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AIDS Effects in Liver and Biliary Tract by using Ultrasound in Sudan

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Background and study aim: The human immunodeficiency virus (HIV) is a retrovirus that infects cells of the immune system, destroying or impairing their function. As the infection progresses, the immune system becomes weaker, and the person becomes more susceptible to infections. The United Nations (UN) estimates that Sudan has the highest rate of HIV infection in North Africa and the Middle East. The aim of this study was to describe the effects of AIDS in liver and biliary tract and evaluate the clinical utility of hepatic and biliary tract sonography in AIDS patients in Khartoum state, Sudan.

Patient and Methods: This prospective study was conducted at Omdurman, Elshaab and Bashayer Teaching Hospitals, in Khartoum State, Sudan. It spanned a period of 3 years from January 2009 to January 2012, involving 300 HIV positive Sudanese patients (198; 66% males and 102; 34% females) and aged 6 to 60 years; mean age of 46 ± 6.4 years. Samples proved to have AIDS by enzyme immuno assay test (EIA) and also confirmed by Western blot (protein immunoblot) blood test. Sonography was performed using Toshiba Just Vision 200, Aloka SSD 500 and Siemens Antares fitted with 3.5 MHz convex transducers.

Results: In AIDS patients, ultrasound findings in liver included wide spectrum of abnormalities like hepatomegally (88; 29.3%), portal hypertension (49; 16.3%), periportal fibrosis (20; 6.7%), fatty fibrotic changes (42; 14%), jaundice (18; 6%), extra hepatic ducts dilatation (24; 8%), cholecystitis (33; 11%), cholelithiasis (24; 8%) and Kaposi sarcoma (18; 6%).

Conclusion: Abdominal ultrasound is a simple and cost effective tool and can be used as a baseline imaging modality in AIDS infected patients. Liver enlargement and gall bladder wall thickening are common effects develop due to AIDS infection in population at Khartoum state, Sudan.

INTRODUCTION

Human immunodeficiency virus infection/Acquired immunodeficiency syndrome (HIV/AIDS) is a disease of the human immune system caused by the human immunodeficiency virus (HIV) [1]. The illness interferes with the immune system, making people with AIDS much more likely to get infections, including opportunistic infections and tumors that do not usually affect people with working immune systems. This susceptibility increases as the disease worsens [2].

Patients with AIDS present a wide variety of clinical manifestations through involvement of various organs. Ultrasonography is easy to perform, safe, inexpensive, not invasive and repeatable. Ultrasound can investigate most of the organs affected in AIDS and can guide biopsies, allowing the cytohistological and microbiological investigations need for a definitive diagnosis [3].

The aim of this study was to describe the effects of AIDS in liver and biliary tract and evaluate the clinical utility of liver and biliary tract sonography in AIDS patients in Khartoum state, Sudan. Despite the fact that the epidemiological data is limited, also absence of any data determines the sonographic findings of AIDS in various body organs of the affected samples.

Ultrasound findings in patients with AIDS were studied by Langer et al. where ultrasonographic findings of 43 patients with AIDS and ARC were analyzed. In 63% an enlarged liver, in 66% an enlarged spleen, partially with focal lesions, and in 21% enlarged abdominal lymph nodes were diagnosed [4]. Non-Hodgkin's lymphomas (NHL) are the second most frequent malignancies in AIDS patients. The majority of NHL associated with AIDS involves extra nodal sites, especially the digestive tract and the central nervous system. Primary liver lymphoma (PLL) is an uncommon neoplasm among these patients. Ultrasonography and computed tomography scans may be helpful in the diagnosis of focal hepatic lymphoma [5].

AIDS was most prevalent in the 4th decade with an incidence of 40.4% compared with the HIV negative individuals. Ultrasonography is optimally suited for its clinical management especially in Africa. Its accuracy and sensitivity may be much improved with clinico-pathologic correlation which may not be readily available in developing countries; further studies may provide this much needed diagnostic algorithms [6]. Ultrasonographic screening for liver enlargement associated with opportunistic infections in patients with human immunodeficiency virus infection revealed that liver enlargement in 63.75% of HIV patients. In 40.7% the right lobe size varied from 140 mm to 160 mm. Of those with hepatomegally, 60.7% had AIDS. Liver enlargement is common in HIV infected patients mostly in association with hepatitis C and B viruses and Mycobacterium tuberculosis [7].

Abdominal ultrasonography and computed tomography were performed in two patients with acquired immunodeficiency syndrome (AIDS) and necropsy proved hepatic Kaposi sarcoma. At ultrasound, small (5-12 mm) hyperechoic nodules and dense periportal bands were seen in the liver. These lesions appeared hypo attenuated on baseline and dynamic CT scans and enhanced on delayed scans after a bolus injection of contrast material. Although nonspecific, these features strongly suggest tumor involvement in the liver in patients with AIDS and Kaposi sarcoma [8].

PATIENTS AND METHODS

Abdominal ultrasound scans were prospectively performed over a 3 years period (January 2009 to January 2012), involving 300 HIV positive Sudanese patients (aged 6 to 60 years; mean age of 46 ± 6.4 years) selected from the outflow of patients at Omdurman Teaching Hospital, Elshaab Teaching Hospital and Bashayer Teaching Hospital, Radiology and Medical Imaging Department, Khartoum State, Sudan. At inclusion, complete medical history, routine clinical and diagnostic procedures including an ultrasound examination of the abdomen, and standard enzyme immuno assay test (EIA) was used to check for antibodies to HIV. If this antibody test states that sample is positive, a confirmatory blood test; Western blot (protein immunoblot) test performed in all patients. The medical records of such 300 qualified patients were analyzed with regard to age, sex, demographic data, liver ultrasound findings, medical history and symptoms. Other samples, which do not apply to them these features, were excluded from the study. An informed consent was obtained from all the subjects before scanning but, in addition, a review and authorization of the study protocols was done by the Ethical Committees available at the Radiology and Medical Imaging Departments of Omdurman, Elshaab and Bashayer Teaching Hospitals. Abdominal sonography was performed using Toshiba Just Vision 200, Aloka SSD 500 and Siemens Antares fitted with 3.5 MHz convex array transducers. For sonograms printing, ultrasound machines used were connected with digital graphic printer, 100 V; 1.5 A; and 50/60 Hz, with serial number of 3-619-GBI-01 and made by Sony Corporation- Japan. All measurements were obtained by electronic calipers available in the ultrasound machines. Prior to exam, Patients should be subjected to abdominal preparation to avoid gas buildup in the intestine. Patient should take nothing by mouth for 8 hours. If fluid is essential to prevent dehydration only water should be given. In case of acute symptoms, examination was proceeding immediately. Infants' clinical condition, permitting was given for nothing by mouth for 3 hours preceding the examination. For most ultrasound exams, the patient is positioned lying

face-up (supine) on an examination table that can be tilted or moved. A clear water based gel is applied to the abdomen to help the transducer make secure contact with the body and eliminate air pockets between the transducer and the skin that can block the sound waves from passing into patient body. The highest frequency transducer permitting adequate penetration is used such as phased array sector probe with its small footprint permits subcostal and intercostal scanning. Scans were performed in the sagittal and transverse planes from the anterior subcostal and intercostal approach to study the liver and biliary tract to diagnose the various findings related to AIDS effects. Various maneuvers may enhance demonstration of the liver and biliary tract including left posterior oblique left lateral decubitus or setting semierect to erect lateral oblique positions. Coronal right lateral subcostal and intercostal approach with different breathing technique is recommended depending on liver shape and patient respiration [9,10]. The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1964. Results statistical analysis, overviewed in a form of tables and graphs by using Microsoft Office Excel package depend on the frequencies and the percentages of categorical variables of the scanned samples.

RESULTS

A total of 300 HIV positive Sudanese patients (198; 66% males and 102; 34% females) aged from 6 to 60 years; mean age of 46 ± 6.4 years, proved to have AIDS by enzyme immuno assay test (EIA) and also confirmed by Western blot (protein immunoblot) blood test. In the sample, AIDS is common in the age group 41 to 50 years; 4th decade with a frequency of (229; 76.3%) **(Table 1** and **Figure 1)**.

Table 1: Age group distribution in AIDS patients

Age group (year)	Frequency	Percentage (%)
< Than 20 years	2	(0.7%)
20-30	18	(6%)
31-40	24	(8%)
41-50	229	(76.3%)
51-60	27	(9%)
Total	300	100%

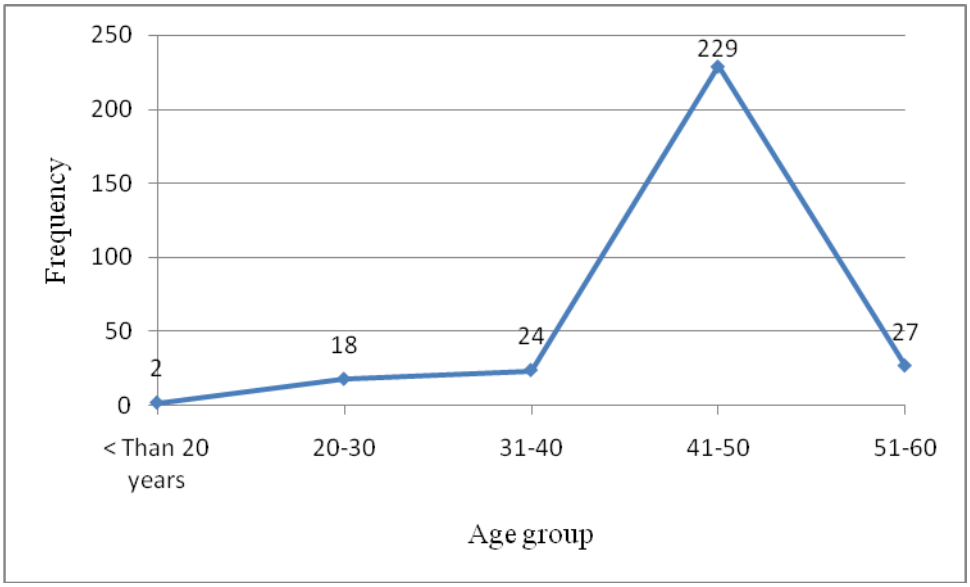

Figure 1: Age group distribution in AIDS patients

The duration of acquired HIV infection is varied with mean of 7.5 ± 3.8 years in the sample. Where (15; 5%) cases were diagnosed in the time period of 2000 to 2002; HIV positive for 12 to 10 years, (17; 5.7%) cases were diagnosed in the time period of 2003 to 2005; HIV positive for 9 to 7 years and (268; 89.3%) cases were diagnosed in the time period of (2006 to 2012); HIV positive for 6 to 1 years (**Table 2** and **Figure 2**).

Table 2: Duration of acquired AIDS infection

Date of diagnosis	HIV positive duration	Frequency in patients	Percentage (%)
2000-2002	12-10 years	15	(5%)
2003-2005	9-7 years	17	(5.7%)
2006-2012	6-1 years	268	(89.3%)
Total	12 years	300	(100%)
Mean ± SD	7.5 ± 3.8 years	-	-

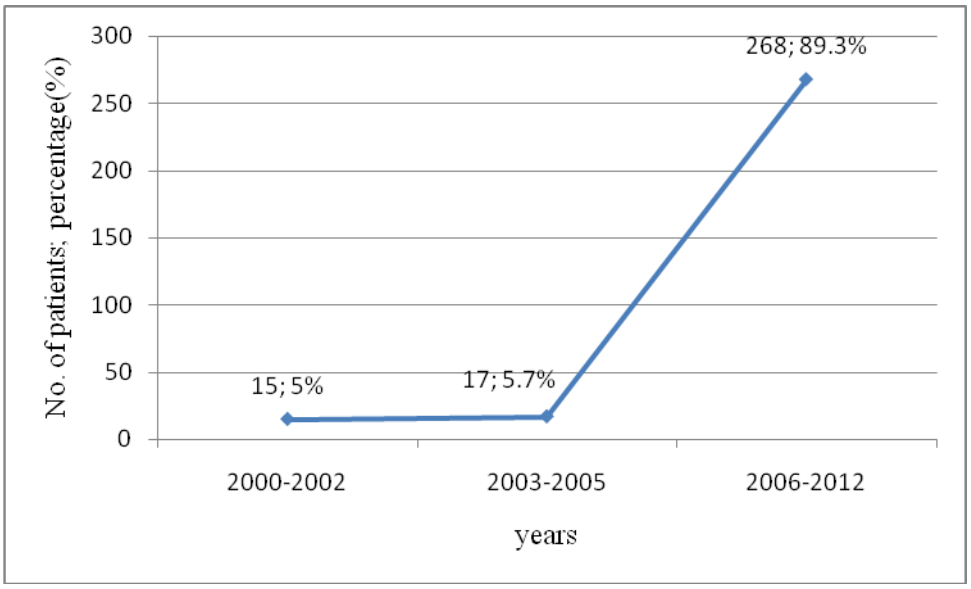

Figure 2: AIDS incidence per year in the sample

Ultrasound findings in liver and biliary tract of AIDS patient are shown in (**Table 3** and **Figure 3**). Out of the study population (88; 29.3%) presents hepatomegally, (49; 16.3%) presents portal hypertension, (20; 6.7%) presents periportal fibrosis, (42; 14%) increased liver echogenicity; fatty fibrotic changes, (18; 6%) presents jaundice, (24; 8%) extra hepatic ducts dilatation, (33; 11%) thick wall gallbladder; cholecystitis, (24; 8%) cholelithiasis and (18; 6%) periportal dense bands and small hyperechoic nodules; Kaposi sarcoma.

Table 3: Ultrasound findings in liver and biliary tract of AIDS patients

Ultrasound findings	Frequency	Percentage (%)
Hepatomegally	88	(29.3%)
Portal hypertension	49	(16.3%)
Fatty fibrotic changes	42	(14%)
Cholecystitis	33	(11%)
Cholelithiasis	24	(8%)
Extra hepatic ducts dilatation	24	(8%)
Periportal fibrosis	20	(6.7%)
Jaundice	18	(6%)
Kaposi sarcoma *	2	(0.7%)
Total	300	100%

* Confirmed by liver biopsy; verification by histological laboratory exam.

