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Contents

Goitre by Using Scintigraphy and Biopsy in Sudan

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Key words:
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Background and study aim: Goitre has been recognized in Sudan as a public health problem since the 1950's. Nationwide surveys on goitre were launched and various approaches in the quest for a solution to the problem were applied. Still, the number of goitrous individuals continues to grow each year. This article sheds lights and reiterates the frequency of goitre in Sudanese in the basis of scintigraphy and biopsy. The statistical findings should be especially useful to professionals in endemic medicine and endocrinology.

Patients and methods: The study was conducted at the Radiation and Isotope Center (RICK), Khartoum . A total of 400 patients received 2 mCi of Tc ^{99m} Pertechnetate intravenously. Imaging was performed using a Nucline gamma camera computer system with general purpose parallel hole collimator. Biopsy was carried out by Fine Needle Aspiration Biopsy (FNAB) with the guidance of ultrasonography.

Results: Goitre was more among females (88.8 %) as compared to males (11.2%) with a female: male ratio of 8:1. Goitre increased in the age group 20-40 with an average age of 35 years. Scintigraphy revealed diffuse goitre in 57.5% , multi nodular goitre in 37.5% and a single (solitary) thyroid nodule in 5% of the sample studied. Toxic goitre was reported in 4% of the patients while the rest were diagnosed as non-toxic goitre . The biopsy results showed that 8% of nodular goitres were malignant and the rest had cystic or degenerative changes only.

Conclusion: The study suggests that goitre in its major kinds, diffuse or nodular have the same frequency scintigraphically. Thyroid malignancy within nodular goitre remains in the minimum level. The goitre sill remains a major public health issue in Sudan and implementing iodine prophylaxis programs must be more activated.

INTRODUCTION

Goitre is an enlarged thyroid gland [1]. Goitre is a major manifestation of iodine deficiency, it is a world-wide problem [2,3,4,5,6,7]. Iodine deficiency not only causes goitre, the obvious sign of inadequate iodine intake, but may also result in irreversible brain damage in the fetus and infant, and retarded the psychomotor development in the child [8]. Goitre is classified anatomically into: diffuse and nodular (single "solitary" nodule and multinodular goitre), which is easy to detect sonographically by the outer shape. Also goiter can be divided physiologically into simple and toxic which can be detected by scintigraphy. The diffuse or nodular nature of goitre can be diagnosed by clinical examination, as well as by using scintigraphy and sonography [3,9,10,11,12,13]. Nuclear medicine imaging of thyroid provides useful information about the shape, size and site of thyroid tissue, the function of thyroid nodule, and functioning thyroid tissue in patients with thyroid carcinoma. Either iodine-123 (I^{123}) or technetium-99m (Tc^{99m}) may be used [14].

Goitre has been recognized in Sudan as a public health problem since the 1950's [15]. This study was aimed on the scintigraphic and biopsy examinations of frequency of goitre in Sudanese population.

PATIENTS AND METHODS

The primary data and the family history for a total of 400 patients referred in to the department of Nuclear Medicine at the Radiation and Isotope Center(RICK), Khartoum were reported. Kind of goitre was studied by clinical examination to the neck and the classification of goitre was estimated.

Sample was separated into four groups: bellow 20, 20-40,41-60,and those above the age of 60 years.

Scintigraphic examination for the sample involved the intra-venous injection of 2 mCi of Tc^{99m} pertechnetate, followed by 15 minutes (150 K counts) at the neck area using a general purpose parallel holes collimator and a Nucline gamma camera computer system, with the patient in a supine position. The examinations were sufficient to determine the kinds of goitre in the patients.

Fine Needle Aspiration Biopsy (FNAB) for the nodular goitres by a plastic disposable syringe and a glass slide was used to carry out the laboratory test. The aspiration was used with the guidance of a 7.5MHz linear probe transducer attached to a General Electric (GE) medial ultrasound system.

RESULTS

Goitre was found to affect females in the reproductive age group less than 40 years, with a female to male ratio of 8:1. The average age was 35 years. The peak was among females between 20-40 years of age presenting the percent of 54.3%.

The majority of patients were from Khartoum state (35.5%), Al Gezera and Central Sudan (29%), Kordofan and Darfur at Western Sudan (24.2%), Northern Sudan (7.8%), Southern Sudan (2.0%) and Eastern Sudan (1.5%) (**Figure 1**).

Clinically, 166 (42%) patients showed moderate goitre, large goitres in 81 (20%), small goitres in 95 (24%) and huge goitres in 29 (7%) (**Figure 2**).

Scintigraphy revealed diffuse goitre in 57.5%, multinodular goiter in 37.5% and a single "solitary" thyroid nodule in 5% of the sample studied. Toxic goitre (**Figure 3**) was reported in 4% of the patients while the rest were diagnosed as non-toxic goitre (**Figure 4**).

Biopsy revealed that 8% of the histopathological findings of the nodular goitre were malignancies, whereas 92% were benign cytologic findings. Among the malignancies 21% were follicular adenocarcnioma and 79% were lymphoma. Histopathological results of benign nodules included 30% benign nodular goitre and 70% were nodular goitre with cystic or degenerative changes.

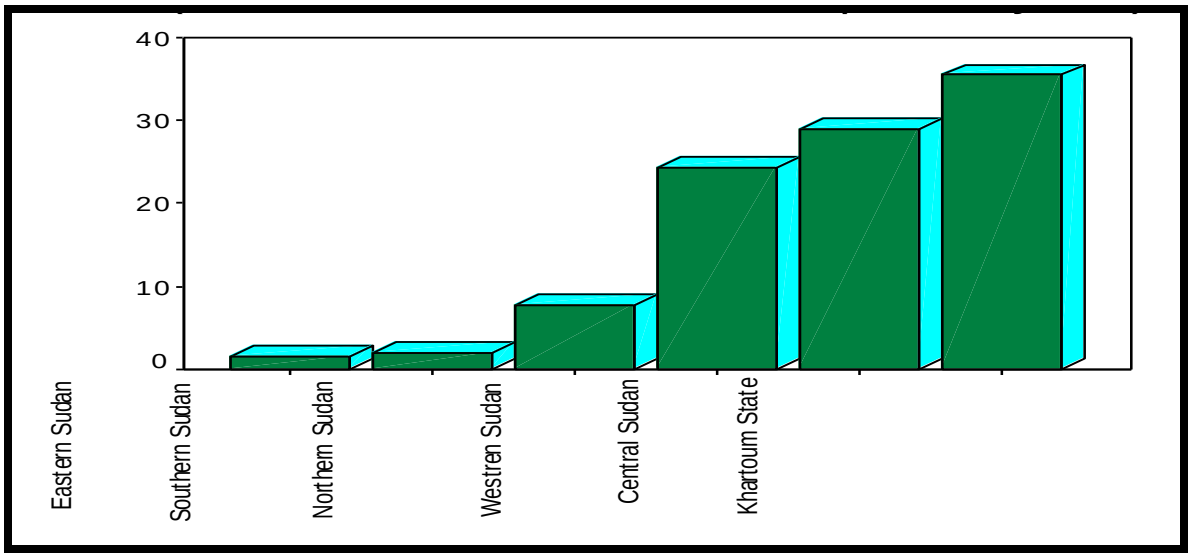

Figure 1: Geographical area

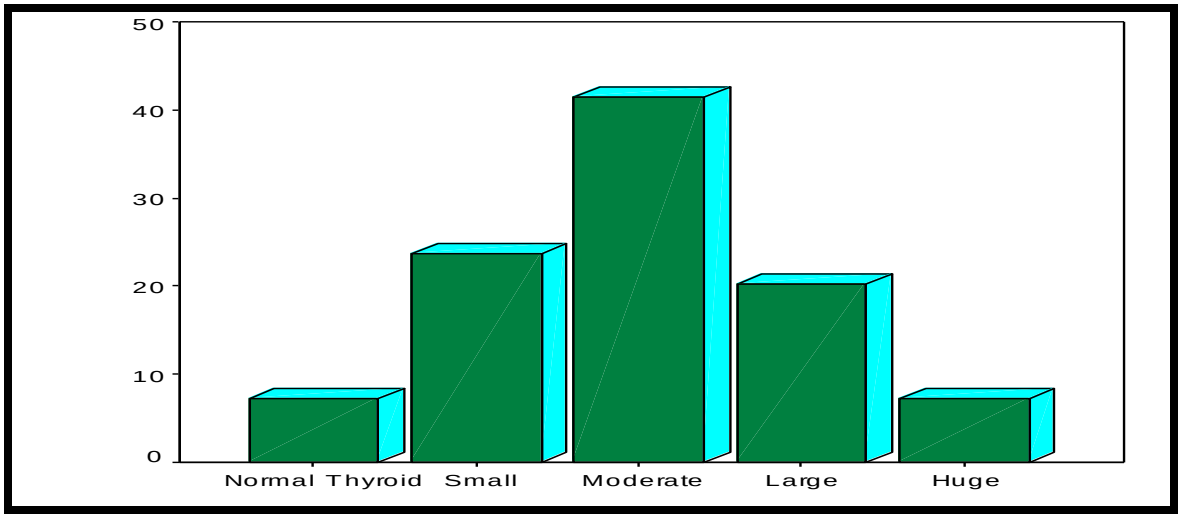

Figure 2: Clinical classification of goiter

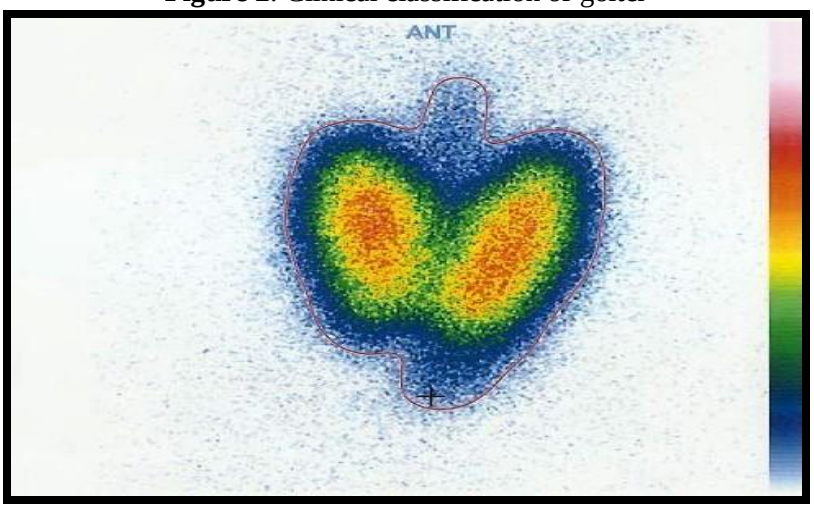

Figure 3: Scintigraphic features of diffuse toxic goitre. The static Spot of the anterior neck was acquired 15 min. following administration of 2 mCi of Tc^{99m}. The thyroid gland is enlarged with no suprasternal extension. Intense homogenous radiotracer uptake portrayed all over the gland with deprivation of extra thyroid tissue from their normal tracer share denoting the activity of the gland to the tracer uptake, with the appearance of pyramidal lobe.

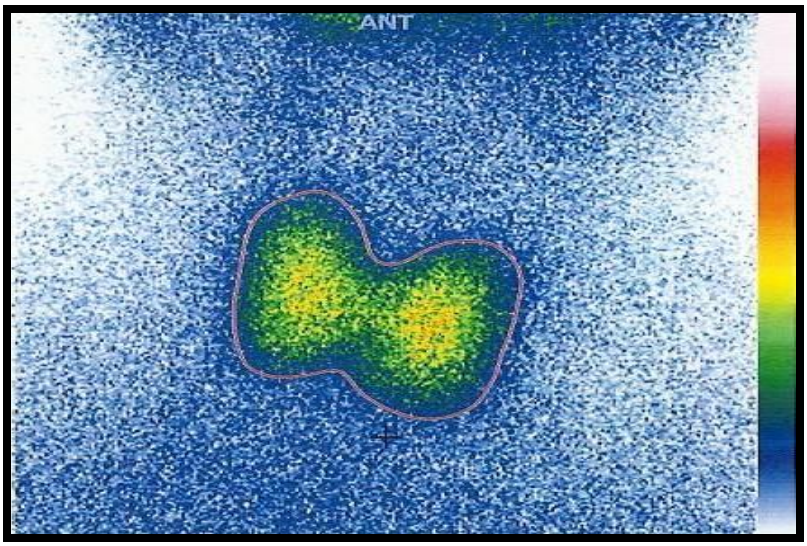

Figure 4: Scintigraphic features of diffuse non toxic goitre. The static spot view of anterior neck was acquired 15 min. following administration of 2 mCi of Tc^{99m} . The thyroid gland is enlarged with no evidence of retrosternal extension. Homogenous radiotracer uptake portrayed all over the gland with normal extra thyroidal tissue tracer uptake.

