Original Article

Viral hepatitis markers screen in children with Acute Lymphoblastic Leukemia Experience of Children Welfare Teaching Hospital

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Summary:

Background:
Patients treated for pediatric malignancy are at high risk of parenterally transmitted viral hepatitis.
Objectives:
To detect the seroprevalence of hepatitis B and C viral infections in children with Acute Lymphoblastic Leukemia & identify some variables that could affect its prevalence in these patients.
Patients and Methods:
One hundred fifty pediatric acute lymphoblastic leukemia patients, presented to Children Welfare Teaching Hospital, Baghdad, during the period from March 11th 2007 to July 31st 2007 were enrolled in this study; they were 103 males, 47 females, aged (2.25 months- 16 years). Sera of these patients were investigated for hepatitis markers including HBsAg and Anti HCV antibody.
Results
The majority of patients were from Baghdad 104 (69.33%). Almost all children received 3 doses of hepatitis B vaccine according to the national Iraqi vaccination schedule & 111 (74%) of them received another course of vaccination during their admission to the oncology unit at CWTH.
Screening for hepatitis B virus infection was positive in 54 (36%) of cases while for hepatitis C virus infection was positive in 4 (3.25%) of cases.
Conclusion
Multiple blood transfusions and prolonged duration of observation of patients showed significant statistical impact on the incidence of HBV infection.
Key word:
Hepatitis screen, Acute Lymphoblastic Leukemia, Pediatrics

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Introduction:

Since the early nineteen sixties, blood banks, as well as plasma manufacturing industries, have aggressively pursued strategies to reduce the risks of Transfusion Transmitted infections (TTI). Today, donor evaluation, laboratory screening tests and pathogen inactivation procedures are considered crucial tools to reduce the risk of TTI, but do not completely eliminate all risk.

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At the same time these advances have moved transfusion medicine towards increasingly safer products, at steadily escalating costs and thus leading to major differences in transfusion product safety between wealthy and poor countries. (1)

There is a risk of viral hepatitis for children with cancer. Both hepatitis B virus (HBV) and hepatitis C virus (HCV) infections in countries with high prevalence cause major problems in the management of cancer patients. (2) Hepatitis virus infection through virus reactivation has a high risk of mortality in

patients with hematological malignancies receiving chemotherapy. (3)

Patients and Methods:

A prospective study done from March 11th, 2007 to July 31st, 2007; included 150 patients with ALL seen in the consultation oncology clinic/CWTH after at least two months from first admission to the hospital were enrolled in the study.

Information regarding (name of patient, date of birth, sex, date of diagnosis, hepatitis screen at time of diagnosis, number of hepatitis vaccines given at home, number of hepatitis given at hospital, date of recent of hepatitis screen and its result, number and place of blood transfusion, history of jaundice and contact number of each patient) were obtained.

These data were taken by questionnaire of the parents & from the patient's own medical notebook (provided to parents during first hospitalization) which contain all information regarding inpatient & outpatient visits with the detailed management.

The blood samples were taken from the patients in the laboratory of CWTH and sent for screening of hepatitis B and C markers in the Teaching Laboratory of Medical City complex in Baghdad.

Hepatitis B surface Ag (HBs Ag) and Anti-HCV Antibody were investigated by commercially available ELISA diagnostic kit techniques.

For Hepatitis B; Hepanostika HBsAg ultra 576T (Biomerieux) for screening tests & hepanostika HBsAg ultra 25T for confirmatory tests.

For Hepatitis C; Bioelisa HCV 480T (Biokit) for screening tests but no confirmatory tests were available.

The positive results of hepatitis B (HBsAg) adopted by confirmatory tests.

Multi-transfused patients (MTP) defined as those patients who have received more than three units of bloods.

Statistical Analysis:

Statistical analysis was performed using GraphPad Instat 3 for windows (1999 GraphPad Software, Inc., San Diego CA). Descriptive statistics were reported. Chi-Squared test and Fisher's exact test were used to compare the proportions between groups.

Statistical significance was set when $P<0.05$

Results:

This study included 150 patients who met the criteria for selection.

The detailed characteristics of patients are listed in table 1.

The median age was 8.3 years (range from 2.25 to 16 years), 103 (68.6%) were males and 47 (31.3%) were females. Male:Female ratio 2.19:1.

The patients were referred from different parts of Iraq; however the majority were from Baghdad 104(69.33%).

All children (except one) received three doses of hepatitis B vaccine according to national Iraqi vaccination schedule as the parents claimed during questionnaire.

One hundred eleven (74%) children received hepatitis B vaccination on first admission to hospital as a part of the infection control policy used in the oncology unit.

