Original article

The prevalence of Hepatitis C Virus Infection in sample of Iraqi Patients With Non –Hodgkin's Lymphoma

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ABSTRACT

Background: Non –Hodgkin's Lymphoma (NHL) are monoclonal proliferation of lymphoid cells that may be of B-cell (70%) or T-cell (30%) origin and include many discrete entities with characteristic morphological ,immunophenotypical , genetical and clinical features . Hepatitis C Virus (HCV) is a hepatotropic and lymphotropic virus, several studies showed that HCV may chronically infect patients with Non–Hodgkin's Lymphoma with or without producing liver damage.

Objectives: To assess the prevalence of hepatitis C virus infection in Iraqi patients with non-Hodgkin's lymphoma and to compare with two control group healthy control and patients had general medical illnesses (unhealthy).

Material and method: A prospective case control study included 40 Iraqi patients with NHL. They were tested for the presence of anti-HCV using Enzyme Linked Immunosorbent Assay (ELISA). Positive results were subjected to confirmatory test using Recombinant Immunoblot Assay (RIBA).The diagnosis of NHL cases was confirmed by two consultants histopathologist and they were classified according to the Working formulation system (WF) .Two control groups was applied ; the first included 250 healthy individuals who were age and sex matched while the second group included 50 patients suffering from general medical diseases who were age and sex matched.

Result: Four out of 40 patients with Non–Hodgkin's Lymphoma (10%) were positive for anti-HCV, whereas only 1out of 250 healthy individuals (0.4%) were positive for HCV and no patient was positive in the unhealthy control group.

Conclusion : The current study revealed the high prevalence of HCV infection in Iraqi patients with Non-Hodgkin's Lymphoma compared to the healthy and unhealthy control groups which support the lymphogenetic role of chronic HCV infection in the pathogenesis of NHL.

Key word: NHL ,HCV , ELISA ,Immunoblot

Introduction:

Non-Hodgkin lymphomas (NHLs) are a heterogeneous group of malignancies arising from lymphoid tissue, with varied clinical and biological features [1]. According to the Iraqi Cancer registry for the years 2008 and 2012, NHL ranked the sixth most common cancer among the ten most common cancers in Iraq, with male predominance in all age groups. The majority of cases arise within the 6th and 7th decades.[2] Non-Hodgkin lymphoma (NHL) is more common in the developed countries with the highest incidence rate in USA, Australia and New Zealand and Europe and the lowest in Eastern and South Central Asia. [3]

However the ratios of mortality to incidence rate are higher in developing countries [4] .Moreover those countries display intermediate and high grade diffuse aggressive or peripheral T-cell NHL, extra nodal disease which is closely associated with Epstein-Barr virus (EBV) and Human T-cell Leukemia/ lymphoma virus type I (HTLV-1) infections. [5]

These findings indicate region specific differences in exposure to environmental factors especially infections and chemicals and coupled to a broad range of genetic polymorphism[4].

The etiology of many cases of NHL remain largely unknown, however many suggested and established risk factors have been applied including a positive family history of lymph proliferative malignancies , hereditary immune deficiency disorders , acquired immunosuppression such HIV and organ transplantation , some infectious agents such as EBV and H. pylori and some autoimmune disorders. [3] Thus more detailed studies and investigations should be performed to determine the exact mechanisms of the disease initiation and induction of molecular and genetic changes. [6]

Hepatitis C virus (HCV) is a positive, single-stranded RNA virus, member of the Flaviviridae family, during its replicative cycle it goes through a negative-stranded RNA, but not DNA, intermediate, so that integration of HCV nucleic acid sequences into the host genome seems unlikely. As such, it lacks a pivotal property of classical oncogenic retroviruses. The HCV genome produces a single polyprotein that is proteolytically processed by viral and cellular proteases to produce structural (nucleocapsid, E1, E2) and nonstructural (NS) proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) [7]

The laboratory tests used to diagnose hepatitis C virus (HCV) infection consist of serologic assays that detect human antibodies against HCV (anti-HCV) and molecular assays that detect HCV nucleic acid. [8]