DISCUSSION

Goitre has been sufficiently investigated in many of its manifestation, world-wide. Williams determined that goitre is 7 to 9 times more common in women than in men [16]. The prevalence of goitre in women has been supported in the studies done in Turkey [5,17,18,19,20]. However, the female/male ratio varies among the researchers: 4.5 was reported by Kologlu [5]; 3.2 by Urgancioglu[18] ; 4.0 by Karpuzoglu [19]; 5.2 by Yilmaz [20]. In Sudan also, the prevalence of goitre in women has been supported by Mohamed et al, study [21]. Mohamed and his colleagues study showed the frequency of 81% were females. The current study result of 8.1 and 88.8% is within Williams and Mohamed range of values respectively. Still women suffer from goitre more than men in Sudanese patients.

Many of the researchers point out that the incidence of goitre increases during puberty, and that the upward trend continues thereon, especially in women, becoming most frequent in both sexes in the age group 20-40 [5,17,22,23].

The current study has shown that the frequency of goitre was higher in women, and that for both sexes goitre was highest, in the age group of 20-40 while Erkan reported that the frequency of goitre is highest in the age group of 15-30 [24].

Greig et al. reported a frequency of goitre of 12.2% in Khartoum region. He considered Khartoum to be an endemic area according to the World Health Organization (WHO) committee which regarded any area in which the frequency of goitre exceeding 10% to be considered as an endemic area [25].

Geographical area distribution in this study points to where the patient used to live within the past 20 years permanently. The majority of our patients (35.5%) were from Khartoum State. Those from Gazira and Central region constituted 29% of our patients. Kordofan and Darfur at western Sudan, being known endemic areas of goitre were presented by 24.2% of our patients.

Also the current results confirmed diffuse goitre in 57.5% , multinodular goiter in 37.5% and a single "solitary" thyroid nodule in 5% of the sample studied. This results near to matches with Mohamed's study who revealed that 58% of patients had nodular goitre while the rest had diffuse goitre [21].

Malignancies with the nodular goitre types was only 8% of the histopathological findings. This result is near to Mohamed's study from Sudan who revealed that 14% of the nodular goitre showed a type of malignancy[21].Also, Kapur from India reported a frequency of 10 % malignancy among patients with solitary thyroid nodule [26].

CONCLUSION

Goitre still remains a major problem in Sudan. The geographic isolation and the socioeconomic, cultural, and political factors in Sudan contribute

to the technical difficulty in implementing iodine prophylaxis programs. Scintigraphy is an important method to identify the morphology of the thyroid gland as well as monitoring and curing the disease.

Funding: Non

Conflicts of interest;Non.

Ethical considerations: Special consideration was given to the right to confidentiality and anonymity of all patients. Anonymity was achieved by using numbers for each patient that will provide link between the information collected and the participants. In addition confidentiality was ensured by making the collected data accessible only to the researchers. The right to equality was ensured by giving each patient the same number and type of scintigraphy and laboratory procedures.

Justice and human dignity was observed by treating selected patients equally when offering them an opportunity to participate in the study. The patients are free to decide whether to participate or not. The patient was given informed consent that will be signed after explanation of the purpose, possible outcomes of the study and conditions applying to their participation. Permission to conduct the study was obtained from the hospitals directors as well as the superintendent radiographer in the nuclear medicine department at RICK.

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Assessment of Tumor Markers in Bile in Patients With Pancreaticobiliary Malignancies: ERCP- Based Study

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Carcinoembryonic antigen, carbohydrate antigen, CA19-9, hepatopancreatobiliary disease, bile.

Background and study aim: The value of serum tumor markers carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) in the differential diagnosis of obstructive biliary disease is a matter of debate. We aimed to define their role prospectively.

Patients and methods: Thirty five cholestatic patients , 14 malignant group, their age ranged from 47-72 years (mean: 58.43 ± 9.08 years), 14 benign group, their age ranged from 46-72 years (mean: 60.21 ± 10.45 years) and 7 calcular, their age ranged from 48-71 years (mean: 60.14 ± 7.78 years) who were referred for endoscopic retrograde cholangiopancreatography examination for obstructive jaundice were included. Bile was obtained through cannulation of ERCP. Serum samples were taken from all patients at the time of acquisition of bile. Serum and bile samples were stored at -80 °C until they were tested. CEA and CA19-9 levels were measured with enzyme immunoassay methods in serum and bile samples by using Immunospec CA 19-9 and Immunospec CEA kits, respectively (Canoga Park,CA, 91303).

Results: In 14 patients with malignant disease, serum CEA levels were 36.77 (23.33-124.92) ng/ml and CA19-9 were 418.07 (1.23-483.47) U/ml, while in 14 patients with benign disease the serum CEA levels were 15.43 (0.38-30.80) ng/ml and CA19-9 were 144.6 (3.99-471.15) U/ml. The difference for both values was significant ($p<0.05$). In malignant disease bile CEA and CA19-9 levels were 5.05 (0-124.84) ng/ml, 455.61 (0.07-483.80) U/ml respectively, while in benign disease the corresponding levels were 3.22 (0-121.81) ng/ml for CEA and 421.45 (0-485.06) U/ml for CA19-9. The differences were not significant in this case ($p>0.05$).

Conclusion: It was concluded that serum CEA and CA19-9 levels are increased both in malignant and benign obstructive biliary diseases. However, levels of serum CEA are markedly increased and mostly restricted to malignant diseases. Measurement of these markers in bile was of no clinical significance.

INTRODUCTION

Tumor markers are antigens and bioactive substances produced by tumor cells because of the abnormal expression of correlated genes. They are either not produced or only minimally produced, in normal tissues, and can be detected in tissues, body fluids and excreta of patients with cancer [1]. Both carbohydrate antigen 19-9 (CA19-9) and serum carcinoembryonic antigen (CEA) are produced by epithelia of the pancreas, stomach, colon, liver, and biliary tract [2]. Small tumors produce detectable levels of the CEA or CA19-9 antigen only in the body fluids such as bile, and this was suggested as a useful tool in the patient with otherwise occult liver metastasis [3] or with primary tumors [4]. CEA and CA19-9 have long been used as tumor markers in gastrointestinal malignancies to help detect the primary tumor and determine tumor stage and prognosis [5]. They also allow

monitoring of therapeutic efficacy and tumor recurrence. Although CEA and CA 19-9 have been studied extensively in pancreatic head cancer [6]. Their roles in other nonpancreatic periampullary cancer have not been clearly established [7]. The present study has aimed to determine the role of both serum and biliary CA19-9 and CEA levels in the differential diagnosis of benign and malignant pancreaticobiliary diseases.

PATIENTS AND METHODS

Between July 2009 and September 2011, 35 patients (18 female, 17 male, median age: 54 yr, range: 19-81) with diagnosis of obstructive jaundice were studied. (The diagnosis was based on ultrasonography (US) and/or computerized tomography (CT) findings). Endoscopic retrograde cholangio-pancreatography (ERCP) was performed. Bile was obtained through cannulation of ERCP. Serum samples were taken from all patients for CEA and CA19-9 at the time of acquisition of bile. Serum and bile samples were stored at -80 °C until they were tested. Bile samples were mixed with 0.1 M. acetic acid at 70 °C for 15 minutes followed by centrifugation for 10 minutes at 3000 g for the elimination of bile pigments and other proteins before the measurements [8]. All CEA and CA19-9 bile samples were tested after dilution at titers of 1/10, 1/20, 1/40 and 1/80. The distinction between malignant and benign was based on clinical or radiological findings (US and/or CT, ERCP). The diagnosis of primary sclerosing cholangitis (PSC) was based on compatible cholangiographic features, biochemical and clinical findings. Histopathology was available in three-fourth of the patients as well. Alkaline phosphates, ALT, AST, total bilirubin levels and white blood cell (WBC) count were studied in all patients. CA19-9 and CEA levels were measured with enzyme immunoassay methods in serum and bile samples by using Immunospec CA 19-9 and Immunospec CEA kits, respectively (Canoga Park, CA, 91303). Serum upper limit of normal for CA19-9 was 37 ng/ml and for CEA was 5 ng/dl. Normal levels are undefined for bile samples .

Those subjects were selected from patients attending the outpatient clinic and inpatient unit of Tropical Medicine and Endoscopy Unit, Cairo University, and informed consents were obtained from each subject before blood samples were collected and pathological specimen were taken.

STATISTICAL ANALYSIS

Statistical analysis was performed using the SPSS 17(Statistical package for social science) . The quantitative data were presented in the form of mean, standard deviation, and range. The qualitative data were presented in the form of number and percentage. Pearson Moment Correlation tests were used to study the relation between variables. ROC curve was constructed for CA 19-9 and CEA. The sensitivity and specificity values of CA 19-9 and CEA were calculated with one cut-off value level. Significance was considered when P value less than 0.05.

RESULTS

Thirty five cholestatic patients, 14 malignant group [8 males and 6 females, their age ranged from 47-72 years (mean: 58.43 ± 9.08 years)], 14 benign group [6 males and 8 females, their age ranged from 46-72 years (mean: 60.21 ± 10.45 years)] and 7 calcular [3 males and 4 females, their age ranged from 48-71 years (mean: 60.14 ± 7.78 years)] who were referred for ERCP examination for obstructive jaundice were included. The etiology of malignant disease was cholangiocarcinoma in 2, papillary carcinoma in 2 and pancreatic head carcinoma in 10. Mean serum CEA level in the malignant group was 36.77 (23.33-124.92) ng/ml while bile CEA level was 5.05 (0-124.84) ng/ml, the area under ROC curve for serum CEA in malignant group was 0.964 (P<0.001) with cutoff value 26.72 ng/ml, the sensitivity and specificity was 92.9% and 85.7% respectively, while the area under ROC curve for biliary CEA level in malignant group was 0.561 (P=0.581) with cutoff value 3.99 ng/ml ,the sensitivity and specificity was 64.3% and 50% respectively. The levels of serum and bile CA19-9 levels in malignant group was 418.07 (1.23-843.47) U/ml and 455.61 (0.07-483.80)U/ml, respectively, the area under ROC curve for serum CA19-9 in malignant group was 0.0.765 (P=0.017) with cutoff value 290.005 U/ml the sensitivity and specificity was 85.7% and 71.4% respectively, while the area under ROC curve for biliary CA19-9 level in malignant group was 0.566 (P=0.55) with cutoff value 215.965 U/ml, the sensitivity and specificity was 71.4% and 42.9% respectively. The benign diseases were hydatid disease related biliary stricture in 1, post surgical biliary stricture in 7, primary sclerosing cholangitis in 4, cholangitis due to *Fasciola hepatica* in 1 and stricture

followed liver transplantation in 1. Serum CEA value in benign disease was 15.43 (0.38-30.80) ng/ml, while the area under ROC curve for serum CEA level in benign group was 0.036 (P<0.001) with cutoff value 28.675 ng/ml ,the sensitivity and specificity was 14.3% and 21.4% respectively, while CA19-9 was 144.6 (3.99-471.15) U/ml with area under ROC curve for serum CA 19-9 level in benign group was 0.235 (P=0.017) with cutoff value 346.73 U/ml ,the sensitivity and specificity was 28.6% and 35.7% respectively. The serum CEA for the malignant group (36.77 [23.33-124.92] ng/ml) was significantly higher than for the benign group (15.43 [0.38-30.80] ng/ml) (P<0.001). Serum CA19-9 concentration was significantly higher in patients with malignant disease (418.07 [1.23-483.47] U/ml) in comparison to patients with benign diseases (144.6 [3.99-471.15] U/ml) (p=0.017).

In benign group, mean bile CEA and CA19-9 levels were 3.22 (0-121.81) ng/ml, 412.45 (0-485.06) U/ml, respectively with area under ROC curve for biliary CEA level was 0. 439 (P=0.0.581) with cutoff value 12.74 ng/ml the sensitivity and specificity was 42.9% and 42.9% respectively and area under ROC curve for biliary CA 19-9 level was 0.434 (P=0.550) with cutoff value 312.2 U/ml ,the sensitivity and specificity was 57.1% and 35.7% respectively. Biliary CA19-9 levels in patients with malignant diseases were not significantly different from those in the patients with benign disease (p=0.550).

In malignant group, there was no correlation in malignant group between Age, Hb%, WBCs, platelet count, serum levels of ALT, AST and serum CEA (P=0.647; P=0.318; P=0.085; P=0.976; P=0.164 and P=0.191) respectively, but there is significant correlation also in malignant group between serum bilirubin, alkaline phosphate, serum CA 19-9 and serum CEA (P=0.007; P=0.002 and P= 0.023). Also, there is inversely correlation between serum albumin and serum CEA in malignant group (P=0.002). There was no correlation in malignant group between Age, Hb%, WBCs, platelet count, serum levels of albumin, ALT, AST and serum CA 19-9 (P=0.788; P=0.946; P=0.670; P=0.464; P=0.308; P=0.064 and P=0.318) respectively, but there is significant correlation also in malignant group between serum bilirubin, alkaline phosphate and serum CA 19-9 (P=0.030 and P= 0.035).

