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Hepatitis B and C Viruses in Egyptian Children with Malignancy

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To study the magnitude of hepatitis B and C infections in children with malignant diseases and its correlation with different risk factors, one hundred children with malignancies attending the pediatric Departments at Damietta Cancer Institutes and Cairo University hospitals were enrolled. They were subjected to history taking and clinical evaluation, in addition to liver function tests, HCV Ab by ELISA (3rd generation), HCV RNA by PCR in seropositive cases, HBsAg assay and HBV DNA by PCR in seropositive cases. The seropositivity for HBsAg was 9% among whom HBV DNA was detected in 7/9 cases. HCV Ab was present in 43% among whom HCV RNA was detected in 33/43 cases. There is a significant relation between HCV (Ab and RNA) and history of transfusions. There was a statistically significant increase in the number of platelets units transfused with the HBV seropositivity. Evidences of HBV and HCV infection were present in around half of cases with childhood malignancies. Blood and platelet transfusions were the most identifiable risk factors for the acquisition of HCV and HBV.

Key words: HBV, HCV, malignancy, cancer, children, Egypt

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INTRODUCTION

Survival of children with cancer has improved dramatically in correlation with advances in therapy. These children require multiple transfusions during intensive therapy and are at increased risk for blood transmittable infections such as HBV, HCV and HIV infections (Kebudi *et al.*, 2000).

Although the HBV contact rate is similar both in multiply transfused children with benign hematological disorders and in cancer; the HBs Ag positivity was found to be much higher in children with cancer. Survivors treated before adequate blood donor screening for Hepatitis C Virus (HCV) was initiated in the early 1990s, are at risk for chronic liver disease (Castellino *et al.*, 2004).

Prevalence of circulating HCV-RNA in Acute Lymphoblastic Leukemia (ALL) patients treated before 1990 ranges from 6.6-49%, with an unknown and likely sizable percentage of survivors never having been tested or aware of their risk (Strickland *et al.*, 2000).

Aggressive chronic HCV infection has also been observed in survivors co-infected with hepatitis B and in those treated with hematopoietic stem cell transplantation (Peffault de Latour *et al.*, 2004). Reports of decompensated cirrhosis and hepatocellular carcinoma in childhood cancer survivors with chronic HCV suggest that this population is at increased risk of liver-related morbidity and mortality (Castellino *et al.*, 2004).

This study aimed at detecting the prevalence of hepatitis B and C viral infections in children with malignant diseases and its correlation with different risk factors.

MATERIALS AND METHODS

One hundred patients proved to have pediatric malignancy attending to the pediatric oncology departments at Damietta Cancer Institute and at Cairo University Hospitals were investigated. The age ranged from 0.5 to 14 years with a mean age of 6.9 ± 4.2 years and male to female ratio 1.6:1.

Cases whether having hematological or solid tumors were subjected to full history taking and clinical evaluation with special emphasis on history of blood transfusion, parenteral therapy, previous operation and history of hepatic dysfunction, in addition to liver biochemical profile, HCV Ab. by third generation ELISA (INNOTEST kit; Innogenetics, Innogenetics N.V., Zwijnaarde, Belgium). In HCV Ab. positive cases, HCV RNA was assessed by PCR (RNA extraction was performed using QIAamp Viral RNA Mini Kits (QIAGEN Inc., Chatsworth, CA, USA) Reverse transcriptase

reaction and PCR were carried out with superscript one-step RT-PCR system supplied by (Life Technologies Gibco BRL, USA).

HBsAg assay (Murex HBsAg kit version 3; Murex Biotech, Dartford, Kent, United Kingdom) and in HBsAg positive cases, HBV DNA detection by PCR was performed (DNA extraction using QIAGEN DNA isolation Kits (QIAGEN Inc., Chatsworth, CA, USA). HBV-DNA in the serum was investigated by the primers detecting pre-S region of the genome (Boehringer-Mannheim).

Statistical analysis: Collected data were coded and arranged to facilitate its manipulation. Data were analyzed and computed using Statistical Package for Social Sciences (SPSS) version 10. Simple statistics as frequency, arithmetic mean and standard deviation were used. Bivariate relationships displayed in cross tabulation were carried out. Chi square test and student t test were used for comparison.

RESULTS

Figure 1 shows that HbsAg was present in 9% (5% HBsAg alone + 4% co-infected with HCV) of patients, while HBV-DNA was detected in 7 out of the 9 patients with HBsAg (77.8%). HCV-Ab was present in 43% (39% HCV-Ab alone + 4% co-infected with HBV) of patients among whom 33 (76.8%) were +ve for HCV RNA. None of the cases was proved to have co-infection according to the PCR results. There was no significant relation between sex and age in relation to hepatitis markers. The seromarker status of parents is not known. The type of malignancy whether hematological or solid and hepatitis markers were not significantly related.

