MEDICAL CHANNEL

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ASSOCIATION OF HEPATITIS C VIRUS INFECTION TO NON HODGKIN'S LYMPHOMA: IS THE VOLCANO NEAR TO BURST?: A CASE CONTROL STUDY

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ABSTRACT

Introduction & Objectives: Limited data is available on association of HCV infection and NHL in Pakistan. This was a matter of particular interest. So, this study was conducted to determine the association between HCV infection and NHL.
Duration & Design: This 2 year observational, case control study was started on 1st January 2009 at C.M.C Larkana, after approval of ethical review committee.
Methods & Materials: After informed written consent, blood samples from 292 NHL cases and 1168 age and sex matched controls meeting our selection criteria, were sent for detection of Anti-HCV Ab on 3rd generation ELISA. Pearson's chi-square test was applied to compare Anti-HCV Ab seropositivity in cases and controls and odd ratios were computed using SPSS.10. P value of .05 was taken as statistically significant.
Results: 52 (17.8%) of cases, 45 (7.7%) controls in group 1 and 50 (8.6%) in group 2 were Anti-HCV Ab positive, with odd ratio of 2.59 (95% CI; 1.69 – 3.97) for control group 1 and 2.31 (95% CI; 1.52 – 3.50) for control group 2. P value was .000 for both. Beside, Anti-HCV Ab positive NHL cases were more likely to be in middle age (40-60) years with odd ratio of 3.68 (95% CI; 2.07 – 6.50) and p value of .000.
Conclusion: Anti-HCV Ab seropositivity is associated with 2.5 times the risk of development of NHL and seropositive cases tend to be in middle age category than seronegative.

Key Words: Hepatitis C virus (HCV), Anti-HCV Ab, ELISA, Association, Non Hodgkin's Lymphoma (NHL).

INTRODUCTION

Hepatitis C virus (HCV) is the most common cause of chronic liver disease in Pakistan.[1] Globally an estimated 170 million persons are infected with HCV and around 3-4 million are newly infected each year with HCV.[2] Currently the prevalence of HCV in Pakistan is 4-7%.[3] HCV is a hepatotropic virus, causing chronic hepatitis, cirrhosis and hepatocellular carcinoma.[4]

Recent reports have documented that HCV is also lymphotropic and by infecting peripheral mononuclear cells can predispose to lymphoproliferative disorder as Non-Hodgkin Lymphoma (NHL). Persistence of chronic HCV in immune secreting lymphocyte along with genetic and environmental factors is postulated.[5-10]

Increased frequency of B-cell clonality, t(14:18), bcl-2 and Bcl-6 gene translocation, STAT3 activation, up-regulation of ERK and p38 MAPK signaling pathways, is seen in patients of NHL infected with HCV and HBV. Possible mechanisms for malignant transformation are clonal proliferation of B-cells, inhibition of apoptotic cell death or both.[11-14]

Survival after developing NHL at present standard therapy is 64.5% at 5years depending on stage, grade, histological diagnosis, age and thus decrease the overall survival.[15] NHL associated with HCV has excellent response to Interferon-?2 and 75%-100% become disease free after treatment. Thus with early detection and treatment of HCV infection,

subsequent development of NHL can be prevented and survival improved.[16,17]

There are limited studies conducted in our region on this issue. This was a matter of particular interest for us, given the documented association between non-Hodgkin's lymphoma and hepatitis C virus infection. So, this case control study was carried out to determine the current frequency of HCV infection in patients of NHL as compared to other non-hematological malignancies and diseases free relatives of NHL patients. This study will not only provide the magnitude of HCV in patients with NHL but will also provide useful information regarding the association of HCV infection and NHL.

MATERIAL AND METHODS

This two year case control study was started on 1st January 2009, after the approval of ethical review committee of C.M.C Larkana. All cases and controls were enrolled after informed written consent from Departments of C.M.C Hospital Larkana and LINAR (Larkana Institute of Nuclear Medicine And Radiotherapy).