In benign group, There was no correlation in benign group between Age, Hb%, WBCs, platelet count, serum levels of albumin, ALT, AST and serum CEA (P=0.664; P=0.177; P=0.599; P=0.474; P=0.581;P=0.122 and P=0.182) respectively, but there is significant correlation also in benign group between serum bilirubin, alkaline phosphate, serum CA 19-9 and serum CEA (P=0.001; P=0.010 and P= 0.019). There was no correlation in benign group between Age, Hb%, WBCs, platelet count, serum levels of albumin, ALT, AST and serum CA 19-9 (P=0.358; P=0.823; P=0.714; P=0.418; P=0.881;P=0.064 and P=0.084) respectively, but there is significant correlation also in benign group between serum bilirubin, alkaline phosphate and serum CA 19-9 (P=0.033 and P= 0.037).

Table (1): Serum tumor marker in different groups

	Malignant group (n = 14)	Benign group (n = 14)	Control group (n = 7)	P value
CEA (ng/ml)	36.77 (23.33 – 124.92)	15.43 (0.38 – 30.80)	1.20 (0.29 – 5.28)	P1 < 0.001 P2 < 0.001 P3 = 0.037
CA19-9 (U/ml)	418.07 (1.23 – 483.47)	144.6 (3.99 – 471.15)	8.40 (3.99 – 104.78)	P1 = 0.017 P2 = 0.004 P3 = 0.021

P1: Malignant versus Benign, P2: P1: Malignant versus Control, P3: Benign versus Control

Table (2): Biliary levels of tumor marker in different groups

	Malignant group (n = 14)	Benign group (n = 14)	Control group (n = 7)	P value
CEA (ng/ml)	5.05 (0 – 124.84)	3.22 (0 – 121.81)	0.38 (0 – 1.14)	P1 = 0.581 P2 = 0.020 P3 = 0.044
CA19-9 (U/ml)	455.61 (0.07 – 483.80)	421.45 (0 – 485.06)	9.13 (0 – 189.58)	P1 = 0.550 P2 = 0.006 P3 = 0.012

P1: Malignant versus Benign, P2: P1: Malignant versus Control, P3: Benign versus Control

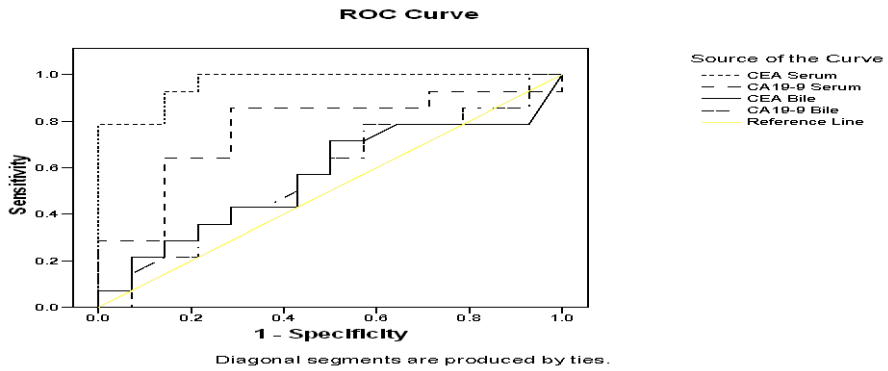

Figure (1): ROC curve analysis of serum and biliary tumor markers in malignant group

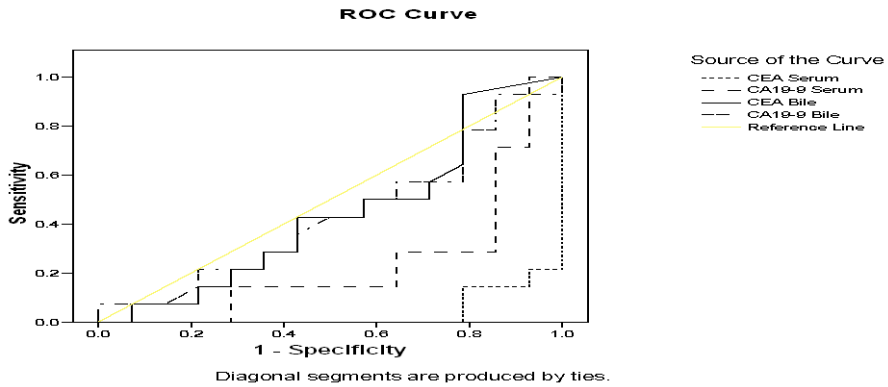

Figure (2) :ROC curve analysis of serum and biliary tumor markers in benign group

Table (3): Correlation between serum and biliary tumor markers with other variables in malignant group

	Serum				Biliary			
	CEA		CA19-9		CEA		CA19-9	
	r	p	r	R	r	p	r	p
Age (years)	0.134	0.647	0.079	0.788	0.334	0.244	0.145	0.620
Hb% (gm/dL)	0.288	0.318	-0.020	0.946	0.227	0.434	0.064	0.829
WBCs(10^3/µL)	-0.477	0.085	-0.125	0.670	-0.143	0.625	-0.196	0.503
Platelet (10^3/µL)	0.009	0.976	-0.213	0.464	0.117	0.690	-0.381	0.179
Bilirubin (mg/dL)	0.684	0.007*	0.579	0.030*	0.676	0.008*	0.590	0.026*
Albumin (gm/dL)	-0.645	0.013*	-0.294	0.308	-0.435	0.120	-0.259	0.372
alkaline phosphatase (IU/L)	0.745	0.002*	0.565	0.035*	0.744	0.002*	0.547	0.043*
ALT (IU/L)	0.393	0.164	0.508	0.064	0.550	0.042*	0.556	0.039*
AST (IU/L)	0.371	0.191	0.288	0.318	0.373	0.189	0.516	0.059
CA19-9 (U/mL)	0.209	0.474	-	-	0.600	0.023*	-	-

Table (4): Correlation between serum and biliary tumor markers with other variables in benign group

	Serum				Biliary			
	CEA		CA19-9		CEA		CA19-9	
	r	p	r	R	r	p	r	p
Age (years)	-0.128	0.664	-0.266	0.358	-0.112	0.703	0.117	0.691
Hb% (gm/dL)	-0.383	0.177	0.066	0.823	-0.180	0.537	0.403	0.153
WBCs	0.154	0.599	0.108	0.714	0.253	0.383	0.198	0.497
Platelet (10^3/µL)	0.209	0.474	-0.235	0.418	0.433	0.122	0.042	0.887
Bilirubin (mg/dL)	0.770	<0.001**	0.572	0.033*	0.638	0.014*	0.631	0.016*
Albumin (gm/dL)	-0.162	0.581	0.044	0.881	0.212	0.466	-0.217	0.457
alkaline phosphatase (IU/L)	0.662	0.010*	0.560	0.037*	0.552	0.041*	0.846	<0.001**
ALT (IU/L)	-0.433	0.122	0.508	0.064	0.099	0.737	0.420	0.135
AST (IU/L)	-0.379	0.182	0.478	0.084	-0.020	0.946	0.333	0.245
CA19-9 (U/mL)	0.618	0.019*	-	-	0.209	0.474	-	-

DISCUSSION

Carbohydrate antigen (CA19-9) and carcinoembryonic antigen (CEA) are tumor markers for the diagnosis of gastrointestinal cancers [9]. Carbohydrate antigens are a saccharide complex derived from certain tumor tissues or tumor cell lines. More than ten types of such antigens have been discovered to increase in the serum of cancer patients. They can be used in diagnosis and treatment of tumors as biological markers if they are highly sensitive and specific. A large number of potential tumor markers have been evaluated in pancreatic cancer, but none has been sufficiently sensitive or specific in detecting pancreatic cancer [10]. CA19-9 has been studied intensively in diagnosis of pancreas cancer for many years [11,12].

In this work, serum CA19-9 levels were found to be elevated in patients with malignant biliary group (P=0.004). This is agree with Safi et al [13] ; Goonetilleke and Siriwardena [14] who approved that elevations in serum CA 19-9 appear to be useful in the diagnosis of adenocarcinoma of the upper gastrointestinal tract and in monitoring of colonic carcinoma, its greatest sensitivity is in the detection of pancreatic adenocarcinoma. Also this is in accordance with Kau et al.,[15] who approved that CA 19-9 is superior in the diagnosis of pancreatic cancer and is often considered the standard marker for pancreas cancer.

In the present study, serum CA19-9 levels were found to be elevated in patients with benign biliary diseases (P= 0.021). This is consistent with Ong et al.[16] and Morris-Stiff et al.[17] who reported that serum level of Ca19-9 have

been found elevated in some benign diseases, such as pancreatitis, cholangitis, hepatitis and cirrhosis. This is due to the presence of jaundice itself leading to up regulation of CA19-9 in benign diseases.

As regard, CA 19-9 antigen is synthesized both by the epithelial cells of the normal biliary tract and by the tumor cells and excreted within the bile [9]. It is suggested that the CA 19-9 antigen, which is high in concentration in the bile of the patients with benign and malignant obstructive jaundice, refluxes into the bloodstream due to the increase in the permeability between bile and blood, secondary to the bile stasis; moreover, it is stated that there can be an inability to degrade the antigen in the liver due to a hepatic dysfunction[18]. Therefore, remeasurement of CA 19-9 after the jaundice subsides can be useful in differential diagnosis of some CA 19-9 positive patients with obstructive jaundice, and if the concentration is still high, then the malignancy potential is high[19]. Moreover, Marrelli et al.,[20] reported that in the presence of successfully drained obstructive jaundice, CA19-9 serum levels that remain unchanged or measure more than 90 U/mL are strongly indicative of a malignant cause of obstruction. However, the real clinical utility of this marker remains controversial.

In this work, elevation of serum Ca 19-9 was higher in malignant group than in benign one (P=0.017). This is in line with McLaughlin et al.,[21]; Morris-Stiff et al.,[17] who approved that CA19-9 levels were significantly lower in patients with benign pathology than those with malignant pathology.

In addition, ROC analysis is a graphic method to determine the optimal threshold for evaluation of sensitivity and specificity profiles of serum tumor markers[22]. In our study, ROC curve analysis of serum CA 19-9 in malignant group (table 19) shows the area under the ROC curve was 0.765 with (p=0.017). For these patients, serum CA19-9 proved to be useful. At a cutoff value of 290.005 U/ml, sensitivity and specificity were 85.7% and 71.4%, respectively for diagnosis of biliary malignancies. This is accordance with Bedi et al.,[23] who reported that ROC analysis has shown that at a threshold level of 300 U/mL, CA 19-9 has 100% specificity in diagnosing pancreatic carcinoma in patients with idiopathic chronic pancreatitis. Similar cut-off level has been proposed by Nouts et al.,[24] in a study comparing de novo pancreatic cancer and chronic pancreatitis.

Moreover, Morris-Stiff et al.,[17] founded that the CA19-9 levels were significantly greater for malignant than for benign disease, A ROC analysis provided an area under the curve for CA19-9 of 0.871 (0.820-0.922), giving an optimal CA19-9 of 70.5 U/ml for differentiating benign from malignant pathology. Using this cut-off, the sensitivity was 82.1%, while specificity improved to 85.9%. When standard radiology was included (US/ CT/MRCP) in the decision process, the results improved to 97.2% and 88.7% respectively.

In the present study, combined correlation between alkaline phosphatase and serum bilirubin with serum CA 19-9 in malignant group (r=0.565; P=0.035) and (r=0.579; P=0.030) respectively and also (r=0.560; P=0.037) and (r=0.572; P=0.033) in benign group respectively. This is in accordance with McLaughlin et al.,[21] who founded that there was a significant correlation between serum CA19-9 levels and alkaline phosphatase, ALT, AST, bilirubin, in obstructive jaundiced patients. Also, Ni et al.,[25] showed that there was a significantly positive correlation between serum CA19-9 and bilirubin, which suggested that obstructive jaundice, might result in increasing of CA19-9 levels and provided some fault positive results. In contrast, Haglund et al.,[26] and Bedi et al,.[23] who found that there is no correlation between serum CA 19-9 and bilirubin or alkaline phosphatase levels were detected. In addition, Morris-Stiff et al.,[17] found that for benign disease, the CA19-9 correlated directly with the serum bilirubin, but for malignant disease, CA19-9 levels were elevated independent of the bilirubin level. On other hand, there is no correlation between ALT and AST with serum CA 19-9 in malignant (r=0.508; p=0.064) and (r=0.288; P=0.318) respectively and also in benign groups (r=0.508; p=0.064) and (r=0.478; P=0.084) respectively in our study.