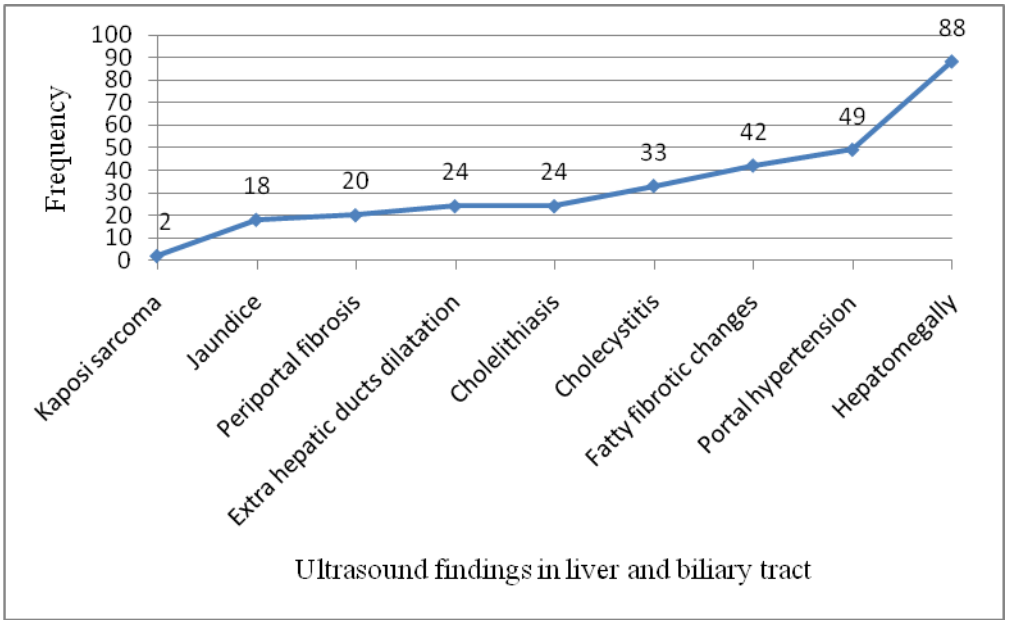

Figure 3: Ultrasound findings in the liver and biliary tract of AIDS patients

DISCUSSION

The prevalence of AIDS out of 300 samples was 198; 66% males and 102; 34% females. The scarce data useful for documenting gender differences in HIV prevalence or incidence come from the very few serologic surveys in national populations, and from local, population based studies, most of them longitudinal. It is worth noting that this age pattern of prevalence is similar to that of tuberculosis (more young women, more older men), another lethal persistent infection, which is also a common opportunistic infection for HIV [11-13].

The study reported an increase in the prevalence of AIDS per year in Khartoum state population (**Table 2**). Understanding the increase prevalence of cases per year was described in base case scenarios, predicted HIV prevalence increases of 24%-38% in 10 years. Reducing the transmission rate by 50% within 10 years reduces incidence by 40%; prevalence increases 20% to an estimated 1 329 000 persons living with HIV. Halving the transmission rate within 5 years reduces incidence by 46%; prevalence increases 13%, to 1 247 000. Although in 10 years incidences is similar regardless of the intervention time frame, more infections are averted when halving the transmission rate within 5 years [14]. The back-calculation (n = 1.230 million HIV/AIDS cases reported by the end of 2006) yielded an estimate of 55 400 (95% CI, 50 000-60 800) new infections per year for 2003-2006 and indicated that HIV incidence increased in the mid-1990s, then slightly declined after 1999 and has been stable thereafter [15]. In addition, analysis indicates that 571 000 new HIV infections occurred in the population 2 years and older during the year 2005 in South Africa [16].

Various diseases associated with human immunodeficiency virus (HIV) infection are often difficult to diagnose. A poor immune response, atypical presentations and opportunistic pathologies all contribute to this difficulty [17]. Imaging plays an important role in the detection of various pathologies associated with AIDS. In many cases, the radiologist is often the first clinician to suspect the possibility of human immunodeficiency virus (HIV) or AIDS in a patient's diagnostic work-up and it is, therefore, important that radiologists are familiar with the imaging features of this disease and its complications [18].

The obtained results reveal wide spectrum of complications in liver and biliary tract related to positive AIDS infection where hepatomegally and cholecystitis are the commonest hepatic and biliary tract complication, such findings was supported by Langer et al. [4] and Uygur et al. [19] where the most common abdominal ultrasound findings in HIV patients were hepatomegally. Dominique et al. [20] reported that cholecystitis is the commonest pathology detected in the biliary tract of positive AIDS patients, which in turn support our findings.

Ultrasound findings such as cholecystitis and hepatomegally, can be explained in the basis that the most common cause of acute cholecystitis is gallstones, which block the cystic duct. This leads to gallbladder irritation and inflammation. Acalculous cholecystitis, though rare, is most often seen in patients hospitalized in intensive care wards of hospitals. In these cases there are no gallstones. Complications of other diseases, like AIDS or diabetes, cause inflammation. While in hepatomegally, certain viral infections can also result in the liver becoming enlarged, as infections with hepatitis A or B, infectious mononucelosis and HIV.

CONCLUSION

In conclusion, abdominal ultrasound is a simple and cost effective tool and can be used as a baseline imaging modality in AIDS infected patients. Its accuracy and sensitivity is invaluable in the assessment of the disease sate and in monitoring of therapy. Liver enlargement and gall bladder wall thickening are common effects develop due to AIDS infection in population at Khartoum state, Sudan. Further studies are required to define patterns of clinical findings, pathologic and laboratory correlates with ultrasound to develop and refine diagnostic algorithms for clinical use.

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Conflicts of interest: The authors declare that there is no conflict of interest.

Ethical approval: The protocol of the study was approved by the Ethical Committees of the Radiology and Medical Imaging Departments of Omdurman, Elshaab and Bashayer Teaching Hospitals. Informed consents were obtained from all patients. The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1964.

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Study of Brain Changes in Chronic Hepatic Encephalopathy by Using MR Imaging

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Brain Changes; Hepatic Encephalopathy; Magnetic resonance imaging; Manganese; Ammonia;Myoinsitol.

Background and study aim: Hepatic encephalopathy (HE) reflects a spectrum of neuro-psychiatric abnormalities. The aim of this study was to evaluate MR imaging of the brain in different grades of chronic HE and its correlation with clinical neurological abnormalities.

Patients and Methods: Sixty patients were included, 40 patients with chronic HE were divided into group I (GI) chronic persistent HE (n=20), group II (GII) chronic relapsing HE (n=20), another 20 patients with early compensated cirrhosis were chosen as control group (GIII), all patients were subjected to full clinical and laboratory investigations, estimation of serum ammonia and Manganese level in the blood, psychometric tests ,conventional MRI and MRS.

Results: A statistically significant increase in serum level of ammonia and manganese in GI (162.1±55.8 µmol/L, 3.35±0.34 ug/dl, respectively) when compared to other groups. By conventional MRI there was statistically significant increased signal intensity of T1 and T2 in group I compared with other groups. By MRS, there was statistical significant increase of glutamine in GI (3.9±0.17 pp,) when compared to GII (3.7±0.13 ppm) & GIII (2.48±0.3 ppm) and significant reduction of both choline and myinstol among GI 1.98±0.17 ppm, 2.19±0.20 ppm, when compared to GII 2.29±0.17 ppm, 2.74±0.17 ppm and GIII 2.59±0.019 ppm, 3.15±0.11 ppm. Moreover there was significant elevation in signal intensity of T1 corresponding to elevation of serum manganese (2.91±0.6 ug/dl) and significant elevation of signal intensity in T2 corresponding to elevation of serum ammonia (116.9±49 µmol/L), as well as highly significant positive correlation between serum ammonia and glutamine (r = 0.86) and highly significant negative correlation between serum ammonia and choline (r = -0.42) and myinstol (r = -0.47).

Conclusion: Changes in brain metabolites as detected by MRS may be sensitive markers for clinical monitoring of brain dysfunction and cognitive impairment in patient with chronic HE.

INTRODUCTION

Hepatic encephalopathy (HE) is a syndrome of neuropsychiatric dysfunction disease [1]. Hepatic encephalopathy is a common complication of advanced cirrhosis. Between one third to one half of hospitalizations for cirrhosis are related to HE. The frequency of hospitalization for HE has nearly doubled over the last decade, with lengths of stay between 5 and 7 days [2]. Patients with HE often have other manifestations of end-stage liver disease, such as ascites, jaundice, or gastrointestinal variceal bleeding. Hepatic encephalopathy can also develop as an isolated manifestation of decompensated cirrhosis. Hepatic encephalopathy usually signals advanced liver failure, and is often considered a clinical indication for evaluation for liver transplantation . HE may disable the patient from employment , driving and self-care, and require involvement of family or household members in the care of affected patients [3].

HE is clinically classified into three major categories, according to the underlying hepatic condition. Type A occurs in patients with acute liver failure. Type B occurs in patients without intrinsic liver disease but with large, noncirrhotic, portosystemic shunting. Type C is related to underlying cirrhosis with portosystemic shunting. Type C is the most common form, It can be divided to episodic or persistent[4].

MRI may help in diagnosis of hepatic encephalopathy, the most frequent conventional MR finding in hepatic encephalopathy is T1 weight image that gives high signal intensity of the basal ganglia caused mainly by deposition of manganese [5] and T2 caused by diffuse brain edema which seems to play an essential role in the pathogenesis of hepatic encephalopathy, which is believed to be related to the porto-systemic shunt and increase level of ammonia, Changes found in conventaial MRI T1and T2 have no quantitative relation to severity of HE [1,2]. MR spectroscopy (MRS) provides a measure of brain chemistry and metabolic changes which occurs in the astrocytes in patients with chronic liver cell failure which showing increase in glutaminel glutamate signal intensity and decrease myo-inosital and choline signal intensity to prevent massive cerebral edema. Changes seen on MRS imaging usually correlate with severity of hepatic encephalolpathy [6].

PATIENTS AND METHODS

This study was conducted in the Tropical Medicine and Radiology Departments, Zagazig University Hospitals from January 2009 to January 2012. The study included 60 patients with liver cirrhosis; 40 of them were chronic hepatic encephalopathy, (20 patients persistent hepatic encephalopathy with grade I-II after taking treatment and 20 patients relapsing hepatic encephalopathy, showed normal psychometric test), 20 of them early compensated cirrhosis as a control. The patients diagnosed for hepatic encephalopathy using psychometric tests and Grading of the symptoms of hepatic encephalopathy is performed according to the West Haven classification system.

All patients were divided into three groups:

Group I: (Chronic persistent hepatic encephalopathy)

It included twenty patients, 15 out of them were males, 5 patients were females, their ages ranged between 43-73 years.

Inclusion criteria for this group (GI)

Patients had changes in consciousness, intellectual function and behavior. Gait abnormalities and flapping tremors (grade I, II by west haven criteria) were included in this group.

Group II (Chronic relapsing hepatic encephalopathy)

It included twenty patients (14 patients were males, 6 patients were females) their ages ranged between 46-67 years.

Inclusion criteria for this group (GII)

Patients who had frequent episodes of acute HE and after examination. The patients were perfectly alert don't showed any sign of cognitive dysfunction and psuychometric test for all patients negative.

Group III: (early compensated cirrhosis)

It included twenty patients with no previous episodes of HE in these group, 16 were males and 4 were female their ages ranged between 42-67 years (G III).

Exclusion Criteria:

Patients with neuropathological evidence of trauma, tumor, Cerebrovascular accident or neurodegenerative disease (Alzheimer's disease or Parkinson diseases) will be excluded from the study regardless the presence of liver disorder.

All patients were undergone:

1- Full history taking and physical examination

2- Routine investigations: liver function tests, kidney function tests, prothrombine time, complete blood picture and pelvi-abdominal ultrasonogrophy

3-**Ammonia and manganese (MN) measurement**

Fasting arterial blood samples were obtained from each patient to measure ammonia concentration (μmol/L), MN concentration (ug/dl).

4-Complete neuropsychological assessment by using psychometric tests (number connection test (NCT), circle connection test (CCT) [7].

5) Magnetic resonance imaging

Routine MRI was done for all patients. MR imaging consisted of transverse nonenhanced T1-weighted spin-echo and T2-weighted fast spin-echo sequences. The imaging parameters were 500/14 msec (repetition time msec/echo time msec), FOV: 230x 230 mm and a 2-minute acquisition time for T1-weighted imaging and 5000/86 msec (TR/TE msec), FOV: 230x 230 mm and a 3-minute acquisition time for T2-fast spin echo sequences. The section thickness was 5 mm with an intersection gap of 1 mm.

- *^{1}H MR spectroscopy:*

A-Localization and data acquisition: was achieved by acquiring three orthogonal. (sagittal, transverse, and coronal) gapless, HASTE sequences. Before recording the spectrum, the homogeneity of the magnetic field over the volume of interest was optimized by shimming. Suppression of water signal was performed by using three preceding Gaussian pulses (60-Hz bandwidth). Then multi-voxel PRESS technique using the following parameters TR: 1500 msec, TE: 30 msec and FOV: 230x 230mm was done.

B-Post processing: Spectral postprocessing consisted of zero filling Gaussian apodiztion for noise reduction with base line and phase correction.

C-Spectral analysis: Measurement was performed at following resonance myoinositol (mI) (3.5 ppm), glutamate or glutamine (Glx) (3.75 ppm), creatine (Cr} (3.03 ppm),Cho (3.22 ppm), and N-acetylaspartate (NAA} (2.0 ppm). Those metabolites were measured in the in deep white matter in the medial part of the occipital lobe, parietal. lobe and basal ganglionic regions. Metabolic ratios were calculated for mI/Cr, Cho/Cr, Glx/Cr and Na/Cr ratios in all our subjects.

Statistical analysis

Statistical were calculated using SPSS windows (version 10). Qualitative variables were expressed by means of frequency and percentiles, and were analyzed using the X^2 test. Quantitative results are expressed as means±SD. Groups were compared by using paired t-test, ANOVA or thine Wilcoxon signed-rank test.

RESULTS

There were no significant difference in epidemiological and biochemical parameters among patients of the three groups of the study apart from statistical significant increase in serum bilrubin and reduction in serum albumin and prothrombine concentration in GI compared to other groups. Child score was high among patient in GI (11.5±1.54) in comparison to group II (9.5±2.6) and III (5.5±0.5) with P<0.001 (Table 1).

There was significant increased in signal intensity of T1 and T2 in group I compared with other groups with complete absence of signal among GIII with (P<0.001) (Table 2).

Regarding MR spectroscopy in different groups, there was statistical significant increase of glutamine in GI (3.9±0.17 ppm) when compared to GII (3.7±0.1 ppm) & GIII (2.48±0.3 ppm) with P<0.001) and significant reduction of both choline and myinstol among GI (1.98±0.17 ppm) (2.19±0.20 ppm) when compared to GII (2.29±0.17 ppm) (2.74±0.17) and GIII (2.59±0.19) (3.15±0.11) respectively with P<0.001 (Table 3, Figure 1&2).

