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Contents

The Effect Of Antioxidants On The Side Effects Of Pegylated Interferon/Ribavirin Combination Therapy In Patients With Chronic Hepatitis C

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Background and study aim: Combined anti-hepatitis C therapy with pegylated interferon and ribavirin is associated with numerous side effects that affect both patients' compliance and response rates. These side effects are believed to be, in part, due to oxidative stress induced by ribavirin. In the present work, we tried to evaluate if antioxidants can ameliorate the side effects of this therapy in patients with chronic hepatitis C (CHC).

Patients and methods: Two hundred selected patients with CHC were randomized to receive either the standard antiviral combination therapy (Peginterferon and Ribavirin) plus antioxidant mixture (slow release vitamin C 500 mg daily, vitamin E 400 mg daily, silymarin 420 mg daily and N-acetylcystein 600 mg daily) for 48 weeks (study group, group I) or to receive the standard combination therapy only for 48 weeks (control group, group II). Patients were followed up during the whole treatment course to assess the occurrence of subjective and laboratory side effects of the combined antiviral therapy.

Results: The results of our study revealed statistically significant difference between both groups regarding changes in hemoglobin concentration along therapy from week one onwards as reduction in hemoglobin concentration along therapy was significantly lower in group I; p = 0.001. The frequency of significant anemia (Hb < 10 gm/dl) in group I was significantly lower than Group II from week twelve onwards; p value ranged from 0.013 to 0.001. Moreover, the frequency of fatigue was significantly lower in group I from week two onwards till the end of therapy; p value ranged from 0.027 to < 0.001. On the other hand, all other checked side effects either subjective or laboratory revealed a non significant statistical difference between both groups.

Conclusion: The use of an antioxidant mixture with antiviral combination therapy in patients with CHC can improve treatment associated anemia and fatigue but has no effect on other treatment associated side effects.

INTRODUCTION

Hepatitis C virus (HCV) is one of the main causative agents of chronic viral hepatitis which can progress to cirrhosis and eventually to hepatocellular carcinoma over a period of 20 to 30 years [1]. According to WHO, Egypt has a very high prevalence of HCV which is higher than neighboring countries as well as other countries in the world with comparable socioeconomic conditions and hygienic standards. Approximately 20% of Egyptian blood donors are anti-HCV positive [2].

The mechanisms by which HCV causes cell damage are not well understood. Different mechanisms have been suggested including immunological liver damage, direct cytotoxicity and oxidative stress induction. The theory of "oxidative stress induction" is supported by several lines of evidence including the presence of increased levels of lipid peroxidation products and diminished levels of reduced glutathione in peripheral blood. [3].

Combined antiviral therapy with pegylated interferon and ribavirin has been recommended as a standard therapy for patients with CHC. These drugs are given for either 48 weeks (HCV genotypes 1, 4, 5, and 6) or for 24 weeks (HCV genotypes 2 and 3) [4]. Unfortunately, this combination therapy is associated with numerous side effects that affect both patients' compliance and response rates [5]. Such effects are, in part, due to oxidtive stress induced by ribavirin. Ribavirin exerts its antiviral activity after intracellular phosphorylation to its monophosphate, diphosphate and triphosphate which are the pharmacologically active forms; resulting in relative deficiency of adenosine triphosphate (ATP) within red blood cells [6]. This deficiency of ATP may affect the antioxidant defence mechanisms indirectly and hence increase susceptibility to oxidative damage and extravascular hemolysis [7].

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which can start chain reactions and lead to cell damage or death. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being themselves oxidized [8]. Although increased ROS levels in patients with CHC may be beneficial by suppressing HCV replication [9], many studies have demonstrated the beneficial effects of antioxidants on these patients [10].

Aim of the work

The present study aims at evaluating the role of certain antioxidant mixture (slow release vitamin C 500 mg daily, vitamin E 400 mg daily, silymarin 420 mg daily and N-acetylcystein 600 mg daily) in amelioration of the side effects of pegylated interferon/ribavirin combination therapy in patients with CHC.

PATIENTS AND METHODS

This is a randomized controlled study carried out at the Department of Tropical Medicine, Zagazig University Hospitals from August 2009 to July 2012. This study comprised 200 patients with CHC under pegylated interferon/ribavirin combination therapy. They were all candidates to begin combination therapy according to guidelines of the National Committee for Control and Prevention of viral Hepatitis "C" in Egypt. Patients were selected by stratified random sample and assigned into two equal groups; **Group I (study group)** included 100 patients received combined antiviral therapy (Peginterferon and Ribavirin) plus antioxidants (slow release vitamin C 500 mg daily, vitamin E 400 mg daily, silymarin 420 mg daily and N-acetylcystein 600 mg daily) for 48 weeks and **Group II (control group)** included 100 patients received combined antiviral therapy (Peginterferon and Ribavirin) only for 48 weeks.

All the studied patients were subjected to the following:

- Complete history taking.
- Complete physical examination.
- Body mass index (BMI).
- Investigations: including complete blood count, liver function tests, kidney function tests, prothrombin time & INR, fasting blood sugar (and Hb A1c if diabetic), pregnancy test in females, anti-HCV, HBs Ag, ANA, TSH (Before treatment and every 12 weeks), alfa feto protein, fundus examination, ECG, pelvi abdominal ultrasound, quantitative HCV RNA assessment (before treatment, after 12 weeks, after 24 weeks and at the end of the course) and liver biopsy for histological staging and grading of chronic HCV.

Treatment regimens: The dose of peginterferon alpha-2a is 180 ug subcutaneously around the umbilicus once per week together with ribavirin using 1000 mg/day for those ≤ 75 kg in weight and 1200 mg/day for those >75 kg in weight. The dose of peginterferon alph-2b is 1.5 ug/kg body weight subcutaneously around the umbilicus once per week together with ribavirin at dose of 800 mg/day for those weighting <65 kg, 1000 mg for those >65 kg to 85 kg, 1200 mg for those >85 kg to 105 kg and 1400 mg for those > 105 kg .

Patient Monitoring: All patients were assessed at weeks 0, 1, 2 and 4 of treatment and thereafter monthly. At each review, laboratory tests were performed including serum ALT and AST, bilirubin, full blood count and serum creatinine. Body weight and symptom checklist were recorded at each visit and dose modifications to the peginterferon or ribavirin were made when appropriate.

Statistical Analysis: Data were checked, entered and analyzed using (SPSS version 15). Data were expressed as arithmetic mean (**X**) ± standard deviation (**SD**) for quantitative variable, number and percentage for qualitative one. Chi-square (X^2) and t test were used when appropriate. P value less than 0.05 was considered significant.

RESULTS

There was a non-significant statistical difference between patients who received combined antiviral therapy (Peginterferon and Ribavirin) plus antioxidants (group I) and patients who received combined antiviral therapy only (group II) regarding age and sex (table 1).

The frequency of fatigue was significantly lower in group I from week 2 onwards till the end of therapy; p value ranged from 0.027 to < 0.001 (table 2, figure 1).

Significant statistical difference was noticed between the two groups regarding changes in hemoglobin concentration along therapy from week 1 onwards (table 3, figure 2) as reduction in hemoglobin concentration along therapy was significantly lower in the group I; p = 0.001 (table 4). The frequency of significant anemia (Hb < 10 gm/dl) in group I was significantly lower than Group II from week 12 onwards; p value ranged from 0.013 to 0.001 (table 5).

Percentage of discontinuation of the treatment, due to either absence of EVR or hematological complications, was comparable between the two studied groups. There was a non-significant statistical difference between both groups (table 6).

Table (1): Age and sex distribution among the studied groups.

	Group I (Study group) (N=100)		**Group II (Control group) (N=100)**		**t**	**P**
Age (years)						
X ± SD	37.8 ±9.4		39.5 ± 9.6		1.56	0.12
Range	19 - 55		19 -58			
Gender	**N°**	**%**	**N°**	**%**	**X^2**	**P**
Male	52	52	52	52	0.0	1.0
Female	48	48	48	48		

Table (2): The percentage of Fatigue in both groups during treatment.

	Week 1	**Week 2**	**Week 4**	**Week 8**	**Week 12**	**Week 16**	**Week 20**	**Week 24**	**Week 28**	**Week 32**	**Week 36**	**Week 40**	**Week 44**	**Week 48**
Group I (%)	64	57	42	34	24	20	14	13	12	4	8	2	4	2
Group II (%)	72	72	62	58	46	46	38	37	38	26	30	26	22	20
X^2	1.47	4.91	8.0	11.6	10.6	15.29	15.0	15.36	18.03	18.98	15.7	23.92	14.32	16.52
P	0.23	0.027	0.005	0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Group I (Study group), Group II (Control group)

Figure (1): Shows the difference between both groups regarding the percentage of fatigue.

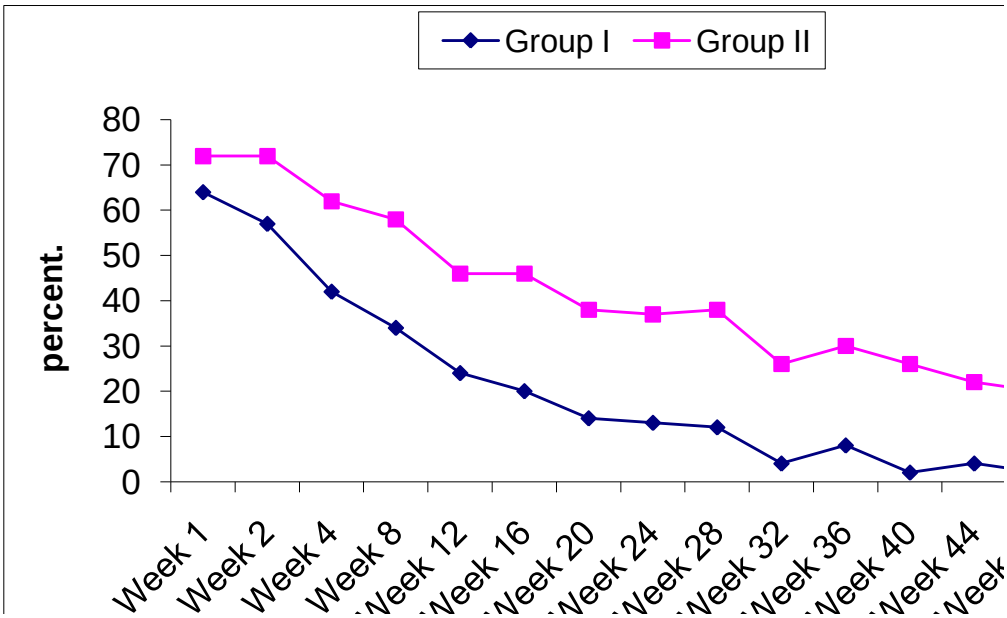

Table (3): Changes in hemoglobin concentration in both groups during treatment.

