

Duration of study: October 2016 to November 2017.

Sample technique: Non-probability consecutive.

Sample selection

All subjects from any socioeconomic class and ethnic group with major thalassaemia diagnosed on clinical features and confirmed by haemoglobin electrophoresis, between the age of 3 to 5 years had history of 3 or more blood transfusions were included. While subjects who had history of vaccination before transfusion (verified by history & vaccination card), born to parents with hepatitis B and C, having history of hepatitis, jaundice or already diagnosed as Hepatitis B and C were excluded from study.

Data Collection

Current study was carried out subsequent to the authorization from hospital ethical review committee PMCH Nawabshah. Subjects with Thalassaemia major, who meet the inclusion criteria-attending outpatient department of Paediatrics, were recruited in the study. Written informed permission was obtained from the patients/parents. 5 ml of blood was withdrawn and sent to the laboratory. Blood was screened by latex agglutination method of HBsAg and anti HCV antibodies and positive cases were confirmed on ELISA. All this information i.e. age, gender, ethinicity, socio-economic status, frequency of transfusion (weekly, monthly, yearly), total number of transfusion, source of blood supplied for transfusion from blood bank (hospital, private or both), donor (family, exchange), viral hepatitis (hepatitis B or C or both) were recorded on a proforma by the researcher himself.

Data Analysis

Data was analyzed through SPSS version 20.0. Frequency, percentages were computed for socio-economic status, ethnicity, gender, source of blood (hospital, private or both) and frequency of blood transfusion, donor (exchange, family) and viral hepatitis (B, C or both). Mean and SD was computed for age, number of transfusion. The variables of age, gender, socio-economic status, ethnicity, frequency of transfusions, and number of transfusions, donor and source of blood (from hospital or private blood bank) were controlled by stratification.

RESULTS

One hundred and twenty nine subjects after meeting the terms of inclusion standards were recruited in study. From total of 129, male were 83 (63.6%) and 47 (36.4%) subjects were female. Figure 1 The average age with standard deviation of study population was 6.9 ±3.4 years of 3.4 years ranging from 3 years to 15 years.

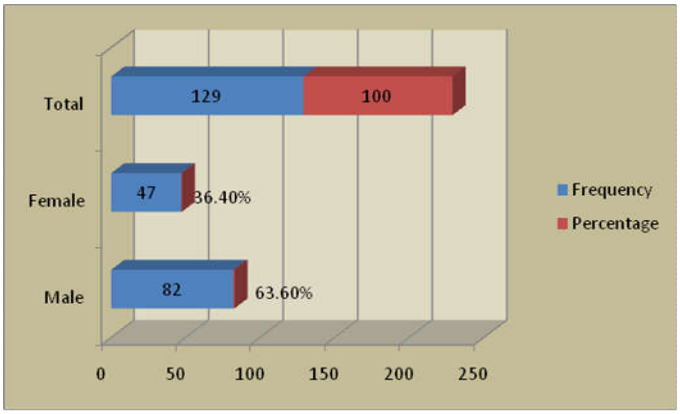

Figure 1. Frequency & %age of gender

The mean of total number of transfusions was 67.9, minimum 12 while maximum 162. Table 1. The mean number of transfusions in subjects with HBsAg was 94.80 SD 27.84, in HCV 59.29 SD 34.89, in HBsAg and HCV positive cases was 55.66 SD 14.01(p=0.402). In subjects with negative HBsAg and HCV mean blood transfusions were 68.98 SD 42.52 and the mean of total number of transfusions in all cases was collectively 67.87 SD 40.60 as shown in Table 2. Different demographic levels were assessed and data show that there were 02 (1.6%) patients belonging to high socioeconomic class, 52 (40.3%) to middle and 75 (58.1%) to low socioeconomic class. 126 (97.7%) muslim while only 03 (2.3%) were non- muslim patients. in study only 5 (3.9%) subjects were those who received blood transfusions every week, whereas a big portion [124 (96.1%)] of subjects were those who received blood transfusion each month. According to analysis about the source of blood we observed that only 25 (19.4%) subjects had blood transfusion from hospital blood bank, 17 (13.2%) had blood transfusion only from private blood banks and 87 (67.4%) had source of blood transfusion both from hospital as well as private blood bank. Majority of patients had exchanged blood transfused [119 (92.2%)], while only 10 (7.8%) had blood transfusion from family donor. Figure 2.