A significant high serum level of ammonia in GI (162.1±55.8 μmol/L) and high serum level of manganes in GI (3.35±0.34 ug/dl) was noticed when compared to other groups with P<0.001 (Table 4).

Relation between Mn level in blood & T1 signal image in hepatic patients, there was significant elevation in signal intensity in T1 in corresponding to elevation of level of Mn in blood (2.91±0.6 ug/dl) with P<0.001 (Table 5).

A relation between ammonia level in blood and T_2 signal intensity in hepatic patients, there was significant elevation in signal intensity in T2 in corresponding to elevation of level of ammonia in blood (116.9±49 μmol/L) P<0.001 (Table 6).

A correlation between ammonia and chemical metabolities we found, MR spectroscopy revealed highly significant positive correlation between ammonia in blood and glutamine in the brain (r = 0.86, P<0.001) as well as highly significant negative correlation between ammonia in blood and Choline (r = -0.42, P<0.01) or Myinstol (r =- 0.47, P<0.001) in the brain (Table 7).

Table (1): Clinical and laboratory findings in the studied groups.

	Chronic persistent hepatic encephalopathy (GI) N = 20	Chronic relapsing hepatic encephalopathy (GII) N = 20	Compensated early cirrhosis (GIII) N = 20	P
Male/Female Mean age (years)	15/5 56.1±8.5	14/6 54.1±4.9	16/4 53.1±9.2	 0.46
T.bill (mg/dl)	1.83±0.3	1.4±.3	1.5±0.5	0.002*
D.Bill. (mg/dl)	0.76±0.1	0.58±0.1	0.9±0.2	0.001**
T.protein(g/dl)	6.6±0.5	6.9±0.3	6.9±0.5	0.25
S. albumin(g/dl)	3.3±0.6	3.2±0.5	3.5±0.2	0.001**
Proth. Conc.	50.6±8.95	70.42±7.4	90.2±4.1	<0.05
SGPT (IU/L)	52.8±11	45.5±12.1	47.6±17.1	0.22
SGOT (IU/L)	58.5±14.6	48.3±15.8	50.5±16.9	0.1
Child score A 20 B 14 C 26	11.5±1.54	9.5±2.6	5.5±0.5	
Ultrasounds Shrunken Enlarged Average	 20 0 0	 4 16 0	 0 0 20	 <0.001
Ascitis	20	16	0	<0.001
Splenomegaly	20	14	0	<0.001
Portosystemic collateral	20	9	0	<0.001

* Significant P<0.05 ** Highly significant P<0.001

Table (2): T_1 and T_2 weighted images in different groups of patients.

	GI (n = 20)		GII (n = 20)		GIII (n = 20)		X^2	P
T_1								
Positive	20	100.0	13	65.0	0	0.0	41.6	<0.001
Negative	0	0.0	7	35.0	20	100.0		
T_2								
Positive	13	65.0	7	30.0	0	0.0	19.05	<0.001
Negative	7	35.0	13	65.0	20	100.0		

G= group

Table (3): MR spectroscopy metabolite and ratios in different groups.

	Chronic persistent hepatic encephalopathy (GI) N = 20	Chronic relapsing hepatic encephalopathy (GII) N = 20	Compensated early cirrhosis (GIII) N = 20	P
Glutamine/ Glx/Cr	3.9±0.17 1.85±0.59	3.7±0.13 1.34±0.49	2.48±0.3 1.18±0.50	<0.001
Choline Cho/Cr	1.98±0.17 0.68±0.10	2.29±0.17 0.80±0.12	2.59±0.19 0.91±0.11	<0.001
Mynstol Mi/Cr	2.19±0.20 0.32±0.12	2.74±0.17 0.75±0.16	3.15±0.11 0.97±0.13	<0.001

Measurements in ppm, G=group.

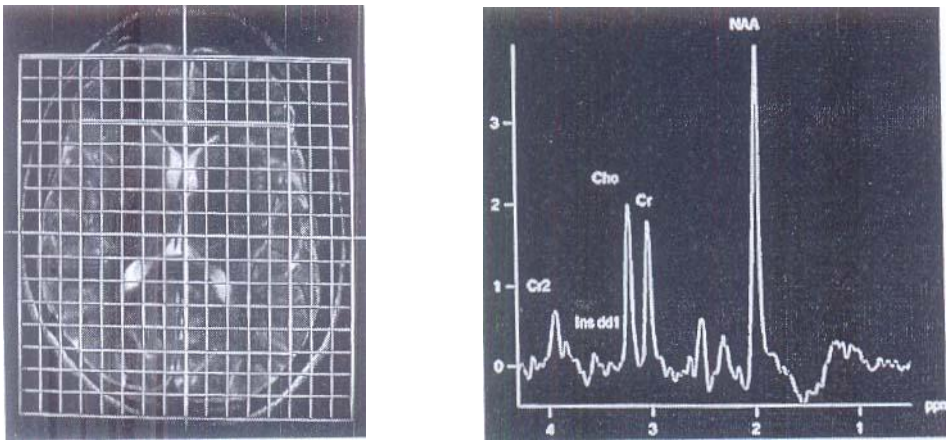

Figure (1): ^{1}H-spectroscopy in control subject (early cirrhosis). VOI is placed in the white matter of right occipital lobe showed the normal resonance of myo-inositol (mI), choline (Cho), creatine (Cr) and N-acetylaspartate (NAA).

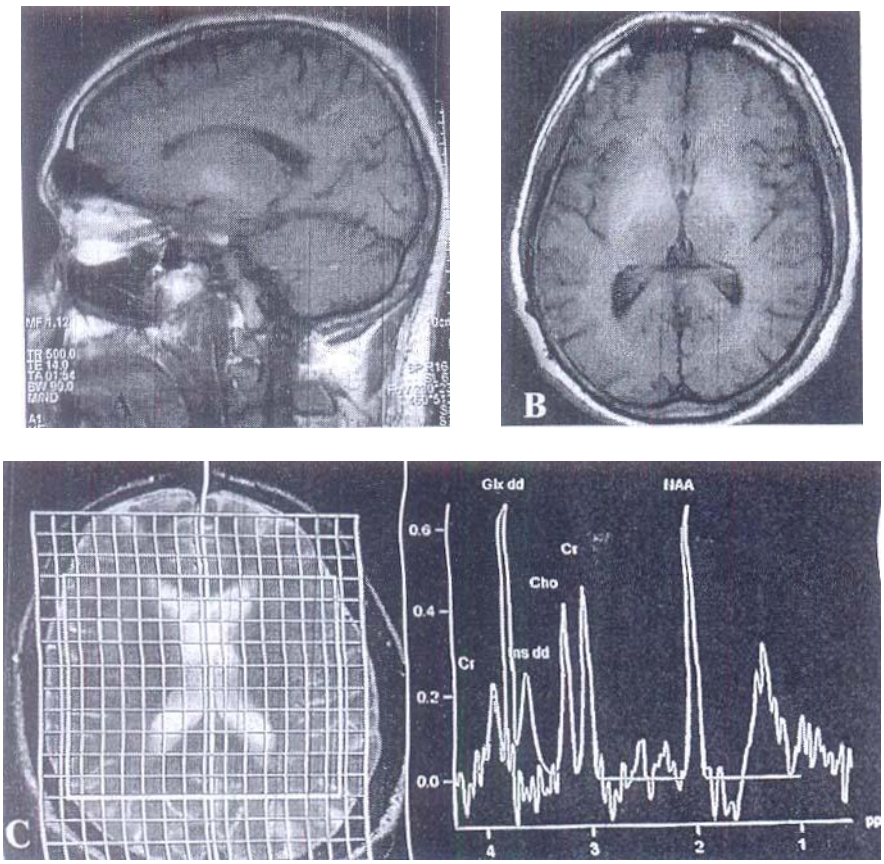

Figure (2): MR imaging and MR ^{1}H-spectroscopic finding in patient with liver cirrhosis and overt hepatic encephalopathy. (A and B): Sagittal and axial T1 WI (TR/TE; 500/14 msec) showed bright intensity in the basal ganglionic region. (C) CSI ^{1}H-spectroscopy, VOI is placed in the white matter of the right parietal region, showed marked increased in glutamate or glutamine (Glx) and decrease in Mi and Cho compared to the healthy control subject. No change as regard the N-acetylaspartate.

Table (4): Serum level of ammonia and manganes in different groups of patients.

	GI	GII	GIII	F	P
Ammonia(µmol/L)					
$\bar{X}$SD	162.1±55.8	95.8±12.7	51.2±9.9	56.2	<0.001
Range	85-220	75-115	33-65		
Manganes (ug/dl)					
$\bar{X}$SD	3.35±0.34	2±0.6	1.3±0.16	45.8	<0.001
Range	2.7-3.9	0.7-2.8	0.9-1.5		

G= group

Table (5): Relation between Mn level in blood & T1 signal image in hepatic patients.

T_1	Mn (ug/dl) $\bar{X}$SD (Range)	T	P
Positive	2.91±0.6 (1.7-3.9)	8.3	<0.001
Negative	1.6±0.6 (0.7-2.8)		

Table (6): Relation between ammonia level in blood and T_2 signal intensity in hepatic patients.

T_2	Ammonia(µmol/L) $\bar{X}$SD (Range)	T	P
Positive	71.1±21.9 (33-105)	10.4	<0.001
Negative	116.9±49 (75-220)		

Table (7): Correlation between ammonia & other parameter.

	r	P	
Glutamine (ppm)	0.86	<0.001	HS
Choline (ppm)	-0.42	<0.001	HS
Myinstol (ppm)	-0.47	<0.001	HS

DISCUSSION

Hepatic encephalopathy includes a spectrum of neuropsychiatric abnormalities occurring in patients with liver dysfunction. Most cases are associated with cirrhosis and portal hypertension or portal-systemic shunts, but the condition can also be seen in patients with acute liver failure and not associated with intrinsic hepatocellualr disease [8]. Chronic HE can be subclassified into relapsing HE and persistent HE. Relapsing HE manifests as frequent episodes of acute HE that may be due to precipitating factors, these patients can be perfectly alert and don't show any sign of cognitive dysfunction. However, a careful neurologic examination and neuropsychological tests may reveal subtle abnormalities. Persistent HE refers to manifestations that do not reverse despite adequate treatment [3].

The liver and brain interact in numerous ways to ensure normal brain function. By using MR imaging for diagnosis there was increase in substances that under normal circumstances are efficiently metabolized by the liver. Classic MR imaging abnormalities include on T_1-weighted images due to high signal intensity in the globus pallidum caused by increased tissue concentrations of manganese, as well as elevated glutamine/glutamate peak coupled with decreased myoinositol and choline signals on proton MR spectroscopy representing disturbances in cell-volume homeostasis secondary to brain hyperammonemia to protect astrocyte [9].

In the present study we found high statistically significant increase in serum level of ammonia and manganese in chronic persistent hepatic encephalopathy when compared to other groups. So serum ammonia and manganese increase with the degree of brain affection these results agreed with that reported by Butterworth et al. [10] and Rose et al. [11].

Our results showed significant increase signal intensity of T_1 and T_2 in chronic persistent HE compared to chronic relapsing HE that agreed with Weissenborn et al. [12], also significant elevation of signal intensity of T2 and T1 corresponding to elevation of serum level of

ammonia and manganese respectively were detected .This agreed with Rovira et al. [3] who found that liver transplantationt provides normalization of both the MRI abnormalities and Mn levels seem to confirm the suspicion of manganese being the responsible agent for the hyperintensity of T1-weighted images .

Signal alterations in T2-weighted MR images in chronic HE are less frequently reported. However, there have been studies showing T2 hyperintensity along the cortico-spinal tract in the brain of cirrhotic patients which were reversed after liver transplantation using the fast-fluid attenuation inversion recovery (FLAIR) sequence [13].

Moreover we found MRS that showed different metabolic changes of the brain in these patients, significant increase in glutamine and significant reduction in choline and myomositol in patients with chronic persistent HE compared to other groups .Our result agreed with those of Ross et al. [14]; Kreis et al. [15] and Rovira et al. [3] and disagree with this study Kostter [16] and Lee et al. [17] in which no difference in 1H-MR spectroscopy finding between patients with and without HE.

Correlation between ammonia and chemical metabolites found in MR spectroscopy revealed highly significant positive correlation between ammonia in blood and glutamine in the brain as well as highly significant negative correlation between ammonia in blood and Choline or Myinstol in the brain were seen .

In chronic hepatic encephalopathy decreased urea cycle activity result in increased level of ammonia with increase synthesis of cerebral glutamine in astrocyte. The effects of increased intracellular glutamate include a reduction in K uptake an increased in Cl uptake which leads to reduction in Myinstol and Choline to prevent more brain edema and astrocyte damage [18].

The current study showed that level of ammonia and manganese correlate with MRI images and with MRS metabolites moreover this study showed significant difference in different grades of hepatic encephalopathy.

Diagnosis of patients of chronic persistent hepatic encephalopathy can be easily done by measuring the chemical parameters so by collectively, MRI, MRS, and estimation of serum manganese and ammonia can told us to prioritize patients of chronic hepatic encephalopathy for liver transplantation.

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Conflicts of interest: The authors declare that there is no conflict of interest.

Ethical approval: The protocol of the study was approved by the committee of Faculty of Medicine, Zagazig University. Where the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1964 Informed consents were obtained from all patients.

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Effect of Intrapulmonary Inhalation of Honey, Nigella Sativa and Curcumin on Liver Function in Patients with Chronic Liver Disease

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Background and study aim: Evidence indicates, that honey, nigella sativa and Curcumin can exert several health-beneficial effects. The aim of this study was to examine the efficacy and safety of honey, curcumin and nigella sativa inhalation on liver function in patients with chronic liver disease.

Patients Methods: The study was conducted in 58 Patients (group1) with chronic liver disease due to hepatitis C and or B infection, with abnormal liver function test regarding liver enzymes, serum albumin, serum total bilirubin and international normalized ratio (INR). Another 43 patients(group2) with chronic liver disease with abnormal liver functions test as a control diseased subjects.

Patients were subjected to inhalation of honey solution diluted with water with total 5 ml solution with 1 ml of curcumin solution, then adding of 2 drops of nigella sativa oil , the patients subjected to 2 times inhalation per day for 3 months. The other 43 control subjects received inhalation of 10% dextrose for the same duration.