Inclusion Criteria

1. All consecutive biopsy proven and newly diagnosed cases of NHL
2. Patients of either sex.

Exclusion Criteria

1. Patient not consenting.
2. Previously diagnosed HCV patient.
3. History of exposure to radiations and chemo therapy.
4. Patient receiving treatment for NHL either palliative or curative.
5. Patient who has received or under gone any blood transfusion and major surgical procedure in last six months.
6. Previously diagnosed as a case of medical disorder requiring parenteral medications e.g. Hemophilia, thalessemias, etc.
7. Patient co-infected with Human Immunodeficiency Virus (HIV) or HBV or HBV with HDV (delta antigen).
8. Patients suffering from immunodeficiency state and auto immune disorders.

Selection of Cases and Controls

A total of 378 NHL patients were seen during our study duration. 5 patients were excluded as they had received blood transfusion or medications or undergone surgical intervention in last 6 months. 1 patient had past history of irradiation exposure was also excluded. 4 HIV, 74 HBV and 2HDV infected patients were also excluded from the patients. After exclusion, remaining 292 treatment naïve cases of NHL were selected for study and labeled as case group. Two sex and age (± 5 yrs) matched control groups were formed. Control group 1 (n=584) consisted of 2 first degree relatives of each case, that also matched cases by their district of origin (native place). Control group 2 (n=584) consisted of 2 age and sex matched patient suffering from non hematological malignancies admitted in different wards of C.M.C Hospital Larkana and LINAR. Controls in group 2 were also matched for date of admission (? 7 days). Patients of Hepato-cellular carcinoma were excluded from control group 2.

DATA COLLECTION

Blood samples from all cases and controls were withdrawn, coded and sent for detection of anti-HCV Ab on 3rd generation ELISA at Pathology Department of C.M.C Hospital, Larkana. The results were collected and transferred to predesigned performa by principal researchers.

DATA ANALYSIS

Data obtained from total of 292 NHL cases and 584 controls in each control group was transferred and analyzed using SPSS version 10. HCV infection was compared by Chi square test in both cases and controls and Odd Ratios with confidence intervals were computed in general and for age categories. P value of less than 0.05 was taken as statistically significant.

RESULTS

A total of 292 cases and 1168 controls were enrolled in the study. The cases consisted of 171 men (58.6%) and 121 women (41.4%) and their demographic characteristics are summarized in Table 1. The cases and controls were similar with respect to sex and age. Anti-HCV Ab was positive in 52 of 292 patients (17.8%) with non-Hodgkin's lymphoma, as opposed to 45 of 584 relatives (7.7%) in control group 1 and 50 of 584 subjects (8.6%) in control group 2. A higher risk of non-Hodgkin's lymphoma was found in anti-HCV Ab positive than in the HBsAg-negative subjects. The odds ratios were 2.59 (95% CI; 1.69 – 3.97) versus control group 1 and 2.31 (95% CI; 1.52 – 3.50) versus control group 2 with p value of 0.000 for both. The association between HCV infection and non-Hodgkin's lymphoma remained significant with mean Odd risk of 2.44 (95% CI; 1.69 – 3.52). (Table 2)

The anti-HCV Ab -positive rate was consistently higher for non-Hodgkin's lymphoma patients in every age group. Risk of non-Hodgkin's lymphoma was most evident in the 40-60 years & < 20 years anti-HCV Ab positive groups. The odds ratios of non-Hodgkin's lymphoma were 3.35 (95% CI; 1.33-8.40), 1.26 (95% CI; 0.47- 3.35), 3.68 (95% CI; 2.07- 6.50) and 2.42 (95% CI; 1.07-5.45) in the < 20year, 20-40 years, 40-60 years > 60 years age groups, respectively (Table 3).

DISCUSSION

To our knowledge, this is first study of its type conducted in Pakistan to observe the association of HCV to NHL. Considering the low incidence of NHL of 12 cases/100,000 population, a cohort for at least a decade or two will be required to definitely establish the impact of HCV infection on development of NHL. This was very difficult to perform in resource depleted countries as Pakistan. So, we opted for a case control study.

In this study, all efforts were made to exclude NHL cases that had exposure of any known risk factor for lymphoma development as irradiation exposure, HIV infection, HBV infection, exposure to cytotoxic or immunosuppressive drugs, autoimmune disorders and connective tissue disorders. Though smoking is a well known carcinogen, but its role in development of lymphoproliferative disorders is not well established, so we intentionally did not evaluated this aspect in our cases. Similarly alcohol intake is extremely rare in our society, so it was also not considered.