In our work, biliary level of CA19-9 levels were found to be elevated in patients with malignant biliary diseases (P=0.006) (table 12) and also elevated in benign group (P=0.44). This is agree with Akdoğan et al.,[27] and Duraker et al.,[19] who approved that the CA 19-9 antigen, which is high in concentration in the bile of the patients with benign and malignant obstructive jaundice.

CEA is a member of the immunoglobulin superfamily which was originally identified in human fetal colon and colorectal cancer. It is widely used as a tumor marker. However, little is known about its function except that it acts as a homotypic adhesion molecule that is implicated in cell aggregation. It is over-expressed in numerous human cancers where it is present on the surface of cancer cells[28].

CEA is mainly secreted by digestive glandular cancers and their metastases. It is also found in other types of cancer such as breast, lung, ovary, thyroid, etc[29,30].

In this work, serum CEA levels were found to be elevated in patients with malignant biliary diseases (P<0.001). This is in accordance with Ni et al., [25] who reported that levels of serum CEA and CA19-9 in patients with pancreatic cancer were higher than that of other malignant diseases and benign pancreatic diseases.

In the present study, serum CEA levels were found to be elevated in patients with benign biliary diseases (P= 0.037). This is consistent with Duraker et al.,[19] who reported that CEA increase in the serums of patients with benign biliary obstruction as follows: CEA-like substances are normally produced endogenously in small amounts; the degradation and excretion of these substances by the liver may be impaired in biliary obstruction. However, in malignant biliary obstruction, since CEA is produced in large amounts by the tumor, usually it will not return to the normal levels after the recovery of jaundice.

As regard to previous observations, ROC curve for serum CEA in malignant group shows area under the curve was 0.964 (p<0.001) with cutoff value 26.72, the sensitivity and the specificity were 92.9% and 85.7% respectively, while ROC curve for serum CEA in benign group shows area under the curve was 0.036 (p<0.001) with cutoff value 28.675, the sensitivity and the specificity were 14.3% and 21.4% respectively . This corresponds with the view of Groblewska et al.,[31]; Liao et al.,[32]; Mroczko et a.l,[33] who reported that carcinoembryonic antigen was the first marker used for clinical diagnostics in the seventies and eighties. Throughout the past 20 years, CEA has been replaced by markers with higher diagnostic performance such as CA 19-9. Nevertheless, there are many studies on CEA which can be detected at low levels in fetal and normal adult tissue while high serum levels indicate presence of pancreatic cancer. The main problem of this biomarker is its low sensitivity of 25–56% at a high specificity of 82–100% for discriminating carcinoma from controls.

In this work, elevation of serum CEA was higher in malignant group than in benign one (P<0.001). This is consistent with Akdoğan et al.,[34] who reported that serum CEA level, in particular, can be elevated in some benign conditions such as cholangitis and biliary obstruction, which can cause confusion when it is used as a diagnostic test for gastrointestinal malignancy. Akdoğan et al.,[27] observed that some patients with benign disease had an elevated level of CEA; however, these levels in the malignant group were markedly higher.

In the present study, combined correlation between alkaline phosphatase and serum bilirubin with serum CEA in malignant group (r=0.745; P=0.002) and (r=0.684; P=0.007) respectively and also (r=0.662; P=0.010) and (r=0.770; P=0.001) in benign group respectively. This is in accordance with McLaughlin et al.,[21] who founded that there was a significant correlation between serum CEA levels and alkaline phosphatase and bilirubin in cholestatic patients. In contrast, Ni et al.,[25] serum CEA and had no significant correlations with serum bilirubin in cholestatic studied groups.

In this work, biliary level of CEA levels were found to be elevated in patients with malignant biliary diseases (P=0.020) and also elevated in benign group (P=0.044). This is agree with Duraker et al.,[19] who approved that the level of CEA in the bile of patients, both with and without malignancy, were high and widely distributed.

As regard, in this study, there is no correlation between biliary CEA and CA 19-9 levels in patients with malignant diseases (r=0.338; P=0.238) and also no correlation between biliary CEA and CA 19-9 levels in patients with benign diseases (r=0.0.209; P=0.474). This is consistent with Akdoğan et al.,[27] who reported that the levels of CEA or CA19-9 in the bile of patients, both with and without malignancy, were high and widely distributed. Biliary CEA levels in patients with malignant diseases tended to be higher when compared to benign group; however, both markers' bile levels failed to discriminate between benign and malignant disease as there is no correlation. Furthermore,

bile levels have a poor discriminatory value in comparison with serum levels.

In addition, in this work, there is correlation between serum CEA and CA 19-9 levels in patients with malignant diseases (r=0.600; P=0.023) and also between serum CEA and CA 19-9 levels in patients with benign diseases (r=0.618; P=0.019). This is consistent with McLaughlin et al.,[21] who founded that there was a significant correlation between serum CA19-9 levels and CEA in cholestatic studied groups.

One limitation of our study can be only single determination of these markers. The measurement of serial serum and bile levels after relief of obstruction may be of advantage in order to decrease the influence of cholestasis on the CEA and CA19- 9 levels[35]. Nevertheless it has been reported that removal of the obstruction of the biliary tract in patients with carcinoma did not result in a marked decrease of the marker bile levels. The authors suggested that CEA or CA19-9 levels in the bile were more influenced by marker production by the cancer than by the hepatobiliary factors [36]. In our study, no difference was found in the levels of these tumor markers in the bile between patients with malignant and benign disease. It has also been reported that the measurement of these antigens in bile seemed to be of little diagnostic value in the differentiation between malignant and benign diseases[37].

In conclusion, serum CA19-9 levels and CEA are increased both in malignant and benign obstructive biliary diseases. However, an increase in serum CEA is mostly restricted to malignant diseases. Measurement of these markers in the serum of obstructive jaundice may help in the detection of early tumor and determination of tumor stage, prognosis and recurrence. Unfortunately, no tumor markers are accurate enough to provide reliable information about tumor diagnosis and prognosis. In addition, measurement of these markers in the bile appears to be of no value in differentiation between benign and malignant biliary disease.

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Conflicts of interest: Non .

Ethical approval: The protocol of the study was approved by the ethical committee of Faculty of Medicine, Mansoura University. Informed consents were obtained from all patients.

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Corroboration of Serum Apolipoprotein J (Clusterin) as a Biomarker for Evaluating Hepatocellular Carcinoma

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Background and study aim: Hepatocellualr carcinoma (HCC) is an increasing problem in Egypt. Clusterin has been reported to play a significant role in tumorigenesis. The aim of this study is to evaluate clusterin as a marker for evaluating diagnosis and metastasis potential of HCC.

Patients and Methods: Eighty patients with HCC, 30 patients with liver cirrhosis, 30 patients with chronic hepatitis and 30 healthy controls were enrolled in study. The diagnosis of HCC patients was based on computed tomography. Estimation of serum clusterin was done by enzyme linked immunosorbent assay.

Results: Serum clusterin levels were significantly increased in patients with HCC ($P<0.001$). Serum clusterin reached the lowest significant levels in cirrhotic patients. Serum clusterin was highly increased in patients with poorly differentiated tumor and in those with capsular infiltration; also it was significantly related with portal vein invasion and lymph node infiltration. In addition, serum clusterin levels were significantly increased according to the progression of Barcelona Clinic Liver Cancer and Tumor-Nodes-Metastasis staging systems. However, these findings were not observed with alpha fetoprotein (AFP). Receiver operator characteristic curve showed that clusterin had a greater area under curve value (0.95) than that of AFP (0.85). At cutoff value 128 ug/ml, serum clusterin yielded 90% sensitivity and 87% specificity for predicting HCC. While at cutoff value 100 ng/ml, serum AFP had 75% sensitivity and 80% specificity.

Conclusion: We concluded that serum clusterin is a promising useful marker for diagnosis of HCC. Higher level of clusterin was closely related to capsular infiltration, venous invasion, lymph node metastasis and poorly differentiated tumor suggesting that clusterin might be deemed as a useful marker for predicting the progression and metastasis potential of HCC.

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide [1]. Hepatocellular carcinoma is currently the main cause of death in patients with hepatitis C virus (HCV) related cirrhosis, and the issue of HCC in Egypt is extensively increasing. In 2001, HCC was reported to account for about 4.7% of chronic liver disease (CLD) patients [2]. Over a decade, there was nearly two fold increase of the proportion of HCC among CLD in Egypt with significant decline of hepatitis B virus and slight increase of HCV as risk factor [3]. Hepatocellular carcinoma is often diagnosed at advanced stage where effective therapies are lacking, so the surveillance of patients at risk is necessary [4].

Clusterin (apolipoprotein J) is a highly conserved multifunctional glycoprotein present in almost all mammalian tissue and most human body fluid [5,6]. It has a wide degree of conservation and a wide degree of tissue distribution suggesting that it has a fundamental biological role. Its action resembles that of small heat

shock protein (sHsPs) since it binds to exposed hydrophobic regions of unfolded protein and inhibit aggregation by stabilizing them in an adenosine triphosphate independent manner [7,8]. Clusterin is implicated in various physiological processes including lipid transport, reproduction, complement regulation, tissue remodeling, senescence and cell interaction [9,10]. It has also been reported to play a significant role in stress response [11], apoptosis [12] and tumorigenesis [9]. In many diseases including human cancers, the expression status of clusterin might change at mRNA and protein levels. Some reports documented that a decrease of clusterin was observed in non-melanoma skin cancer [13], esophageal cell carcinoma [14], prostatic carcinoma [15]. However, in the majority of other human cancer such as, breast [16], lung [17], bladder [18] and colon cancers [19], upregulated expression of clusterin was detected. These reports suggested that changed expression of clusterin whether upregulated or downregulated may play an important role in tumorigenesis.

OBJECTIVE

This work aimed to investigate the role of serum clusterin as a biomarker for evaluating diagnosis and metastasis potential of HCC.

PATIENTS AND METHODS

This study was done in Tropical Medicine, General Surgery, Medical Biochemistry and Pathology Departments, Faculty of Medicine, Zagazig University. One hundred and seventy subjects were enrolled in this study. The subjects were classified into 4 groups:

Group 1: This group included 80 patients with established HCC (60 males and 20 females) with a main age of 55.5±6.4 years. The diagnosis of HCC was based mainly on typical imaging study and histopathology study (if available) according to American Association for Study of Liver Disease (AASLD) guideline [20]. Hepatocellular carcinoma tissue was diagnosed histologically by 2 expert pathologists when the surgical liver specimens were available (25 patients) in case of respectable hepatocellular carcinoma, while the remaining HCC patients were diagnosed according to imaging. Hepatocellular carcinoma tissues from 25 patients were histologically graded into one of three categories; well differentiated, moderately differentiated, or poorly differentiated according to criteria proposed by Liver Cancer Study Group of Japan [21]. The Barcelona Clinic Liver Cancer (BCLC) staging system was obtained which accounts for different factors of performance status, tumor burden, and hepatic function and categorizes patients into 5 stages which then help select the ideal candidates for the therapies currently available [22]. Tumor-Nodes-Metastasis (TNM) stage of HCC determined by The American Joint Committee on Cancer/United International Consensus Committee (AJCC/UICC) staging system for HCC [23] was obtained on the basis of imaging studies.

Group 2 : This group included 30 patients with viral related liver cirrhosis (16 males and 14 females) with mean age of 54.7±7.4 years. The diagnosis of liver cirrhosis was established on the basis of clinical, laboratory, imaging and histo-pathological examination.

Group 3: This group involved 30 patients(18 males and 12 females and their mean age of 53.9±9.6 years) with chronic viral hepatitis who were diagnosed by persistent elevation of ALT 3 times more than normal value for more than 6 months with no evidence of liver cirrhosis as confirmed by liver biopsy and histopathological examination.

Group 4: This group included 30 healthy age and sex matched controls with normal liver function (20 males and 10 females) with a mean age of 54.5±16 years.

All patients underwent complete history taking and thorough clinical examination, triphasic computed tomography (CT) and liver biopsy (when available) for histo-pathological examination.

Biochemical measurements:

Blood samples were drawn from all subjects after an overnight fast. Sera were separated immediately and stored at −20°C. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), Albumin, Alkaline phosphatase, bilirubin, prothrombin time and creatinine were measured in serum by routine enzymatic methods (spinreact). Serum Alph-fetoprotein (AFP) concentration was measured by ELISA kit (Biosource Europe S.A, Belgium). Serum HBsAg, HCV antibodies were measured by ELISA (Abbott laboratories, North Chicago,IL). Polymerase Chain Reaction (PCR) was used for detection of HCV RNA and HBV DNA.

