Non Hodgkin Lymphoma and Hepatitis C Virus in Egyptian Children: One Centre Study

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Abstract: Non Hodgkin lymphoma (NHL) represents a major health problem in the world. In Egypt, where both, hepatitis C virus is highly endemic and NHL has high incidence, questions arise about the association of both diseases especially in children.

The primary aim of this study was to describe the epidemiological situation of NHL in children associated with HCV in Mansoura, Egypt.

Pediatric patients provisionally diagnosed as suffering from NHL were recruited from hematology unit at Mansoura University children hospital (MUCH) from January 2013 to March 2014. Hematologists collected demographic characteristics, clinical history, and laboratory and treatment data.

Blood samples were collected from each participant in the study and subjected to complete virological profiles to detect hepatitis C specific IgG, hepatitis B Surface antigen (HBsAg) and anti hepatitis B core IgM (HBcIgM) and HIV specific immunoglobulin G. Sera samples were subjected to real time polymerase chain reaction to determine active infection.

The present study was carried out on 92 children with NHL. They were mainly males (69.9%) with mean age 6.9± 3.7 years. The lymphoma was mainly Burkitt type (54.3%). The patients were mainly in stage 3 (80.5%). HCV IgG was positive in 41.3% of the patients and HCV viremia was detected in 43.5% of the patients, while none of the control subjects had any positive HCV markers.

In comparative study between NHL associated with active HCV and those not associated with active HCV, there was statistically significant elevations of ALT (P=0.002), AST (P=0.03) and bilirubin (P=0.005). History of blood transfusions, though was common among NHL associated with HCV (75%) than those not associated with HCV (42.3%), this was statistically insignificant difference (P=0.13). HCV viremia was associated with 30% of patients with recent incidence of NHL. There was statistically significant association of HCV IgG with HCV viremia (90%-P=0.0001).

The results presented here describe a strong association of chronic HCV infection with NHL. Much remains unknown about the natural history of HCV infection and its possible contribution to carcinogenesis; however, our data suggest that NHL may be among the malignant complications of chronic HCV infection in children. It is strongly recommended to screen all children, in endemic areas for HCV, affected with NHL for HCV IgG and when positive to evaluate them by PCR.

Keywords: NHL, Egyptian children, HCV.

INTRODUCTION

Hematological malignancies are groups of neoplasm widely spread that compromise wide varieties of malignancy. Their origin is from two groups of immunological cell types mainly myeloid and lymphoid cells. They compromised wide varieties of diseases mainly leukemias, lymphomas, plasma cell tumors myelodysplastic syndromes and mastocytosis. Lymphoma is classified mainly as Hodgkin and Non-Hodgkin's lymphoma (NHL). NHL has much higher incidence than Hodgkins lymphoma (HC) with different geographical distribution across the world. It is reported that in developed countries in Europe and in west the rates of NHL is more than 10 per 100,000, while parts of South and East Asia and Africa reported rates of less than 5 per 100,000. Egypt exceptionally has high incidence of lymphoma [1].

Egypt has been claimed to have high incidence of NHL that is higher than developed countries and united states [2, 3]. In Egypt, NHL represented the second most common cancer in adults and lymphoma is the most common cancer in children [4].

NHL has high incidence seen in patients with immunocompromised conditions or in association with viral infections like hepatitis C virus (HCV), Epstein–Barr virus, or HIV [5,6]. There is also limited evidence suggesting association of NHL with some environmental conditions like higher exposure to ultraviolet light [7], pesticides [8], ionizing radiation and alkylating agents such as those used for chemotherapy [9-14].

In Egypt where high prevalence of HCV infection is reported to be in range of 13.9%- 15.8% of the healthy population [15], this high rate of HCV infection may predispose to NHL, especially B-cell type NHL.

It is well known that HCV infection affects basically B lymphocytes leading to lymphoproliferation and

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mixed cryoglobulinemia [16,17]. HCV has been claimed to be the etiological pathogen for the development of NHL and certain reports determined that the risk for development of NHL is a two to four times higher among HCV-positive patients [18, 19]. The association of HCV infection and NHL is clear in Egypt since the pattern of incidence of lymphomas is quite similar to the pattern of incidence of hepatocellular carcinoma [20].

The primary aim of this study was to describe the epidemiological situation of NHL in association with HCV in children in Mansoura, Egypt.

MATERIAL AND METHOD

Pediatric patients provisionally diagnosed as suffering from NHL were recruited from hematology unit at Mansoura University children hospital (MUCH) from January 2013 to March 2014. Hematologists collected demographic characteristics, clinical history, and laboratory and treatment data. In addition, seventy nine healthy children matched in age and sex were included as control group.