	Group I (Study group) (N=100)	Group II (Control group) (N=100)	t	P
Week 0	13.8±1.0	13.75±1.0	0.14	0.88
Week 1	13.77±0.97	13.2±1.0	3.5	0.001
Week 2	13.2±0.97	12.6±1.03	4.1	0.001
Week 4	12.5±1.1	11.99±1.17	3.29	0.001
Week 8	11.9±0.9	11.5±1.1	2.9	0.004
Week 12	12.0±1.1	11.3±1.1	4.65	0.001
Week 16	12.0±1.1	11.2±1.1	5.13	0.001
Week 20	11.9±1.2	11.0±1.2	4.88	0.001
Week 24	11.85±1.2	10.9±1.3	5.26	0.001
Week 28	11.63±1.4	10.9±1.3	3.5	0.001
Week 32	11.6±1.3	10.9±1.3	3.5	0.001
Week 36	11.6±1.4	10.9±1.4	3.6	0.001
Week 40	11.63±1.4	10.89±1.3	3.5	0.001
Week 44	11.6±1.39	10.9±1.4	3.5	0.001
Week 48	11.5±1.3	10.67±1.2	4.5	0.001

Figure (2): Shows changes in hemoglobin concentration in both groups during treatment.

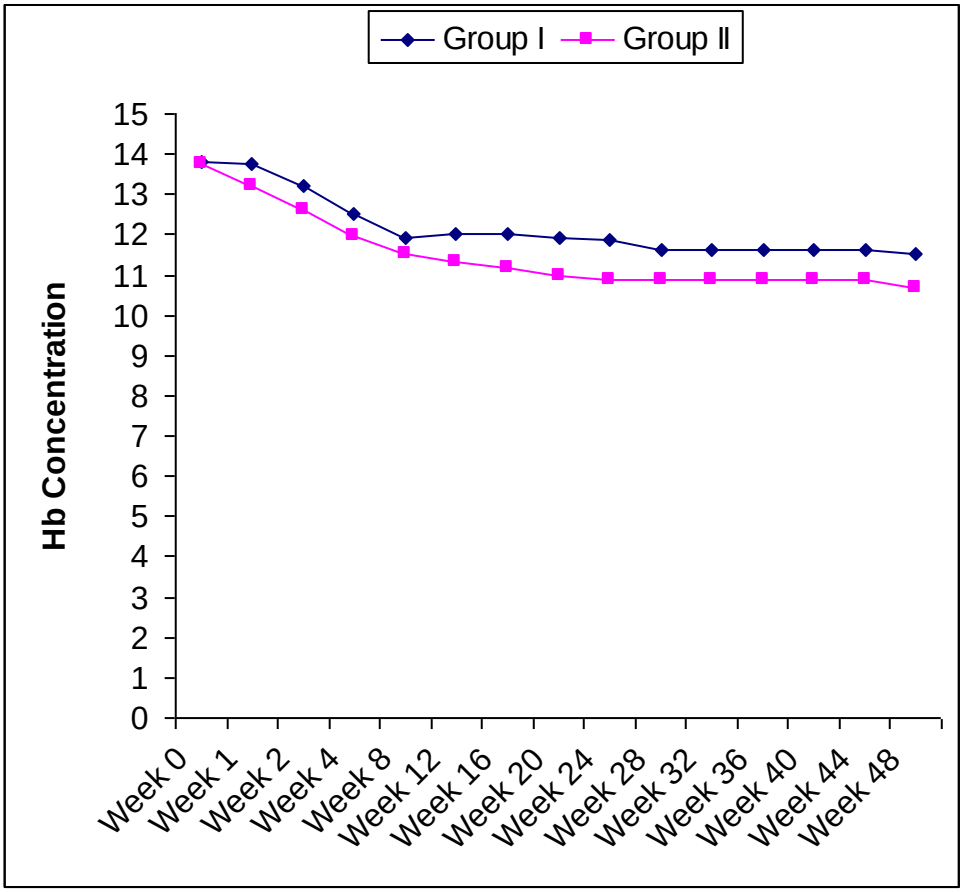

Table (4): Mean reduction in hemoglobin concentration in both groups along therapy.

	Group I (Study group) (N=100)	Group II (Control group) (N=100)	t	P
X ± SD	2.4 ± 1.5	3.2 ± 1.6	3.7	0.001
%	16.5	22.3		

Table (5): The percentage of significant anemia (Hb < 10 gm/dl) in both groups.

	Week 1	Week 2	Week 4	Week 8	Week 12	Week 16	Week 20	Week 24	Week 28	Week 32	Week 36	Week 40	Week 44	Week 48
Group I (%)	0	0	0	0	0	3	2	9	4	3	4	7	9	8
Group II (%)	0	0	0	0	11	14	15	23	21	24	14	20	27	24
X^2	0.0	0.0	0.0	0.0	11.64	7.78	10.87	7.29	13.2	18.88	6.12	7.24	10.98	9.52
P	1.0	1.0	1.0	1.0	0.001	0.005	0.001	0.007	0.001	0.001	0.013	0.007	0.001	0.002

Group I (Study group), Group II (Control group)

Table (6): The percentage of discontinuation of treatment in both groups.

	Group I (Study group) (N=100)		Group II (Control group) (N=100)		X^2	P
	N	%	N	%		
Discontinuation:						
Total	8	8	6	6	0.31	0.58
No EVR	5	5	3	3	0.52	0.47
Anemia	1	1	2	2	0.34	0.56
Neutropenia	2	2	1	1	0.34	0.56

DISCUSSION

Side effects of CHC treatment with Pegylated interferon and Ribavirin are believed to be, in part, due to oxidative stress induced by ribavirin.

In the present work we have studied the effect of antioxidants administered in conjunction with Pegylated interferon and Ribaverin in the amelioration of subjective and laboratory side effects of the combined therapy. We have used a mixture of four antioxidants with average therapeutic doses (slow release vitamin C 500 mg daily, vitamin E 400 mg daily, silymarin 420 mg daily and N-acetylcystein 600 mg daily).

The results of our study revealed significant difference between both groups regarding changes in hemoglobin concentration along therapy from week one onwards as reduction in hemoglobin concentration along therapy was significantly lower in group 1 (study group); p = 0.001. The frequency of significant anemia (Hb < 10 gm/dl) in group 1 was significantly lower than Group 2 (control group) from week twelve onwards; p value ranged from 0.013 to 0.001. This in turn resulted in much lower needs for dose modifications and hence more adherence to therapy in group 1.

The etiology of anemia with antiviral therapy in CHC is multifactorial. It is caused mainly by ribavirin induced hemolysis. Interferon also may lead to suppression of the normal compensatory bone marrow response. The exact mechanism of ribavirin induced hemolytic anemia is unclear. Intracellular accumulation of phosphorylated metabolites of ribavirin leading to ATP depletion and oxidative damage of RBCs was suggested by De Franceschi et al. [7].

These results were consistent with Brass and Piken [11]**;** who have reported that administration of 1000 mg of vitamin C and 800 mg of vitamin E daily together with antiviral therapy can reduce ribavirin induced hemolytic anemia. However, the number of patients included in their study was very low as it was just a pilot study that included twelve patients with CHC in the study group and fourteen patients in the control group whereas, the present study included 100 patients in each group. In the above mentioned study, the authors used standard INF alpha 2b whereas, in the present study pegylated INF was used. Moreover, their patients were followed just for twelve weeks while in our study patients were followed for the whole 48 weeks.

These results were also consistent with Kawaguchi et al. [12]; who stated that administration of 2000 mg of vitamin C and 2000 mg of vitamin E daily together with antiviral therapy can reduce ribavirin induced hemolytic anemia. Doses of vitamins E and C used in the above study are much more than those used in our study. In the present work, two additional antioxidants namely silymarin and N-acetylcystein (NAC) were given. Besides, the preparation of vitamin C used in the present work was a slow release form that allows prolonged effect of the vitamin. The number of patients included in the above study was also low, 21 patients in the study group and 21 patients in the control group, while our study included 100 patients in each group. Moreover, INF used was standard INF alpha 2b while in our study we used pegylated INF.

Saeian et al. [13]**;** reported that the administration of 800 IU of vitamin E twice daily together with antiviral therapy did not reduce ribavirin-induced hemolytic anemia. This study included forty seven patients with CHC (27 vitamin E /20 controls). INF used was standard INF alpha 2b. By the end of this study, the

authors recommended adding other antioxidants, like vitamin C or NAC, to vitamin E to augment its antioxidant effect; which was done in our study. The results of the above study as well as the present work suggest that vitamin E alone is not effective in reducing ribavirin-induced hemolytic anemia and that the combination employed in the present work is more effective.

Neither of the previously mentioned studies has been conducted on patients with genotype four which is the predominant type in Egypt. Whether this observation has any relevance to the outcome of the studies remains to be elucidated.