Table 1. Descriptive statistics

	Minimum	Maximum	Mean	Std. Deviation
Age/years	3	15	6.9	3.4
Total number of transfusions	12	162	67.9	40.6

Table 2. Analysis of mean number of blood transfusions among the viral hepatitis

Hepatitis B &C status	Total number of transfusions	
	Mean	Std. Deviation
Hepatitis B	94.8000	27.84241
Hepatitis C	59.2917	34.89672
Hepatitis B & C	55.6667	14.01190
None	68.9897	42.52291
Total	67.8760	40.60577
	P=0.402	

On analysis of frequency of viral hepatitis in our study population we observed that 05 (3.9%) [2 male, 3 female] had hepatitis B, 24 (18.6%) [19 male, 5 female] had Hepatitis C, 03 (2.3%) [1 male, 2 female] had both Hepatitis B & C (p=0.170). On further analysis we detected that from 5 subjects of hepatitis B positive, only 1 (20%) belonged to high, 1 (20%) from middle and 03 (60%) subjects were from low socioeconomic class.

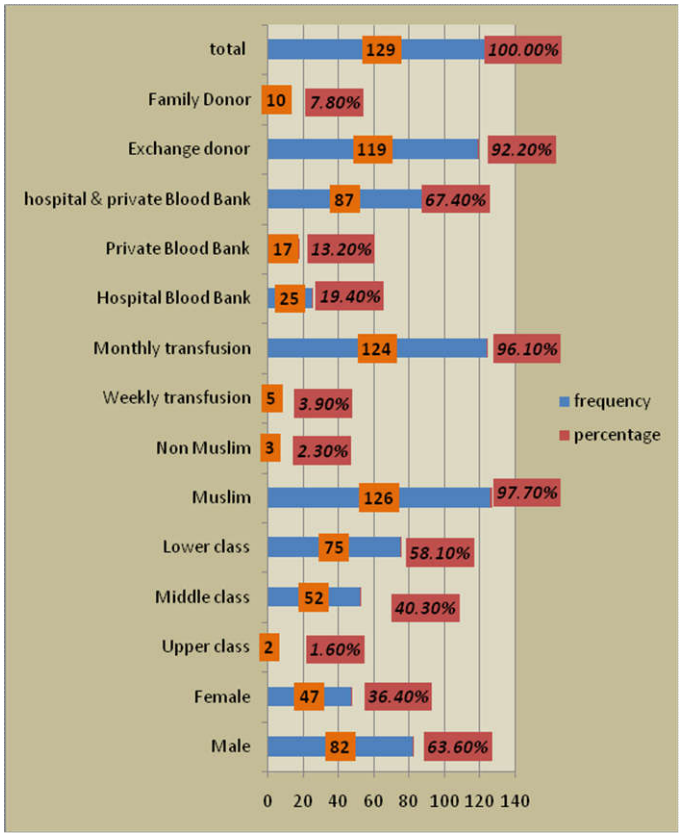

Figure 2. Demographic Variables

Out of 24 hepatitis C positive patients 09 (37.5%) belonged to middle socioeconomic group and 15 (62.5%) to low socioeconomic group**.** Out of 03 patients who were hepatitis B & C positive 01 (33.3%) belonged to middle socioeconomic group and 02 (66.7%) to low socioeconomic group**.** (p=0.054). On analysis of frequency of viral hepatitis among the ethnic groups we observed that out of 5 hepatitis B positive patients 4 (80%) were muslim and 1 (20%) was non-muslim (p=0.046**).** On analysis of frequency of blood transfusions amongst the subjects of positive viral hepatitis we detected that all the 5 subjects of hepatitis B had monthly transfusion of blood, out of 24 hepatitis C positive patients 22 (91.7%) had monthly blood transfusion and only 02 (8.3%) had weekly transfusion and all of 03 patients positive for both hepatitis B & C had monthly transfusion (p=0.623).

