Prevalence of HCV among Yemeni patients with Non-Hodgkin’s lymphoma at Al-Thawra Teaching Hospital.

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Abstract

Background

Epidemiological studies in different parts of the world have revealed controversial results on the association between HCV infection and Non-Hodgkin’s lymphomas. This discrepancy suggests that HCV lymphotropism and its effect on host lymphocytes may be influenced by regional and racial factors as well as by viral genomic variations.

Objectives

To determine the prevalence of HCV infection in Yemeni patients with NHLs diagnosed and treated in our hospital and to evaluate the association between the two diseases.

Methods

All consecutive patients with NHL treated in our Haematology/Oncology Unit between January 2005 and January 2007 were screened for anti-HCV antibodies by enzyme immunoassay. The prevalence of HCV infection in patients with NHLs was compared to that in a non-lymphomatous control group consisted of all patients checked for HCV infection with several acute medical conditions in the same hospital who were coming from different parts of the country.

Results

A total of 192 patients with NHLs were tested for anti-HCV antibodies. One hundred seventeen patients (60.94%) were males and 75 patients (39.06%) were females. The mean age of the patients was 41.30±19.15 SD and range between 5-80 years. Twenty-nine (15.1%) of the 192 patients were found positive for HCV infection. Twenty-one (17.6%) of 2 positive patients were male and only 8 (10.7%) patients were female. The mostly involved age group was 41-60 representing 24.6%. A total of 2039 patients from different departments were investigated for anti-HCV antibodies and used as a control. Of the 12274 control (60.4%) were males and 8055 (39.6%) were females. A total of 814 (4%) controls were found positive for HCV antibodies.

Of the positive controls, 529 (4.3%) were males and 285 (3.5%) were females. The association between HCV infection and NHLs was assessed using logistic regression models. There was a significant association of HCV with NHLs, the unadjusted OR [4.27, 95% CI 2.86-6.37] which differ slightly by gender in males the OR [4.86, 95% CI 3.01 - 7.85] and in female the OR [3.26, 95%CI 1.55- 6.84].

For separate NHL subtypes, the numbers of HCV infected cases were limited. Nonetheless, positive associations were noted for indolent type [OR=4.49, 95% CI 1.87-10.78] and for aggressive type as well [OR=4.215% CI 2.69-6.59].The prevalence of HCV antibodies in cases and controls was more frequent in males and in the age group above 40 years.

Conclusion

In conclusion the results of the present study indicate a higher prevalence of HCV infection among Yemeni patients with NHL than among persons in the control group. Therefore our study demonstrates an association between HCV infection and NHLs. HCV infection may play a role in causing NHLs.

Key words

Hepatitis C virus, Non-Hodgkin’s lymphoma, prevalence, Yemen.

Introduction

Lymphomagenesis is a multifactorial process in which environmental, genetic and infectious
underlying the disease (1-15).

Epidemiological studies performed in different parts of the world in Italy, Japan and Israel (16) as well as from USA (8,16) revealed a non-random association between HCV infection and NHLs in a significant number of cases, although other studies in Europe failed to confirm this association (16).

Objectives of the study

1. To determine the prevalence of HCV infection among Yemeni patients with NHLs diagnosed and treated in the Haematology/Oncology unit at Al-Thawra Teaching Hospital.
2. To compare the prevalence of HCV infection in Yemeni patients with NHLs and in non-lymphomatous patients admitted to the same hospital in the same period.
3. To evaluate the risk of association between HCV infection and development of NHLs.

Methods

The study was conducted in the Haematology/Oncology Unit at Al-Thawra Teaching Hospital–Faculty of Medicine–Sana’a, which is a referral hospital for all diseases requiring highly specialized health care from all parts of Yemen including patients with malignant diseases.

All patients included in the study were Yemeni Nationals and from different governorates.

Patients of the study group

A total of 192 unselected patients with NHLs were enrolled in the study during the period January 2005-January 2007.

Patients of the control group

A total of 20329 patients from general medical, surgical, orthopaedic, gynaecological, intensive care units, emergency room and healthy blood donors were randomly selected and included in the study. The data of the control group who had been tested for HCV infection was collected from the laboratory record during the same period of the study and in the same hospital. The inclusion and exclusion criteria were same for both groups.

**Inclusion criteria**

1. Confirmed histopathological diagnosis of NHL.
2. Absence of history of blood and/or blood components repeated transfusions.
3. Absence of past history of chronic liver disease.

**Exclusion criteria**

1. Patients with stigmata of chronic liver disease and history of HCV infection.
2. Patients with history of hemodialysis.
3. Patients without histopathological confirmation of the diagnosis of NHL.
4. Patients with positive HIV infection

**Pathological diagnosis of NHL**

NHLs were diagnosed on the basis of morphological evaluation of lymph node biopsies including bone marrow specimen or extranodal tissue taken surgically, endoscopically or by trephine bone marrow biopsy in the case of primary extranodal disease. Histochemical staining was carried out according to the standard practice. For classification and grading of the lymphomas, the modified working formulation classification for Non-Hodgkin’s lymphomas was used (17), because it is the only available classification system for Non-lymphomas in Yemen as the WHO and REAL classification system is not applicable by the Yemeni pathologist till present.