Patients and Eligibility

All patients met the following study criteria: children with age below 18 years diagnosed with Burkitt or non Burkitt (according to the Revised European-American Lymphoma Classification/or the World Health Organization Classification). The diagnosis and staging was established according to physical examination, a full blood count (FBC), a metabolic evaluation, chest X-ray scan, a MSCT scan of neck, thorax, abdomen and pelvis to evaluate the extent of the spreading of the disease and staging, a CT brain scan where indicated. A written informed consent was required from the patients' parents. The study was approved by ethical committee of Mansoura Faculty of Medicine, Egypt.

Blood samples were collected from each patient and control children included in the study and subjected to complete virological profiles to detect hepatitis C specific IgG (Diapro-Italy), hepatitis B s antigen and anti core IgM and HIV specific immunoglobulin G (BIO-RAD HBsAg, FRANCE). Sera samples were subjected to real time polymerase chain reaction to determine active infection either in the presence of HCV IgG or in its absence.

Quantitative HCV PCR by COBAS AMPLICOR HCVMonitor Test, Version 2.0 (HCM) Principle

The Cobas Amplicor HCV Monitor Test v2.0 includes steps involving specimen preparation, reverse transcription of the target RNA to generate complementary DNA (cDNA), PCR amplification of HCV target RNA, hybridization of the amplified products to oligonucleotide probes specific to the target, and detection of the probe-bound amplified product by colorimetric determination.

The HCV standard used as quantitation standard (QS) is a non-infectious RNA transcript that is incorporated into each individual specimen at a known copy number and it is carried through the same processes performed by the test. It contains the identical primer binding sites as the HCV RNA target and a specific probe binding region that allows QS amplicon to be distinguished from HCV amplicon.

Cobas analyzer calculates the HCV RNA levels in the sample by comparing the HCV signal to the QS signal for each specimen. Thus QS compensates for effects of inhibition and controls for the amplification process to allow the accurate quantitation of HCV RNA. The Amplicor HCV Monitor quantitates virus titers from 6 ×102 to 6 ×107 viral particles (IU) per ml of serum or plasma.

Statistical Analysis

The statistical analysis of data done by using excel program and SPSS program (Statistical package of social science) version 16. For qualitative data (frequency & proportion) chi – squire test was used. For quantitative data (mean SD) t – test was used for comparison of two groups and one way anova was used for comparison of more than 2 groups. N.B P is significant if ≤ 0.05 at confidence interval 95 %.

RESULTS

The present study was carried out on 92 children with NHL. They were mainly males (69.9%) with mean age 6.9 3.7. The control children were with similar sex and age distribution. The lymphoma was mainly Burkitt type (54.3%). The patients were mainly in stage 3 (80.5%). HCV IgG was positive in 41.3% of the patients and HCV viremia was detected in 43.5% of the patients, while none of the control subjects had any positive HCV markers, Table **1**.

In comparative study between NHL associated with HCV and those not associated with HCV, there was statistically significant elevations of ALT (P=0.002), AST (P=0.03) and bilirubin (P=0.005). History of blood transfusions, though was common among NHL associated with HCV (75%) than those not associated

Table 1: Clinical and Laboratory Data of the Studied Children

Parameter	Patients (n=92) (%)	Control (n=74) (%)
Sex Male Female	 64(69.9%) 28(30.4 %)	 44(59.5 %) 30(40.5 %)
Age mea± SD(y)	6.9 ±3.7	6.4±4
Pathology Burkitt B-Lymphoblastic Large cell Small cells Others	 50 (54.3%) 18(15.2%) 6(6.5%) 6 (4.3%) 14(15.2%)	 -
Staging Stage 2 Stage 3 stage 4	 10(10.9%) 74(80.5%) 8(8.7%)	 -
HCV IgG PCR Positive	38 (41.3%) 40(43.5%)	0(0%) 0(0%)

Table 2: Clinical and Laboratory Findings in Children with NHL According to Presence of HCV

PCR Positive (n=40) (%)	PCR Negative (n=52) (%)	P-value
Pathological Burrkits 26 (65%) Lympho 10 (25%) Large cell lymphoma 4 (10%) Small cell lymphoma 2(5%) Other 0 (0%)	 24 (46.1%) 8 (15.4%) 2 (3.8%) 4 (7.7%) 14 (26.2%)	 P=0.09
ALT 80± 5.0 AST 120± 2.0 Bilirubin 1.5 ±0.8 Blood transf. 30 (75%)	40±2.0 45±2.0 1.4±0.0 22 (42.3%)	P=.002* P=0.03* P=0.005* P=0.13
Chemotherapy 20 (50%)	10 (19.2%)	P=0.07*
Recent 12 (30%)	32 (61.5 %)	P=0.003*
1gG+ve 36 (90%)	2 (3.8%)	P=0.000*

*Statistically significant.

with HCV (42.3%), this was statistically insignificant difference (P=0.13). HCV viremia was associated with 30% of patients with recent incidence of NHL. There was statistically significant association of HCV IgG with HCV viremia (90%-P=0.0001), Table **2**.