Assem and Yousri [14]**;** stated that Pentoxifylline 800 mg and vitamin E 1000 IU daily can ameliorate RBV associated hemolytic anemia. These results are consistent with the results of our study. Pentoxifylline, TNF alpha antagonist, increase erythrocyte ATP and erythrocyte deformability resulting in reduction of RBCs hemolysis and augmenting vitamin E protective effect. This study was conducted in Egypt on genotype four using peginterferon α-2 b. This study was also conducted on 200 patients (100 in each group) which is comparable to the present study. However, their study has not focused on the other subjective side effects induced by antiviral therapy. To the date we have planned our study in 2009, these data were not yet published.

During antiviral therapy with Peginterferon and ribavirin, fatigue is a well documented side effect. The exact mechanism of fatigue is not clear; however, it is most likely multi-factorial, including the systemic effects of increased cytokines and treatment-related side effects such as anemia [15].

The results of the present study revealed significant difference between both groups regarding the frequency of fatigue from week two onwards till the end of therapy. The frequency was much lower in group I; p value ranged from 0.027 to < 0.001. This can be explained, in part, by the higher mean of hemoglobin concentration and the lower frequency of significant anemia in group I.

The results of our study revealed a non-significant statistical difference between both groups regarding neutrophilic count or the mean reduction in neutrophilic count along therapy. Regarding platelet count, there was also a non-significant statistical difference between both groups in all weeks of follow up during therapy apart from weeks sixteen and twenty eight where changes were significant. Moreover, there was a non-significant statistical difference between both groups regarding thyroid dysfunction (all cases reported were hypothyroidism).

Neutropenia and thrombocytopenia occurring with interferon therapy are mostly due to bone marrow suppression [16], although autoimmune related thrombocytopenia may also occur [17]. Thyroid dysfunction with INF therapy is mostly due to autoimmune mechanism. Oxidative stress seems to play no role with all these parameters and this may explain why they were not affected by antioxidants.

The results of our study revealed also a non significant difference between both groups in all other checked symptoms throughout the period of treatment including fever, headache, musculoskeletal symptoms (myalgia, arthralgia, bony pains), nausea, anorexia, weight loss, dry cough, insomnia, irritability, depression, alopecia (hair loss), injection site reactions and dermatological side effects (Itching, Rash, Dermatitis). The exact mechanisms of these side effects are not well understood. Oxidative stress has no clear role in these side effects and this may explain lack of benefit of antioxidants on these side effects.

Withdrawal from the study was comparable between the studied groups. Most of cases withdrawn due to lack of EVR, the rest due to hematological complications. Antioxidants had no effect on the frequency of treatment discontinuation.

Some previous studies have declared that erythropoietin can improve anemia caused by peginterferon and ribavirin therapy and is more effective than dose reduction at improving quality of life during treatment. However, erythropoietin has not yet been approved by FDA for the use in patients with HCV infection, in addition to its expensive costs [18].

In conclusion, it can be recommended to use the mixture of the four antioxidants used in the present study to ameliorate anemia and fatigue during treatment of CHC with pegylated interferon and ribavirin, as this can improve the quality of life and adherence to therapy.

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Clinical and Epidemiologic Study of Hepatitis C Virus Genotype 4 Infection among Patients with B cell non Hodgkin's Lymphoma

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Background and study aim: Many recent studies showed that chronic infection with hepatitis C virus (HCV) is associated with increased risk for B-cell non-Hodgkin's lymphoma (NHL). The aim of this study is to evaluate the frequency of HCV infection in a series of de novo B cell non Hodgkin's lymphoma (NHL) patients and to correlate virological findings with clinico-histological features.

Patients and methods: 50 patients with B cell NHL diagnosed by histopathology and immunophenotyping were recruited from Tropical medicine department and Oncology unit affiliated to Internal medicine department, Zagazig University hospitals. Gender and age matched controls (N = 50) were volunteers selected from relatives of patients. Study participants were subjected to history taking, clinical examination, routine and specific laboratory tests. Anti-HCV antibody was determined by ELISA for all study participants. HCV RNA PCR was done for all cases and HCV antibody positive controls. Appropriate radiologic examinations were performed.

Results: Frequency of HCV infections were statistically significantly higher in B cell NHL patients than in controls ($p = 0.004$). ALT levels were statistically significantly higher in HCV positive patients than in HCV positive controls ($p < 0.001$) and HCV negative patients but without statistically significant difference ($p = 0.067$). There was no statistically significant difference in histologic types, grades and stages of NHL between HCV positive patients and HCV negative patients. Cryoglobulinemia showed no significant difference between studied groups.

Conclusion: HCV has a strong association with de novo B cell NHL, not complicating essential mixed cryoglobulinemia (EMC).

INTRODUCTION

The World Health Organization (WHO) estimates that 170 million people are infected with hepatitis C virus (HCV) [1]. An estimated 12–15% of Egyptians, have serological evidence of HCV infection (up to 99 % genotype 4), with higher rates in older age groups and residents of rural areas in lower and middle Egypt. There is evidence for a large-scale iatrogenic transmission of HCV during the parenteral anti-schistosomal treatment campaign carried out from the 1920s through the 1980s [2]. Continued transmission in Egypt has been associated with transfusion of unscreened blood, invasive surgical procedures, including Caesarean section and abortion; injections by informal health care providers and haemodialysis [3, 4].

Since its identification, HCV has been added to the roster of tumour-associated viruses because of its role in hepatocarcinogenesis. It has also been linked to extrahepatic disease manifestations [5]. One of the extrahepatic diseases in which HCV has been implicated is B cell non Hodgkin's lymphoma (NHL). HCV associated lymphomas have been observed, but whether they are caused by HCV remains to be shown definitively.

There is a suggestion that some B-cell NHL associated with HCV arise from clonal expansion of B-cells with particular immunoglobulin gene rearrangements specific for the E2 protein of the HCV envelope [6]; which is consistent with the hypothesis that lymphomas develop when B cells proliferate in response to antigen. However, no biological mechanism of HCV-associated lymphoma genesis has been definitively elucidated [7].

Most of the studies reported to date failed to find an association of HCV with NHL were conducted in areas where the prevalence of HCV was extremely low, leaving open the possibility that such an association actually exists but could not be detected because neither cases nor controls had adequate opportunity for exposure [8,9,10]. Working in a population with high prevalence of HCV allowed us to conduct a case-control study with adequate statistical power to assess the question of whether there is an association of chronic HCV infection with NHL [11].

Some studies investigated the sequel of HCV infection on the liver of patients with B cell NHL [12,13]. In the present study, we try to evaluate the frequency of HCV infection in a series of *de novo* B-cell non-Hodgkin's lymphoma (B NHL) patients and to correlate virological findings with clinico-histological features.

PATIENTS AND METHODS

50 patients with B cell NHL were collected from Tropical medicine department and Oncology unit affiliated to Internal medicine department. The diagnosis of B cell NHL was based on histopathology and confirmed by immunophenotyping. Of these cases, 25 were admitted at Tropical medicine department, because of generalized lymphadenopathy (7 patients), FUO (5 patients), anaemia (5 patients), persistent vomiting (3 patients), ascites (2 patients), anorexia and weight loss (2 patients) and bleeding tendency (1 patient). The other 25 cases were patients already diagnosed as B cell NHL and coming to receive chemotherapy in the Oncology unit.

The control group included 50 volunteers selected from relatives of patients admitted at Tropical medicine department and the Oncology unit. Controls were frequency-matched to cases by the 5-year age category and gender. Control subjects were representative of the source population of cases by region; since all cases and controls were from the region of Sharkia governorate. Controls were matched to cases as regard other risk factors of NHL; namely smoking and occupational exposure to industrial or agricultural pesticides. That is to have a more accurate assessment of HCV infection as a risk factor for NHL.

HCV infection among cases and control was defined as: positive HCV RNA PCR test with or without positive HCV antibody test. Positive HCV antibody test alone was not adopted to, minimize the false positive results of HCV infection among cases and controls in patients who catched the virus and cleared it, and minimize the false negative results of HCV infection among cases in patients who are immunocompromised and can not sustain antibody response to HCV infection. In order to further minimize the false negative results of HCV infection among cases, cases were selected at the time they were diagnosed before starting treatment, so, immunocompromisation complicating chemotherapy excluded.

After an informed consents were obtained from all subjects before enrollment, study participants were subjected to: detailed history taking and thorough clinical examination, routine laboratory. investigations **(**CBC, LFT, KFT, INR and urine analysis) and special lab. investigations(detection of anti-HCV antibodies using a third generation enzyme-linked immunosorbent assay (ELISA),determination of HCV RNA PCR in cases and HCV positive controls using COBAS® AmpliPrep/COBAS® Taqman® HCV Test. Detection of cryoglobulinin in cases and HCV positive controls, [14] and determination of LDH, in all patients [15]. Pelviabdominal ultrasonography was done for all cases and for HCV positive controls to determine the sonographic state of the liver, spleen, abdominal lymph nodes and to detect the presence of ascites. Pelviabdominal and chest CT and MRI of brain and spinal cord were done for all cases for staging [16]. Histopathologic examination and immunophenotyping were performed at Pathology department, faculty of medicine, Zagazig University. The slides were reviewed blindly without knowledge of virologic state of the patient. Formalin-fixed tissues were stained with haematoxylin and eosin and examined by light microscopy. Type and grade of B cell NHL were defined according to the 2008 World

Health Organization (WHO) classification of tumours of haematopoietic and lymphoid tissues[17]. Low grade B-NHL included: marginal zone lymphoma (nodal, splenic and extranodal), lymphoplasmocytoid lymphoma and grade 1-2 follicular lymphoma. Intermediate and high grade B-NHL included: diffuse large cell lymphoma, mantle cell lymphoma, Burkitt's lymphoma and grade 3 folliclar lymphoma.

All slides were subjected to immunophenotyping for B- and T-cell markers. Identification of B- and T-cell surface markers was carried out using pan-B (CD-20) and pan-T (CD-45) monoclonal antibodies with the DAKO EnVision System (Code No. K4006, DAKO, Carpinteria, CA, USA). Patients with samples that tested positive for the B-cell marker were considered cases, while those positive for the T-cell marker were dropped from the study, regardless of previous classification based on histological examination of haematoxylin and eosin-stained slides.