The years of diagnosis of ALL for the selected patients were between 1999& 2007 but most of the patients entered the study were diagnosed between 2003 & 2006 .

Duration of assessment from the date of diagnosis till last hepatitis screen ranges from 3m-95m with a median of 30 months.

Blood transfusions range from 1-15 units with a median of 7 units and most of the transfusions were given in CWTH.

Table 2 shows that Hepatitis screening tests (HBs Ag & anti HCV antibody) were done for all patients at time of diagnosis. The results were negative for both hepatitis B & C apart from two cases were missed from the records (one for both viruses and another for hepatitis C only).

Hepatitis B surface Ag screen was done for all patients at time of reassessment and it was positive (screening & confirmatory tests) in 54 (36%) cases, while anti HCV antibody screen was done for 123 patients only and was positive in 4/123 (3.25%) of cases (screening test only), the other 27 cases missed the chance of screening for hepatitis C due to lack of laboratory material.

History of clinical jaundice was reported in 19(12.66%) cases, 12/19(63.15%) got HBsAg positive and none got positive results for Anti HCV antibody.

We studied some Variables that might have a significant impact on hepatitis B infection. Variables like Age at reassessment, year of diagnosis, residence of the patients, and

vaccination status at hospital showed no statistical significant impact on the incidence of hepatitis B infection as shown in Tables 3-6, while multiple blood transfusions (> 3 units) & prolonged duration of observation have a significant impact table 7-8.

Table 1 Demographic & clinical data of 150 patients with ALL

Patient's characteristics	**No.**	**%**
Overall	150	100
Age (years)		
1-6	55	36.66
>6 -16	95	63.33
Sex		
Males	103	68.66
Females	47	31.33
Residence		
Baghdad	104	69.33
Other governorates	46	30.66
Number of hepatitis B vaccination doses at home		
Three doses	149	99.33
Less than three doses	none	0
Not given	1	.66
Hepatitis B vaccination doses at hospital		
Three doses	111	74
Less than three doses	15	10
Not given	24	16

Table 2 Viral hepatitis markers screening profile

Hepatitis screening tests	**No.**	**%**
Overall	150	100
At time of diagnosis		
Hepatitis B screening test		
Hepatitis B surface Antigen positive	None	0
Hepatitis B surface Antigen negative	149	100
Not done/not recorded	1	
Hepatitis C screening test		
Anti hepatitis C virus Antibody positive	none	0
Anti hepatitis C virus Antibody negative	148	100
Not done/not recorded	2	
At time of reassessment		
Hepatitis B screening test		
Hepatitis B surface Antigen positive	54	36
Hepatitis B surface Antigen negative	96	64
Not done	None	
Hepatitis C screening test		
Anti hepatitis C virus Antibody positive*	4	3.25
Anti hepatitis C virus Antibody negative	119	96.74
Not done	27	

*valid percentage was calculated excluding those who missed the screening

Table 3 Impact of patient's age on the prevalence of Hepatitis B infection

Patient's age at time of reassessment	Hepatitis BsAg negative	Hepatitis BsAg positive	Total
1-6 years	39	16	55
>6-16 years	57	38	95
total	96	54	150

Fisher's Exact Test
P = 0.2178

Table 4 Impact of years of diagnosis on the prevalence of Hepatitis B infection

Years of diagnosis	Hepatitis BsAg negative	Hepatitis BsAg positive	Total
1999-2003	13	9	22
2004-2007	84	44	128
total	97	53	150

Fisher's Exact Test
P =0.6354

Table 5 Impact of patient's residence on the prevalence of Hepatitis B infection

Residence	Hepatitis BsAg negative	Hepatitis BsAg positive	Total
Baghdad	64	40	104
Other governorates	32	14	46
total	96	54	150

Fisher's Exact Test
P =0.3639

Table 6 Impact of vaccination status at hospital on the prevalence of Hepatitis B infection

Vaccination at hospital	Hepatitis BsAg negative	Hepatitis BsAg positive	Total
Full vaccination	84	44	128
None or inadequate	12	10	22
total	96	54	150

Fisher's Exact Test
P =0.3428

Table 7 Impact of Number of blood transfusion on the prevalence of Hepatitis B infection

Blood transfusion	Hepatitis BsAg negative	Hepatitis BsAg positive	Total
1-3 units	66	26	92
> 3 units	30	28	58
total	96	54	150

Fisher's Exact Test
P =0.0151

Table 8 Impact of duration of observation on the prevalence of Hepatitis B infection