There are three different types of assays that can detect antibodies to hepatitis C virus: enzyme immunoassay (EIA), chemiluminescent assay (CIA) and recombinant immunoblot assay (RIBA). The EIA test is now the dominant HCV screening test used in clinical practice and this assay detects antibodies against epitopes derived from the HCV core, nonstructural 3, nonstructural 4, and nonstructural 5 regions. The RIBA originally was developed as a high-specificity confirmatory test for patients with a positive EIA result, but the importance of the RIBA has diminished with the marked improvement in specificity of the 3rd generation EIA tests and with the more widespread use of molecular assays. The recombinant immunoblot assay (RIBA) identifies the specific antigens to which antibodies are reacting in the EIA, and the results are interpreted as positive (2 or more antigens) [8,9]

Over the past 2 decades considerable evidence has accumulated on the association between hepatitis C virus (HCV) and hepatitis B virus (HBV) and several hematologic malignancies, most notably B-cell non-Hodgkin lymphoma (NHL) [10]. The aim of this study was to assess the prevalence of hepatitis C virus infection in Iraqi patients with non-Hodgkin's lymphoma and to compare with two control group healthy control and patients had general medical illnesses (unhealthy)

Patients' material and methods :

This case control study was conducted on 40 adult patients (> 15 years) with diagnosis of NHL who were attending Imamein Kadhimein Medical City and Baghdad Teaching Hospital during the period from first February 2014 to October 2014 .Eight patients were newly diagnosed with NHL and the rest were seen during follow up visit.

The diagnosis of NHL was confirmed by two consultant's histopathologist and was classified according to the Working Formulation .Consent for the participation in the research was obtained from each patient. All patients were married with no history of alcohol abuse and were not receiving interferon therapy. Any patient who was diagnosed with HCV after the diagnosis of NHL and those who were HCV seropositive NHL patient with overt high risk for viral infection were excluded (ex: drug abuser , patient on dialysis , patients who had received blood product after diagnosis of lymphoma and patient with significant cirrhosis)

Two control groups were applied; the first included 250 age and sex matched healthy individuals while the second included 50 age and sex matched patients having general medical diseases such as diabetes mellitus, cardiac disease, dyslipidemia; this group was most matched with the patient group to exclude any possible risk of hospitalization or effect of treatment or the disease on the result of the

study. For both control groups the same exclusion criteria to that used in patients group was applied

Sample collection: From each patient and control, three milliliters of venous blood were aspirated and transferred to sterile plain tube. The sample was centrifuged at 1000 rpm for 5 minutes to separate the serum which was dispensed into tightly closed Eppendorf tubes and stored at -20 C° until the time of the assay.Anti HCV was tested for all NHL patients and control groups in 100 ul of serum using sandwich immunoassay ELISA technique manufactured by CUSABIO BIOTECH co. code reference 4250, LOT: S3C1/4 . The procedure was carried out for patients, control and standard samples in accordance with the manufacturer's instructions of the kit. The concentration of the anti-HCV in the sample was determine by comparing the O.D, of the sample to that on the standard curve by using spectrophotometer at a wave length of 450 ±10 nm . Patients with positive or intermediate results were tested by Recombinant Immunoblot Assay (RIBA) to confirm positive results .

The confirmatory Recombinant Immunoblot Assay (RIBA) test was done following the manual instruction of the Kit of RIBA 3.0 Strip Immunoblot Assay (Chiron RIBA HCV 3.0 SIA, Chiron Corp., Emeryville, CA, USA) 3.0 SIA #930600

Results

This study had revealed that the mean age of NHL patients was 58.2±9.13 years (mean±SD) with range of 35-81 years and it was more common in male than female as shown in table 1 and 2 .

The present study had revealed that 4 out of 40 patients with NHL (10%) were positive for anti-HCV using both ELISA and RIBA technique for diagnosis as shown in table 3. Regarding control groups ; the first healthy control group showed that one out of 250 healthy individuals (0.4%) was positive for Anti –HCV whereas no positive case was detected in the second unhealthy control group (0%). Table 3

By applying Chi square test , both control groups differed significantly from the patient group .Table 3 The distribution of NHL cases according to the Working formulation classification was shown in table 4 , which showed that the most frequent type was the intermediate subtype and the least subtype was the high grade. Regarding HCV positive NHL patients , three of them had intermediate grade and the other one had low grade lymphoma .P>0.05.

Two of the four HCV positive NHL patients gave history of previous blood transfusion and were diagnosed with HCV more than 10 years before the diagnosis of NHL. Whereas the other patient did not give any history suggesting the route of infection and was diagnosed with HCV six years before the diagnosis of NHL . Any patients who were diagnosed with HCV after the diagnosis of NHL were excluded from the study. This study revealed that HCV was detected in 5 out of 340 (1.47 %) Iraqi individuals from Baghdad.