Estimation of serum clusterin concentration in patients and controls:

Estimation of serum clusterin using Sandwich ELISA method according to manufacturer's instructions (Human clusterin ELISA, Bouendor laboratories Ltd Modrice Czech Republic) All diluted samples, quality controls were incubated in microtitration wells pre-coated with monoclonal antihuman clusterin antibody. After 10 min and washing, biotin-labeled second monocloncal antihuman clusterin antibody was added and incubated with captured clusterin for 60 min. After another washing, streptavidin-horse radish peroxidase (HRP) conjugate was added. After 30 min incubation and the last washing step, the remaining conjugated was allowed to read with substrate solution hydrogen peroxide and tetramethylbenzidine (TMB). The reaction was stopped by the dilution of acidic solution (0.2 M H_2SO_4) and absorbance of the resulting yellow product was measured spectrophotometrically at 450 nm. The absorbance was proportional to the concentration of clusterin. A standard curve was constructed by plotting absorbance value versus clusterin concentration of standards, and concentrations of unknown samples are determined using this standard curve.

Statistical analysis:

Data were analyzed with SPSS for version 15.0 (statistical package for the Social Science, Chicago, IL). Data were expressed using descriptive statistic (mean and standard deviation, and percentage and were analyzed using "*t*" and Chi-square tests. One way analysis of variance (ANOVA) test was done to compare of different parameters between more than two groups. AFP was expressed as median (range) and data analysis was done using Mann Whitney and Kruskal-Wallis tests. Pearson correlation coefficient was used to measure the association between clusterin and the other studied parameters. The receiver operator characteristic (ROC) curve with 95% confidence interval (CI) was performed to determine cutoff values for serum clusterin and AFP. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were determined. *P*-value was considered significant if <0.05 and highly significant if <0.001.

RESULTS

Clinical characteristic of HCC patients showed that most of them were males (n = 64), their mean ages (55.5±6.4) and most of them had hepatitis C virus (80%) (Table1). Serum clusterin levels were significantly elevated in HCC patients when compared to other groups (P<0.001). Cirrhotic patients had the lowest significant level of serum clusterin when compared to other groups (Table 2 and Fig. 1).

Serum clusterin levels were investigated according to various clinico-pathological features. There were no significant differences of serum clusterin levels according to size and number of tumor nodules (P>0.05). However, serum clusterin level was significantly overexpressed in patients with capsular infiltration, portal vein invasion, lymph node infiltration and poorly differentiated tumor. On the other hand, apart from significant increased of AFP according to the progression of size and increased numbers of tumor nodules, there were no significant differences according to other clinico-pathological parameters (Table 3).

Serum clusterin levels were significantly increased according to the progression of BCLC (Table 4) and TNM (Table 5) staging systems. However, these findings were not observed with AFP.

Correlation studies between serum clusterin and other parameters showed that serum clusterin had positive correlation with AFP with absence of correlation with Child- Pugh scores (Table 6).
Clusterin had greater area under curve (AUC) = 0.95 (CI; 0.90-0.99) than that of AFP = 0.85 (CI; 0.76-0.94) (Fig. 2). At cutoff value 128 ug/ml, serum clusterin had 90% sensitivity and 87% specificity. While at cutoff value 100 ng/ml, AFP had 75% sensitivity and 80% specificity (Table 7).

Table (1): Clinical characteristic of patients with HCC, cirrhosis and chronic hepatitis.

Parameters	HCC (N = 80)	Cirrhosis (N = 30)	Ch. hepatitis (N=30)	*P*
Age (years) X±SD	55.5±6.4	54.7±7.4	53.9±9.6	0.06 (NS)
Sex (n & %):				
Males	64(80%)	20 (66.7%)	21(70%)	0.06 (NS)
Females	16(20%)	10(33.3%)	9(30%)	0.06 (NS)
Viral cause (n & %):				
HCV	64(80%)	25(83.3%)	27{90%)	0.46 (NS)
HBV	12(15%)	3(10%)	2(7%)	0.4 (NS)
Both	4(5%)	2(6.7%)	1(3%)	0.8 (NS)
Child classification (n & %):				
A	42(52.5%)	15(50%)	30(100%)	<0.001 (HS)
B	32(40%)	12(40%)	0(0%)	<0.001 (HS)
C	6(7.5%)	3(10%)	0(0%)	0.2 (NS)
Alanin aminotransferse ALT (U/L)	73.2±23.5	59.2±19.5	85.2±24.5	<0.001 (HS)
Aspartate aminotransferse AST (U/L)	71.1±21.5	60.6±17.5	79.5±22.5	<0.001 (HS)
Alkaline phosphatase (U/L)	300.3±20.3	133.2±34.2	73.1±11.3	<0.001 (HS)
INR	1.2±0.4	1.34±0.3	1.1±0.3	0.29 (NS)
Albumin (g/l)	3.5±1.1	3.4±1.3	4.1±1.4	0.02 (S)
Bilirubin (mg/dl)	2.5±0.8	1.9± 0.5	1.2±0.4	<0.001 (HS)

HS=Highly significant S=Significant NS= Non significant

Table (2): Serum clusterin and AFP levels in HCC, cirrhosis, chronic hepatitis and healthy controls.

Groups	Serum clusterin level M±SD	Serum AFP level Median (range)
(G1) HCC (n = 80)	198.5±55.8	209(10-570)
(G2) Cirrhosis (n = 30)	44.4±6.9	35(4-210)
(G3) Ch. Hepatitis (n = 30)	117±18.5	10(1.7-135.1)
(G4) Healthy controls (n = 30)	113.1±18.3	2.3(1.5- 5.1)
F	122.3	106.7
P	<0.001(HS)	<0.001(HS)

AFP was expressed as median (range) and data analysis was done using Kruskal-Wallis test

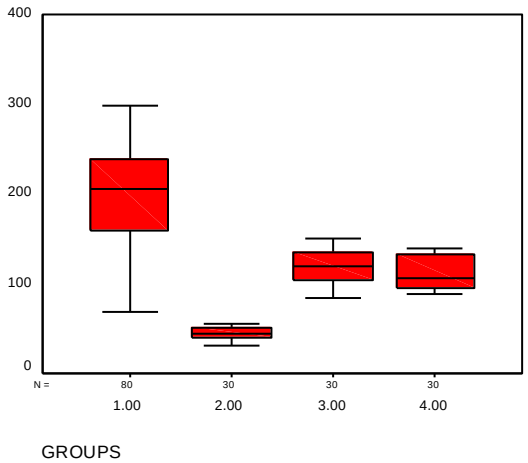

Fig. (1) : Serum clusterin in HCC, cirrhosis, chronic hepatitis and healthy controls

Table (3): Serum clusterin and AFP levels according to different clinico- pathological features.

Parameters	serum clusterin Mean ±SD	*P*	Serum AFP Median (range)	*P*
Tumor size:				
< 5 cm (n = 62)	198.9±55.9	0.73 (NS)	197(10-500)	0.005 (S)
≥ 5 cm (n = 18)	201.1±51.2		518(150-570)	
No of nodules:				
< 2 nodules (n = 66)	197.6±53.8	0.85 (NS)	200(10-518)	<0.001 (HS)
≥ 2 nodules (n = 14)	201±67.2		528(208-570)	
Infiltration of Glisson capsule:				
With capsular infiltration (n = 23)	240.3±31.5	<0.001 (HS)	200(18-570)	0.09 (NS)
Without capsular infiltration (n = 57)	180.5±54.6		238(10-538)	
Portal vein invasion:				
With portal vein invasion (n = 25)	242.3±28.4	<0.001 (HS)	286(15-570)	0.41 (NS)
Without portal vein invasion (n = 55)	177.4±53.6		208(10-538)	
Lymph node metastasis:				
With lymph node metastasis (n = 12)	260.1±58.19	<0.001 (HS)	249(20-570)	0.051 (NS)
Without lymph node metastasis (n =68)	183±51.1		207(10-528)	
Degree of differentiation (25 specimens):				
Well (n = 7)	145.5±41.5	<0.001 (HS)	169(21- 375)	0.16 (NS)
Moderate-poor (n = 18)	227.03±39.2		220 (31-538)	

NS = Non significant HS = Highly significant

AFP was expressed as median (range) and data analysis was done using Mann Whitney test

Table (4): Serum clusterin and AFP levels according to Barcelona classification.

Groups	Serum clusterin level M±SD	Serum AFP level Median (range)
Very early(n=38)	155.1±42.9	195(10-570)
Early (n=12)	207.5±19.9	323(10-570)
Intermediate (n=4)	211.5±7.5	315(130-500)
Advanced (n=16)	245.7±18.4	312.5(21-538)
Terminal (n=10)	271.8±16.6	375(19-528)
F	37.8	9.02
P	<0.001(HS)	0.06(NS)

HS= Highly significant NS= Non significant

AFP was expressed as median (range) and data analysis was done using Kruskal-Wallis test

Table (5): Serum clusterin and AFP levels according to TNM classification.

Groups	Serum clusterin level M±SD	Serum AFP level Median (range)
Stage I(n=40)	166.7±42.07	197.5(10-528)
Stage II(n=10)	171.8±56.2	208(15-570)
Stage III(n=18)	241.4±20.1	286(19-538)
Stage IV(n=12)	262.5±28.1	291.5(120-570)
F	29.13	5.2
P	<0.001(HS)	0.15(NS)

HS=Highly significant NS= Non significant

AFP was expressed as median (range) and data analysis was done using Kruskal-Wallis test

Table (6): correlation between serum clusterin and other clinical parameters.

Parameters	r	*P*
Child -Pugh score	0.27	0.08 (NS)
AFP	0.57	<0.001 (HS)
Size of tumor	0.08	0.62 (NS)
No of tumor nodules	0.22	0.16 (NS)

NS = Non significant HS = Highly significant

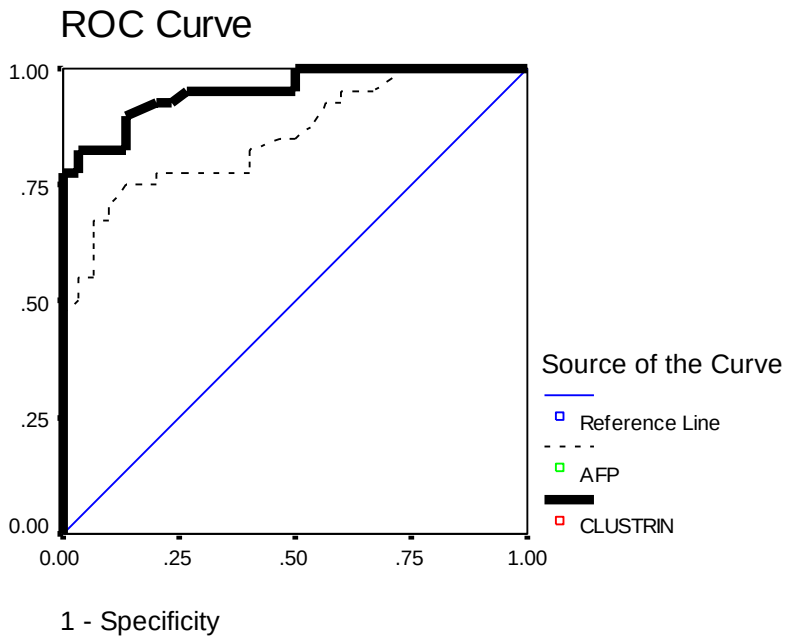

Fig. (2): ROC Curve of serum clusterin and AFP
AUC of clusterin= 0.95(CI: 0.90-0.99)
AUC of AFP= 0.85 (CI: 0.76-0.94)

Table (7): Validity of serum clusterin and AFP in the diagnosis of HCC.

	Sensitivity	Specificity	PPV	NPV
Clusterin	90%	87%	87.8	89.7
AFP	75%	80%	76.9	78.2

Clusterin cutoff value = 128 ug/ml
AFP cutoff value =100ng/ml
Cutoff values of both AFP and clusterin were determined by AUC
PPV = Positive predictive value NPV = Negative predictive value

DISCUSSION

Hepatocellular carcinoma is the sixth most common cancer and the third cause of caner related mortality in all over the world [24]. Screening of such higher risk patients with 3-6 month interval, using ultrasound (US) and AFP assay is generally recommended [25]. However, AFP has a limited role in HCC surveillance as it may increase in serum of some benign chronic liver disease patients or it may not increase in serum with some HCC patients [26]. However, El-Zayadi et al. [3] considered that α-fetoprotein is the most widely used tumor marker, but has poor diagnostic accuracy and ethnic variability. Although AFP improves detection of HCC, a significant number of HCC patients present without elevated AFP, and therefore additional markers are needed to increase the sensitivity and specificity. Therefore, early detection of HCC to improve its prognosis is an important issue for research.

Clusterin in human is a single-copy gene located on chromosome 8 p21-p12 that exhibition almost ubiquitous tissue expression pattern both during development and in adult [27]. Although number of reports has purported to explain clusterin functions in various cell types and tissue, including senescent and cancer cells, an understanding of clusterin function has remained elusive, especially in term of apoptosis and tumorigenesis [9].