**Data collection**

Patients were interviewed by the same physician using a standardized questionnaire that consisted of 3 sections: socio-demographic characteristics, medical history and history of behavioural and environmental exposure including occupational exposure to toxins. The patients were interviewed at the time of admission to our unit. Informed consent was obtained from all patients.

**Assessment of HCV infection**

Antibodies to HCV were detected in lymphoma patients and controls sera using IV generation ELIZA Diasorin Spain.

Serum separation, handling and testing were carried out according to the standard practice and manufacturer recommendations.

**Statistical assessment**

The prevalence (%) of HCV infection among patients and controls was determined by dividing the number of patients positive for HCV in each group into the total number of patients in the same group, multiplied by 100. The significance of the differences in prevalence of HCV in cases and controls was estimated using the Pearson’s Chi-square (X2) and P-value. Odds ratios (OR) and corresponding 95% confidence intervals (CI) were computed using logistic regression models to evaluate the risk of association between HCV infection and NHLs. Collecting, calculating and analysing of data were performed using Microsoft Access and Excel 2003, Medcalc programme 2006 and SPSS programme version 15, 2006.

**Results**

The study population consisted of 192 patients diagnosed with NHL in the Haematology/Oncology Unit during the study period. Out of the 117 patients (60.94%) were males and 75 (39.06%) were females. Male: female ratio was 1.56:1. The mean age of the patients was 41.30±19.15 SD and range between 5-80 years. A total of 29 (15.1%) of 192 patients were found positive for HCV infection, that the prevalence of HCV infection among patients with NHL is 15.1%. Out of the 29 positive patients 21(17.6%) were male and only 8 (10.7%) patients were female with a male: female ratio of 2.6: 1. The mostly involved age group was 41-60 representing 24.6%. [Table 1]

The control group consisted of 20329 persons from different departments of the same hospital excluding those with high risk, patients with chronic liver diseases, history of HCV infection, hemodialysis and repeated recipient of blood transfusions. Of the total control population studies 12274(60.4%) were males and 8055 (39.6%) were females. The distribution of the controls according to sex was the same as cases. A total of 814 patients were positive for HCV antibodies, that the prevalence of HCV between the controls was 4%. Of the positive controls 529
(4.3%) were males and 285 (3.5%) were females. On distribution of the controls to age groups we faced a problem, that the age of many of the controls was missed in 888(4.7%). 67(4.1%) of the positive controls and 8516(5.%) of the negative controls. So the distribution of the controls among the age groups was made according to available data only as shown in Table 1. The mostly involved age group in the control was above 60 years group representing 7.6% followed by the 41-60 in 6.6%.

No patient was immunosuppressed either because of concurrent HIV infection or because of immunosuppressive therapy. Histological types of NHL were low grade in 8 cases (small lymphocytic 24, follicular small cleaved , follicular mixed small cleaved and large cells 8 and maltoma in ), intermediate grade in 107 cases (follicular large cells 1, diffuse small cleaved cell , diffuse mixed small and large cells 5 and diffuse large cells 80) and high grade in 47 cases (immunoblastic 1, lymphoblastic 14, small non-cleaved Burkitt and Burkitt-like 20 and Mycosis Fungoides in ). HCV prevalence by types of NHL is shown in Table 2.

Diffuse large cell type was mostly diagnosed, present in 80(41.7%) patients. Patients with Follicular mixed small cleaved and large cell were mostly involved by HCV infection in 37.5%.

According to histological grade HCV was positive in 6 (6/32), patients with low grade, 15 (15/92) patients with intermediate grade and 8(8/39) patients with high grade.

During statistical analysis due to the small number of positive cases in each type of NHL, we grouped NHLs into indolent (low grade) in 38(19.8%) patients, 6 of them were HCV positive and aggressive (intermediate and high grade) in 154(80.2%) patients, 23 of them were HCV positive. Sixteen of 23 patients were males and 7 were females.

The mostly involved age group with lymphoma was 41-60 year, in 69 patients (35.1%) followed by 21-40 group in (29.7%) as shown in Table 1.

P-value was ≤0.0001) indicating highly significant association.

The majority of the positive cases in the patients group were males as well as in the control group (17.9% versus 4.3%, the P-value was ≤0.0001). In the female group the difference between the positive cases in patients and controls was also very significant (10.7% versus 3.5%, the P-value was ≤0.001).