We also excluded cases with history of transfusion or parenteral medication, or surgical procedure in last 6 months, as well as known cases of hemophilia or thalessemias, as they may falsely elevate the number of anti-HCV Ab positive group. After exclusion, total of 292 patients met our selection criteria.

For cases we took age and sex matched controls. The advantage in selecting 1st degree relatives as control group 1 was that, the relative belonged to same locality of origin, plus environmental

Table: NO: 1. DEMOGRAPHIC PROFILE OF CASES AND CONTROLS

Characteristics	Case(N=292)	Control 1[a] (N=584)	Control 2[b] (N=584)	P Values*
AGE (years)				.000
Mean ±SD, y	44.23 ± 20.23y	45.11±19.50y	46.83 ±19.12y	
Range	70 (4-74)	66(8-74)	64(9-73)	
AGE CATEGORY, n (%)				.243
< 20years	34(11.6%)	83(14.2%)	82(14.0%)	
20 – 39 years	82 (28.1%)	136(23.3%)	137(23.5%)	
40 – 59 years	71 (24.3%)	161(27.6%)	161(27.6%)	
> 60 years	105 (36.0%)	204(34.9%)	204(34.9%)	
GENDER				1.000
Males	171 (58.6%)	342 (58.6%)	342 (58.6%)	
Females	121 (41.4%)	242 (41.4%)	242 (41.4%)	
HCV SEROPOSITIVITY				.000
POSITIVE	52 (17.8%)	45(7.7%)	50(8.6%)	
NEGATIVE	240 (82.8%)	539(92.3%)	534(91.4%)	

a. Control group 1= age and sex matched 1st degree relatives.

b. Control group 2= age and sex patients of non hematological malignancies.

*Pearson Chi – Square. (2-sided) Significance

TABLE: NO: 2. ODD RISK AND P VALUES FOR ASSOCIATION OF HCV AND NHL IN CASE AND CONTROLS

CHARACTERISTIC	CONTROL 1		CONTROL 2		TOTAL(1+2) *	
	OR[a]	95%CI	OR[b]	95%CI	OR[c]	95%CI
ANTI-HCV AB						
POSITIVE	2.59	(1.69 – 3.97)	2.31	(1.52-3.50)	2.44	(1.69-3.52)
NEGATIVE	1.00		1.00		1.00	
P values[d]	.000		.000		.000	

a. Risk of NHL taking control group 1 as reference

b. Risk of NHL taking control group 2 as reference

c. Risk of NHL taking control group 1&2 as reference

d. Pearson Chi – Square. (2-sided) Significance

TABLE: NO: 3. Anti-HCV Ab POSITIVE RATES OF CASES AND CONTROLS ACCORDING TO AGE GROUPS WITH ODD RATIOS AND P VALUES

CATEGORY	<20yrs	20-40yrs	40-60yrs	>60yrs
CASES				
n	9/34	6/82	27/71	10/105
%	26.47%	7.31%	38.03%	9.52%
CONTROL 1				
n	7/83	8/136	22/161	8/204
%	8.43%	5.88%	13.66%	3.92%
OR[a]	3.909	1.263	3.877	2.579
95%CI	(1.31 – 11.58)	(0.42 – 3.77)	(2.01 – 7.48)	(.986 – 6.74)
P values[b]	.010[c]	.676	.000[c]	.046[c]
CONTROL 2				
n	9/82	8/137	24/161	9/204
%	10.97%	5.84%	14.91%	4.41%
OR[d]	2.92	1.273	3.50	2.28
95%CI	(1.04 – 8.175)	(.426 – 3.80)	(1.83 – 6.68)	(.897 – 5.88)
P values[b]	.036[c]	.665	.000[c]	.076
CONTROL 1&2				
n	16/165	16/273	46/322	17/408
%	9.69%	5.86%	14.28%	4.16%
OR[e]	3.35	1.26	3.68	2.42
95%CI	(1.33-8.40)	(.47- 3.35)	(2.07- 6.50)	(1.07-5.45)
P values[b]	.007[c]	.631	.000[c]	.028[c]