Our result demonstrated that, clusterin levels were significantly higher in HCC patients than that in other different groups. Serum clusterin reached the lowest significant levels in cirrhotic patients without HCC. However, there was no significant difference of serum clusterin levels between healthy subjects and chronic hepatitis. Our data indicated that upregulated serum clusterin level in HCC patients might play a role in tumorigenesis and it could be used as a marker for early detection of cirrhotic liver that progressed to HCC. Significant lower level of serum clusterin in cirrhotic patients may be due to reduced liver cells mass or regenerating nodule can not be able to express clusterin like normal and malignant cells.

Wang et al. [28] reported that serum clusterin levels in HCC patients were significantly lower than in those with chronic hepatitis and healthy subjects, but it was higher than in those with cirrhosis. This result disagreed to our result as serum clusterin level in our study was significantly higher in patients with HCC than in healthy subjects and chronic hepatitis patients. However, it was not surprising that serum clusterin levels were higher in HCC patients when compared to healthy subjects and chronic hepatitis patients as clusterin is normally present in all tissue and human body fluid [6] and it can also be overexpressed in human neoplasm cells including HCC. So, clusterin level could be overexpressed in HCC patients when compared to other groups. Overexpression of clusterin has also been reported in HCC in other studies [29,30]. Moreover, Kang et al. [29] studied the immunoreactive pattern of clusterin in patients with HCC and found two distinct pattern of clusterin immunoreactivity namely cytoplasmic and canalicular. They also found that cytoplasmic overexpression might be an independent predictor of poor survival, as compared with the canalicular overexpression.

Upregulated serum clusterin was also reported in other tumor like lung cancer [31], colorectal carcinoma [32], urinary bladder cancer [33], and endometrial adenocarcinoma [34]. In other type of human cancer, such breast cancer [35] and esophageal squamous carcinoma [14], down-regulated serum clusterin was frequently observed.

In this study, we investigated serum clusterin levels according to different clinico-pathological features, we found no significant difference of serum clusterin levels according to size of tumor (either more of less than 5 cm) and according to numbers of tumor nodules. This indicated that clusterin could differentiate even small HCC (≤ 5 cm) from cirrhotic liver, which is of great importance as other marker can not distinguish between early small HCC and liver cirrhosis. On the other hand, AFP was significantly increased according to the progression of size and increased numbers of tumor nodules. This indicated that small size tumor or single tumor nodule can be missed if we depend on AFP for its diagnosis as it may not rise significantly in this situation.

Serum clusterin was highly expressed in patients with capsular infiltration compared to those without, also was significantly increased in patients with portal vein invasion and lymph node infiltration. Also, we found significant higher level of serum clusterin in patients with poorly-moderately differentiated tumor than in those with well differentiated tumor. Furthermore, serum clusterin was significantly increased with the

progression of BCLC and TNM staging systems of HCC. On the other hand, these findings were not observed as regard AFP. This result indicated that clusterin overexpression could point to HCC progression and enhanced metastasis potential of HCC.

Moreover, in our study we did not observe correlation between serum clusterin and the degree of deterioration of functional liver status with advancement of Child-Pugh score which indicated that increased serum clusterin levels in HCC patients could be related to the process of carcinogenesis rather than cirrhosis or fibrosis.

Our current data also showed that the sensitivity and specificity of serum clusterin in differentiation of HCC patients from cirrhosis were 90% and 87% respectively using a cutoff value of 128 ug/ml, while at cutoff value of 100 ng/ml AFP had 75% sensitivity and 80% specificity. Analyzing of AUC showed that serum clusterin had greater AUC (0.95) than that of AFP (0.85) which suggested that serum clusterin level might be superior to AFP in diagnosis of HCC and differentiating it from cirrhosis. This result agreed with that of Wang et al. [28] who found that at cutoff value 50 ug/ml, serum clusterin had 91% sensitivity and 83% specificity. The area under the ROC curve was 0.937 for clusterin versus 0.781 for AFP.

We concluded that serum clusterin was up regulated in HCC and more sensitive and specific than AFP for differentiating HCC patients from those with cirrhosis. It was closely related to capsular infiltration, portal vein invasion, lymph node metastasis and poorly differentiated tumor suggesting that clusterin might be deemed as a useful biomarker for diagnosis and predicting the metastasis potential of HCC.

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Conflicts of interest: None.

Ethical considerations: Special consideration was given to the right to confidentiality and anonymity of all patients.The patients are free to decide whether to participate or not. Informed consent from each patient was taken**.**

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Predictors of HCV Response to Treatment with Pegylated Interferon and Ribavirin in Sharkia Governorate

A retrospective study among patients involved in the National Campaign for Treatment of Chronic Hepatitis C in Sharkia Governorate in Egypt

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HCV, Pegylated interferon-α, PEG-INF, response predictors, ribavirin.

Background and study aim: The current standard of care (SOC) for chronic hepatitis C (CHC) in Egypt is pegylated interferon and ribavirin (PEG-INF/RBV) for 48 weeks, which is expensive, can be difficult to tolerate and with high failure rate. Additional information about predicting sustained virologic response (SVR) may be helpful in making proper decisions of treatment. This is a retrospective observational cohort study which was designed to identify predictors of SVR to PEG-INF/RBV among a cohort of Sharkian patients in Egypt

Patients and methods: 2991 patients with CHC fulfilling all inclusion and exclusion criteria for treatment were enrolled in this study. Patients were allocated randomly to treatment with either PEG-INF α2a/RBV (67.8%) or PEG-INF α2b/RBV (32.2%). Virologic monitoring was planned to be tested basically and at weeks 12, 24, 48 and 72 weeks after initiation of treatment.

Results: Analysis of data could be applied to 795 (26.6%) patients of the cohort because of their adherence to protocol and availability of treatment and follow up data. The remaining participants of the study (73.4%) were excluded because of lack of essential data and drop out for various reasons. Overall SVR was encountered in 404 (50.8%) patients and in 54.9% and 44.2% for patients treated with PEG-INF α2a and PEG-INF α2b respectively (p< 0.0001). Responders had statistically significant lower levels of fasting blood sugar, ALT, AST, indirect bilirubin, serum albumin, α fetoprotein, hemoglobin, stage of liver fibrosis and HCV RNA and higher values of alkaline phosphatase, prothrombin time , absolute neutrophil and platelet counts. In multivariate linear regression analysis, early virologic response (EVR) and receiving PEG-INF α2a (rather than PEG-INF α2b) were predictors of SVR.

Conclusion: Overall SVR among Sharkian patients with CHC, treated with SOC therapy is 50.8%. EVR is the best independent predictor of SVR. The form of PEG-INF may also predict the treatment response. None of the initial pretreatment variables could predict the SVR to treatment with the current SOC for CHC.

INTRODUCTION

Hepatitis C Virus (HCV) infection is a global health problem with worldwide prevalence of about 3% [1, 2]. The highest prevalence of infection in the world is recorded in Egypt. According to Egypt Demographic Health Survey (EDHS) published in 2009, 14.7% of Egyptians between 15 and 59 years old are positive for HCV antibodies and 9.8% have active infection with positive HCV RNA viremia [3]. This report of EDHS provides a precise national prevalence estimate and includes additional data on patterns of HCV prevalence by gender, age, urban vs. rural, and between different regions of the country. Age was the strongest and most consistently associated factor to HCV prevalence and HCV RNA positivity.

In Sharkia Governorate the prevalence of HCV infection is estimated to range from 4.8% among people < 20

years old to 41.9% among people > 40 years old, with an average prevalence of 25.8%[4]. The significant predictors of HCV infection, according to the same group of investigators, were previous parenteral therapy for Schistosomiasis among people more than 20 years old, blood transfusion, invasive procedures (surgery and endoscopy) and use of contaminated syringes as well as shaving at community barbers [4].

Validated mathematical models for estimating incidence from age-specific prevalence of HCV infection among Egyptians were used. The modeled incidence from the national study and collectively from the modeled incidence from the previous community studies was 6.9/1,000 [95% confidence interval (CI), 5.5–7.4] per person per year and 6.6/1,000 (95% CI, 5.1–7.0) per person per year, respectively. Projected to the age structure of the Egyptian population, more than 500,000 new HCV infections per year were estimated [5].

There are 6 major genotypes of HCV [6] and more than 50 subtypes [7, 8]. About 91% of the Egyptian patients are infected with genotype 4 [9]. Each genotype differs from the others by 30%-35% of its nucleotide site sequence and also exists as numerous genetically distinct isolates [6,10].

Each HCV genotype is unique with respect to its nucleotide sequence, geographic distribution, and response to therapy. Thus, each genotype can be considered a phylogenetically distinct entity requiring its own specific clinical appreciation. Knowledge of the epidemiology of HCV genotypes is essential not only for epidemiological reasons but also from a clinical standpoint. The infecting HCV strain is known to be one of the main independent factors that influence the outcome of antiviral therapy [7, 10]. Genotypes 1, 2, and 3 are common throughout the United States and Europe [7, 11] and have thus become the focus of much interest and research. The clinical presentation and management of infections arising from these viral genotypes has advanced rapidly. In contrast, genotype 4, which is prevalent in Egypt, has not been adequately studied; therefore, the management strategies for patients infected with this genotype are not as well developed .

The major problem of HCV infection, regarding its natural history, is the high rate of chronicity after viral infection. More than 80% of infected patients become chronic [12]. Chronic HCV infection leads to liver cirrhosis in 10-20% of cases within 20 years, with some studies showing estimates up to 50% [13, 14, 15, 16]. About 5% of HCV cirrhotic patients are at risk of decompensation every year [14]. Hepatocellular carcinoma is common in HCV patients with cirrhosis, with an estimated risk of up to 3% per year [17, 18].

The current standard of HCV therapy in Egypt is based on combination of pegylated interferon and ribavirin for 48 weeks. There are 3 kinds of pegylated interferon in the Egyptian market; Pegasys (Pegylated interferon α2a with 40 KD side chain) by Roche, PEG-Intron (Pegylated interferon α2b with 12 KD side chain) by Schering and Reiferon Retard (Pegylated interferon α2a with 20 KD side chain) by Minapharm.

Sustained virological response (SVR), defined as undetection of HCV RNA in patient's serum for 6 months after end of treatment, is ranging from 42.9% to 69% [19-28] in patients with genotype 4. This means that between 31-57% of patients fail to respond to the current therapy. This failure of response is likely because of a combination of viral and host factors.

Several studies, including pivotal trials, assessed baseline host and viral predictors such as body weight, ethnicity, liver histology, genotype, and viral load [29, 30]. In order of descent, viral load, ethnicity, fibrosis, steatosis, diabetes mellitus, and alanine aminotransferase (ALT) were found to have significant impact on sustained virological response in a multivariate regression analysis [31]. Viral load remains the most important single variable prior to therapy, but one that cannot be altered. Other variables, however, may be modified prior to treatment. It is clear that metabolic factors such as elevated fasting glucose and the histologic finding of steatosis are important negative predictors [32].

Considering the high failure rate of response in addition to the very high cost and significant side effects of the current treatment, predicting the possibility of response to treatment prior to initiating therapy would be very useful.

We hypothesize that identification of validated pretreatment predictors of response and failure among our unique patients would help in forwarding our national guidelines of HCV

treatment for better clinical outcomes and more favorable economic impact.

The objective of this study is to find out what are the reliable baseline predictors of treatment response unique to Egyptian patients with chronic hepatitis C infection. Another objective is to identify the SVR rate among our patients.

PATIENTS AND METHODS

This is a retrospective observational study analyzing data of patients selected for SOC therapy for CHC in "Al-Ahrar Center for Treatment of Hepatitis C Infection". This center is located in Zagazig City, the capital of Sharkia Governorate, one of the Eastern Delta Governorates of Egypt. This center, and many others all over Egypt, was established in February 2008 by the "National Committee for Treatment of Viral Hepatitis". Patients were enrolled in the study from March 12, 2008 to June 3, 2009 with follow up to November 4, 2010. All patients were treated under full sponsorship by Ministry of Health.

Patients were selected according to the inclusion and exclusion criteria of the national protocol for the treatment of CHC and were subjected to: thorough history taking, complete clinical examination and undergone the pre-enrollment investigations in the form of fasting blood glucose level and HbA1C for diabetic patients, serum creatinine, serum albumin, AST and ALT serum level, serum alkaline phosphatase level, total & direct bilirubin levels, hemoglobin concentration, WBC's count and platelets count, prothrombin time , INR , PTT , PC, pregnancy test for female patients, hepatitis B surface antigen, anti HCV antibody, quantitative HCV RNA (PCR) assay, serum α-fetoprotein level, ANA titer, TSH level, pelvi-abdominal ultrasonography, ECG for male patient over 40 years, female patient over 50 years, Ocular and fundus examination, Anti-schistozomal antibody and liver biopsy with histopathological examination using Metavir Scoring System for assessment of stage of fibrosis and grade of inflammatory activity.