Regarding the distribution of different types of malignancies among cases, out of the 100 patients, 8 cases had leukemia while 23 patients were documented to have non-Hodgkin lymphoma and 14 had Hodgkin's

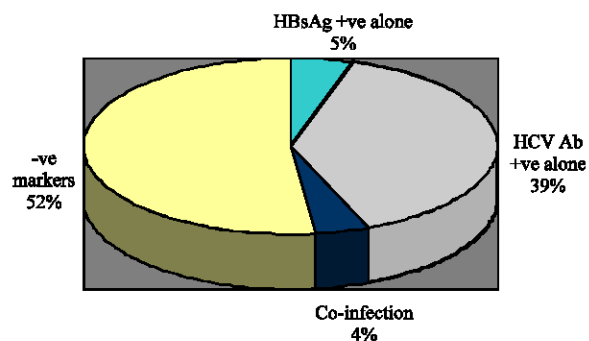


Fig. 1: Hepatitis markers of 100 children with malignant diseases

disease. The remaining 55 patients had solid tumors (mainly neuroblastoma and soft tissue sarcoma). The type of malignancy whether hematological or solid and hepatitis markers were not significantly related.

Past history of blood products transfusion (whole blood, red cells, platelets or plasma) was found in 72% of patients with a mean number of 9.9±5.9 units. The relation between hepatitis markers and history of blood transfusion is presented in Table 1. There is a significant relation regarding history of transfusion and HCV Ab (p = 0.001) and HCV RNA (p = 0.007). No significant relation was present between hepatitis markers positivity and the number of blood units transfused.

Past history of platelet transfusion was present in 48% of patients with a mean number of units 20.06±10.98. It was noticed that 47% of patients had been exposed to surgery during therapy. There was a positive association between the mean number of platelet transfusions and the presence of HBsAg (p<0.05). There was no significant relation between history of surgical intervention and hepatitis markers.

Regarding liver functions tests, 43% had elevated alanine aminotransferase (ALT) levels (at least 1.5 folds

the upper limit of normal). ALT levels were significantly higher in HCV Ab positive than negative cases (p = 0.003) (Table 2).

DISCUSSION

Results of this study showed that the seropositivity for HBV in cases with pediatric malignancy to be 9% for HBsAg (Fig. 1). A higher prevalence (18.2%) was reported by Mostafa *et al.* (2003) among Egyptian children with cancer and in India (60%) (Arora *et al.*, 2003). Present results are near to those obtained by Turkish groups, who stated that the prevalence of HbsAg among children with malignancy is 10% (Kebudi *et al.*, 2000) and 11.6% (Sevinir *et al.*, 2003). Present results are slightly higher than those obtained in Saudi children with malignancy (6%) (Bakir *et al.*, 1995). While, there was no HBsAg seropositivity among cases in the United States (Monteleone *et al.*, 1994).

The difference can be explained by the fact that the global prevalence of HBV varies widely from low <2% as in Western Europe, North America and Japan to high >8% as in Africa, Southeast Asia and China (El Khouri and dos Santos, 2004). Egypt is considered to be a region of intermediate prevalence for HBV infection ranging from 3.2% (Zaki *et al.*, 2003) to 4.3% (El-Gilany and El-Fedawy, 2006).

Previous studies for HBs Ag positivity gave very high results (40%) and the recent decline in incidence owes to the use of more sensitive screening methods for blood and blood products, as well as the recent application of compulsory mass vaccination against HBV (Kebudi *et al.*, 2000).

Seven percent of our cases were HBV DNA positive, a result similar, but slightly higher than that reported by Sevinir *et al.* (2003) (5.5%).

Regarding HCV, 43% of our cases were found to be positive for anti-HCV antibodies (Fig. 1). HCV PCR was performed for all antibody-positive cases and infection was confirmed in 33% of cases. These results are much higher than the general population as El-Raziky (2007) reported; that the seroprevalence HCV infection is 2.02% in Egyptian children. Previous reports from Egypt demonstrated a prevalence of 3-9% in children (Medhat *et al.*, 2002; Habib *et al.*, 2001), respectively.

An earlier study from Italy, reported that 43% of children with Acute Lymphoblastic Leukemia (ALL) had an evidence of HCV infection and this is similar to the results of the present study (Arico *et al.*, 1994). Other studies show much lower rates of HCV infection among cancer patients. In Italy, Cesaro *et al.* (1997), have

Table 1: Relation between hepatitis markers and history of blood products transfusion

Hepatitis markers	History of transfusion		p-value
	Yes	No	
HBs Ag			
-ve	66	25	>0.05
+ve	6	3	
HBV DNA			
-ve	8	2	>1
+ve	6	1	
HCV Abs			
-ve	32	25	<0.05*
+ve	40	3	
HCV RNA			
-ve	6	4	<0.05*
+ve	33	0	

*Significant at p<0.05

Table 2: Relation between hepatitis markers and ALT

Hepatitis markers (No.)	Mean±SD X ULN	p-value
HBs Ag		
-ve(91)	1.70±1.04	>0.05
+ve(9)	2.00±1.00	
HBV DNA		
-ve(2)	2.40±1.30	>0.05
+ve(7)	2.00±1.00	
HCV Ab		
-ve(57)	1.46±0.70	<0.05*
+ve(43)	2.20±1.20	
HCV RNA		
-ve(10)	1.80±0.90	>0.05
+ve(33)	2.20±1.30	

SD: Standard Deviation; *Significant at p<0.05; X ULN: Times the upper limit of normal

detected HCV infection in 17.8% of pediatric malignancy patients. Prevalence of HCV infection among Italian children is 0.36% (Cesaro *et al.*, 1997). In a clinical study done by Strickland *et al.* (2000) to detect HCV infection among survivors of childhood cancer in, 6.6% of patients had evidence of HCV infection. Also in the US it was reported that, 9.8% of pediatric malignancy patients were HCV positive, while the United States childhood HCV incidence is 0.2 to 0.4% (Monteleone *et al.*, 1994).