a. Risks of non-Hodgkin's lymphoma among HBsAg-positive patients within specified age groups using control groups 1 as reference groups.
b. Pearson Chi – Square. (2-sided) Significance.
c. Statistically significant ($p < .05$).
d. Risks of non-Hodgkin's lymphoma among HBsAg-positive patients within specified age groups using control groups 2 as reference groups.
e. Risks of non-Hodgkin's lymphoma among HBsAg-positive patients within specified age groups using both control groups 1 and 2 as reference groups

factors and genetic makeup was supposed to be nearest to cases as compared to any other group of controls. Control group 2 consists of non hematological malignancy, to compute the odds of NHL in HCV as compared to other non hematological malignancies. Our study found statistically significant (p value 0.000) odd ratios for HBV in NHL cases. The odds ratios were 2.59 (95% CI; 1.69 – 3.97) versus control group 1 and 2.31 (95% CI; 1.52 – 3.50) versus control group 2. When taking both controls as reference, odd ratio was 2.44 (95% CI; 1.69 – 3.52) with p value of 0.000. From data, we can assume that HCV infected patients are 2.5 times more likely to develop NHL as compared to HCV non-infected patients. Beside, HCV infected patients are at more risk to develop NHL at any age, but the risk is more for young age < 20 years and middle age group 40-60 years having odd ratio of 3.35 (95% CI; 1.33-8.40) with p values of 0.007 and 3.68 (95% CI; 2.07-6.50) with p values of 0.000 respectively, as compare to other age groups as 20-40 years with odd ratio of 1.26(95% CI; 0.47-3.35) with p value of 0.631 and > 60 years with odds of 2.42 (95% CI; 1.07-5.45)

Cowgill et al. at Egypt studied the association of NHL to HCV

in 227 NHL cases as compared to controls of 227. He found statistically significant unadjusted association of HCV RNA with NHL of 2.3 (95% CI; 1.5-3.5). After adjustment statistically significant risk was found to be 2.9 (95% CI; 1.9, 4.5) with p value of 0.028.[18]

Seve et al. at France evaluated the role of hepatitis C virus (HCV) infection in the pathogenesis of non-Hodgkin's lymphoma (NHL). They compared anti-HCV antibodies in 212 consecutive patients with B-cell NHL with a group of 974 patients tested for HCV before transfusion at the same hospital during the same period. There was a positive but non-significant trend towards an association between NHL and HCV infection with odds ratio of 1.31 (95% CI; 0.51-3.36).[19]

Spinelli et al.at Canada investigated Hepatitis C virus (HCV) seropositivity and the risk of non-Hodgkin lymphoma (NHL) in 795 cases and 697 control subjects. HCV seropositivity was 2.4% in cases and 0.7% in controls with odd ratio of 2.6 (95% CI; 0.9-7.4).[20]

Takeshita et al. at Japan found that 45 (11.3%) of 400 B-cell lymphoma cases were HCV antibody (Ab) positive, which was significantly ($P < 0.01$) higher than the blood donors (2.5%). Nieters et al. in a European multicenter case-control study EPILYMPH and El-Sayed GM et al. in 2006 at Egypt also showed similar results.[21-23]

The above discussion based on the international literature has showed that Hepatitis C and Non Hodgkin Lymphoma have definite association. Despite that these two diseases are also very common in our region and country, there are very few studies available exploring the issue. The current trend of both these disorders is on remarkable increase. This put the public at risk of epidemic of NHL, which can result in decreased life expectancy, loss of gains achieved in last century due to improvement in health facilities and loss of productive part of nations.

CONCLUSIONS

1. HCV infection is associated with risk of development of NHL. HCV infected patients are 2-3 time more likely to develop NHL than non-infected patients and infected subjects are likely to develop NHL at young and middle age.
2. The results of our study provide a strong rationale from where other studies could build their hypothesis and massive epidemiological studies may be conducted.
3. Our study results would also provide the health policy makers and decision making bodies to pursue this as an issue in our country to control this combined epidemic.

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