When comparing HCV prevalence among patients and controls by the degree of histological type of NHLs (indolent or aggressive), adjusting for possible confounding factors such as age or sex, the unadjusted Odds Ratio (OR) was 4.27 with corresponding 95% confidence intervals between (2.86-6.37) in all types of NHLs indicating excessive risk of association. When adjusted for gender the association was still significant in males [OR=4.27, 95%CI 2.86-6.37] and in females [OR=3.26, 95%CI 1.55-6.84]. Same for the types of lymphoma the Odds ratio was calculated and found to have the same risk as shown in Table 4.

The relative risk of association between HCV infection and NHLs was calculated and found to be 3.76. This large relative risk shows that the HCV +ve group have a much higher risk of developing NHLs than HCV-ve group.

Discussion

The present study shows high prevalence of HCV infection among patients with NHL than that in the control group which included patients from different departments of the same hospital. A high prevalence of HCV infection among patients with NHL has been reported in some Italian studies (18,19), USA (20), Romania (21), Saudi Arabia (22), Egypt (23) and Japan (24,25) but has not been confirmed by data from Lebanon (26) and the United Kingdom (27, 28). To our Knowledge, our study is the first report of this kind from Yemen. The 15.1% prevalence of HCV infection that we found in our cohort of patients with NHL goes with most of the results reported from different parts of the world, ranging from 6-37% (29). The highest prevalence was reported from Italy (29) where the prevalence of HCV infection in the general population is very high. Although the
The exact prevalence of HCV infection in the Yemeni population at present is unknown. Prevalence of 1.56% in blood donors (30) and 4.2% in healthy persons (31) has been suggested based on limited number of community based surveys and a pool of healthy blood donors. However, the prevalence of HCV infection in our control group (4%) was within the prevalence range of those reports. Thus, it seems that the prevalence of HCV infection in the control group falls within the estimated population rate, because our control group contains a large number of patients of different departments of the hospital and from different parts of the country that nearly representing the normal distribution of Yemeni population. Approximately two thirds of the lymphoma patients and HCV +ve infected patients and controls were males, consistent with a known higher incidence of the two diseases among males than females (32). Our results do not indicate an absolute predominance of any particular lymphoma type in association with HCV infection despite the fact that the low-grade category has been described more frequent than the others in some reports (32).

Diffuse large cell type was mostly described in this study (41.7%) of lymphomas type but not the mostly involved by HCV infection.

Most of our patients were in the age group 41-60 years and above and this is going with international reports about an increasing incidence of lymphomas and HCV infection by increasing age. The association between HCV infection and NHLs that was found in this study raises the possibility that HCV plays a pathogenetic role in lymphoma development.

Several lines of biological evidence point to HCV as a cause of NHLs. HCV infection is nearly universally present in essential (MC) (6), a low-grade lymphoproliferative disorder that can evolve into NHL. Furthermore, HCV-infected individuals frequently harbor circulating B-lymphocytes with chromosomal translocations involving the bcl-2 oncogene (33). Persistently detectable bcl-2 translocations are associated with a high risk for progression to frank NHL and successful treatment of HCV infection results in loss of these translocations (34). Although HCV can establish persistent infection with B-lymphocytes, HCV does not integrate into the host genome and does not possess oncogenes (35).

Thus, the mechanism of HCV-mediated lymphomagenesis may involve chronic B-lymphocyte stimulation through specific immune related interactions (36). Immunoglobulins produced by NHLs in some cases recognize the HCV E2 envelope protein (36, 37). Furthermore, HCV binds to CD81 on the surface of B-lymphocytes (38), which might facilitate B-lymphocyte activation. Finally, HCV-infected patients with splenic marginal zone lymphoma, Interferon-based therapy leads to resolution of HCV viridian (39).

In this study there are two limitations; first the anti-HCV anti-bodies were detected by ELIZA technique only without confirmation by RIBA or PCR technique due to limited laboratory resources.

Second, approximately one third of the controls, their age have not been written in the laboratory records leading to incomplete matching of the age groups between patients and controls.

Conclusions

In conclusion, the actual study identifies a strong association between HCV infection and NHLs in Yemeni patients.

Chronic HCV infection may predispose to a broad range of NHL subtypes through prolonged stimulation of B-cell proliferation. Additional large scale studies will be needed to confirm these results and for a better understanding of this apparent association.

Recommendations

1. A large study of lymphoma and HCV infection including a big size sample from different parts of the country to determine the real incidence and prevalence of the two diseases and to identify if there is any geographical variations.

2. A national population-based study of HCV incidence and prevalence among Yemeni population should be performed in appropriate ways.

3. Confirmation of the positive HCV antibodies by RT-PCR as well as of negative high risk group persons who are more risky for the general population.

4. Strict preventive measures against HCV and other viruses should be applied in all hospitals, medical units, and in society.

5. Immunohistochemical testing to differentiate B-cell from T-cell NHLs should be introduced to the referral hospitals to apply the WHO and REAL modern classification system of NHLs.

6. Further work is necessary to elucidate the full pathogenetic mechanisms of lymphoma in the setting of HCV.

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References