HCV positive cases and controls with no clinical, laboratory and sonographic evidence of liver cirrhosis underwent a liver biopsy taking. Hepatitis grading and staging were evaluated according to the METAVIR scoring system [18, 19].

Patients were excluded form the study if the had any of the following criteria: patients with B-cell NHL who started treatment for patients collected from the oncology unit, patients who turned out to be T-cell lymphoma by immuno phenotyping, for those collected from tropical medicine department, age less than 15 years old, patients not physically and mentally capable of understanding and completing interview, hepatitis B surface antigen positive patients and HIV patients.

Statistical Analysis

Data were checked, entered and analyzed using SPSS version 15 for data processing and statistics. Data were expressed as numbers, and percentage for qualitative variables and mean (x) ± standerd deviation (SD), and range for quantitative variables. Student, t, test, and chi-square (X^2) were used when indicated to assess significance, $p < 0.05$ was considered significant and highly significant if $p < 0.001$.

RESULTS

The present study included 50 patients with B-cell NHL and 50 age and sex matched control.

Characteristics for studied groups were represented in table (1). There was statistically significant increase in the percentage of NHL patients who had positive HCV Ab and HCV RNA than control (p, 0.02, p, 0.004 respectively), also viral loads were higher in HCV infected patients than HCV infected control without statistically significant difference (p, 0.79) table (2). Liver enzymes and serum bilirubin were significantly increase in HCV positive cases when compared with HCV positive control ($p < 0.001$, P, 0.005 respectively), table (3). Elevated LDH levels were more common in HCV positive patients (14 patients out of 18 patients) than HCV negative patients (20 control out of 32 control) without statistically significant difference (p, 0.26 data not shown). Apart from, lymphomatous infiltration in 3 HCV positive patients, and one HCV positive patient with liver cirrhosis, no significant differences in necro inflammatory activity grading and staging of fibrosis between HCV positive patients and HCV positive control were recorded (p, 0.24 data not shown).

There was no statistically significant difference in staging, grading and types of NHL between HCV positive patients and HCV negative patients table (4). As regard cryoglobulonimia and cryoglobulinemic manifestations, no significant difference between HCV positive patients and HCV positive control was recorded, table (5).

Table (1): Demographic data of both studied groups

Demographic character	Patients (N=50)		Controls (N=50)		X2	P value
	Number	%	Number	%		
Age						
15-30	8	16.0	9	18.0	0.07	0.79
31-45	15	30.0	16	32.0	0.08	0.84
46-60	21	42.0	20	40.0	0.08	0.86
≥61	6	12.0	5	10.0	0.1	0.74
Gender						
Male	31	62.0	26	52.0	1.02	0.31
Female	19	38.0	24	48.0		
Smoking						
Non	33	66.0	29	58.0	0.68	0.4
Active	15	30.0	18	36.0	0.41	0.52
Ex-smoker	2	4.0	3	6.0	0.0	1.0
History of occupational exposure to pesticide						
No	41	82.0	40	80.0	0.06	0.72
Yes	9	18.0	10	20.0		
History of blood transfusion						
No	41	82.0	46	92.0	2.21	0.13
Yes	9	18.0	4	8.0		
History of surgical intervention						
Non	21	42.0	26	52.0	1.0	0.31
Major operation	9	18.0	5	10.0	1.33	0.24
Minor operation (dental manipulation)	20	40.0	19	38.0	0.04	0.83

Table (2): Serology and viremia of HCV infection in both studied groups

Parameter	Cases (N=50)		Controls (N=50)		X^2	P value
	Number	%	Number	%		
HCV Ab						
-ve	32	64.0	42	84.0	5.2	0.02
+ve	18	36.0	8	16.0		
HCV RNA PCR						
+ve	18	36.0	6	12.0	7.89	0.004
Viral load (IU X 10^5)					**T test**	
$\overline{X} \pm SD$	2.61 ± 5.3		2.96 ± 3.8		0.25	0.79
Range	0.001-20.0		0.076 -9.5			

P < 0.05 significant P < 0.001 highly significant

Table (3): Comparison of lab. investigations of HCV positive patients and HCV positive controls

Lab. test	HCV positive cases (N=18)	HCV positive controls (N=6)	T test	P value
Liver function tests				
Total bilirubin (mg/dL)				
X ± SD	2.36 ± 5.2	0.67 ± 0.25	7.59	0.005
Range	0.3-23	0.3-1.2		
Direct bilirubin (mg/dL)				
X ± SD	1.47 ± 4.1	0.3 ± 0.19	5.12	0.02
Range	0.1-18	0.1-0.8		
S. Albumin (gm/dL)				
X ± SD	3.5 ± 0.7	3.9 ± 0.3	2.95	0.004
Range	1.7-4.6	3.2-4.5		
ALT				
X ± SD	68.3 ± 22	36.8 ± 12.5	6.45	<0.001
Range	33-120	18-77		
AST				
X ± SD	70.8 ± 22	35.8 ± 8.6	7.99	<0.001
Range	38-114	21-55		
CBC				
Hb (gm/dL)				
X ± SD	10.9 ± 2.1	12.2 ± 2	2.09	0.04
Range	6.1-12.8	8.6-14.1		
PLT count (x 10^3/mm^3)				
X ± SD	182 ± 75	186 ± 49	0.22	0.82
Range	72-290	97-310		
WBC count (x 10^3/mm^3)				
X ± SD	6.8 ± 2.6	7.1 ± 2	0.49	0.62
Range	3.5-12.5	3.9-10.2		
INR				
X ± SD	1.2 ± 0.2	1.03 ± 0.05	1.9	0.31
Range	1-1.8	1-1.2		

Table (4): Comparison of histopathologic types, grading and staging of B cell NHL in HCV positive patients and HCV negative patients

Type of NHL	HCV positive patients (N=18)		HCV negative patients (N=32)		X^2	P value
	Number	%	Number	%		
Biffuse large B cell lymphoma	6	33.3	15	46.9	0.87	0.35
Follicular lymphoma	3	16.7	7	21.9	0.01	0.94
Small cell lymphoma/ chronic lymphatic leukemia	2	11.1	2	6.3	0.01	0.94
Marginal zone lymphoma	2	11.1	2	6.3	0.01	0.94
MALT lymphoma	1	5.6	3	16.7	0.01	0.94
Mantle cell lymphoma	1	5.6	2	6.3	0.27	0.68
Lymphoplasmacytic lymphoma	2	11.1	0	0.0	1.38	0.24
Burkitt lymphoma	1	5.6	1	3.1	0.11	0.74
Grade of NHL						
Low	6	33.3	13	40.6	0.26	0.61
Intermediate	4	22.2	8	25.0	0.02	0.82
High	8	44.5	11	34.4	0.5	0.48
Staging of NHL						
I	4	22.2	5	15.6	0.04	0.56
II	5	27.8	12	37.5	0.49	0.48
III	3	16.7	10	31.3	0.63	0.42
IV	6	33.3	5	13.6	1.2	0.27

Table (5): Comparison of cryoglobulinemia among HCV positive patients and HCV positive controls

Parameter	HCV positive patients (N=18)		HCV positive controls (N=6)		X^2	P value
	Number	%	Number	%		
Cryoglobulinemia						
No	10	55.6	4	66.7	0.0	1.0
Yes	8	44.4	2	33.3		
Cryoglobulinemic manifestations in the cryo +ve patients	2	11.1	0	0.0	0.73	0.39

DISCUSSION

HCV association with B cell NHL is still a matter of debate, no association between HCV infection and B cell NHL was found [8, 9]. Most of the studies that failed to find an association of HCV with B cell NHL were conducted in areas where the prevalence of HCV is extremely low; leaving open the possibility that such an association actually exists but could not be detected because neither cases nor controls had adequate opportunity for exposure to the virus [10].

Working in a population with the highest prevalence of HCV allowed us to conduct a case-control study with adequate statistical power to assess the question of whether there is an association of chronic HCV infection with B cell NHL [11].

The present study shows that the incidence of HCV active infection among the control group is 12% (6 out of 50 control), 16 % of controls were HCV antibody positive, 2 HCV Ab positive control were HCV RNA PCR negative, that mean they cleared viremia. About 15% of the Egyptian had HCV positive anti bodies while 10% had active infection with positive HCV RNA PCR. [20, 21]

The incidence of HCV infection among cases was 36 % (18 out of 50 cases). All cases were positive for HCV antibodies and for HCV RNA PCR; that mean none of the infected cases could clear viremia. This finding can be explained by the fact that B cell NHL is a sequel of disordered

immune system which can't successfully clear viremia [11].

The occurrence of HCV active infection among cases was statistically significantly higher than in controls (p 0.004). These results suggest a positive association between HCV infection and B cell NHL [11, 22].

Studies which failed to find an association were carried out in communities with low prevalence of HCV infection. Their results may be explained by the smaller sample size of their studies than that required to obtain an adequate statistical power or by that the spread of HCV in those communities is relatively recent and not having enough time to be complicated by NHL [23].

HCV viral loads were higher in HCV associated B cell NHL patients compared to HCV infected controls without statistically significant difference (p, 0.79). This finding is in agreement with that obtained by Karavattathayyil et al., [24] who demonstrated actively replicating virus in HCV-associated lymphomas.

There was a statistically highly significant increase in liver enzymes levels between HCV positive patients and HCV positive controls (p value < 0.001). This finding may be due to:

Lymphomatous infiltration of the liver in HCV positive patients and Chemotherapy induced hepatotoxicity in HCV associated patients [12]. There was no statistically significant difference in liver enzymes levels between HCV positive patients and HCV negative patients. Liver enzymes levels were elevated in both groups but higher values occurred in HCV positive patients [12, 13].