On analysis of frequency of viral hepatitis among the source of blood transfusion we found that out of 5 hepatitis B positive patients only 01 (20%) had source of blood transfusion from hospital, 02 (40%) from private blood bank while another 02 (40%) had source of blood for transfusion both from hospital and private blood bank. Out of 24 hepatitis C positive patients 09 (37.5%) had source of blood transfusion from hospital only, 06 (25%) from private blood bank while another 09 (37.5%) had source of blood for transfusion both from hospital and private blood bank**.** Out of 3 hepatitis B & C positive patients 02 (66.7%) had source of blood transfusion from private blood bank and 01 (33.3%) had source of blood for transfusion both from hospital and private blood bank (p=0.00**).** Out of 5 hepatitis B positive patients 03 (60%) had exchange transfusion while 02 (40%) had family donor. Out of 24 hepatitis C positive patients 22 (91.7%) had exchange transfusion and only 02 (8.3%) had family donor. All of hepatitis B & C positive patients had exchange transfusion (p=0.049) Table 3.

Table 3. Frequency & %age of different variables of Thalassemia subjects. n=129

Variable	Title	HBs Ag positive		HCV positive		HBsAg & HCV +ve		HBsAg & HCV –ve		p-value
		Freq	%	Freq	%	Freq	%	Freq	%	
Gender	Male	2	40.0	19	79.2	1	33.3	60	61.9	0.170
	Female	3	60.0	5	20.8	2	66.7	37	38.1	
SE class	Upper Class	1	20.0	0	.0	0	.0	1	1.0	0.054
	Middle Class	1	20.0	9	37.5	1	33.3	41	42.3	
	Lower Class	3	60.0	15	62.5	2	66.7	55	56.7	
Ethnicity	Muslim	4	80.0	23	95.8	3	100.0	96	99.0	0.046
	Non Muslim	1	20.0	1	4.2	0	0.0	1	1.0	
Frequency of blood transfusion	Weekly Transfusions	0	.0	2	8.3	0	.0	3	3.1	0.623
	Monthly Transfusions	5	100.0	22	91.7	3	100.0	94	96.9	
Source of blood	Hospital Blood Bank	1	20.0	9	37.5	0	.0	15	15.5	0.00
	Private Blood Bank	2	40.0	6	25.0	2	66.7	7	7.2	
	Both Blood Banks	2	40.0	9	37.5	1	33.3	75	77.3	
Blood donor	Exchange Donor	3	60.0	22	91.7	3	100.0	91	93.8	0.049
	Family Donor	2	40.0	2	8.3	0	.0	6	6.2	

DISCUSSION

Subjects of thalassaemia because of multiple blood transfusions are more prone to develop the transfusion related diseases. HBV and HCV are the commonest viral infections among these subjects. Around the world HBV, the commonest viral disease affects more than 300 million populations (Muhammad *et al.,* 2003). Over 20 million people are infected annually with this virus. Globally more than 20 million individuals are infected by this virus annually, and the sums of chronic carriers for HBV are estimated up to 350 million. The carrier rate of hepatitis B virus in Pakistan had estimated as one out of every ten personnel (Ahmad *et al.,* 1998). Transmission of HBV is through family members or close contacts that are positive for HBsAg, comprising those of intravenous drug addicts, subjects with kidney failure, transplantation of organ and migrants of high prevalence carrier rate areas. Fomites, sharing of razors or toothbrushes, scratches, blood and blood products such as concentrates of clotting factor, immunoglobulins, and plasma derived products from unscreened blood source may be the contributing factors for spread of infection (Au *et al.,* 1998). Thalassemic subjects with multiple transfusions also had frequent HCV disease. Subjects with thalassaemia, those on haemodialysis, health care employees and intravenous drug addicts had increased incidence of HCV (Esteban *et al.,* 1992). Presently about the 200 million populations throughout the world are infected with HCV (Schiff *et al.,* 2012). Hepatitis C viral infection is usually transmitted through blood transfusions (Allander *et al.,* 1995).