Results: there was significant difference in patients group before and after mixed solution inhalation for 3 months with decrease in serum level of ALT and less significant decrease in AST, also there was significant decrease in serum total bilirubin and INR level, already with significant increase in serum albumin. Correlation between duration of exposure to the mixed solution inhalation and change of liver functions after one and 3 months duration, showed significant negative correlation between duration and decrease in ALT, AST, INR and serum bilirubin with positive correlation with serum albumin. No significant changes was detected in liver functions in control subjects after inhalation of 10% dextrose for the same duration.

Conclusion: Natural medications like honey, curcumin and nigella sativa administered by new methods like inhalation my contribute in improving liver functions with high safety profile.

INTRODUCTION

Honey has been an ingredient of traditional medicine on account of its dietary and curative properties since ancient times .

Starting in the early 1970 researchers from different scientific fields have investigated the chemical and biological properties of honey, including antibacterial, bacteriostatic, anti-inflammatory, wound and sunburn healing activities .

Recent views propose honey not only as health promoting dietary supplement, but shed light on antioxidant, non-peroxide dependent properties [1].Evidence indicates that honey can exert several health-beneficial effects such as gastroprotective [2], hepatoprotective [3], reproductive[4,5], hypoglycemic[6], antioxidant[6], antihypertensive[7], antibacterial[8], anti-fungal [9] and anti-inflammatory[10] effects.

Honey also contains other bioactive constituents such as organic acids, ascorbic acid, trace elements, vitamins, amino acids, proteins and Maillard reaction products[11]. The data presented suggest that honey, administered alone or in combination with conventional therapy, might be of therapeutic benefits in the management of chronic diseases commonly associated with oxidative stress.

Considering that the bulk of these data emanate from animal studies, it is worthwhile to perform clinical studies that investigate if this antioxidant effect of honey can be extrapolated to human subjects with chronic diseases. The liver plays an important role in many metabolic processes such as glycemic control, detoxification of xenobiotics, synthesis of lipoproteins, hormones and enzymes[12]. Available evidence suggests that the liver is susceptible to oxidative stress and damage; and the beneficial effect of antioxidants on hepatic oxidative stress has been documented[13].

The amelioration of oxidative stress, as a result of honey administration, was accompanied by significant reductions in the size of enlarged hepatocytes and edema, restoration of bile canaliculi dilatation and reduced number of apoptotic cells[14].

Similar hepatoprotective effect of honey was also reported in rats with obstruction of the common bile duct[15], in rats with N-ethylmaleimide (NEM)-induced liver injury, honey supplementation significantly restored the levels of hepatic glutathione, ameliorated the (NEM)-induced congestion and mononuclear cell infiltration in the liver[16].

These findings, generally, suggest that amelioration of oxidative stress in the liver may contribute to the hepatoprotective effect of honey.

Nigella sativa (*N. sativa*) is a herbaceous plant used as a natural food additive. It belongs to the botanical family of Ranunculaceae, grows in the Middle East, Central Europe and Western Asia. Traditionally these seeds are used for the prevention and cure of many ailments for over 2000 years. The principal active ingredient isolated from the volatile oil of *N. sativa* is thymoquinone (TQ), it has been reported that TQ exhibits many pharmacological effects, including antioxidant and protective effects against hepatotoxins[17].Recently conducted clinical and experimental researches have shown many therapeutic effects of NS extracts such as immunomodulator, anti-inflammatory and anti-tumour agents[18].

Since 1900 bc, several therapeutic activities have been attributed to the rhizomes of the plant Curcuma longa for a variety of diseases, including liver disorders. Curcumin, the main active compound obtained from this plant, was first isolated two centuries ago and its structure as diferuloylmethane was determined in 1910. Curcumin has shown anti-inflammatory, anti-oxidant, antifungal, antibacterial and anticancer activities. The pharmacological properties of curcumin were reviewed recently and focused mainly on its anticancer properties. However, its beneficial activity on liver diseases (known centuries ago, and demonstrated recently utilizing animal models) has not being reviewed in depth until now. The curcumin ability to inhibit several factors like nuclear factor-kappa B, which modulates several pro-inflammatory and profibrotic cytokines as well as its anti-oxidant properties [19].

An increasing number of medications are being administered by inhalation. However, proper dosing, frequency, formulation, and the optimal delivery device remain to be determined for many of these agents. Inhalation therapy has many advantages compared with other routes of administration including achieving a high drug concentration in the lung, lack of systemic adverse effects, ease of administration, and patient convenience. A broad range of patients may benefit from this type of drug delivery[20].

Aerosolized medications is better than oral route because within this route nearly most of the medication is delivered to the blood without changes in contrast to oral route, the medications subjected to different factors through the gastrointestinal tract affecting it.

Aerosolized medications may be delivered to the lower airway either through the nasal cavity or the oropharynx, however, the oropharynx route is preferable for several reasons. First, the alveolar region of the lung provides a larger surface area for drug absorption (approximately 75 sq m) compared to intranasal route[21]. Alveolar walls are thin and well perfused, allowing rapid drug absorption. The large surface area coupled with the high amount of blood flow through the pulmonary tissues maximizes drug absorption. Second, pulmonary mucociliary clearance

mechanisms are minimal in the alveolar region of the lungs. Unlike the terminal portions of the lung, however, the mucociliary clearance of the nasal passages are much more effective[22].

Thus, intrapulmonary administration is associated with less rapid elimination of medications compared to intranasal, allowing prolonged deposition time and, for some medications, subsequent systemic absorption. For these reasons, intrapulmonary inhalation seems to be emerging as the optimal route for administration of aerosolized solutions.

In this study, we investigated the effect of honey, curcumin and nigella sativa inhalation on liver function in patients with chronic liver disease.

PATIENTS AND METHODS

After approval from the medical ethical committee, this study was conducted from March to August 2012, the study was conducted in 58 patients (group 1) selected from outpatients of Zagazig university hospital with chronic liver disease due to hepatitis C and or B infection already with abnormal liver function test regarding liver enzymes, serum albumin, serum total bilirubin and international normalized ratio INR. Another 43 patients (group 2) with chronic liver disease with abnormal liver functions test as a diseased control subjects.

Patients were subjected to detailed medical history, thorough physical examination, liver function tests (ALT,AST, INR, serum albumin and bilirubin), renal function tests (urea and creatinine), pulmonary function tests(FEV1, FVC, FEV1\FVC ratio) using spirometry were done directly befor the study and at one and 3 months latter. , abdominal ultrasound, chest X ray.

Inhalation of suspension containing honey solution diluted with water (60|40) [7] with total 5 ml solution with 1 ml of curcumin solution prepared by adding 50 gm of curcumin powder dissolved in 50 (ml) water with adding one sodium bicarbonate tablet of 325 mg to enhance dissolving then filtering all to get clear fluid, then adding of 2 drops of nigella sativa oil which purchased from the market. The honey used is a cotton flower honey; the inhalation device used nebulizer in unit dose presentation to prevent microbial contamination during use, the patients subjected to 2 times inhalation per day for 3 months. These preparations are based on a systematic review of scientific literature edited and peer-reviewed by contributors to the Natural Standard Research Collaboration (www.naturalstandard.com).

Any patients showed irritation or change in pulmonary function tests will be excluded, but no one did.

The control group received inhalation of 10% dextrose 5ml for the same duration.

Data collected after one month and 3 months later.

All subjects included in this study were informed to stop any medication that may affect liver function before the study.

Statistical analysis

The data were presented as means ± standard errors. For the comparison of statistical significance between two groups paired sample t-test was used. Values were accepted as being statistically significant if a P value was less than 0.01. Correlation between duration of exposure to the mixed solution inhalation and change of liver function was done using Pearson correlation.

RESULTS

As shown in Table 1 there was no significant changes in both groups. Table 2 showed significant difference in patients group before and after mixed solution inhalation for 3 months with decrease in serum level of ALT and less significant decrease in AST, also there was significant decrease in serum total bilirubin and INR level, already with significant increase in serum albumin, no significant difference in control patients group 2 before and after 3 months of dextrose inhalation Table 3.

Table 4 showed Correlation between duration of exposure to the mixed solution inhalation and change of liver functions after one and 3 months duration, with significant negative correlation between duration and decrease in ALT, AST, INR and serum bilirubin with positive correlation with serum albumin.

No significant changes was detected in liver functions in control subjects after inhalation of 10% dextrose for the same duration.

Table (1): Patients and control subjects characteristics (mean ± SD)

	Group 1 (*n* = 58)	**Group 2 (*n* = 43)**	***P***
Age (yr)	48 ± 4	46 ± 4	NS
Serum creatinine (mg/dL)	1.2 ± 0.2	1.0 ± 0.1	NS
BUN (mg/dL)	25.5 ± 7.3	23.5 ± 1.2	NS
AST (U/L)	65 ± 8.9	63 ± 8.2	NS
ALT (U/L)	59 ± 14	61 ± 14.1	NS
Total bilirubin (mg/dL)	2.7 ± 0.5	2.8 ± 0.6	NS
Serum albumin (g/Dl)	2.3 ± 0.44	2.4 ± 0.46	NS
INR	1.6 ± 0.2	1.5 ± 0.1	NS

NS=non-significant

Table (2): Mean difference ±SD in patients of group 1 before and after 3 months of mixed solution inhalation.

	Before	**After**	**t**	***P***
ALT(U/L)	59 ± 14	38 ± 8	8.9	0.001
AST (U/L)	65 ± 8.9	55±9	4.8	0.004
Serum albumin (g/dl)	2.2 ± 0.4	3 ± 0.8	-6.2	0.001
Total bilirubin (mg/dL)	2.7 ±0 .5	1.7 ± 0.3	7.7	0.001
INR	1.6 ± 0.2	1.3 ±0.1	6.6	0.001

Table (3): Mean difference ±SD in control patients group 2 before and after 3 months of dextrose inhalation.

	Before	**After**	***P***
ALT (U/L)	61 ± 14.1	63 ± 13.6	NS
AST (U/L)	63 ± 8.2	61±7.8	NS
Serum albumin (g/dl)	2.4 ± 0.46	2.3 ± 0.38	NS
Total bilirubin (mg/dL)	2.8 ± 0.6	2.7 ± 0.5	NS
INR	1.5 ± 0.1	1.6 ±0.3	NS

Table (4): Correlation between duration of exposure to inhalation of the mixed solution and change of liver function.

	Mean (*n* = 58) After one month	**Mean (*n* = 58)** After 3 months	**Correlation**	***P***
ALT (U/L)	44 ± 10	38 ± 8	-.731	0.001
AST (U/L)	60±10	55±9	-.641	0.001
Total bilirubin (mg/dL)	2 ± 0.4	1.7 ± 0.3	-.531	0.001
INR	1.4 ±0.2	1.3 ±0.1	-.481	0.001
Serum albumin (g/dL)	2.7 ± 0.5	3 ± 0.8	+.561	0.001

No significant changes in pulmonary functions tests regarding FVC,FEV1,FEV1|FVC ratio.

DISCUSSION

Honey is a natural antioxidant which may contain flavinoids, ascorbic acid, tocopherols, catalase, and phenolic compounds all of which work together to provide a synergistic antioxidant effect, scavenging and eliminating free radicals[23].

Nigella Sativa treatment decreased the elevated liver enzyme levels and also increased the reduced antioxidant enzyme levels in patients with hepatitis. Previously performed clinical and experimental investigations have shown that NS has a protective effect against oxidative damage in infected hepatocytes. It was found that the fixed oil of NS has both antioxidant and anti-eicosanoid effects greater than thymoquinone which is its active constituent [13]. Furthermore, NS has antioxidant activity by suppressing the chemiluminescence in phagocytes[24].

Curcumin has shown pleiotropic beneficial effects in many iatrogenic organ maladies. It apparently ameliorated the gross and histological alterations in hepatocytes in patients with

hepatitis. It decreased the relative liver/ body weight ratio. It also mitigated pleomorphism, apoptotic bodies, necrosis and inflammation as well as dilatation of central lobular sinusoid. It improved fatty changes with minimal microvesicular steatosis and hepatocytes became more granular. Recently, the protective effect of curcumin on rat liver injury induced by CCl4 was demonstrated by Fu et al. who showed that curcumin administration prevented ALT and AST increases and improved liver function in CCl4-induced liver damage[25].

Our result showed that intrapulmonary administration of honey, curcumin and nigella sativa solution did not cause any adverse effect in all patients receiving this mixed solution, already with improvement in liver enzymes with significant reduction in ALT and AST. Also our results showed significant improvement of INR, serum bilirubin and serum albumin level.

We noticed that there was more improvement in liver functions with increasing period of treatment reaching to 3 months as shown in table 4. In which there was positive correlation between improvement in liver functions and period of exposure to the mixed solution.

Our results are in agreement with Antony *et al*., who reported that honey has a hepatoprotective activity , it was found that honey reduce lipid peroxidation and nitric oxide and greatly improved liver enzymes, referred to its antioxidant activity [26]. Honey have a hepatoprotective activity against methyl nitrosourea (MNU)-induced oxidative stress and inflammatory response by keeping normal defense system and decrease NO [27]. Also honey supplementation significantly restored the levels of hepatic glutathione, ameliorated the (NEM)-induced congestion and mononuclear cell infiltration in the liver.

Improvement of serum bilirubin may be correlated with Kilicoglu et al, who reported that amelioration of oxidative stress, as a result of honey administration, was accompanied by significant reductions in the size of enlarged hepatocytes and edema, restoration of bile canaliculi dilatation and reduced number of apoptotic cells with subsequent decrease in serum bilirubin[14].

Improvement of liver function by adding NS is documented by reports from Turkdogan *et al.* who, observed that Nigella Sativa has a significant hepatoprotective effect in CCl_4-administrated rabbits, and the hepatocellular degenerative and necrotic changes are slight in NS-treated group and also they found that NS can prevent liver fibrosis and cirrhosis, suggesting that NS protects liver against fibrosis possibly through immunomodulator and antioxidant activities[28].

Curcumin inhibits nuclear factor-kappa B (NF-_B) binding activity and the expression of tumor necrosis factor (TNF), interleukin (IL-12), monocyte chemoattractant protein-1 (MCP-1), macrophage inhibitory protein (MIP-2), Cyclooxygenase-2 (COX-2), and nitric oxide (iNOS) in endotoxin treated Kupffer cells, which supports our results in the reduction of liver enzymes which may be consider as markers of liver inflammation, this was proved by Amin et al, who treated Isolated Kupffer cells with vehicle or the indicated concentrations of curcumin for 1 h before treatment with endotoxin lipopolysccharides (100 ng/ml) and found that treatment with curcumin inhibited NF-_B binding activity and the expression of TNF-_, IL-12, MCP-1, MIP-2, COX-2, and iNOS in endotoxin treated cells [29].