There was no statistically significant difference between different histological types of HCV associated lymphomas. In the present study, the commonest types of HCV associated B cell NHL were diffuse large B cell lymphoma which is an aggressive lymphoma followed by follicular lymphoma which may be indolent or aggressive. This is in concordance with Goldman et al. [25], who found that HCV is associated with diffuse large B cell, marginal zone, and follicular lymphomas. While others, found that lymphoplasmacytoid lymphoma/immunocytoma and Waldenströmmacroglobulinemia, were the only NHL associated to HCV, because they studied patients with HCV associated essential mixed cryoglobulinemia (EMC) in whom that types of NHL are a common complication [22, 26, 27]. The results of the present study showed that HCV is associated with de novo NHL not complicating essential mixed cryoglobulinemia (EMC).

The percentage of HCV positive patients who had cryoglobulinemia (44.4%) was higher than those with HCV positive control (33.3%) without significant difference. 2 HCV positive NHL patients had cryoglobulinemic manifestations in the form of purpura, arthralgia and microscopic hematuria for many years before the diagnosis of lymphoplasmacytic lymphoma. However, others found that HCV associated lymphoma, were overt B cell lymphomas that complicate essential mixed cryoglobulinemia (EMC) with up to 30% of cases associated with hepatitis C [22, 26, 27]. Our results showed that HCV is linked to de novo B cell lymphoproliferative disorders not complicating mixed cryoglobulinemia (EMC). The difference form our result is attributed to difference in selection criteria as, they selected patients with EMC as an inclusion criteria.

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Efficacy of Ribavirin to Prevent Hepatitis Reactivation in Hepatitis C Virus-infected Patients Treated for Non-Hodgkin Lymphoma

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Background and study aims: Reports have found an association between B cell non-Hodgkin lymphomas (NHL) and Hepatitis C virus (HCV) infection. However, data on acute exacerbation and reactivation of chronic HCV infection following chemotherapy are very limited. We studied the efficacy of ribavirin to prevent hepatitis reactivation in HCV-infected patients treated for NHL.

Patients and methods: This study was carried out at Medical Oncology & Hematology Department , Zagazig University Hospitals. It included 57 patients with B-cell NHL who were naïve to chemotherapy, among them 24 patients were positive for HCV and 33 patients were negative for HCV (group C). The HCV positive group were subdivided into 11 patients who received ribavirin (group A) and 13 patients did not receive ribavirin (group B). Routine investigations for NHL were done, HCV RNA was measured for HCV positive patients before and after the end of chemotherapy.

Results: HCV infection occurred in 42% of patients with B cell NHL. Acute hepatic enzyme exacerbation occurred in 8 (14%) of all patients with the highest percentage was 29.2 % among HCV infected patients (7/24), while only one patient (3%) in the HCV negative group (p= 0.007). Among the 24 NHL patients with HCV positivity, we compared group A versus group B during chemotherapy as regards to hepatic enzyme flare, it was (27% & 30%, respectively, p= o.6). Five (20.8%) of 24 NHL patients with HCV positivity developed HCV PCR reactivation; 2 patients of group A and 3 patients of group B (18.2% & 23.1%, respectively, p= 0.58). The outcome was comparable between the three groups.

Conclusion: The frequency of HCV infection in patients with B cell NHL is higher than in the general population. Acute exacerbation and reactivation of chronic HCV infection occur in a sizeable subset of patients with NHL during chemotherapy. The use of ribavirin did not decrease hepatic enzyme flare or HCV PCR reactivation during chemotherapy.

INTRODUCTION

Hepatitis C virus (HCV) infection is endemic in Egypt. Many reports have found an association between B cell non-Hodgkin lymphomas (NHL) and HCV infection. The role of HCV infection in lymphomagenesis may be related to chronic antigenic stimulation of HCV [1]. However, little is known about acute exacerbation and reactivation of chronic HCV infection in patients with cancer [2]. Most of the reported cases of liver dysfunction in HCV-infected cancer patients occur in non-Hodgkin lymphomas [3]. Authors have reported a reactivation of HCV replication in patients with CD20-positive B-cell NHL under Rituximab-based chemotherapy [4].

Also, limited studies indicate that episodes of acute exacerbation of chronic HCV seem to be less severe than similar episodes of chronic hepatitis B virus (HBV) exacerbation [5]. As reactivation of the HBV after cytotoxic chemotherapy is common in clinical practice. Thus, prophylactic antiviral (lamivudine) treatment should be started at the initiation of chemotherapy and maintained for at

least 6 monthes following the completion of therapy [6].

In our study, in analogy with the use of lamivudine as prophylaxis against HBV reactivation, we tried to use Ribavirin (a synthetic nucleoside analogue) as a prophylactic antiviral treatment to reduce the risk of HCV reactivation and severe hepatitis flares.

We sought to determine the frequency of HCV infection among B cell NHL patients, also to determine the efficacy of ribavirin to prevent hepatitis reactivation in HCV-infected patients treated for NHL.

PATIENTS AND METHODS

The study is a randomized controlled intervention trial, that was carried out at Medical Oncology and Hematology Department , Zagazig University Hospitals between July 2010 and August 2012. It included 57 patients with NHL who were naïve to chemotherapy, among them 24 patients were positive for HCV infection & 33 patients were negative for HCV infection (group C). The HCV positive group was subdivided into 11 patients who received ribavirin (group A) and 13 patients without ribavirin (group B) (figure1).

Inclusion criteria:

1. Age: > 18 years.
2. Sex: Both sexes were eligible.
3. Pathological proof of B-cell non- Hodgkin's lymphoma.
4. Adequate bone marrow reserve.
5. Adequate liver and kidney functions.
6. Eastern Cooperative Oncology Group performance status (PS) of ≤ 2.
7. All patients were naïve for anti-HCV treatment.
8. All patients were chemotherapy naïve.

Exclusion criteria:

1. Prior or concurrent second malignancy.
2. Pregnant, lactating females.
3. Medical contraindication for receiving the study treatment as patients with active or uncontrolled infection.
4. Positivity for HBsAg or HBcAb or HIV.
5. Non-viral causes of liver affection.

Methods:

Informed consent was obtained from participants. (females must accept to use contraception during treatment). All participants were subjected to:

1. Thorough history taking, clinical examination.
2. Complete blood counts.
3. Serum Lactate dehydrogenase (LDH).
4. Erythrocyte sedimentation rate (ESR).
5. Liver and Kidney function tests (ALT ,AST ,Serum billirubin, serum albumin, INR and serum Creatinine) .
6. Viral markers (HBs Ag, HBcAb , HCV Abs) and HCV RNA in serum by PCR if HCV antibodies were positive.
7. Autoimmune Hepatitis antibodies (antinuclear antibody (ANA), anti-smooth muscle antibody (SMA), liver/kidney microsomal antibody (LKM), anti soluble liver antigen (SLA/LP) and anti-mitochondrial antibody (AMA)).
8. Serum electrolytes (Na, K & Ca), Serum uric acid and fasting blood sugar.
9. Bone marrow Biopsy.
10. Echocardiography and radiological studies were performed including: chest radiograph, CT scans of abdomen and pelvis and CT scans of the neck and thorax if any abnormality is noted or suspected on the routine chest radiograph (for staging).

HCV RNA examination

HCV RNA in serum was done at the beginning and at the end of chemotherapy. It was quantified using a commercially available polymerase chain reaction method (COBAS TaqMan HCV Test; Roche Molecular Systems, Branchburg, NJ) with a quantification range from 43 to 69,000,000 IU/ml [7].

Treatment plan:

Chemotherapy were given based on the pathological sub-types either indolent or aggressive NHL. Detailed history, full clinical examination and Laboratory assessment were performed before each treatment cycle. All patients who ended the first 4 cycles of treatment were eligible for reevaluation within 2 to 3

weeks, and those who responded (complete response [CR] or partial response [PR]) were completed the therapy to a total 6 cycles.

We randomly divided the patients with positive HCV infection into two groups, one group received Ribavirin 1000- 1200 mg daily orally during the course of chemotherapy and the other did not.

Definitions:

Acute exacerbation of chronic HCV infection was defined as a 3-fold or greater increase in serum ALT level in the absence of the use of hepatotoxic drugs (other than chemotherapeutics), or other systemic infections (including hepatitis A, HBV and human immunodeficiency virus infections) [5]**.**

HCV reactivation was defined as an increase in HCV viral load of at least 1 log10 IU/ml over baseline following chemotherapy or immunosuppressive therapy, as chronically infected patients have stable HCV RNA levels that may vary by _0.5 log10 IU/ml [8]**.**

Response criteria:

According to Revised response evaluation criteria in solid tumors (version 1.1) [9].

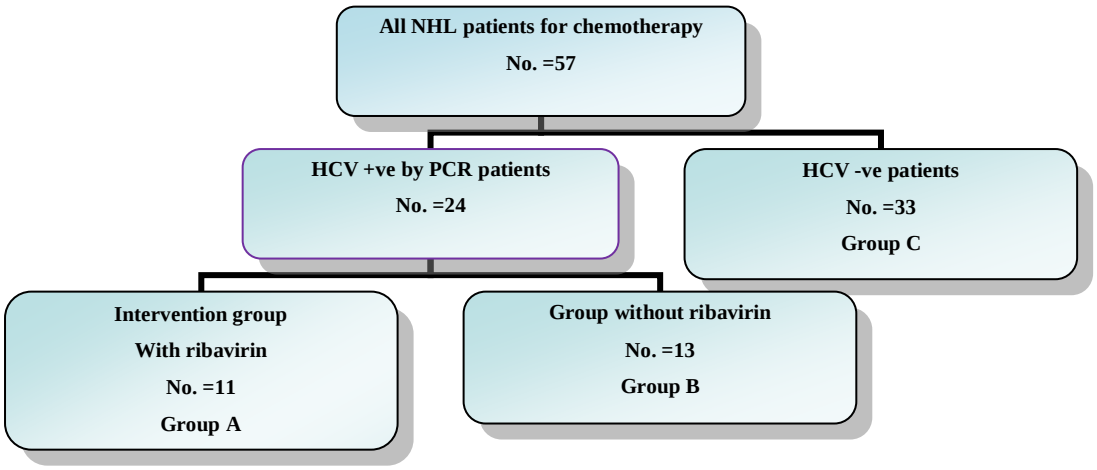
Statistical Analysis

All the data were managed using SPSS-version version 20.0. A two-sided P value of less than 0.05 was considered to indicate statistical significance. The association between categorical data was tested by Chi-square and Fisher exact tests. The t-test was used to assess whether the means of two groups were statistically different from each other. To compare between more than two groups, one way analysis of variance (ANOVA) was used. Survival analysis was done according to Kaplan-Meier method, and compared by log-rank test.