In Pakistan, sero prevalence of HCV had documented as 60-90% in subjects with multiple blood and blood product transfusions (Choudhry *et al.,* 1995). Thalassaemic subjects are amongst those who are mostly transfused in Pakistan. Present study demonstrates the prevalence of both HBsAg and Anti HCV antibodies through ELISA (3rd generation), in subjects of thalassaemia major with history of multiple blood transfusions. The incidence of viral hepatitis B was 3.9%. Cacopardo and his coworkers analysed sero positivity in 08.0% subjects of thalassaemia major with multiple transfusions from that 18.6% subjects were with positive anti HCV antibodies (Cacopardo *et al.,* 1992). A study from Germany by Resti and coworkers on subjects with β thalassaemia show the correlation of Anti HCV status in subjects who developed chronic non A non B hepatitis in a follow up for 13 years, they observed that 83.3% have acute NANB hepatitis and 82.9% were those with chronic NANB hepatitis.

The overall assessment of hepatitis had shown that acute infection due to viral hepatitis C was 56.7% and 50.0% in NANB hepatitis positive and negative cases respectively (Resti *et al.,* 1992). Ni-YH & his associates in a study on 61 thalassaemic subjects with multiple blood transfusions observed that 43% (n=61) had acquired HCV in 4 year duration (Ni *et al.,* 1996). Cacopardo and his coworkers noted 47% (n=152) positivity for anti HCV antibodies in Sicilian with multiple blood transfusions. 60% sero positivity for anti HCV antibodies was observed by Bhatti et al, their findings are near to the current study findings. Ahmad and Shamsi in study from Karachi had observed 46% (n=130) sero positivity thalassaemia major subjects with multiple blood transfusions (Bhatti *et al.,* 1995). Blood transfusion is no more a most important means of conduction of viral diseases in the developed world due to perfect screening. Still in Pakistan, the threat of obtaining viral diseases by the route of transfusion is much more elevated. Transmission of Hepatitis B can be prevented by proper vaccination. In a study by Al-Fawaz and Ramia they observed that 70% of the thalassaemic subjects who were vaccinated against hepatitis B before transfusions were negative for HBsAg regardless receiving multiple blood transfusions (Al-fawaz and Ramia, 1993). Subjects of thalassaemia major are very large public health issue in under developed nations, because of constant need for big amount of appropriately screened blood. Unluckily, in Pakistan the blood transfusion services are not well structured.

The subsequent suggestions are required to improve this big public health issue:

Blood and its products must be screened appropriately by ELISA for viral hepatitis B and C to keep away false negative reports. The public must be given health education especially for blood donation from fit family members, for appropriate screening of blood and to discourage the blood from professional donors. Encourage for proper vaccination of all family members especially children to prevent hepatitis B should be encouraged. Media and other resources should be utilized to educate the public for the risk of hepatitis, hazards of using polluted syringes and needles, educated to demand for to make use of new reliable disposable syringes when needed.

Conclusion

Children with thalassemia are at high risk to acquire hepatitis B and C viral infections due to repeated blood transfusions. Proper screening of donors before collection should be done thru valid screening methods before transfusions.

Recommendations

These infections could be prevented if proper preventive measures are taken as follows:

Blood and its products must be properly and routinely screened for viral hepatitis B and C infections. Professional and other high-risk blood donors must be encouraged for donation of blood. All subjects of thalassaemia should be vaccinated of against viral Hepatitis B before transfusions. Public awareness against the preventable infectious diseases should be encouraged.

REFFERENCES

Ahmad K. Hepatitis B in: Viral Hepatitis. An overview Proceedings of seminar, AFIP Rawalpindi, Pakistan 1998;1:6-19.

Al-fawaz, Ramia S. Decline in hepatitis B infection in sickle cell anaemia and beta thalassaemia major. *Arch Dis Childhood,* 1993;69:594-96.

Allander T, Gruber A, Naghavi M. Frequent patient to patient transmission of hepatitis C virus in a haematology ward. *Lancet*., 1995;345:603-7.