Duration of observation	Hepatitis BsAg negative	Hepatitis BsAg positive	Total
2-6m	13	3	16
7-12m	20	6	26
13-18m	21	9	30
19-24m	9	8	17
25-30m	13	4	17
31-36m	3	11	14
37-42m	7	6	13
43-94m	10	7	17
total	96	54	150

x2 = 8.261
df = 7
P = 0.0108

Transfusion transmitted infections continue to be a threat to the safety of blood supply, (4) and multi-transfused patients (MTP) are at a particularly increased risk of TTI. (5)

Contrary to the recommendation of WHO, many blood transfusions in developing countries are donated by coerced or remunerated donors rather than voluntary donors. (6)

Viral infections cause the major part of mortality and morbidity in blood recipients. The majority of known cases of post-transfusion hepatitis have been caused by hepatitis B (HBV) or hepatitis C virus (HCV).(4)

Although the convenient sample size of 150 subjects does not allow any statistical inferences, it allows describing and exploring the prevalence of viral hepatitis in children with ALL treated in Children Welfare Teaching Hospital. It also provides an outline for further studies with greater sample consistency.

Iraq is a developing country where hepatitis B and C infections are still prevalent. In the present study, seropositivities for HBsAg among patients with ALL were found to be (36%) which is a high figure.

Similar results were obtained in some studies done in other developing countries; a study carried by Mostafa A et al (7)which included 222 pediatric malignancy patients, presented to the national cancer institute, Cairo University during the period from June 2000 to March 2001. They were classified into two groups (I & II). Group I included 111 newly diagnosed cases of pediatric malignancy that were evaluated initially before starting chemotherapy and after six months of treatment. Group II included 111 cases of pediatric malignancy that ended chemotherapy and were already put under follow up from the beginning. The prevalence of HBV & HCV infection in group I was found to be 3.6% and 0.9%, respectively, at diagnosis. It increased significantly to 18.2% (p value = 0.0001) and 13.1% (p value = 0.0001), respectively, after six months of chemotherapy. On the other hand, the seropositivities for HBV and HCV in group II were found to be as high as 34.2% and 39.6%, respectively. (7)

A study done by Meir H. et al (8) which included 105 children with ALL (54 Egyptian and 51 Saudi). All eligible patients had been on maintenance therapy for at least 12 months. Markers for HBV and HCV including HBsAg, anti-HBC, and anti-HCV and for some patients HCV RNA by PCR were studied. The prevalence of hepatitis infection (HBV and/or HCV) among Egyptian children was found to be high (80%). Only five Saudi children had evidence of exposure to HBV (9.8%), P<0.0001. (8)

A study done by kebudi R. et al (9) to determine the prevalence of hepatitis B & C infections, as well as HIV infections in children with cancer at diagnosis and following therapy in Turkey.(9) This study included 50 children with solid tumors who were receiving intensive chemotherapy and multiple transfusions. These children were investigated for HBsAg, anti-HBs, anti-HBc, anti-HCV and anti HIV at diagnosis and at end of therapy.

The seropositivities for HBV and HCV infections were 4% and 2% at diagnosis and it increased significantly to 20% and 14%, respectively after therapy. (9)

A study done in Bangladesh by Mollah AH et al (10) showed that the HBV and HCV-markers were found significantly more often among multi-transfused thalassaemic children than among the controls in terms of HBsAg (13.8% vs 6.5%, $p < 0.04$) and anti-HCV (12.5% vs 0.9%, $p < 0.0001$). (10)

In contrast to our results; a study by Monteleone PM et al (11) was carried out on 45 transfused children with cancer in the United States to determine the prevalence of HCV infection. HBsAg, HBsAb, HBcHb and anti-HCV Ab were assessed.

No seropositivity for HBsAg, HBs Ab, or HBcAb could be detected. However, 9.8% of the patients were positive for HCV antibodies. (11)

On the other hand the prevalence of hepatitis C in our study was 3.25%, which is lower than other studies [Egypt ,(7) Turkey, (9) Bangladesh, (10) Brazil (5) & US (11)] but higher than a previous study done in Iraq for high risk group which showed the prevalence of hepatitis C in adult leukemic patients is 2.2% although the sample size was small 60 patients only. (12)

The rationale behind taking some variables to correlate with the risk of infection with hepatitis is:

To see whether the age has an impact on the prevalence on hepatitis B infection in two directions; first: if the prevalence of hepatitis in the young age group is lower due to high efficacy of hepatitis vaccine, Second: if the chronic hepatitis affected by age group. The risk of developing chronic HBV infection, defined as being positive for HBsAg for more than 6 mo or being negative for IgM anti-HBc and positive for HBsAg, is related inversely to age; the older the age of acquisition, the lower the risk of chronic disease. (13)

Also to see if there is a possibility that hepatitis screening for blood donors was suboptimum in a specific year or period.