Overall survival (OS) was calculated as interval (by months) between date of randomization (pathology date) till date of death or date of last follow up.

Disease free survival (DES) was calculated as the period of time patient lived without evidence of disease relapse (for responding patients) .It is the interval (by months) between date of the complete response till date of disease progression or date of last follow up[1].

Figure (1) shows the distribution of the study groups



RESULTS

The base line characters of all patients are described in (table1). In our study, HCV infection occurred in 42% of patients with B cell NHL. Acute hepatic enzyme exacerbation occurred in 8 (14%) of all patients, and with the highest percentage (29.2 %) among HCV infected patients (7/24), while only one patient(3%) in the HCV –ve group (p= 0.007). Among the 24 NHL patients with HCV positivity , we compare patients who received ribavirin (group A) versus those who did not receive ribavirin (group B) during chemotherapy as regards to hepatic enzyme flare which was (27% & 30%, respectively, p= o.6). Five (20.8%) of 24 NHL patients with HCV positivity developed HCV PCR reactivation; 2 patients of group A and 3 of group B patients (table 2 and 3). There was a significant relation between HCV reactivation and hepatic enzyme flare (p <0.001) (table 5).

Only 3 patients (37.5%) form those who developed hepatic enzyme flare stopped their chemotherapy ,while none from the other group stopped chemotherapy , there is a statistical significant difference (p = 0.002) (table 4).

In this study the overall survival and disease-free survival in the three patients groups were comparable, although many patients among the HCV infected NHL group showed some delay in the treatment schedule, but this follow up was short (figure 2).

In the terms of overall response , 63% of our patients achieved complete response (CR) and 28% partial response (PR); 76% of DLBCL (30/39) patients obtained a CR, while between indolent NHL patients 21% (3/14 patients) achieved CR. While, among HCV positive NHL, 73.3% (11/15 patients) of DLBCL achieved CR, whereas, 33.3% (3/9 patients) of an indolent NHL achieved CR (p-value = 0.07).

Table (1): Patient characteristics of the studied groups.

Variable		HCV positive patients		HCV negative patients	p-value
		Received Ribavirin Group A (11) No (%)	Without Ribavirin Group B (13) No (%)	Group C (33) No (%)	
Age (years)	Mean + SD	45 + 15.9	52 + 6.5	49 + 13.7	0.496
Sex	Male	6 (54.5%)	5 (38.5%)	19(57.7%)	
	Female	5 (45.5%)	8 (61.5%)	14(42.4%)	0.50
PS	PS=0,1	10(90.9%)	11(84.6%)	29 (87.9%)	
	PS=2	1 (9.1%)	2 (15.4%)	4(12.1%)	0.895
Clinical	LN(only)	7 (63.6%)	6 (46.2%)	20 (60.6%)	
	Splenomegaly	3 (27.3%)	5 (38.5%)	5 (15.2%)	0.424
	Extranodal	1 (9.1%)	2 (15.4%)	8 (24.2%)	
Stage	I	0 (0 %)	1(7.7%)	4 (12.1%)	
	II	2 (18.2%)	3 (23.1%)	15 (45.5%)	0.224
	III	4 (36.4%)	6 (46.2%)	9 (27.3%)	
	IV	5 (45.5%)	3 (23.1%)	5 (15.2%)	
LDH	Normal	0(0 %)	0(0 %)	2(6.1 %)	0.471
	High(> 234)	11(100%)	13(100%)	31(90.9%)	
IPI	Low risk	1 (9.1%)	3 (23.1%)	11 (33.3%)	
	Low-intermediate risk	8 (72.7%)	8 (61.5%)	18 (54.5%)	
	High intermediate risk	2 (18.2%)	2 (15.4%)	3 (9.1%)	0.572
	High risk	0(0 %)	0(0 %)	1 (3.0%)	
Pathology	DLBCL	8 (72.7%)	7 (53.8%)	24 (72.7%)	
	Indolent NHL - marginal zone lymphoma - small lymphocytic lymphoma - Mantle cell lymphoma - Lymphoplasmacytic lymphoma	3 (27.3%) 1 (9%) 0 (0%) 1 (9%) 1 (9%)	6 (46.2%) 2 (15.3%) 3 (23%) 1 (7.6%) 0 (0%)	5 (15.2%) 0 (0%) 4 (12.1%) 1 (3%) 0 (0%)	0.130
	Burkitt	0(0%)	0(0%)	4 (12.1%)	
Chemotherapy	CHOP	8 (72.7%)	7 (53.8%)	24 (72.7%)	
	-COP	3 (27.3%)	5 (38.5%)	5 (15.2%))	0.165
	-HyperCVAD	0(0 %)	0(0 %)	4 (12.1%)	
	-FC	0(0 %)	1 (7.7%)	0(0 %)	

LN = Lymphadenopathy, DLBCL =diffuse large B-cell lymphoma, IPI = International Prognostic Index

Table (2): Frequency of hepatic enzyme flare among the three studied groups .

Enzyme flare	HCV positive patients				HCV negative patients		P value
	Received Ribavirin Group A (11)		Without Ribavirin Group B (13)		Group C (33)		
	No	(%)	No	(%)	No	(%)	
No enzyme flare	8	72.7	9	69.2	32	97	0.019*
With enzyme flare	3	27.3	4	30.8	1	3	

Table (3): Comparison between HCV positive patients who received ribavirin (group A) versus those who did not receive ribavirin (group B) regarding hepatic enzyme flare & HCV PCR reactivation

Variable	HCV positive patients				P value
	Received Ribavirin (11)		Without Ribavirin(13)		
	No	(%)	No	(%)	
Enzyme flare:					
-No	8	72.7	9	69.2	0.605
-Yes	3	27.3	4	30.8	
PCR reactivation:					
No	9	81.8	10	76.9	0.585
Yes	2	18.2	3	23.1	

Table (4): Frequency treatment disruption among the studied groups of patients according to hepatic enzyme flare

Variable	Hepatic enzyme flare				P value
	No enzyme flare(49)		With enzyme flare(8)		
	No	(%)	No	(%)	
Chemotherapy course:					
-completed	49	100	5	62.5	0.002*
-stopped	0	0	3	37.5	

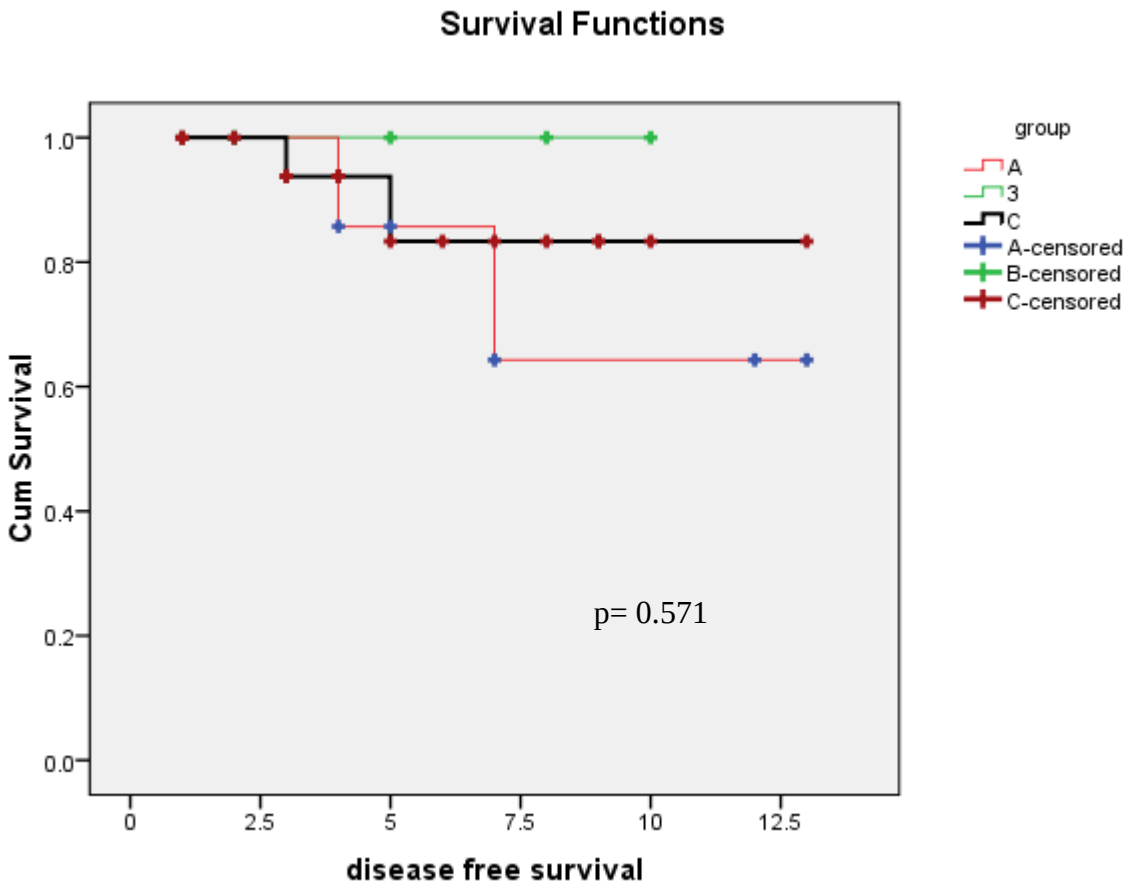
Table (5): Relation between HCV PCR reactivation in NHL patients with HCV positive and hepatic enzyme flare:

VARIABLE	HCV POSITIVE NHL PATIENTS				P VALUE
	No HCV PCR reactivation (N=19)		With HCV PCR reactivation (N=5)		
	No	%	No	%	
Enzyme flare:					
-No	17	89.5	0	0	< 0.001*
-Yes	2	10.5	5	100	

Table (6): Distribution of treatment response among the studied groups of patients according to HCV status

Response	HCV positive patients				HCV negative patients		P value
	Received Ribavirin Group A (11)		Without Ribavirin Group B (13)		Group C (33)		
	No	(%)	No	(%)	No	(%)	
Responders -Complete	8	72.7	6	46.2	22	66.7	0.329
Non-responders	3	27.3	7	53.8	11	33.3	
-Partial	3	27.3	5	38.5	8	24.2	
-Stable	0	0.0	1	7.7	3	9.1	
-Progression	0	0.0	1	7.7	0	0.0	
Total	11	100	13	100	33	100	

Figure (2): One year disease-free survival



DISCUSSION

The percentage of HCV infection among B cell NHL patients is 42 % (24 out of 57 cases) which is in agreement with and equal to results obtained by Cowgill et al.[10] (a study conducted at Cairo University hospitals, Egypt on 220 patients and the prevalence of HCV infection was 42.7 %), while Coppola et al. [11] (a study conducted in Italy on 36 patients and the prevalence of HCV infection was 22 %).

The interesting finding in our study is that percentage (42 %) of HCV infection which is higher than that observed in the general population (15-20%) [12]. This comes in agreement with Nosotti et al. [3], who stated that the prevalence of HCV infection among NHL patients was 9.2%, this prevalence is also higher than that observed in the general population in Italy (3%). This association between B cell non-Hodgkin lymphomas (NHL) and HCV infection may be related to chronic antigenic stimulation of HCV.

In this study, the comparison between HCV positive patients versus HCV negative patients with NHL showed no statistically significant difference as regard age, sex, clinical presentation, stage , IPI score, PS status ,LDH level, pathological type , chemotherapy regimen, ,this comes in concordance with results of Marignani et al.[13]**.** Most of our HCV positive patients (75%) presented with advanced stage (III/IV) compared to 42.5% among HCV negative patients (p-value =0.09), this result was similar to the study of Luppi et al.[14] who reported that the percentage of advanced stage (III/IV) was 74% of HCV positive patients ,while 64% of HCV negative patients.

In the present study, 14 patients (24.5%) had an indolent NHL and 43 patients (75.4%) had an aggressive NHL. The most frequent pathological type was DLBCL. The highest percentage (37.5%) of Indolent NHL type was found among HCV positive NHL cases (9/24 patients), especially marginal zone lymphoma (3 patients). This result is in concordance with Arcaini et al**.** [1] who reported that 37% of HCV positive NHL cases (59/160 patients) had an indolent NHL type, while 62% of the cases had a DLBCL type.

In the terms of overall response , 63% of our patients achieved complete response (CR) and 28% partial response (PR); 76% of DLBCL (30/39) patients obtained a CR, while between indolent NHL patients 21% (3/14 patients) achieved CR. While, among HCV positive NHL, 73.3% (11/15 patients) of DLBCL achieved CR, whereas, 33.3% (3/9 patients) of an indolent NHL achieved CR (p-value = 0.07) . While, Pellicelli et al. [15] showed that 54% of DLBCL achieved CR and 55% of indolent NHL patients achieved CR. In our study, no statistically significant difference was found when we compared the CR rates in the three groups.

Among the 57 NHL patients in our study, patients (14%) developed hepatic enzyme flare. The highest percentage of enzymatic flare was among HCV infected patients (29.2 %) , while only one patient (3%) in the HCV negative group (C). There was a statistical significant difference (p= 0.007) between the two groups. This result comes in agreement with another study which reported that among the HCV-infected subjects, the incidence of hepatitis flares was 26.3% vs 2.1% among the HCV-uninfected individuals [3].

We found that 20.8% of HCV infected NHL patients (5/24 patients) developed HCV PCR reactivation. Also, Parag et al [5] reported that 36.3% of HCV infected NHL patients proved by PCR (8/22 patients) developed HCV PCR reactivation. While, Boyle et al. [16] reported that 66.6% of HCV infected NHL patients(6/9 patients) developed HCV PCR reactivation. This difference between our study and other studies regarding the percentage of hepatic enzyme flare and HCV PCR reactivation can be explained by (1) difference in the definition of hepatic enzyme flare and HCV PCR reactivation between studies (2) different sample size (3) heterogeneity of the histopathology [1] (4) difference in chemotherapy regimens and the use of rituximab (5) different HCV genotype and association with other viral infection (HBV or HIV) (6) difference in duration of chronic HCV infection and the risk of developing cirrhosis.

In our study there was a significant relation between HCV reactivation and hepatic enzyme flare (p <0.001), also Parag et al. [5] agree

with our result , in contrary to Marignani et al. [13] who found no significant relation (p =0.8).

Regarding the toxicities (apart from hepatic toxicity), they were similar in all groups ; there were no statistically significant differences , with only one patient developed Grade 4 anemia in group A (the intervention arm who received ribavirin) but without significant difference

comparing to other groups (p = 0.58) . The hematologic toxicity with the use of ribavirin was to some extent accepted.

In this study the overall survival and disease-free survival in the three patients groups were comparable, although many patients among the HCV infected NHL group showed some delay in the treatment schedule, but this follow up was short.

Conclusion,

Frequency of HCV infection in patients with B cell NHL is higher than in the general population. Acute exacerbation and reactivation of chronic HCV infection occur in a sizeable subset of patients with NHL during chemotherapy.The use of ribavirin did not decrease hepatic enzyme flare or HCV PCR reactivation during chemotherapy, also ribavirin did not affect response to chemotherapy or survival rates.

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Ethical approval: approved.

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Fungal Infections in the Elderly

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Fungi are increasingly recognised as major pathogens in critically ill patients. Several reasons have been proposed for the increase in invasive fungal infections, including the use of antineoplastic and immunosuppressive agents, broad-spectrum antibiotics, and prosthetic devices and grafts, and more aggressive surgery. Patients with burns, neutropenia, HIV infection and pancreatitis are also predisposed to fungal infection Candidiasis is caused by infection with species of the genus Candida, predominantly with *Candida albicans*. *Candida* species are ubiquitous fungi that represent the most common fungal pathogens that affect humans. The growing problem of mucosal and systemic candidiasis reflects the enormous increase in the number of patients at risk and the increased opportunity that exists for *Candida* species to invade tissues normally resistant to invasion.

INTRODUCTION

Candida species are true opportunistic pathogens that exploit recent technological advances to gain access to the circulation and deep tissues. The increased prevalence of local and systemic disease caused by Candida species has resulted in numerous new clinical syndromes, the expression of which depends primarily on the immune status of the host. Candida species produce a wide spectrum of diseases, ranging from superficial mucocutaneous disease to invasive illnesses, such as hepatosplenic candidiasis, Candida peritonitis, and systemic candidiasis.

The management of serious and life-threatening invasive candidiasis remains severely hampered by delays in diagnosis and the lack of reliable diagnostic methods that allow detection of both fungemia and tissue invasion by Candida species.

Advances in medical technology, chemotherapeutics, cancer therapy, and organ transplantation have greatly reduced the morbidity and mortality of life-threatening disease. Patients who are critically ill and in medical and surgical ICUs have been the prime targets for opportunistic nosocomial fungal infections, primarily due to Candida species. Studies suggest that the problem is not under control and, in fact, show it is worsening. On a daily basis, virtually all physicians are confronted with a positive Candida isolate obtained from one or more various anatomical sites. High-risk areas for Candida infection include neonatal, pediatric, and adult ICUs, both medical and surgical[1]. Candida infections can involve any anatomical structure.

Candida

Over 200 species of *Candida* exist in nature; thus far, only a few species have been associated with disease in humans.

The medically significant *Candida* species include the following[2]:

C albicans, the most common species identified (50-60%)

Candida glabrata (previously known as *Torulopsis glabrata*) (15-20%)

C parapsilosis (10-20%)

Candida tropicalis (6-12%)

Candida krusei (1-3%)

Candida kefyr (< 5%)

Candida guilliermondi (< 5%)

Candida lusitaniae (< 5%)

Candida dubliniensis, primarily recovered from patients infected with infected with HIV

C glabrata and *C albicans* account for approximately 70-80% of *Candida* species recovered from patients with candidemia or invasive candidiasis. *C glabrata* has recently become very important because of its increasing incidence worldwide, its association with fluconazole resistance in up to 20% of clinical specimens, and its overall decreased susceptibility to other azoles and polyenes.

C krusei is important because of its intrinsic resistance to ketoconazole and fluconazole (Diflucan); it is also less susceptible to all other antifungals, including itraconazole (Sporanox) and amphotericin B.

Another important *Candida* species is *C lusitaniae;* although not as common as other *Candida* species, *C lusitaniae* is of clinical significance because it may be intrinsically resistant to amphotericin B, although it remains susceptible to azoles and echinocandins.

C parapsilosis is also an important species to consider in hospitalized patients. It is especially common in infections associated with vascular catheters prosthetic devices. Additionally, in vitro analyses have shown that echinocandins have a higher minimum inhibitory concentration (MIC) against *C parapsilosis* than other *Candida* species. The clinical relevance of this in vitro finding has yet to be determined [3].

C tropicalis has frequently been considered an important cause of candidemia in patients with cancer (leukemia) and in those who have undergone bone marrow transplantation.

Oral candidiasis.