All patients must fulfill all the Inclusion criteria which are: male or female patient aged 18 - 60 y, Hb ≥ 12 g/dl (for males) and 11 g/dl (for females), WBC's count ≥ 3,500/µL , neutrophil's count ≥ 1,500/µL and platelet's count≥ 85,000/ /µL, Prothrombin time not more than 3 sec. above the control value, direct bilirubin ≤ 0.4mg/dl or within 20% of ULN, indirect bilirubin ≤ 1 mg/dl or within 20% of ULN, fasting blood glucose ≤ 115 mg /dl or HbA1c < 8.5 for diabetics, serum albumin ≥ 3.5 g/dl, serum creatinine < 1.5 mg/dl, negative hepatitis B surface antigen, negative ANA or less than 1/160, positive anti- HCV antibody, positive HCV– RNA , serum α-fetoprotein less than 100 ng/ml , proper contraceptive methods for both partners(double contraception) and a written informed consent.

Exclusion criteria of the treatment program include : patients with any other cause of liver disease (Hepatitis B, hemochromatosis, Wilson's disease), alcoholic liver disease, fatty liver disease, decompensated liver disease, hypersensitivity to interferon or ribavirin, autoimmune liver or systemic diseases, pregnant or breast feeding female, clinically significant retinal disease, acute coronary syndrome in the last 6 months, drugs related liver disease, patients with CNS trauma that require medical treatment and patients with active seizures.

Candidate patients were allocated randomly for treatment with one of two regimens: (1) Pegylated interferon α2a (180 µg s.c once weekly) plus ribavirin (1000-1200 mg p.o daily for patients with body weight < 75 or ≥ 75 kg respectively). (2) Pegylated interferon α2b (1.5 µg s.c once weekly) plus ribavirin (800-1400 mg p.o daily according to body weight as recommended by the manufacturer).

The protocol for follow up of treated patients included regular clinical evaluation with inquiry about any side effects and laboratory estimation of CBC, ALT, AST, Total bilirubin, serum creatinine at week 1, week 2 , week 4 and every 4 weeks thereafter up to week 48. HCV RNA was tested at weeks 12, 24, 48 and 72 after initiation of treatment. The patient was considered responding and continued treatment if the 12 week HCV RNA was undetected (complete early virologic response) or reduced to less than 1% of pretreatment levels (partial early virologic response). If the HCV RNA didn't drop to less than 1% of baseline level at week 12 or was detected qualitatively at week 24 the patient was considered not responding and his treatment was stopped at that point. SVR was achieved if HCV RNA became undetected at week 24 and remained so up to week 72 of follow up.

STATISTICAL ANALYSIS

Data were checked, entered and analyzed using software computer package (SPSS version 17) for interpretation of the results. Data expressed as number and percentage for qualitative variables and as mean ± standard deviation for quantitative variables. Significance was used as appropriate and P less than 0.05 was considered statistically significant. The t-test for independent samples was used to compare variables of two groups. Chi square was used to compare proportions. Multivariate linear regression analysis was used to identify independent predictors of SVR.

RESULTS

We identified 2991 patients who started treatment with PEG-INF/RBV (fig.1). 2196 (73.4) patients were excluded (drop out) because of many reasons: 1) transfer to other centers (for geographical reasons), 2) intolerability of medications, 3) discontinuation of treatment because of occurrence of serious side effects, 4) loss of follow up mostly with lack of HCV RNA results at end of treatment or at week 72, 5) missing essential data that interfere with proper analysis.

Only 795 (26.6%) subjects of the cohort were available with complete data (basic and follow up) for protocol statistical analysis.

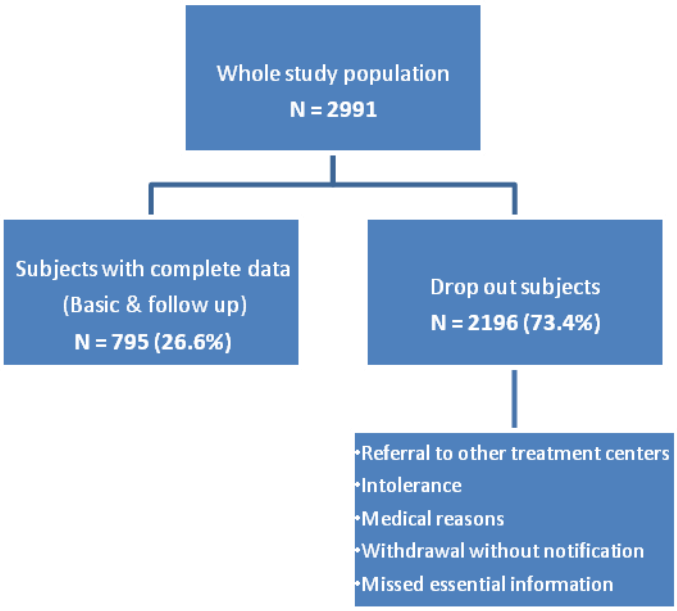

Figure (1): A chart showing subjects enrolled in the study with intension to treat (ITT)

The chart in fig.2 shows the response of these patients to treatment. EVR {defined as ≥ 2 log reduction (pEVR) or undetection of HCV RNA (cEVR) after 12 weeks of therapy} was encountered in 666 (83.8%) patients. 585 (73.6%) and 81 (10.2%) patients achieved cEVR and pEVR respectively.

ETR (defined as undetection of HCV RNA after 48 weeks of therapy was identified in 484 (60.9%) patients. SVR (defined as undetection of HCV RNA 24 weeks after end of treatment was identified in 404 (50.8%) patients as per protocol analysis. Considering the initial number of patients enrolled in the study with intention to treat (ITT), this percentage of patients with SVR drops to 13.5% which raises a questionable conclusion in front of the health policy-makers in Egypt.

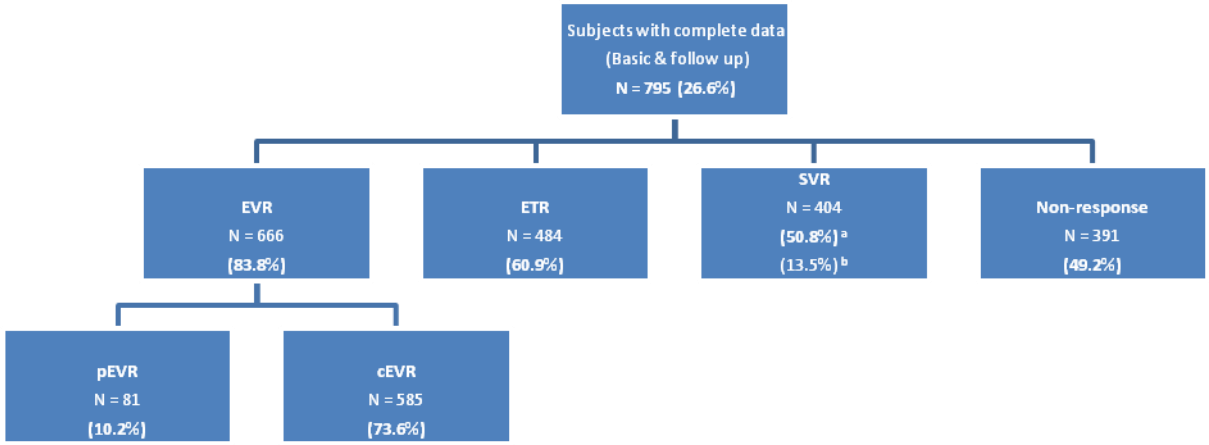

Figure (2) A chart showing Virologic Response to Treatment (EVR = early virologic response, ETR = end of treatment response, SVR = sustained virologic response, pEVR = partial virologic response, cEVR = complete early virologic response). **a:** Per protocol analysis **b:** per ITT analysis

The chart in fig.3 shows the response to treatment with different types of PEG-INF in our protocol. SVR was encountered in 54.8% and 43.9% in patients treated with PEG-INF α2a and PEG-INF α2b respectively (χ^2=24.144, p<0.0001).

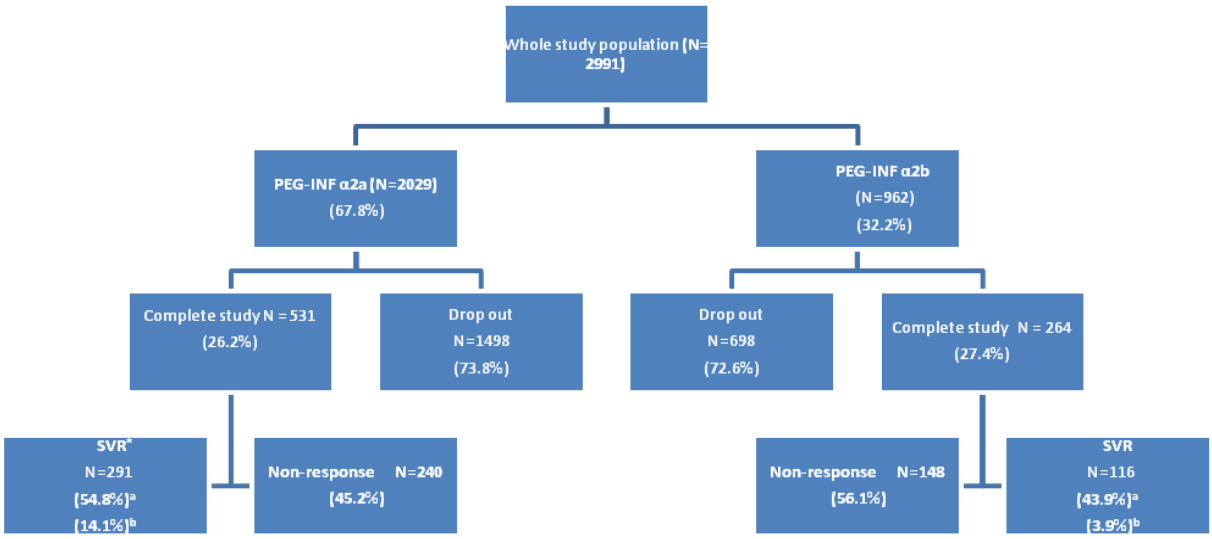

Figure (3) A chart showing Virologic Response to Treatment with PEG-INF α2a & PEG-INF α2b (a: Per protocol analysis **b:** per ITT analysis)
* p<0.0001 (χ^2 =24.144) compared with SVR in PEG-INF α2b arm

Table (1) shows the baseline characteristics of study population. 795 patients were included with average age 41.7 ± 8.8 years, 617 (77.6%) patients were males with average body weight 83±12.4 kg and BMI 30.3±3.86 kg/m^2.

Table (2) compares the mean ± SD of various data between responders and non-responders to treatment with PEG-INF/RBV. Responders were significantly characterized by having more percentage of males, lower levels of FBS, ALT, AST, Indirect bilirubin, serum albumin, α fetoprotein and hemoglobin, but higher levels of serum ALKP, PT, ANC and platelet count. Responders were also characterized by having

milder degrees of liver fibrosis and lower baseline values of serum HCV RNA. There was no statistically significant difference between responders and non-responders regarding age, body weight or BMI.

Table (3) compares the mean ± SD of various baseline data and SVR between patients treated with PEG-INF α2a and PEG-INF α2b. The percentage of males was significantly higher in patients treated with PEG-INF α2a, while the degree of inflammatory activity was milder in patients treated with PEG-INF α2b. Regarding other baseline variables, there was no statistically significant difference between both groups.

SVR was encountered in 54.9% and 44.2% of patients treated with PEG-INF α2a and PEG-INF α2b respectively (χ^2 =24.144, p<0.0001).

Using multivariate linear regression analysis (table 4), EVR was the best independent predictor of SVR (B±SE=0.67±0.118، 95% C.I =0.436 - 0.903, p 0000) followed by PEG-INF α2a (rather than PEG-INF α2b) (B±SE=0.378±0.108، 95% C.I =0.163 - 0.593, p 0.001).