Residence of patient might have an impact on infection as there might be inadequate measures for screening the blood for hepatitis in other governorates but this study shows no difference in the prevalence.

This study shows that being vaccinated at home or in the hospital doesn't prevent the infection which might raise the question about efficacy of the vaccines given & the reliability of the history taken from the parents.

A study done in India by Goyal S. et al (14) in which active immunization against hepatitis B virus infection was carried out in 162 patients with acute lymphoblastic leukemia attending the outpatient department at Tata Memorial Hospital. Recombinant DNA vaccine was given in three doses at 0, 1 and 2 months followed by a booster 1 year after the first dose. Antibodies to hepatitis B surface antigen could be detected in 19.7% of patients following vaccination, of these only 10.5% had titers in the protective range. (14)

Meral A. et al (15) held a study in Turkey between 1993, and 1998, a total of 151 children with leukemia, lymphoma and solid tumor, were screened for hepatitis B virus (HBV). Children with negative serology received active and/or passive immunization. The vaccine dose was 40 µg. Children with solid tumor and lymphoma received recombinant hepatitis B vaccine at diagnosis, repeated at months 1, 2, and 12. Hyperimmunoglobulin was administered monthly in children with leukemia during the intensive chemotherapy period. They were then vaccinated following the third month of maintenance therapy with the schedule described above. Anti-HBs titers were measured every 3 months, and titers above 10 mlU/ml were accepted as protective. Anti-HBs positivity after the first three doses was 77% in solid tumors, 88% in acute leukemia, and 48% in lymphomas. Anti-HBs positivity with respect to diagnosis in children completing the vaccination schedule was 94% in solid tumor, 90% in leukemia, and 74% in lymphoma ($P > 0.05$). Thirty-three percent of children have not received the fourth dose yet. In total 78% of the children developed protective antibody titers with or without the fourth dose, and none was infected with HBV during 3 years of follow-up. Ten (39%) of twenty-six children who remained unresponsive to immunization were infected with HBV. (15)

In an earlier study done in Tata Memorial Hospital in India, only 10.5% of 162 patients developed protective levels of antibody (anti-HBs) to a series of three double dose immunization with Hepatitis B virus (HBV) vaccine (Engerix B, Smith Kline Beecham). A second study was conducted by Somjee S. et al (16) giving five primary doses at monthly

intervals followed by a booster 1 year after the first dose. Serum antibodies were detected in 30% of patients who received all six doses of vaccine, and in only 19% were antibody levels protective. Infection with HBV occurred in 43% of patients. (16)

The impact of multiple blood transfusions on the prevalence of hepatitis B infection might point to an inadequate hepatitis screen in the national blood bank centre but doesn't' explain why the children got infection while they have a recent vaccines in the hospital unless there might be inadequate antibody production after vaccination.

The period of follow up shows a statistical significant correlation with incidence of hepatitis which might again give explanation to the possibility of receiving infected blood during the course of maintenance rather than exposure to infected unsafe procedures from the paramedical staff during early few months of treatment which needs frequent admissions to the hospital.

Laboratory error in form of poor quality of kits or human error needs to be thoroughly investigated as it might be the sole cause of this high figure of hepatitis B, yet absence of high results of HBsAg in other newly diagnosed cases make this possibility weak.

Lower prevalence of hepatitis C compared to hepatitis B infection might raise the question about the accuracy of hepatitis C screening which depends on the method & quality of kits in the laboratory department or reflects better screening of hepatitis C in the national blood bank centre.

Limitation of the study:

1. The choice of selection of the patients was not strict as some patients from far governorates refused to do the reassessment screens.
2. There might be some patients who missed screening for hepatitis C in the initial evaluation due to shortage of kits or the concentration was mainly on the results of hepatitis B screen in our registry.
3. There should be a better assessment of the number of admissions to hospital that might give a hint to the unsafe practices of the paramedical staff during invasive procedures or drug injections.
4. The study might be more convincing if a control group was taken from newly diagnosed malignancy in the same period of recent hepatitis screening to assess the possibility of laboratory or human error.
5. Lack of a proper registry for the number of platelets given to the patients.

The study recommends improving the infection control policy in the oncology unit by adopting safe invasive procedures for aspiration of blood, bone marrow and drug delivery by the doctors and other paramedical staff, implementation of safe way for hepatitis B vaccination in our hospital by monitoring (the storage of hepatitis B vaccine in the stocks, the route of administration of the vaccine and the number of doses given) and refer the laboratory preliminary results to the central disease control (CDC) center in Baghdad to investigate and adopt more strict policy for donor screening of blood samples in the laboratories by using nucleic acid technology.

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