Conclusion: Natural medications like honey, curcumin and nigella sativa administered by new methods like inhalation my contribute in improving liver functions with high safety profile.

Recommendation: Such studies have to be applied for a larger numbers of patients with a longer period and study if such medications can affect hepatitis B or C viral load.

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Conflicts of interest: The authors declare that there is no conflict of interest.

Ethical consideration: Informed consents were obtained from all patient. The study was performed according to the ethical standards for human experimentation and was approved by the scientific committee of Faculty of Medicine , Zagazig University.

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Role of Toxoplasmosis in Acute Flaccid Paralysis among Children

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Background and study aim: With the eradication of poliomyelitis, Guillain-Barré syndrome (GBS) is the most common cause of Acute Flaccid Paralysis (AFP) in children. The present study aimed at assessment of how far Toxoplasmosis contributes to the cases of Acute Flaccid Paralysis (AFP) among children in Sharqiya governorate, Egypt.
Patient and Methods: Over years from April 2010 to September 2012, one hundred children with non-polio acute flaccid paralysis were selected, after their parent written consent, out of children monitored in Sharqiya Governorate by the Project of Acute Flaccid Paralysis (AFP) Surveillance, the Ministry of Health and Population, Egypt. As they underwent treatment by appropriate therapy for AFP, anti-Toxoplasma IgM and IgG antibodies, anti-*Campylobacter jejuni* IgM and IgG antibodies and tumor necrosis factor alpha (TNF-α) were sought quantitatively in their sera by ElISA.
Results: Anti-Toxoplasma IgM and IgG were respectively detected among 3 (3%) and 42 (42%) of them. Anti-campylobacter IgM and IgG were respectively detected among 25 (25%) and 54 (54%) of them. TNF-α absorbance values were 0.95±0.35 among 3 patients with symptomatic acute toxoplasmosis (positive IgM and IgG), 0.22 ±0.11 among 39 patients with chronic toxoplasmosis (with positive anti Toxoplasma IgG only), and 0.21±0.12 among patients without toxoplasmosis. The 3 cases of acute flaccid paralysis due to acute toxoplasmosis did not respond to the ordinary treatment of AFP treatment; but dramatically responded to Sulfadiazine and Pyrimethamine.
Conclusion: These results may make the study hypothesize that Toxoplasma may exert its pathogenic effect on nerve myelin directly via TNF-α. Thus approaching Acute Flaccid Paralysis, higher index of suspicion is needed so as to do not miss cases with toxoplasmic etiology.

INTRODUCTION

With the eradication of poliomyelitis, Guillain-Barré syndrome (GBS) is the most common cause of Acute Flaccid Paralysis (AFP) in children [1].

Guillain-Barré syndrome (GBS) is an acute disease of the peripheral nervous system of humans, characterized by ascending paralysis, conduction block with segmental demyelination of the nerves, macrophage and lymphocytic infiltration of the nerves, and elevated protein with no cells or very few cells in the cerebrospinal fluid [2]. GBS has been shown to be associated with viral or bacterial infections, including *Campylobacter jejuni* [3,4], *Borrelia burgdorferi* [5], *Brucella melitensis* [6], or infection with the protozoan parasite,*Toxoplasma gondii*[7,8],or following vaccinations, including rabies [9] and swine influenza [10].

The present study aimed at assessment of how far Toxoplasmosis contributes to the cases of Acute Flaccid Paralysis (AFP) among children in Sharqiya governorate, Egypt.

PATIENTS AND METHODS

This study was conducted during the period between April 2010 and September 2012 at the Departments of

Pediatrics, Tropical Medicine, Microbiology and Parasitology, Faculty of Medicine, Zagazig University. The study was carried out, after written and oral consent from the parents of 100 cases of non-polio acute flaccid paralysis proved in time to be copro-negative for Polioviruses by stool cultivation that was made by the Project of Acute Flaccid Paralysis surveillance of the Ministry of Health and Population, Egypt.

The patients were subjected in time to the following: thorough history taking and clinical examination and blood sample collection so as to separate and store sera at 2-8 ^{o}C to seek anti-Toxoplasma IgM and IgG antibodies, anti-*Campylobacter jejuni* IgM and IgG antibodies and Tumour necrosis factor-α .

According to anti-Toxoplasma seropositivity; the cases were classified into 3 groups of AFP:

1. Acute flaccid paralysis with acute toxoplasmosis on basis of anti-Toxoplasma IgM seropositivity.
2. Acute flaccid paralysis with chronic toxoplasmosis on basis of anti-Toxoplasma IgM seronegativity and IgG seropositivity.
3. Acute flaccid paralysis without toxoplasmosis on basis of negative anti-Toxoplasma serology.

Management of the cases was carried out by: corticosteroids, intravenous human immunoglobulin (IVIG) or by plasmapheresis if recommended. Specific anti-Toxoplasma therapy was applied to the cases with Toxoplasma seropositivity after failure of afore- mentioned measures. Pyrimethamine was given as loading dose: 2 mg/kg/24 hours for the first 2 days of treatment and Maintenance dose of 1 mg/kg/24 hours altogether with Folinic acid: 20 mg three times a week or even daily depending on the leukocyte count [11].

Case definition:

The diagnosis of acute toxoplasmosis was established by the presence of a serum Toxoplasma IgM titre of 1/8 by ELISA together with the clinical triad of fever (chills or documented fever), headache and lymphadenopathy, in addition to history of contact with cats [12,13].

The cases of AFP who met the following criteria were regarded to be induced by acute toxoplasmosis: Anti-Toxoplasma IgM seropositivity, significantly high level of Tumour necrosis factor (TNF-α), anti-*Campylobacter jejuni* IgM seronegativity, and good response to anti-Toxoplasma treatment.

- **Qualitative Serotesting for antiToxoplasma IgM and IgG antibodies** According to Montoya and Rosso, [14]. Kits of the Onsite Toxo IgG/ IgM Rapid Test- Cassette (Serum/ plasma) Catalog no. R0233C were obtained from CTK Biotech, Inc., 6748 Nancy Ridge Drive. San Diego, CA 92121, USA. This Rapid test depends on a lateral flow chromatographic immunoassay, for the simultaneous detection and differentiation of IgG and IgM anti-*Toxoplasma Gondii* (T. gondii) in human serum or plasma. The reactive specimens with the onsite toxo IgG/IgM Rapid test were confirmed with the quantitative ELISA test. If only the control (C) band is present, the absence of any burgundy color in both T bands (T1 (IgM) and T2 (IgG)) indicates that no anti – *T. gondii* antibodies are detected in the specimen. The result is negative. In addition to the presence of C band, if only T2 band is developed the test indicates for the presence of IgG anti- *T. gondii* in the specimen, the result is IgG positive. If both T1 and T2 bands are developed, the test indicated the presence of both IgG and IgM anti-T. gondii in the specimen.

- **Quantitatve IgM anti Toxoplasma Sero-testing by Enzyme – linked Immunosorbent Assay (ELISA)** according to Johnson and Holliman [15]. Equipment and reagents were purchased from Organon Technica. Briefly, 100 µl of PBST/BSA was added into two wells of each plate to function as the antigen/conjugate and substrate controls. 100 µl of each test serum, one negative, and one positive control sera diluted 1/1000 was mixed well and aliquoted into wells of the micro titration plates pre-coated with human µ heavy chain of IgM. Then incubated for one hour and washed, The Toxoplasma antigen/conjugate were dispensed & mixed in each well. After being covered and incubated at 37 ^{0}C for one hour, the substrate solution (100 mg/10 ml DMSO) was added immediately and rapidly to every well. Then the reaction was stopped by adding 25µl of one M H_2SO_4 to each well. The absorbance (optical density) was measured at a wavelength of 450 mm blanking the plate against the substrate control well using a spectrophotometer. Absorbance value of ≤ 0.4 was considered negative. Absorbance ≥ 0.5 was positive.

• **Detection of *Campylobacter jejuni* IgM and IgG antibodies by** the commercially available (ELISA recomWell Campylobacter) from Microgen, Poland according Schmidt-Ott et al[16]. Kits of the Human *Campylobacter Jejuni* PEB1 ELISA Kit Catalog number: CDN-E0568, were obtained from Creative Diagnostics, CD Bio Sciences, Inc., 45-16 Ramsey Road Shirley, NY11967 USA. Strip plate with micro wells coated with 100 µl of rabbit antihuman µ or γ chain, Creative diagnostics were washed prior to use thrice with PBS/T. Well A1 was left empty (blank). 100 µl of 1/50 dilution (2% casein with PBS) of a *Campylobacter jejuni* IgM or 1/100 IgG serum negative and positive control sera were dispensed into two coated wells. 100 µl of patient's serum (with 1/50 dilution in IgM assay and 1/100 in IgG assay) were dispensed into the other coated wells. The wells were incubated and washed. 100 µl of sonicated *Campylobacter jejuni* organism (Reactive Diagnostics) were dispensed into the wells. After second incubation and coverage, 100 µl of peroxidase-conjugated anti - *Campylobacter jejuni*; Reactive diagnostics were dispensed into each well. Then 100 µl of one M H_2SO_4 were dispensed into each well to stop in reaction. The absorbance value of each well was read in an ElISA strip reader at 450 nm.Values ≥ 0.2 were considered positive for IgM assay and values ≥ 0.4 were considered positive for IgG assay according to the manufacturer.

• **Quantitative ELISA for Estimation of Tumor Necrosis Factor Alpha (TNF-α)** **According to Thomas** [17]. The number of eight well strips needed for the assay were determined and inserted in the frame. Fifty µls of the incubation buffer were added to all wells and the well reserved for chromogen blank were left empty. One hundred µls of the standard diluents buffer were added to the zero standard wells and the well reserved for chromogen blank were left empty. One hundred µls of standards were added to the appropriate micro titer wells and fifty µl of standard diluents buffer were added to each well followed by fifty µl of each test sample. Fifty µl of biotinylated anti- TNF-α (Biotin conjugate) solution were put in each well except the chromogen blank. Then, the plate was incubated and washed. One hundred µl of Streptavidin-HRP working solution were added to each well except the chromogen blank. After second incubation and washing, one hundred µl of Stabilized Chromogen were added to each well and the liquid in the wells started to become blue. After 30 minutes, one hundred µl of Stop solution were added to each well until the solution in the wells was changed from blue to yellow. Then the absorbance of each well was read at 450 nm having blanked the plate reader against a chromgen blank composed of 100 µl each of Stabilized Chromogen and Stop Solution then the plate was read within 2 hours after adding stop solution.

Results were tabulated and statistical inference on difference between means, were made by t-student test and on difference between proportions by Z test.

RESULTS

Table (1): Age of AFP cases

	Age (Year)	No. of Patients (n=100)	Percentage	P value		
				a V b	b V c	a V c
A	< 2	31	31	< 0.05	< 0.001	<0.05
B	2 – 6	50	50			
C	> 6	19	19			

Table (2): Male/Female ratio in all cases

Sex	No. of Patients (n=100)	Percentage	P value
Male	57	57	> 0.05
Female	43	43	

Table (3): Anti-Toxoplasma IgM & IgG seropositivity among AFP cases

	No. examined patients	Number of Positive Cases	%	P value
Ig M	100	3	3	< 0.01
IgG	100	42	42	

Table (4): The Positivity as referred to positive control and level of (TNF-α) Among Cases

		No. examined	Seropositive	%	Optical density□	
					Mean	SD
A	acute toxoplasmosis	3	3	100*	0.95	0.035□
B	Chronic toxoplasmosis	39	1	2.55**	0.22	0.11□□
C	Without toxoplasmosis	58	1	1.77	0.21	0.12

Table (5): Clinical picture of toxoplasma related manifestations among the cases

Clinical Picture	No. Patients (n=100)	Percentage
• Lymphadenopathy	3	3 %
• Retinochoroiditis	2	2 %
• Pulmonary	0	0
• Hepatomegaly	0	0
• Splenomegaly	0	0

Table (6): Prevalence of: anti- *Campylobacter jejuni* IgG seropositivity among AFP cases

		No. examined	No. IgG seropositive	%
A	Acute toxoplasmosis	3	1	33.3□
B	Chronic toxoplasmosis	39	20	51.28□□
C	Without toxoplasmosis	58	33	56.89

□ P value versus b & c < 0.05 – □□P value versus c > 0.05

Table (7): Prevalence of anti-*Campylobacter jejuni* IgM seropositivity among AFP cases

		No. examined	No. IgM seropositive	%
A	Acute toxoplasmosis	3	0	0*
B	Chronic toxoplasmosis	39	8	20.51**
C	Without toxoplasmosis	58	17	29.31

* P value versus b & c < 0.001 – ** P value versus c > 0.05

DISCUSSION

The incidence of AFP was 2.3-2.39/100.000 among children aged less than 15 years in Sharkiya governorate from April 2010 to September 2012 by the project of acute flaccid paralysis surveillance, the Ministry of Health and Population. Several infections as well as immunizations have been known to precede or to be associated with Guillain-Barré syndrome (GBS) [1]. Only a few cases of acute polyradiculoneuritis have been reported in patients with increasing levels of immunoglobulin G (IgG) and IgM antibodies directed against *Toxoplasma gondii* [18]. Recently, it has been documented that AFP in some dogs, like GBS in some humans, may be triggered by *Toxoplasma gondii infection* [19].

The present study showed that the prevalence of chronic toxoplasmosis was 42% among the study population; the prevalence of symptomatic acute toxoplasmosis was limited to 3%. These prevalence rates, more or less agree with McLeod and Remington who reported that several studies made on random populations have detected significant antibody titers that ranged 50-80% of residents in some localities and less than 5% in others. These authors added

that Toxoplasma infection is one of the most common latent infections of humans throughout the world [11].

This study revealed that the 3 cases AFP were positive for Acute Toxoplasmosis and all the 3 cases were with significant high levels of TNF-α. This finding may suggest that Toxoplasma exerts its pathogenic effect on the nerves via the increased production of TNF-α thus leading to induction of acute flaccid paralysis. The role of toxoplasmosis in production of tumor necrosis factor-alpha (TNF α) has been discussed in many reports [20]. Toxoplasma tachyzoites stimulate macrophages to produce interleukin (IL-12) [21]. IL-12, in turn activates natural killer (NK) cells and T cells to produce interferon- (IFN-) and it is this early produced IFN- that is crucial for resistance [22,23].