DISCUSSION

There is association between HCV and NHL supported by previous studies in various countries, like Italy, and other countries like Thailand [20-32]. There were many shortage in these studies like small sample sizes, absence of control groups, and tested for HCV infection by serological rather than nucleic acid tests. Moreover, there were converses in these studies. Previous studies from Egypt supported this association [33].

The main finding of the current study is a positive association of current HCV infection with NHL with high prevalence of active HCV replication detected by real time PCR.

In the present study, there was high prevalence rate of HCV among children affected with NHL. Serological

markers were positive in 41.3% of children and active viral replication was found in 43.5% of children with NHL. In previous study included adults with NHL overall, 42% of subjects were anti-HCV positive and 33% had HCV RNA [34].

Finding the association only among those currently infected with the virus is on line with theory about how HCV may increase the risk of occurrence of NHL, with a minimum latency period between HCV infection and NHL development. Nevertheless, because NHL is a result mainly of a disrupted immune system, another theory may suggest a lower rate of viral clearance by NHL patients over period of time [34]. However, the young age of our patients does not support the second theory.

The possible source of HCV transmission are blood transfusions , but we did not find a significant difference among number of blood transfusions within children with HCV associated with NHL and those who were negative for HCV. Other exposures like injections and surgeries were not reported in our patients. Still, other sources should be excluded like materno-fetal transmission of HCV in children.

Few studies have indicated the association of HCV with NHL was more common within specific NHL subtypes related to lymphoplasmacytoid lymphoma with mixed cryoglobulinaemia type II, or, with follicular centre, marginal zone, and diffuse large NHL subtypes [35,36]. In the present report, though HCV was common in children with Burkitt lymphoma more than other subtypes of NHL this increase was not statistically significant. This may be attributed to the limited number of the studied children.

There was high prevalence of HCV among children under chemotherapy. This highlights that in areas with high endemic HCV prevalence like Egypt, screening for HCV at diagnosis of all new B-cell malignancies is important to help direct future treatment and to give clue about patients who may develop problems secondary to the HCV during or after therapy [37]. Patient's positive for HCV antibodies should be assessed for HCV viremia by molecular method, although until now there is no linking between baseline viral load and subsequent outcome of treatment. The degree of hepatitis with or without cirrhosis should be determined by liver biopsy, in viremic patients with abnormal liver function tests before therapy begins especially with high-dose chemotherapy.

The majority of lymphomas presenting concurrently with HCV carriage are managed with similar protocol of chemotherapy a similar to HCV-negative patients. For certain low-grade lymphomas there is increasing evidence that treatment of the HCV with antiviral therapy can lead to remission of the lymphoma. The explanation could be attributed to the eradication of underlying B-cell monoclonal proliferation when HCV is treated with interferon-α (IFN-α) [38], and there were reports of long lasting complete remission of lymphoplasmacytoid lymphoma accompanied with eradication of the virus with IFN-α [39]. Further evidence to support this hypothesis arises from a study of patients with splenic lymphoma with villous lymphocytes accompanied with HCV infection and cyroglobulinaemia [40]. In those patients, treatment of the HCV with IFN-α and ribavirin was followed by a complete remession of the lymphoma in the majority of patients while patients with the same lymphoma who were not infected with HCV did not respond to the IFN-α therapy. It is not clear if this line of therapy would be effective to other types of lymphomas. There are no available data on the relative effectiveness of the treatment and the prognosis of other subtypes of lymphoma when associated with HCV.

There was high prevalence of HCV detection by PCR compared to serological methods. These findings highlight the importance of the use of molecular method in those patients to determine the presence of HCV in the presence of disorders of immune system response.

CONCLUSION

The results presented here describe a strong association of chronic HCV infection. Much remains unknown about the natural history of HCV infection and its possible contribution to carcinogenesis; however, our data suggest that NHL may be among the malignant complications of chronic HCV infection in children. It is strongly recommended to screen all children, in endemic areas for HCV, affected with NHL for HCV IgG and when positive to evaluate them by PCR.

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