In a study conducted on Saudi children receiving cycled cancer chemotherapy, the prevalence of anti-HCV was 11% while in the general population is 1% (Bakir *et al.*, 1995). Kebudi *et al.* (2000), reported that 14% of cancer patients are seropositive for HCV antibodies (HCV prevalence in Turkey is less than 2%). A more recent Turkish study reported that 5.5% of children with cancer were anti-HCV positive (Sevinir *et al.*, 2003).

In the current study 33% of our patients were positive for HCV-RNA. In India Arora *et al.* (2003) stated that 38% of cancer pediatric patients were positive for HCV-RNA by PCR (HCV infection in India has a population prevalence of around 1%) (Acharya *et al.*, 2006). In Turkey Sevinir *et al.* (2003) reported a much lower rate of 3.5% in a similar studied population.

So, our high results, in comparison to the international data can be attributed to the high prevalence of HCV among the Egyptian population (Medhat *et al.*, 2002; Habib *et al.*, 2001).

In the current study 4% of children had both HBV and HCV seropositivity (Fig. 1). Higher rates were reported by other authors: 12.3% (Januszkiewicz *et al.*, 1997), 22% (Cesaro *et al.*, 1997) and 26.4% (Arora *et al.*, 2003). A lower rate was reported in Turkey (2%) (Sevinir *et al.*, 2003).

The seroprevalence of HBV and HCV were not statistically different when comparing male and female and this agrees with a study done in Saudi Arabia in 2004 that showed, there was no statistically significant difference in male to female ratio regarding HCV and HBV (El-Hazmi, 2004).

In the current study, as in a previous study, no significant relations were found between the seroprevalence of HBV and HCV and the exposure to the surgical procedures, although invasive procedures represent an important mode of transmission (Mostafa *et al.*, 2003).

On looking for the relation between the number of blood units transfused and the seroprevalence of HBV in the present study, we were able to find that the higher the number of platelets units transfused (but not other blood products) the more is the incidence of HBV infection.

Studies done in Turkey showed that there was no statistically significant relation between the number of blood units and the risk of acquiring HBV (Kebudi *et al.*, 2000; Sevinir *et al.*, 2003).

Regarding HCV prevalence, there is a significant relation regarding history of transfusion and HCV Ab ($p = 0.001$) and HCV RNA ($p = 0.007$) (Table 1), while, no significant relation was found to link them to the number of blood units transfused and these results confirm the previously done work, which showed that, the number of donor exposure was not significantly different between HCV positive and HCV negative patients (Arico *et al.*, 1994). Also the same result was obtained by Kebudi *et al.* (2000) and Sevinir *et al.* (2003). Older studies reported that, anti-HCV positive children had received significantly more blood products transfusions compared to seronegative patient (Fink *et al.*, 1993). Also, a strong relation between the volume of blood infused and the risk for HCV infection, explains the higher prevalence of HCV infection among patients tested before anti-HCV blood screening was available (Strickland *et al.*, 2000).

In comparing the seroprevalence of HBV and HCV with different types of malignancy (i.e. hematological and solid tumors) we were not able to demonstrate a significant difference. Similar results were obtained by Cesaro *et al.* (1997) who showed that prevalence of HCV infection did not show any significant change in the distribution between leukemia/lymphoma and solid tumor patients, even if the former group had a higher exposure to risk factors for HCV infection (i.e., more frequent blood-products transfusions, invasive diagnostic procedures and frequent blood sampling) (Cesaro *et al.*, 1997).

In comparing the seroprevalence of HBV and HCV with ALT (Table 2), we were not able to demonstrate a significant elevation in ALT with cases of HBV seropositivity, while an Egyptian study concluded that children with hematological malignancies have worse liver disease when associated with chronic HBV (El-Sayed *et al.*, 2003).

Our cases expressed significantly higher ALT levels in HCV positive compared to negatives. Similarly Arora *et al.* (2003) found that ALT and AST were significantly higher in the HCV +ve group. Similar results were obtained in a study done in the United States which showed that ALT levels after cessation of treatment and during treatment of children with cancer were significantly higher in the HCV- positive group versus the HCV-negative group (Monteleone *et al.*, 1994). According to a study by El-Raziky *et al.* (2004), HCV infection is not always benign in the Egyptian children as ALT levels are elevated in half of the studied children.

CONCLUSION

Evidences of HBV and HCV exposure were present in around half of cases with childhood malignancies. Blood and platelet transfusions were the most identifiable risk factors for the acquisition of HCV and HBV.

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