IFN- and tumor necrosis factor (TNF) act synergistically to mediate killing of tachyzoites by macrophages. The combination of these two cytokines results in greatly enhanced production of free radicals and nitric oxide (NO) both of which can affect parasite killing [22, 24]. In toxoplasmosis, various cell types including macrophages, microglia, neutrophils, T cells and dendritic cells produce TNF. Production of TNF is induced by IFN-γ in infected cells and the latter cytokine and its receptor have a pivotal role in the control of *T. gondii* in mice [25].

The role of tumor necrosis factor-alpha (TNF α) in acute flaccid paralysis has been stated by many authors; Trojaborg reported that: Other factors of importance in the pathogenesis of GBS: T lymphocytes and macrophages secrete TNF α, which has a toxic effect on myelin, Schwann cells and endothelial cells [26]. There is a close relation between the amount of circulating tumor necrosis factor- α in serum and prolonged distal latency, slow motor conduction velocity and reduced compound muscle action potential (CMAP) amplitude in GBS patients suggesting a role for TNF- α in the pathogenesis of peripheral nerve demyelination. Similar correlations were not observed for serum levels of interleukine-1 or soluble interleukine-2 receptors [27]. Recently, Wu et al., found a significant association between TNF-α and risk of the GBS in Asian population [28]. On the other hand, Prasad et al., stated that TNF polymorphisms may increase susceptibility to axonal GBS subtypes [29]; however, the role of TNF in GBS remains unclear and wants further investigation.

As far as it has been reviewed; the present study in Egypt may be the first to accuse Toxoplasma as a one of the causative agents of AFP in a survey study. The present study recorded that cases of acute flaccid paralysis due to toxoplasmosis, do not respond to the ordinary treatment of AFP treatment. This finding was supported by Bossi et al. [8], who reported that the patient's condition improved with pyrimethamine (50 mg/day), sulfadiazine (4 g/day), and folinic acid (25 mg/day). Fever disappeared within 5 days, lymph node disorders within 10 days, and neurologic disorders and retinochoroiditis within 15 days. The treatment was stopped after 6 weeks. Ten months later, the patients became fully recovered. They reported also that a poor host adaptation to the uncommon highly virulent tropical strains of *T. gondii* can explain these unusual clinical presentations. They attributed the occurrence of Guillain-Barré syndrome among immune competent patient, to infection with a new strain of *T. gondii*. This strain was highly virulent, as confirmed by the rapid death of the mice (within 3 days). Moreover, this strain was not affected by a 10-day treatment with spiramycin (which is ineffective in toxoplasmosis with central nervous system symptoms), and parasitemia remained after this therapy. Parallel poor host adaptation may have occurred with the 3 cases of the present study

The study assessed also the role of *Campylobacter jejuni* as causative agent of AFP. The study recorded that *Campylobacter jejuni* comes as the most common cause of Guillain-Barré syndrome; our study revealed that: 25 AFP cases (25%) were positive for anti-Campylobacter jejuni IgM while 54 AFP cases (54%) were positive for anti- *Campylobacter jejuni* IgG. Kalra et al., in an Indian case-control study reported that 27.7% of childhood GBS cases were associated with *C jejuni* infection [30]. Hughes and Rees (1997) stated that among infectious agents, *Campylobacter jejuni is* the most frequently identified cause of Guillain- Barré syndrome [31].

CONCLUSION

Approaching Acute Flaccid Paralysis, higher index of suspicion is needed so as to do not miss

Cases with toxoplasmic etiology. Toxoplasmic Acute Flaccid Paralysis needs specific treatment in the form of pyrimethamine and sulfadiazine and there is no response to the other forms of treatment. The study hypothesize that Toxoplasma may exert its pathogenic effect on nerve myelin directly via TNF-α. Treatment with specific anti-Toxoplasma chemotherapy may shorten the course of recovery and improve the prognosis of acute flaccid paralysis.

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Conflicts of interest: The authors declare that there is no conflict of interest.

Ethical approval: The protocol of the study was approved by the committee of Faculty of Medicine , Zagazig University. Where the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1964. Informed consents were obtained from all patients.

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Faecal Calprotectin as Reliable Non-invasive Marker to Assess the Severity of Mucosal Inflammation in Patients with Ulcerative Colitis

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Background and study aim: We aimed to evaluate the validity and accuracy of the faecal calprotectin in differentiating patients with IBD from those with IBS and in the assessment of the severity of intestinal mucosal inflammation in patients with ulcerative colitis (UC) which may facilitate in the prognosis and follow.

Patients and Methods: We studied 60 Patients who came to endoscopy unit with lower gastroenterological symptoms. Patients with history of infections, malignancy, gastrointestinal surgery, pregnancy, alcohol abuse or taking non-steroidal anti-inflammatory drugs were excluded from study. All patients subjected to thorough medical history, simple clinical colitis activity index was determined with a score > 4 indicate active UC, complete blood picture, liver, kidney function tests, ESR, CRP, ANCA were done, a stool sample for FC levels determined by a highly sensitive enzyme-linked immunosorbent assay and total colonoscopy with histological examination of intestinal mucosa biopsy were done. The patients divided into 2 groups. Group A: patients with UC, group B: patients with manifestation of irritable bowel syndrome as a control group.

Results: There was a high significant difference between individuals with no pathological activity and other degree of mucosal inflammation as regard simple clinical colitis activity index, endoscopic appearance and faecal calprotectin (p = 0.000). The sensitivity, specificity, positive predictive value and negative predictive value of faecal calprotectin in diagnosis of UC were 93.5%, 89.7%, 90.6%, and 92.9% respectively. The positive predictive value and negative predictive value of simple clinical colitis activity index for diagnosis of UC were 76.5% and 80.8% respectively. The positive predictive value and negative predictive value of endoscopic appearance for diagnosis of UC were 100%, and 85.3% respectively. There was a high significant difference and positive correlation between faecal calprotectin, score of colonic pathological activity, endoscopic appearance and simple clinical colitis activity index.

Conclusion: Faecal calprotectin is highly useful for the diagnosis and disease monitoring of patients with UC as it is easy, non invasive, reliable tool.

INTRODUCTION

The cause of ulcerative colitis (UC) is currently under examination. It is believed that the 2 idiopathic forms of inflammatory bowel disease (IBD), ulcerative colitis and Crohn's disease (CD), develop secondary to complex interactions among genetic predispositions, environmental risk factors, and the immune system.

Several genes likely play a role; their products, when combined with environmental factors and dysfunctional immunity, result in a disease spectrum with heterogeneous manifestations and many unique phenotypes [1].

The determination of inflammatory activity is crucial for patients with IBD for the diagnosis, monitoring and step up of therapy. Colonoscopy is the accepted gold standard for investigation of the colon, but is invasive and associated with risks [2]. Among objective clinical features; bloody stool frequency, body temperature and heart rate are good predictors of outcome. Laboratory markers have been studied intensively with varying degrees of success. The widely used acute phase protein C-reactive protein in this respect is a less good marker for assessing disease activity in UC than Crohn's disease [3].

More recently, faecal markers have demonstrated promising results. The most studied markers are faecal calprotectin and lactoferrin have shown accuracy at detecting colonic inflammation [4].

Calprotectin is a calcium-binding protein that is derived predominantly from neutrophils and, to a lesser extent, from monocytes and reactive macrophages [5].

It is worth noting that fecal calprotectin concentrations correlate more closely with histological than macroscopic (endoscopic) findings, suggesting that this biological marker is more sensible than endoscopy in evaluating IBDs activity [6].

The present study aimed at evaluation of the accuracy of faecal calprotectin and correlate it with clinical scores, common serum markers and endoscopy in the assessment of the severity of intestinal mucosal inflammation in patients with ulcerative colitis.

PATIENTS AND METHODS

This present study was conducted in the Tropical medicine department and gastrointestinal endoscopy unit, faculty of medicine, Zagazig University during the period from January 2011 to March 2012.

Our study included 31 patients with ulcerative colitis. The control group comprised 29 patients with manifestation of IBS matched for age and sex with patient's group. Written informed consents were obtained prior to participation in this study.

Patients with history of infections (recent respiratory or urinary tract infections within 1 month), malignancy (current), gastrointestinal trauma or surgery (within 1 month), or regularly taking aspirin, anticoagulants, or non-steroidal anti-inflammatory drugs, pregnancy and history of alcohol abuse were excluded from this study.

All patients should be subjected to the following:

- Thorough medical history taking.
- Simple clinical colitis activity index (SCCAI).
- Thorough clinical examination.
- Complete blood picture.
- Liver and kidney functions tests.
- Blood sample for estimation of ESR, and of CRP.
- Determination of ANCA in serum.
- Thorough stool examination.
- Quantitative measurement of faecal calprotectin levels were measured by a highly sensitive enzyme-linked immunosorbent assay (PhiCal^{TM}).
- Total colonoscopy with histological examination of intestinal biopsy specimens.

Statistical analysis:

Comparisons between means of several groups of mucosal inflammation were done by one way Anova (F test) and LSD when there was a significance difference between means. Comparison between median were done by non-parametric test (Krurskal wallis-H test) followed by Mann-Whitney u test. Receiver operating curve characters were used to develop best cut off value in estimating the validity of different parameter in diagnosis of ulcerative colitis. Kappa measurement of agreement was done to test agreement between studied parameters and degree of mucosal inflammation. P value was considered significant when P value is less than 0.05.

RESULTS

Table (1): Demographics distribution among the examined patients

Degree of colonic mucosal inflammation		Number	Sex (F/M)	Age		f	p
				Mean ± SD	Range		
Group B	No mucosal inflammation	29	15/14	32.2±9.6	19-49	0.32	0.81
Group A	Mild	6	3/3	29.83±5.56	25-39		
	Moderate	9	7/2	29.89±9.97	19-44		
	Severe	16	9/7	29.63±10.52	19-49		

Table (2): Relation between degree of colonic mucosal inflammation SCCAI, endoscopic appearance and faecal calprotectin

	Degree of colonic mucosal inflammation			
	Group B		Group A	
	No mucosal inflammation	Mild	Moderate	Severe
	Median / Range	Median / Range	Median / Range	Median / Range
SCCAI	3 (2-6)*	6 (6-11)	7 (6-11)	7 (4-12)
Endoscopic appearance	0 (0-1)*	1 (1-3)	2 (1-3)	3 (2-3)*
Fecal calprotectin (µg/g)	30 (10-176)*	108 (10-176)*	165 (149-190)	190 (170-215)

* Highly significant, **SCCAI** =Simple clinical colitis activity index

Table (3) Relation between degree of mucosal inflammation, CRP value and ESR

	Group B	Group A	t	p
CRP ± SD	**6.76 ± 3.86**	**9.94 ± 5.09**	2.711	0.009**
ESR ± SD	**23.14 ±13.23**	**26.94 ± 10.36**	1.242	0.219

** Significant difference

Table (4): Validity of SCCAI, endoscopic appearance and faecal calprotectin in relation to pathology

	Sensitivity	Specificity	Positive predictive value	Negative predictive value
SCCAI	83.9%	72.4%	76.5%	80.8%
Endoscopic appearance	83.9%	100%	100%	85.3%
Faecal calprotectin	93.5%	89.7%	90.6%	92.9%

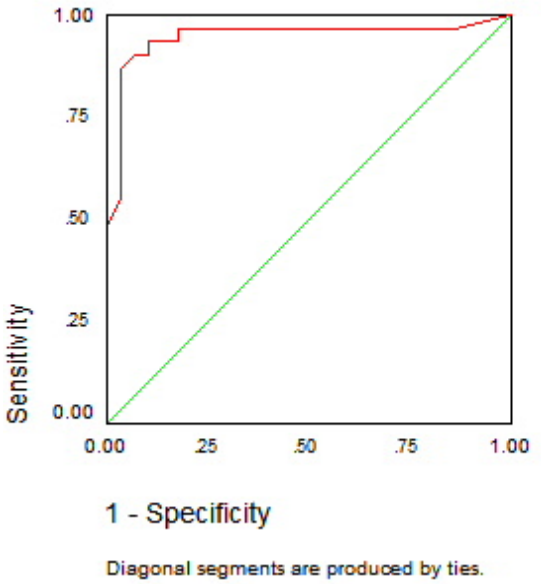

Fig (1): ROC curve of faecal calprotectin in predicting ulcerative colitis

DISCUSSION

Inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) are common entities. Both conditions may present with similar clinical features such as diarrhea and abdominal pain. Patients with IBD oscillate between periods of active and inactive disease and may even present with concomitant functional IBS [7].

Most patients with quiescent IBD have low-grade inflammation and it is possible that symptomatic relapse occurs only when the inflammatory process reaches a critical intensity. Furthermore, because inflammation is a continuous process, direct assessment of the level of inflammatory activity may provide a quantitative presymptomatic measure of impending disease relapse [8].

Calprotectin is a valuable marker at the very early stage of inflammatory reactions in human beings [9].

Faecal calprotectin assessment is that it is a measure of mucosal inflammatory activity that may be detected at a level insufficient to cause an increase in ESR and CRP [10].

In the current study, more intense levels of inflammations are associated with elevated level of faecal calprotectin value, demonstrating a significant correlation between calprotectin and the severity of inflammation. Furthermore, faecal calprotectin had a high correlation with the histologic grading as that observed for endoscopy. Its sensitivity was 93.3%, specificity was 89.7%, and also it had a high negative predictive value. The results were the same as those obtained by Bunn et al., [6] who claimed that faecal calprotectin concentrations predicted the severity of colorectal inflammation, with advanced histological grades of colorectal inflammation.

Inflammation is the basis for many signs and symptoms of IBD, making its detection and monitoring fundamental to clinical management [5].

One means to assess inflammation that has been discussed in recent years is the analysis of the infiltration of neutrophil in the intestinal mucosa and their transmigration to the lumen [11].

Calprotectin is derived predominantly from neutrophils and, to a lesser extent, from monocytes and reactive macrophages [5].

Therefore the presence of calprotectin in faeces is directly proportional to neutrophil migration towards the intestinal tract [1].

When intestinal inflammation occurs, the calprotectin levels correlate closely with histological evaluation than macroscopic findings, suggesting that this biological marker is more sensible than endoscopy in evaluating IBDs activity [12].

Our study revealed that level of faecal calprotectin was higher in IBD patients than in non-IBD patients (by 205 µg/g), which is matched by a study conducted by von Roon et al., [13] who stated that fecal calprotectin was

higher in IBD patients than in non-IBD patients (by 219 µg/g), and showed excellent pool sensitivity and specificity rates in distinguishing between these groups (95% and 91%, respectively).

In our study faecal calprotectin resulted the most accurate tool to assess the presence of active mucosal inflammation when compared to C-reactive protein, erythrocyte sedimentation rate. These results had matched with Tibble et al. [10].