Table 1: The age distribution of the three studied group

Groups	Mean age ±SD (Years)	P-Value
Patients group n =40	**58.2±9.13 (35-81 years)**	=
1st control group n =250	**57.9±8.5 (40-71 years)**	**>0.05***
2nd control group n = 50	**58.7±10.5 (40-68 years)**	**>0.05***

P-Value > 0.05 non- significant .

Table 2 : The gender distribution of the studied groups

Groups	Male		Female		P value
	no.	%	No.	%	
Patients	29	72.5	11	27.5	<0.05
Control 1st group	155	62	95	38	>0.05
Control 2nd group	32	64	18	36	>0.05

P-Value > 0.05 non- significant

Table 3- The incidence of HCV in the study groups

	Patients N=40		1st control N=250		2nd control N=50		P value
	no.	%	no.	%	no.	%	
HCV +	4	10	1	0.4	0	0	<0.0001
HCV_	36	90	249	99.6	50	100	<0.02

Table 4 : The distribution of the cases according to the WF classification of NHL

Grading	NHL n= 40	HCV +ve n= 4	%	p-value
Low	13	1	7.7	> 0.05
Intermediate	21	3	14.2	
High	6	-	-	
Total	40	4	10	

Discussion:

This study had revealed that NHL was more common in adult male than female which was consistent with Iraqi cancer registry and other Iraqi and non-Iraqi studies [2]

This study had demonstrated that the prevalence of HCV infection in sample of NHL Iraqi patients was significantly high reaching 10% as compared to normal healthy control population (0.4%) and unhealthy control patients (0%). A positive association between HCV and NHL was first described by Ferri et al [10] and Pozzato et al [11] , and was further confirmed in a large number of studies Mele et al [12] Duberg et al [13] and Anderson et al [14] as well as in Mezzaro et al study in 2005[9] ; those studies had found that the prevalence of hepatitis C virus infection in non-Hodgkin's lymphoma had ranged between 7.4 and 37.0%.

Since the pathogenic mechanism involved in hepatitis C virus-associated lymphomas remains considerably unknown, thus it is assume that the virus may exert its oncogenic potential via an indirect mechanism or utilizes other pathways directly and that several different pathogenic mechanisms may operate in the etio-pathogenetic role of hepatitis C virus in non-Hodgkin's lymphoma [9]

Zignego et al and Giordano et al studies had found that HCV-induced lymphomagenesis through three pathways ; (A) Chronic antigenic stimulation of a B cell through the interaction of B cell surface Igs with the cognate HCV antigen. (B) HCV-E2 protein will engage its high-affinity receptor CD81 which is expressed on B cells. (C) Direct infection of a B cell by HCV. [15,16] In the current study the NHL associated HCV cases were of the intermediate and low grade subtype ; this was similar to Mazzaro et al study who had state that lymphoproliferative disorders related to hepatitis C virus usually include the intermediate -grade lymphoma, and the more common indolent, low-grade lymphoma .[9]

The present study had tested HCV in 340 subject and it was positive in 5 of them (1.47 %) which can be considered as a limited number of community base study. HCV Infection occurs

throughout the world, however the incidence of HCV on a global scale is not well known, because acute infection is generally asymptomatic A review study was done by Hanafiah et al to estimate age specific sero-prevalence curves in 1990 and 2005, it found that globally the prevalence and number of people with anti-HCV has increased from 2.3% to 2.8% between 1990 and 2005. Central and East Asia and North Africa/Middle East are estimated to have high prevalence (>3.5%); South and Southeast Asia, sub-Saharan Africa, Andean, Central, and Southern Latin America, Caribbean, Oceania, Australasia, and Central, Eastern, and Western Europe have moderate prevalence (1.5%-3.5%); whereas Asia Pacific, Tropical Latin America, and North America have low prevalence (1.5%) [18] It is recommended that wide scale survey should be done to study the prevalence of HCV in Iraq .

Even though Hepatitis C virus (HCV) has been recognized as a potential cause of B-cell lymphoma but the management of them is still similar to that of conventional lymphoma ; thus recently trial therapy with antiviral therapy was applied for treatment of low grade hepatitis C viral –related lymphomas which had lead to regression of them.[17]

Conclusion:

References This study had show greater prevalence of HCV infection in NHL patients compared to that reported for the normal population .Moreover the intermediate and low grades are the most frequent subtypes

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