White plaques that are present on the buccal, palatal, or oropharyngeal mucosa and can easily be removed are the typical lesions seen in patients with oral candidiasis.Some patients develop painful cracks at the corns of the mouth [angular cheilitis].Factors that predispose patients to the development of thrush include xerostomia,the use of broad-spectrum antibiotics, inhaled corticosteroids, and diminished cell-mediated immunity. Age alone is not sufficient for the development of oral candidiasis .In an older adult ,oral thrush-in the absence of an obvious cause or extension to involve the esophagus-may herald underlying immuno-suppression in the form of cancer or AIDS. The presence of xerostomia has been shown to correlate with both increased colonization of the oropharynx and increase oral mucosal lesions due to yeast [4]. In older adults ,the presence of systemic diseases and a multiplicity of medications can frequently cause xerostomia, which may than place patients at risk of developing oral thrush.

Denture stomatitis.

Denture stomatitis, a variant of oral candidiasis, presents as chronic mucosal erythema beneath a denture. This may affect up to 65% of all patients who wear dentures, occurring particularly in those with full sets[5].Patients who do not remove the dentures at night and who have poor oral hygiene are the most likely to be affected.

Skin and nail infections.

Candidal infection of the skin under the breasts, a pannus,or the perineum occurs when these areas become macerated. Lesions are almost always pruritic, erythematous, and have a distinct border. Small, flat, erythematous satellite lesions provide an additional clue to the diagnosis of candidiasis.

Paronychia and onychomycosis.

Candida uncommonly causes infection in the periungual area and underneath the nailbed. Inflammation leads to thickening and even loss of the nail. The disease occurs most often in persons who frequently immerse their hands in water and has not been a major problem in older patients. However, one group of older adults in whom onychomycosis can have serious consequences in those with diabetes mellitus. In comparison with nondiabetic patients, patients with diabetes have both increase susceptibility to and worse outcome from bacterial infection of the feet. Onychomycosis can contribute to difficulty cutting the toenails, predisposing these patient to trauma and thus bacterial foot infection. Onychomycosis is found more frequently in the elderly and more often in males than females. There are four types of onychomycosis; distal subungual onychomycosis, proximal subungual onychomycosis, white superficial onychomycosis, and candidal onychomycosis.

Vulvovaginal candidiasis (VVC)

This is the second most common cause of vaginitis. The patient's history includes vulvar pruritus, vaginal discharge, dysuria, and dyspareunia. Approximately 10% of women experience repeated attacks of VVC without precipitating risk factors. Physical examination findings include a vagina and labia that are usually erythematous, a thick curdlike discharge, and a normal cervix upon speculum examination [6].

Candiduria often presents a dilemma to the clinician as it may represent contamination, colonization,or infection. Contamination may be detected by repeating collection and culture of the urine. However, straight catheterization of the urethra may be needed to obtain an uncontaminated urinary specimen in older women. In the asymptomatic patient,some clinicians consider the presence of any yeast in the urine to represent infection ,whereas some authors suggest that <10,000 cfu/mL may represent only colonization[7].The presence of a urinary catheter limits the usefulness of colony quantitation. Most authors would agree that the presence of symptoms such as suprapubic discomfort, dysuria, or frequency usually comprise infection, rather than colonization. Asymptomatic candiduria: Most catheterized patients with persistent candiduria are asymptomatic, similar to noncatheterized patients. Most patients with candiduria have easily identifiable risk factors for *Candida* colonization. Thus, invasive disease is difficult to differentiate from colonization based solely on culture results because approximately 5-10% of all urine cultures are positive for *Candida* [8].

Candidemia

Candida species are currently the fourth most commonly isolated organism in blood cultures, and *Candida* infection is generally considered a nosocomial infection [9]. The patient's history commonly reveals the following:

Several days of fever that is unresponsive to broad-spectrum antimicrobials; frequently the only marker of infection

Prolonged intravenous catheterization

A history of several key risk factors

Possibly associated with multiorgan infection

Physical examination results may include the following:

-Fever.

-Macronodular skin lesions (approximately 10%).

-Candidal endophthalmitis (approximately 10-28%).

-Occasionally, septic shock (hypotension, tachycardia, tachypnea).

CONCLUSION

Infections in the elderly are more common than in younger individuals.They are more complicated by the multiple medications used to control the diseases that accompany normal ageing. Understanding the diseases an elderly patient might suffer enhances their health and well being.

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Video Case :Extraction of a Pin from the Stomach of a 13 Years Girl

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Comment

A pin was accidentally ingested by a 13 years Girl . Repeated X rays over one month revealed impacted pin in the upper abdomen. Upper gastrointestinal endoscopy using Olympus GIF-Q160 endoscope was performed.

5 mg midazolam was administered intravenously . Endoscopy revealed penetrated pin in the antrum of the stomach. Extraction using shark tooth forceps was done.

Image Case:Sub-capsular Pyogenic Splenic Abscess: Feasibility of Ultrasound Guided Drainage

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We reported a 58-year old female with chronic liver disease, type 2 diabetes, ischemic heart disease and hypertension presented by left hypochondrial dull aching pain. She denies fever, malaise, nausea, vomiting, bowel habit disturbance and dysuria. On examination she looked mildly toxic, blood pressure 140/80, pulse 68 regular, temperature 37 C. Ultrasonographic examination revealed subcapsular collection in the spleen that was further confirmed in abdominal CT scan (figure 1). Ultrasonographic guided aspiration (figure 2) and broad spectrum antibiotics including cefoperazone and ciprofloxacin to the isolated gram negative bacilli depending on the culture and sensitivity testing achieved complete response.

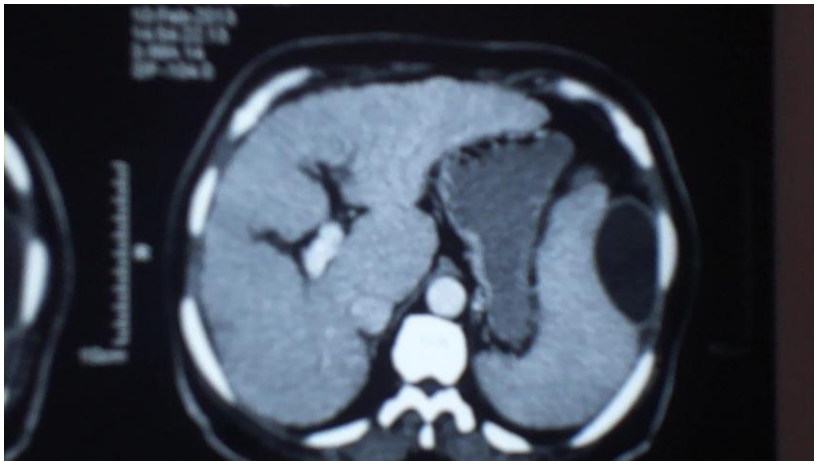

Figure 1: Abdominal CT scan showing splenic subcapsular abscess

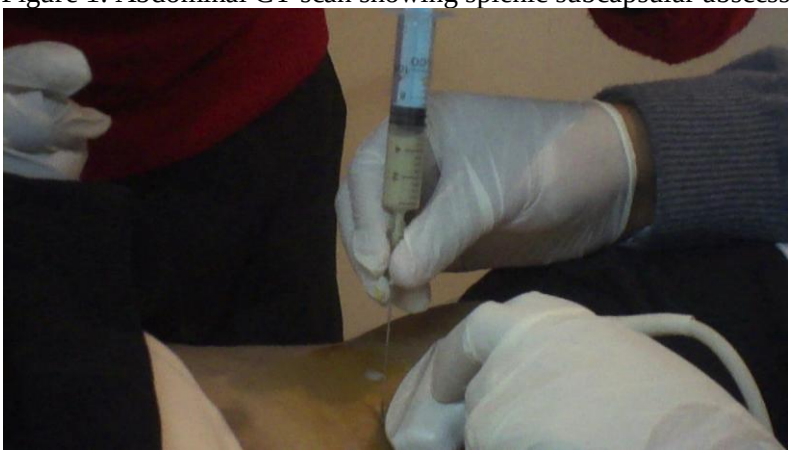

Figure 2: Ultrasound guided drainage with pus seen in the syringe

Image Case:Loculated Abdominal Collection following Leaking Hepatocellular Carcinoma

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A 60 years old women presented by progressive abdominal distension and abdominal pain. When presented ultrasound guided abdominocentesis confirmed intraperitoneal hemorrhage further assessment by abdominal-pelvic CT scans showed leaking sub capsular hepatic focal lesion consistent with hepatocellular carcinoma. The patient was treated conservatively and 2 months later she experienced recurrent abdominal pain and sub acute intestinal obstruction. Ultrasonographic examination showed intrabdominal loculations(figure 1). The differential diagnosis of this condition includes intraperitoneal hemorrhage due to other causes, abdominal TB and recurrent spontaneous bacterial peritonitis in ascetic patients..

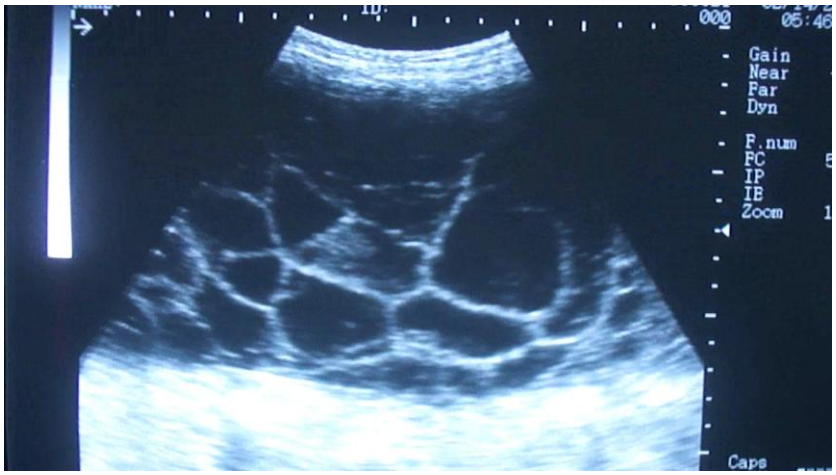

Figure 1: Intraabdominal fibrotic loculations