Our study showed that faecal calprotectin concentration above 72 µg/g, gave a sensitivity of 93.5%, a specificity of 89.7%, a positive predictive value (PPV) of 90.6%, and a negative predictive value (NPV) of 92.9% in predicting UC.

The data obtained by our study revealed that there is a good agreement between faecal calprotectin, and endoscopic appearance. These results showed that fecal calprotectin at a concentration above 72 µg/g was in agreement with Simple clinical colitis activity index when it was above 4 of about 46%, while with endoscopy when the score above 1 the agreement was about 80%.

Faecal calprotectin allows a non-invasive monitoring of disease activity, especially when the repeated measurements are considered, among UC patients, as better identifying controlled disease activity.

In most clinically quiescent IBD, residual mucosal inflammation is still present to some extent. When disease activity increases, clinical symptoms are usually not present during the early relapse stage. Faecal calprotectin seems to be able to detect subclinical mucosal inflammation, and thus might earlier identify those patients at risk for IBD relapse [14].

We can conclude that measurement of faecal calprotectin is highly useful for the diagnosis and disease monitoring of patients with ulcerative colitis, and might additionally predict disease outcome. It is a sensitive and direct biomarker of intestinal inflammation with a better performance than the traditional non-invasive tests. It is both easily carried out and reliable, which makes it suitable for use as a first-level test for the diagnosis of organic ulcerative colitis as well as for the activity monitoring of UC.

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Conflicts of interest: None.

Ethical approval: Informed consents were routinely obtained from patients. The study was performed in accordance with the ethical standards on human experimentation and with the Helsinki Declaration of 1964.

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A Study of the Effect of the Use of Antioxidants in Patients with Hepatitis C Receiving Interferon/Ribavirin Therapy on the Response to Therapy

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Background and study aim: Treatment of HCV with interferon takes a long duration and has many side effects. The use of antioxidants with interferon/ribavirin therapy is believed to minimize the side effects and improves adherence and hence improves response to therapy. Reactive oxygen species are part of the human defense mechanisms towards infection and they increase due to hepatitis C virus infection. In this study we aim to study the impact of the concomitant use of antioxidants with interferon/ribavirin combination therapy for HCV on response as regards enzymes level, rate of viral clearance as well as liver histopathology..

Patients and methods: 240 patients on interferon/ribavirin therapy for chronic hepatitis C divided in two groups. The test group received concomitant antioxidant combination while the control group received only interferon/ribavirin. Follow up of liver function tests, complete blood count, viral load by PCR and post treatment histopathology by liver biopsy were performed.

Results: Liver enzymes level in test group achieved a larger and faster decline than in control group. Hematological parameters were significantly higher in the test group all through period of follow up. Viral load and histopathology showed no significant difference between the two groups.

Conclusion: concomitant use of antioxidants with interferon/ ribavirin therapy minimizes complications of therapy and rapidly normalizes the liver enzymes level without affecting the rate of response to therapy or histopathology of the liver.

INTRODUCTION

Pegylated interferon α (peginterferon α, peg-IFNα) in combination with weight-based doses of ribavirin (RBV) is currently recommended as the first-line "standard-of-care" treatment for chronic hepatitis C virus (HCV) infection. [1]

A recent trend in the treatment strategy of chronic HCV infection is the development of individualized treatment regimens based on strong predictors of SVR to IFN-based treatment, such as HCV genotype [2] and the initial virologic response to treatment.[3] Meanwhile, alternative options, such as modified regimens with currently available medications, novel modified IFNα and RBV or combinations with specifically targeted antiviral therapy for HCV (STAT-C) agents, are currently being investigated for the growing number of patients for whom current "standard-of-care" treatment has failed. For the foreseeable future, however, peg-IFNα and RBV appear to remain the backbone of "standard-of-care" treatment. [4]

Dose: Peg INF alpha 2a 180ug/week subcutaneous injection + ribavirin 800 mg /day oral may be increased to 1000-1200mg daily according to the

body weight.Peg INF alpha 2b 1-1.5mg/kg weekly subcutaneous injection + ribavirin at same previous dose. [5].

So many studies were done to evaluate the beneficial effects of the use of antioxidants in hepatitis C virus. Schizandrae chinensis, a potent anti-oxidant, lowers ALT levels in patients with chronic viral hepatitis**.** [6] A combination of three potent antioxidants (alpha-lipoic acid, silymarin, and selenium) induced marked clinical, laboratory and histologic improvement in chronic HCV patients. [7]

Another study observed that high vitamin E supplementation improves the aminotransferase status in patients who have chronic HCV. [8] A retrospective study examining the effects of stronger neo-minophagen C (SNMC), which contains glycyrrhizin as an active component, revealed that treatment with this agent reduces the long term relative risk of developing hepatocellular carcinoma by a factor of 2.49. [9] A randomized double-blind trial of thioctic acid (alpha-lipoic acid) in chronic hepatitis patients showed that 55% patients have significant improvements in mean ALT levels, and 77% patients have histological improvements on liver biopsy.[10] Intravenous glycyrrhizin was tested in patients with chronic HCV infection, and lowered ALT levels (26% ***vs*** 6% with placebo) within 4 wk were noted. The effect disappears after cessation of therapy. [11]

Administration of glutathione to patients with chronic hepatitis significantly decreases GSH-Px activity of catalase (CAT), and increases superoxide dismutase (SOD) activity. [12] A Cochrane systematic review of trials of medicinal herbs in HCV, reported that silybinin significantly reduced serum AST and GGT levels in only one trial, with no firm evidence for the use of herbal medicines in this condition. [13]

It was demonstrated that antioxidant vitamin (E and C) supplementation during interferon alfa-2b prevented decrease in eicosapentaenoic acid of mononuclear cell phospholipids. [14] The results of another more recent study showed a modest reduction in liver enzymes at the end of 24 wk of treatment in patients receiving the combined intravenous and oral protocol. [15]

So many studies also evaluated the concomitant use of antioxidants with interferon. It was reported that a combination therapy of interferon (IFN) with glycyrrhizin induces normalization of serum ALT levels in 64.3% of non-responders with serum HCV RNA disappeared in 38.5%. [9] It has been demonstrated that vitamin E-treated patients have a 2.4 times higher chance of obtaining a complete response and a more significant reduction in viral load than patients not treated with vitamin E. [16]

The use different antioxidants regimens prior and with interferon therapy lead to decline in liver enzymes. The liver histology wasn't affected and liver enzymes levels re-rise after stoppage of therapy in non-responders to interferon. This was confirmed by many studies which used different and complex antioxidant combination of oral and intravenous preparations. These studies also declared that antioxidants improved patients' quality of life during interferon therapy. [17, 18] The efficacy of antioxidants in European studies was less than that reported on Japanese subjects, which can be explained by the genetic polymorphism in drug metabolism. [19]

Side effects of interferon are so many but most important of them are the hematologic effects. They are the most recurrent abnormal laboratory values that can lead to dosage reductions and premature treatment termination. [20] Neutropenia is defined as an absolute neutrophil count less than 500 cells/mm^3 when using pegylated interferon α-2a. [21]

In most clinical trials, neutropenia is treated with dose modification. Interferon dose reduction occurs in about 17% to 20% of patients and treatment termination in 2% to 3% of patients**.** [2] Another option for patients who develop neutropenia from interferon therapy is the use of granulocyte colony-stimulating factor (GCSF)**.** [22] Another interferon-induced hematologic adverse effect is thrombocytopenia. It has been shown that platelet count can fall up to 50% of pretreatment count. [23] Eltrombopag, a thrombopoietin receptor agonist, to effectively increase platelet counts to greater than 250,000/mm^3 in thrombocytopenic patients with hepatitis C virus. [24]

Ribavirin also has so many serious side effects most important of them is the hematologic effects. The signature adverse effect of ribavirin is anemia, occurring in up to 30% of treated individuals. Ribavirin-related anemia is one the most common reasons for dosage reduction or discontinuation of the drug, resulting in 9% to 22% of patients requiring dosage reduction. [4] The mechanism of ribavirin-associated hemolytic

anemia is unclear, but is believed to be related to impaired antioxidant defenses and red blood cell oxidative damages through its metabolites .[25] Erythropoietin use in early-onset anemia minimized treatment discontinuation and led to higher sustained viral response rates. [26]

PATIENTS AND METHODS

The study was conducted in Tropical Medicine Department, Zagazig University Hospitals and patients were selected randomly from patients attending the hepatitis viruses out patient clinic of Alahrar General Hospital.

Total number of 240 patients, all having chronic HCV infection, were included in the study and were randomly divided on two groups.

Test Group: consists of 120 patients received:

1- Pegylated INF-α2a 180µg/wk SC.

2- Ribavirin 15 mg/kg/day orally.

3- Antioxidant combination regimen (vitamin E 400 mg, silymarin 420 mg, N-acetylcysteine (NAC) 600mg, vitamin C 500mg) orally daily.

Control group: consists of 120 patients received:

1- Pegylated INF-α 2a 180µg/wk SC.

2- Ribavirin 15mg/kg/day orally.

All patients were subjected to the following: Careful history taking and thorough clinical examination with calculation of the body mass index (BMI).The following laboratory tests: complete blood count, liver function tests, coagulation profile, kidney function tests, thyroid function tests, serologic markers for hepatitis B virus, anti-nuclear antibodies, quantitative polymerase chain reaction for HCV RNA, fasting blood glucose level and glycosylated hemoglobin A1C if fasting blood glucose is elevated.

Liver biopsy was performed to all patients before start of their therapy protocol in Tropical Medicine Department. Biopsies were prepared and examined in the Pathology Department in Faculty of medicine, Zagazig University and were interpreted according to METAVIR score.

Follow up:

Patients repeat liver function tests, serum creatinine level, complete blood count and PCR for HCV RNA at 4 weeks, 12 weeks, 24 weeks, and 48 weeks. Post-treatment liver histopathology was performed by number of patients after they stopped therapy ie after being declared non-responders or at end of therapy at 48 weeks.

RESULTS

Table (1): Demographic data

	Test group (n=120)		Control group (n=120)		X^2	P	Significance
	No.	%	No.	%			
Gender							
Male	84	70.0	85	70.8	0.02	0.88	NS
Female	36	30.0	35	29.2			
Diabetes	1.6	13.3	18	15	0.14	0.75	NS
Age (years)							
$\overline{X}$ ±SD	35.0±6.8		33.3±7.8		t=1.88	0.06	NS
Range	21-49		20-50				

NS: non-significant

Table (2): Rates of responses throughout period of therapy.

	Test group (n=120)		Control group (n= 120)		X^2	P	Significance
	No.	%	No.	%			
RVR	56/120		51/120				
Negative PCR	27	49.1	26	51	0.04	0.81	NS
EVR							
Negative PCR	95	79.16	92	76.6	0.22	0.64	NS
Incomplete responders (>2log decline)	5	4.16	7	5.8	0.35	0.55	NS
24wks							
Negative PCR	83	69.1	82	68.3	0.02	0.88	NS
48wks							
Negative PCR	80	66.6	80	66.6	0	1	NS

NS: non-significant

Table (3): Incidences of breakthroughs through out the period of treatment.

Items	Test group (n=120)		Control group (n=120)		X^2	P	Signif.
	No.	%	No.	%			
Total	16	13.3	14	11.6	0.15	0.69	NS
24wks	Test n = 100/120		Control n = 99/120				
	13	13	12	12.1	0.03	0.85	NS
48wks	Test n = 83/120		Control n = 82/120				
	3	3.6	2	24	0.001	0.98	NS

NS: non-significant

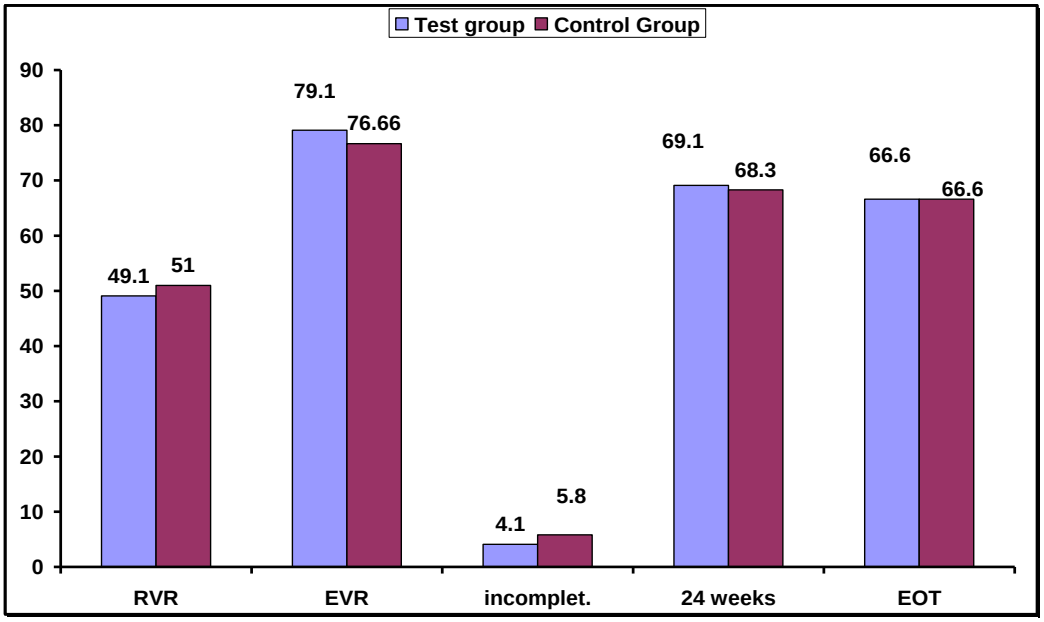

Figure (1): Represents the rates of responders all through the period of therapy.