Amin MJ, Yousuf MH, Mumtaz A, Iqbal M. Prevalence of hepatitis B surface antigen and anti hepatitis. C virus; among general population in Lahore. *Professional Med J.,* 2004;11:334-7.

Au S, Tariq WU, Khan A. Viral hepatitis in children. Proceedings of seminar, AFIP Rawalpindi, Pakistan, 1998;20-24.

Bhatti FA, Amin M, Saleem M. Prevalence of antibody to hepatitis C virus in Pakistani thalassaemics by partial agglutination test utilizing C200 and C223 viral antigen coated particles. *J Pak Med Assoc.,* 1995; 45(10):269-71.

Cacopardo B, Russo R, Fatuzzo F, Cosentino S, Lombardo T, Larosa R. HCV and HBV infection among multitransfused thalassaemics from eastern Sicily. *Infection* 1992; 20(2):83-5.

Choudhry GM, Noor AN, Qazi AW. Frequency of acute viral hepatitis cases in Nishtar Hospital Multan. *I Coil Physicians Surg Pak.,* 1995;5(1):6-9.

Esteban JI, Genesca I, Alter HI. Hepatitis C molecular biology, pathogenesis, epidemiology, clinical features and prevention. In: Boyer JL, Ockner RK,eds.progress in Liver Diseases, vol. 10, Philadelphia W.B Saunders,1992. p. 253-82.

Jafri W, Jafri N, Yakoob J, Islam M, Tirmizi SF, Jafar I, et al. Hepatitis B and C. prevalence and risk factors associated with sero positivity among children in Karachi Pakistan. *BMC Infect Dis.,* 2006;6:101.

Kapoor C, Hanif M, Iqbal M. Polytransfused thalassaemia patients; prevalence of viral markers and malarial parasite. *Professional Med J.,* 2007;14:177-80.

Karimi M, Ghavanini AA. Seroprevalence of hepatitis B, hepatitis C and human immunodefieiency virus antibodies among multitransf used thalassaemic children in Shiraz, Iran. *J Paediatr Child Health,* 2001;37:564-6.

Khokhar N, Gill ML, Malik GJ. General seroprevalence of hepatitis C and hepatitis B virus infections in population. *J Coil Physicians Surg Pak.,* 2004;14:534-6.

Lee WS, Teh CM, Chan LL. Risks of seroconveriosn of hepatitis B, hepatitis C and human immunodeficiency virus in children with multitransfused thalassaemi major. *J Paediatr Child Health,* 2005;41:265-8.

Mohammad J, Hussain M, Khan MA. Frequency of hepatitis B and hepatitis C Virus infection in thalassaemic children. *Pak Pediatr J.,* 2003;27:161-4.

Muhammad J, Hussain M, Khan MA. Frequency of Hepatitis B & Hepatitis C infection in thalassaemic children. *Pak Paed Journal,* 2003;27:161-64.

Ni YR. Chang MH, Lin KH. Hepatitis C viral infection in thalassaemic children: Clinical and molecular studies. *Pediatr Res.,* 1996; 39(2):323-8.

Resti M, Azzari C, Rossi ME, Vullo C, Zammarchi E, Vierucci A. Hepatitis C virus antibodies in along term follow up of beta thalassaemic children with acute and chronic NANB hepatitis. *Eur J Paediatr.,* 1992; 151(8):573-6.

Schiff E. Hepatitis Central, Current information on hepatitis C and treatment for the medical profession, University of Miami 2002;1-2.

Shah SM, Khan MT, Zahour Ullah, Ashfaq NY. Prevalence of hepatitis B and C virus infection in multitransfused thalssaemia major patients in North West Frontier Province. *Pak J Med Sci.,* 2005;21:281-4.

Vento S, Cainelli F, Cesario F. Infections and thalassaemia. *Lancet infect Dis.,* 2006;6:226-33.

Zaidi A, Raziq F, Alam N, Haider KA. Screening of beta thalassaemia trait using red cell indices and red cell count. *Pak J Pathol.,* 2004; 15:54-9.

****