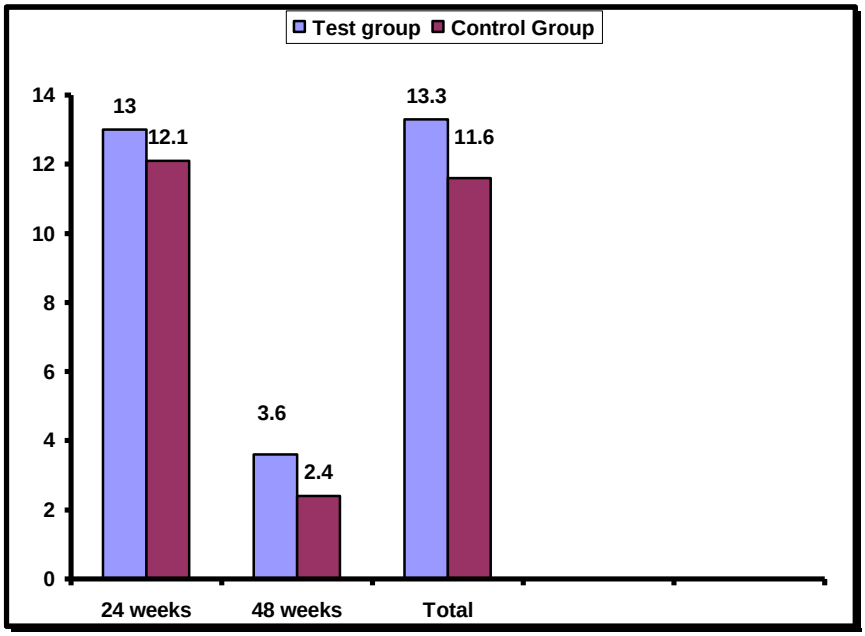

Figure (2): Represents the rates of break through all through the period of therapy

Table (4): Test groups liver function tests, creatinine and hematological parameters all through period of follow up.

Items	Base line	4 weeks	12 weeks	24 weeks	48 weeks	Weekly rate of change
ALT (IU/ml)						
$\overline{X}$ ±SD	61.9±31.4	56.3±27.5	54.8±27.6	41.1±18.2	24.4±11.5	0.78 IU/ml/wk
AST (IU/ml)						
$\overline{X}$ ±SD	50.2±23.9	49±22.3	41.1±17.3	37.2±15.4	25.9±14.7	0.50 IU/ml/wk
Alkaline phoshpatase (IU/ml)						
$\overline{X}$ ±SD	89.3±44.1	89.6± 58	83±22.4	77.2±17.5	75.1±13.3	0.29 IU/ml/wk
Bilirubin (mg/dl)						
$\overline{X}$ ±SD	0.6±0.17	0.9 ± 0.2	1.3±0.3	1.5±0.2	1.9±0.5	0.03 mg/dl
Albumin (g/dl)						
$\overline{X}$ ±SD	4.5±1.2	4.4±1.2	4.1±0.7	3.9±0.6	3.8±0.7	0.014 g/dl/wk
Cr (mg/dl)						
$\overline{X}$ ±SD	0.83±0.2	0.85±0.15	0.86±0.2	0.9±0.1	0.9±0.9	
WBC (cells/ml)						
$\overline{X}$ ±SD	6.1±1.6	5.5±1.7	4.6±1.6	4±1.4	3.5±1.6	54 cells/ml/wk
Hb (g/dl)						
$\overline{X}$ ±SD	13.9±1.1	13.1±1.4	12.4±1.2	11.8±1.3	11.7±1.3	0.045 g/dl/wk
PLT (cells/ml)						
$\overline{X}$ ±SD	177.8±41	162.2±45.2	14.8±46	137.1±43	121.1±47	1181 platelets/ml/wk

Table (5): Control group liver function tests, creatinine and hematological parameters all through period of follow up.

Items	Base line	4 weeks	12 weeks	24 weeks	48 weeks	Weekly rate of change
ALT (IU/ml)						
$\overline{X}$ ±SD	68.7±45.4	51.8±27.9	50.6±36	53.4±27	31.4±10	0.77 IU/ml/wk
AST (IU/ml)						
$\overline{X}$ ±SD	49.7±23.4	53.6±32	47.5±20.5	46.8±20.6	28.1±9.3	0.45 IU/ml/wk
Alkaline phoshpatase (IU/ml)						
$\overline{X}$ ±SD	85.6±39.2	91.2 ± 62.1	86.5±30.2	82±12.3	80.3±10.1	0.11 IU/ml/wk
Bilirubin (mg/dl)						
$\overline{X}$ ±SD	0.7±0.23	1.6± 0.4	2.1±0.5	2.7±1	2.7±0.6	0.04 mg/dl/wk
Albumin (g/dl)						
$\overline{X}$ ±SD	4.4±1.1	4.5±1.5	4±1.02	3.8±0.8	3.5±0.5	0.02 g/dl/wk
Cr (mg/dl)						
$\overline{X}$ ±SD	0.86±0.14	0.89±0.2	0.87±0.14	0.9±0.12	0.87±0.15	
WBC (cells/ml)						
$\overline{X}$ ±SD	6.5±1.74.6±1.2	3.7±1	3.2±1	2.7±1	79	79 cells/ml/wk
Hb (g/dl)						
$\overline{X}$ ±SD	14.2±1.5	12.5±1.4	11.8±1.4	11.1±1.36	11±1.3	0.066 g/dl/wk
PLT (cells/ml)						
$\overline{X}$ ±SD	180.7±50.4	156.4±39.7	142.5±37	123.7±38	105.6±31	1560 cells/ml/week

Table (6): The percent of change that occurs in liver function tests and hematological parameters.

Items	Test group (n=120)	Control group (n=120)	P	Significance
ALT	-56.6%	-40.0%	0.035	S*
AST	-41.02%	-38.0%	0.7	NS
Alk.phos.	-15.9%	-6.2%	<0.001	HS**
Bilirubin	+192%	+285%	<0.001	HS**
Albumin	-22%	-20.4%	0.7	NS
WBC	-38.6%	-56.6%	<0.001	HS**
HB	-15.8%	21.4%	0.012	S*
PLT	-34.3%	-39.8%	0.25	NS

NS: non-significant S: significant HS: highly significant

Table (7): Comparison between pre-treatment and post-treatment biopsy of test group.

Items	Pre-treatment (N=120)		Post-treatment (N=26)		*McNemar's test of Significance*	
	No.	%	No.	%		
A						
0	0	0	1	3.8	1	NS
1	41	34.2	12	46.1	0.24	NS
2	58	48.3	11	42.3	0.57	NS
3	21	17.5	2	7.6	0.3	NS
4	0	0	0	0	1	NS
F						
0	4	3.3	0	0	0.7	NS
1	46	38.3	11	42.3	0.68	NS
2	44	36.7	10	38.4	0.78	NS
3	26	21.7	5	19.2	1	NS
4	0	0	0	0	1	NS

NS: non-significant **NB:** McNemar's test ignores statistically all the subjects with missing data.

Table (8): Comparison between pre-treatment and post-treatment biopsy of control group.

Items	Pre-treatment (n=120)		Post-treatment (n=32)		McNemar's test of Significance	
	No.	%	No.	%		
A						
0	1	0.8	0	0	0.77	NS
1	38	31.7	11	34.4	0.67	NS
2	65	54.2	16	50	0.96	NS
3	16	13.3	5	15.2	1	NS
4	0	0	0	0	1	NS
F						
0	0	0	0	0	1	NS
1	35	29.2	5	15.2	0.12	NS
2	53	44.2	18	56.2	0.22	NS
3	32	26.6	9	28.1	0.86	NS
4	0	0	0	0	1	NS

NS: non-significant

DISCUSSION

The response to interferon that we evaluate in our study has three parameters: biochemical; level of ALT and AST, virological; measured at 4 weeks (RVR), 12 weeks (EVR), 24 weeks, 48 weeks (EOT),(the rate of SVR isn't assessed in this study), and histopathology using liver biopsy to assess the degree of activity and fibrosis.

The biochemical response: We found out as regards ALT levels; they fall gradually over the period of follow up so by the end of treatment most patients had normal ALT level. The level of ALT showed non significant difference between the two groups in the early period of follow up but became significantly lower in test group starting from week 24 to end of therapy. Although the weekly rate of ALT levels decline was calculated in both groups [.78 IU/ml/week in group I and .77 IU/ml/week in group II] and there was no significant difference between two groups, the percent of change that occurred in ALT levels was calculated in both groups at end of therapy and showed that test group I achieved a higher percent of decline than the control group. Our results disagree with Seeff et al, 2008 and Par et al, 2009 who used silymarin with long term interferon therapy found that it had no favorable effects on the liver enzymes or on viral load. In our study we used other antioxidants (vitamin E and C) with silymarin which have superoxide-scavenging activity so the results for liver enzymes were favorable. [27, 28] On the contrary, our results are supported by the study of Emerit et al, 2005 who used a phenol-rich processed grain food with superoxide-scavenging properties. They found a manifest decline in liver enzymes but no effect on viral load or biopsy was noted. [29] Our results also agree with Murakami et al, 2006, who found that vitamin E and C supplementation during INF therapy prevented the drop in level of Ecosapentaenoic acid in the cell membrane phopholipids in monomuclear cells. This keeps the integrity in those cell membranes and the level of ecosapentaenoic acid level was inversely co-related to the ALT level. [14]

AST level is different from ALT level being less specific to hepatocyte injury; AST is elevated also in hemolysis. The drop in AST level expressed as a weekly decline rate and as percent of change showed non-significant difference between the two groups. However, AST level was significantly lower in a specific point of time which is 24th week. Being non-specific to liver injury, AST doesn't seem to follow ALT strictly; AST achieves a slower decline than ALT.

In our study we threw light at other biochemical parameters such as; alkaline phosphatase level, albumin and bilirubin. As regards alkaline phosphatase level it showed no significant differences between the two groups until the 24th week when it became significantly lower in test group and then highly significantly lower in 48th week. When we calculated its weekly rate of decline and percent of change, we found a highly significant difference between the two groups. This means that, like the other liver enzymes, alkaline phosphatase achieves a faster and greater decline with antioxidants added to INF/RBV therapy than with INF/RBV therapy alone.

The bilirubin level seamed to be affected the most by the antioxidants. It became highly significantly higher in the control group starting from week 4 to the end of therapy. This can easily be explained on the background that antioxidants abolish the ribavirin-induced hemolysis. The rise in bilirubin was also associated with a decline in Hb concentration in both groups. This emphasizes, without doubt, that the hyperbilirubinemia is due to hemolysis. The weekly rate of rise as well as the percent of change of bilirubin level was highly significantly higher in the control group.

Among all the biochemical parameters monitored in our study, the albumin level seams to be the least affected by addition of antioxidants to therapy. This can be easily rationalized by the facts that albumin has a long half life, the synthetic function of the liver is good and the dyspepsia associated with interferon therapy is not severe enough to affect nutritional status of the patients.

The virological response: There was no significant difference between two groups as regards the rate of virological responses; RVR, EVR, 24 weeks and EOT. There were also no significant differences between two groups as regards rates of incomplete response and rate of breakthroughs at 24 weeks and at end of therapy. Most studies that studied the impact of use of antioxidants on the viral load with or without interferon therapy came to a common result that antioxidants don't affect the viral load [15, 17, 27, 28, 29] Our results disagree with Kalantari et al, 2011 who found that using an antioxidant (silymarin alone) use can decrease the viral load. This study used the antioxidant without interferon and involved a small number of patients. The period of follow up was shorter 24 weeks only in both studies. [30]

The histological response: There was non significant difference between two groups as regards post-treatment biopsy. There was no significant difference between pre and post-treatment biopsy as regards both degrees of fibrosis and activity in each group. Hence, we can say that the decline in liver enzymes doesn't mean an actual decline in the degree of activity in biopsy. This also means that the degree of fibrosis showed neither progression nor regression during the period of follow up. Here, we can mention that the period between the pretreatment and the post treatment biopsies ranged from 3 months in null-responders to 12 months in responders (EOT), we can also mention that 3-month period may not be enough to manifest the change. The previous studies that recorded histological changes ranged from 12 to 26 weeks and also found no evidence of histological changes. [15, 18, 31, 32] We recommend a longer duration up to 2 years to manifest if there will ever be significant histological changes with antioxidant use vs placebo.

Complications of interferon therapy are so many, and they are not the main topic that this thesis was designed to study, however we managed to describe and evaluate some of them, being difficult to totally ignore. Most important of these complications that we studied are; hematological complications, seen clearly in the CBC. The mean Hb concentration was non-significantly different in two groups before treatment began, then became significantly higher in test group all through the period of follow up. The weekly rate of decline of Hb concentration was significantly higher in control group; the percent of change was also significantly higher in the control group. Our results are in agreement with DeFranceschi et al, 2000 who demonstrated the central role of oxidative stress in the ribavirin-induced hemolysis. [25] Our results also agree with Murakami et al, 2006 and Hino et al, 2006 who concluded that vitamin E and C supplementation helped stopping RBC's premature destruction through keeping the ecosapentaenoic acid level in their cell membranes from falling with interferon therapy. Hino et al, also assured that the oxidative damage in erythrocyte membrane plays an important role in ribavirin induced anaemia. [14, 33]

Mean WBC count was also non-significantly different in two groups before treatment began, then became highly significantly higher in test group all through the period of follow up, the weekly rate of decline and percent of change were significantly higher in control group.

Mean platelet count was non-significantly different in two groups before treatment began and all through period of therapy, however the weekly rate of decline was significantly higher in control group. Our results are comparable to those of Rustgi et al, 2002 who found that platelet count falls over a longer period than the other hematological parameters. [34]

Conclusion: We ascertain that antioxidants slowed the deterioration of hematological parameters that occurs with INF/RBV therapy. This effect is central in the maintaining the patients adherence to therapy. They also helped to achieve a faster decline in liver enzymes without affecting the virological response to therapy or liver histopathology.

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Conflicts of interest: The authors declare that there is no conflict of interest.

Ethical approval: approved.

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Video Case: Endoscopic Extraction of a Large Piece of Fleshy Meat from the Esophagus by the Polypectomy Snare

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Comment

A fifty five years old female presented with acute dysphasia of 5 hours duration following ingestion of a large piece of fleshy meat. The patient feel a sense of impacted lump at the lower end of the sternum associated with repeated attack of retching. When examined by flexible upper endoscopy under light sedation using intravenous diazepam 10 mg, a large white fleshy piece of meat was seen impacted at the lower end of the esophagus about 35 cm from incisors. Several trials to push the fleshy meat to inside the stomach failed. Then several trials for fragmentation using the biopsy forceps and Dormia basket failed to extract the meat, till finally it was grasped firmly with a polypectomy snare and extracted to outside without any further hazard to the patient.

Image Case: Sausage Shaped Stone Extraction from the Common Bile Duct

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A 60-year old male patient presented with recurrent obstructive jaundice. He gave history of chronic hepatitis C and cholecystectmy. When presented for the current episode of obstructive jaundice his lab was bilirubin total 5.1 mg/dl, bilirubin direct 4.1 mg/dl, prothrombin concentration 77%, Haemoglobin 11 gm%. ERCP showed oblong filling defect involving the lower one third of common bile duct. Extrcation balloon extracted this large stone that appears sausage shaped emerging from the duodenal papilla after precut sphincterotomy.