Table (1): Baseline clinical, biochemical, histopathological and virological characteristics of the study subjects

Parameters	Minimum	Maximum	Mean	Std. Deviation (SD)
Age (years)	18	60	41.7	8.84
Weight (kg)	39	129	83	12.4
Height (cm)	141	190	173	9.11
BMI (kg/m^2)	22.22	39.4	30.3	3.86
Ribavirin (mg)	800	1400	1095.6	139.1
FBS (mg/dl)	55	346	97.1	29.7
Creatinine (mg/dl)	0.3	1.5	0.79	0.17
Albumin (g/dl)	3.5	5.3	4.1	0.44
ALKP (U/L)	11	310	113	43.42
AST (U/L)	2	272	40.9	37.39
ALT (U/L)	4	317	43.0	41.14
T.bilirubin (mg/dl)	0.1	1.5	0.84	0.25
I. bilirubin (mg/dl)	0.01	1	0.4	0.26
WBC ((/µL)	3500	17200	6772	2081
Hb (g/dl)	11	19.8	14.2	1.57
Plt. (/µL)	75000	770000	199850	65574
ANC (/µL)	1500	12300	3750.3	1591.2
PT (Seconds)	9.9	17	12.6	1.23
TSH (mU/L)	.03	17	1.85	1.26
HCV RNA (IU/ml)	97	47600000	534792	213287
AFP (ng/ml)	0.50	85	5.85	9.44
Fibrosis (Fo-4)	0.00	4	2.03	1.04
Activity (A0-3)	0.00	3	1.81	0.79

(BMI = body mass index, FBS = fasting blood sugar, ALKP = alkaline phosphatase, AST = aspartate aminotransferase, ALT = alanine aminotransferase, T.bilirubin = total bilirubin, I.bilirubin = indirect bilirubin, WBC = white blood cells, Hb = hemoglobin, Plt = platelets, ANC = absolute neutrophil count, PT = prothrombin time, TSH = thyroid stimulating hormone, AFP = alpha fetoprotein)

Table (2): Comparison of the mean ± SD of various clinical, biochemical and pathological parameters of the study

Parameters	**Responders (n=404)**	**Non-responders (n=391)**	t	P value
Age (y)	41.2± 8.8	42.1± 8.9	1.44	0.151
Sex M/F n (%)	325/79 (80.4/19.6)	292/99 (74.7/25.3)	X^2= 7.05	0.0079
Body weight(KG)	83.12 ± 12.5	82.9 ± 12.3	0.282	0.778
BMI (KG/m2)	30.2 ± 4.4	30.4 ± 3.5	0.129	0.898
FBS (mg/dl)	93.14 ± 21.7	101.3 ± 35.8	3.885	0.000
Serum creatinine (mg/dl)	0.79 ± 0.15	0.8 ± 0.18	0.913	0.361
Serum albumin (g/dl)	4.19 ± 0.42	4.08 ± 0.45	5.530	0.018
ALT (U/L)	35.9 ± 36.8	50.4 ± 44	4.987	0.000
AST (U/L)	31.4 ± 28.4	50.7 ± 42.8	7.468	0.000
Total Bilirubin (mg/dl)	0.82 ± 0.23	0.85 ± 0.27	1.765	0.078
Indirect Bilirubin (mg/dl)	0.41 ± 0.26	0.48 ± 0.27	2.886	0.006
ALKP (U/L)	117.6 ± 41.9	108.1 ± 44.5	3.028	0.003
Hb (g/dl)	14.09 ± 1.56	14.36 ± 1.58	2.432	0.015
WBC (/µL)	7023.4 ± 2206.6	6509.2 ± 1910.2	3.478	0.001
ANC (/µL)	3977.3 ± 1731.9	3483.8 ± 1368.5	2.404	0.017
Platelets (/µL)	207489 ± 58018	191871 ± 71845	3.349	0.001
AFP (ng/ml)	3.73 ± 4.79	8.12 ± 12.26	6.621	0.000
TSH (mU/L)	1.84 ± 1.136	1.85 ± 1.377	0.099	0.921
PT (seconds)	12.78 ± 1.19	12.48 ± 1.26	3.413	0.001
HCR RNA (IU/ml)	368961 ± 100002	707533 ± 286351	2.228	0.026
Fibrosis degree (F0-4)	1.94 ± 0.95	2.13 ± 1.11	2.453	0.014
Inflammatory activity (A0-3)	1.77 ± 0.79	1.84 ± 0.78	1.152	0.25
Ribavirin dosage (mg)	1091.8 ± 136.2	1099.7 ± 142.1	0.799	0.424

$P < 0.05$ was considered statistically significant.

Table (3): Comparison of the mean ± SD of various clinical, biochemical and pathological parameters of PEG-INF α2a -based therapy vs PEG-INF α2b -based therapy

Parameters	**PEG-INF α2a** (n=521)	**PEG-INF α2b** (n=260)	t	p
Age (Y)	42.1 ± 8.7	40.8 ± 8.9	1.92	0.055
Sex M/F (%)	398/122(76.5/23.5)	207/52 (80/20)	X= 3.89	0.049
Weight (Kg)	83.4±12.39	82 ± 12.46	1.53	0.125
BMI (Kg/m^2)	30.2±3.55	30.45 ± 4.57	0.197	0.844
Ribavirin (mg)	1096.5±136	1093.85 ± 145.33	0.255	0.799
FBS (mg/dl)	97.3±28.7	96.1 ± 29.8	0.55	0.582
Creatinine (mg/dl)	0.79±0.17	0.8 ± 0.16	1.009	0.313
Albumin (g/dl)	4.1±0.43	4.2 ± 0.45	1.832	0.067
ALKP (U/L)	114.15±42.66	111.41± 44.82	0.817	0.414
AST (U/L)	41.3±36.79	39.8 ± 38.78	0.531	0.595
ALT (U/L)	44.5±43.28	39.8 ±36.59	1.5	0.133
T. bilirubin (mg/dl)	0.845±0.26	0.82 ± 0.22	1.110	0.267
I. bilirubin (mg/dl)	0.46±0.27	0.42 ± 0.26	1.69	0.091
WBC (/uL)	6772±2011.9	6808 ± 2210.1	0.229	0.819
Hb (g/dl)	14.21±1.56	14.26 ± 1.6	0.442	0.658
Platelets (/uL)	200350.2±62424	199662.8±72048.7	0.137	0.891
ANC (/uL)	3747.8±1642.8	3757.3 ± 1455	0.041	0.967
PT (seconds)	12.68±1.21	12.55 ± 1.28	1.39	0.165
TSH (mU/L)	1.87±1.35	1.81 ± 1.07	0.571	0.568
RNA (IU/ml)	451824±104650	696031±339466	1.5	0.134
AFP (ng/ml)	6.2±10.53	5.1 ± 6.88	1.6	0.11
Fibrosis (F0-4)	2.02±1.03	2.04± 1.06	0.169	0.866
Activity (A0-3)	1.87±0.80	1.68 ± 0.74	3.07	0.002
SVR	54.9%	44.2%	χ^2 =24.144	<0.0001

P < 0.05 was considered statistically significant.

Table (4) Independent predictors of SVR in linear regression analysis

	B±SE	95% C.I	P
EVR	0.67±0.118	0.436 - 0.903	0.000
PEG-INF α2a	0.378±0.108	0.163 - 0.593	0.001

DISCUSSION

Although Baseline predictors are useful tools in assessing the relative difficulty of clearing hepatitis C virus (HCV), they have limited utility for selecting which patient should be considered for therapy and those patients with a reduced likelihood of successful therapy, perhaps sparing them the side effects and cost of therapy. Despite use of multiple variables, still there is no reliable way to ascribe an odds ratio for the chance that a particular patient will respond to therapy.

In chronic hepatitis C, the primary therapeutic goal is SVR, defined as undetectable HCV RNA by a sensitive assay at the end of a 24-week follow-up period after treatment completion. The current combination therapy consisting of pegylated (PEG) IFN plus ribavirin (RBV) for at least 48 weeks may be accompanied by numerous potentially dose-limiting side effects and SVR rates are still unsatisfactory with only approximately 50%[33,34] .

In the present study, responders were significantly characterized by having lower

levels of serum α fetoprotein. Moreover, there was a statistically significant difference between responders and non-responders regarding FBS. Many studies reported that diabetic patients achieved a lower SVR rate than that in nondiabetic subjects [35, 36]. It is clear that metabolic factors such as elevated fasting glucose and the histologic finding of steatosis are important negative predictors [32]. Moreover, higher baseline serum AFP levels predicted a lower SVR rate among patients with chronic hepatitis C [37]. However, we have shown that neither diabetes mellitus nor AFP levels were found to be independent predictors by multivariate regression analysis. These factors need to be assessed in HCV-4 patients in well-designed prospective studies.

Furthermore, responders were significantly having low ALT and AST [31]. A study by Zechini et al showed a statistically significant positive correlation of baseline aminotransferase values with the hepatitis activity index and fibrosis score, liver biopsies carried out in patients with normal transaminases show some degree of hepatic lesion, although, in most cases, histopathology of the lesion is mild and progression to fibrosis lower than in patients with elevated ALT [38].

A significant higher level of serum albumin, serum ALKP, PT, ANC (absolute neutrophil count) and platelet count were found in responder than in non-responder patients. However , further studies will be required to determine whether these findings bear any relationship to differences in rates of response to therapy among HCV patients.

In the present study, SVR was encountered in 50.8% of those who completed treatment. These results are similar to the responses achieved in previous studies that an anticipated SVR in genotype 4 patients is around 50% to 70% [39, 40]. Our study demonstrate a significant difference in SVR rates between pegylated interferon (PEG-IFN)- 2a plus ribavirin and PEG-INF α2b plus ribavirin respectively (e.g., 54.9% versus 44.2%; P = p<0.0001). However, the use of PEG-INF alfa-2 and RBV for 48 weeks lead to a substantial improvement in the rate of SVR [40)]

This study reported that a milder degrees of liver fibrosis predicted favorable response to treatment with pegylated interferon plus ribavirin in an analysis of participants [36, 40]. However, neither the fibrosis stage nor the inflammation grade in the pre-treatment liver biopsy was found to be independent predictor of SVR .

HCV viral load is one of the important factors to influence the response of antiviral therapy and considered as proxy determination of HCV replication [24, 41]. In this study responders were significantly characterized by having lower baseline values of serum HCV RNA. However HCV viral load was not found to be an independent predictor of SVR in our study. This is contrary to what was previously reported by other study [28].

In the present study, we found that EVR was the best independent predictor of SVR, so monitoring the early antiviral response to therapy can help identify those patients who are less likely to achieve SVR and therefore provide critical information for the overall management of patients with chronic hepatitis C [42, 43]. Also, we found that the type of interferon used in treatment was a contributor to the eventual outcome of therapy. Patients treated with pegylated interferon 2a were more likely to achieve viral clearance than those receiving the 2b form.

We can conclude that, the most reliable predictor of successful treatment is Early Virological Response, or EVR. If EVR is attained, treatment is continued, side effects and other factors permitting. If EVR is not attained, the patient is usually withdrawn from treatment, as the risk of taking the medication outweighs the predicted benefit of therapy. Form of PEG-INF may also predict the treatment response. None of the initial pretreatment variables could predict the SVR to treatment with the current SOC for CHC..

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Extrahepatic Manifestations of Hepatitis C Virus: An Extending List

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Hepatitis C virus is a hepato-lymphotropic virus that was also detected in various organs of the body. Furthermore, this form of infection may be presented for the first time by an extrahepatic manifestation. Different extrahepatic manifestations for hepatitis C was described; these extend from strong associations as in mixed cryoglobulinemia to others were anecdotal finding as vitiligo was reported. The list of extrahepatic manifestations of hepatitis C is extending. In this review we tried to shade the light on this expanding list.

INTRODUCTION

Extrahepatic manifestations (EHM) of hepatitis C virus (HCV) include diseases that affect organs other than the liver, and it may be the first presentation of HCV infection. Association between them was first described in 1990 with cryoglobulinemia [1]. Subsequently, nearly all organs were reported as kidney, skin and thyroid [2]. Up to 40-74% of patients infected with HCV might develop at least one extrahepatic manifestation during the course of their disease [3].

PATHOGENESIS

HCV replicates within extrahepatic tissues with expression of viral proteins, leading to EHM. An important feature of HCV is that the virus avoids immune elimination. The consequences are chronic infection, accumulation of immune complexes and auto-immune phenomena. HCV shows lymphotropism in addition to the hepatotropism, which is responsible for many EHM [4].

I) Autoimmunity

- Auto antibodies production

The cellular components leak from the persistent destruction of the infected cells. About 20% with hepatitis C patients are ANA positive [5].

- The molecular mimicry between HCV and auto antigens [6].

- Abnormality of lymphocytic cells

HCV infection and proliferation within lymphocytes leads to functional alteration of lymphocyte and production of excessive auto antibodies and cryoglobulins [7].

II) HCV infection of cells other than hepatocytes

HCV binds several cell surface receptors. Cell tropism required for HCV genome replication are not well characterized [8].

Classification of EHM of hepatitis C virus

EHM classified into four groups according to degree of association recorded to HCV infection.

Table (1): EHM Classification [9].

Group A	Group B	Group C	Group D
Strong association	Significant association	Similar pathologic nature	Anecdotal
- Mixed cryoglobulinemia - B-cell non-Hodgkin's lymphoma	- Monoclonal gammopathies - Porphyria cutanea tarda - Lichen planus - Diabetes mellitus	- Autoimmune thyroiditis - Thyroid cancer - Sicca syndrome - Idiopathic lung fibrosis - Non cryoglobulinemic nephropathies - Erectile dysfunctions - Carotid atherosclerosis - Psychopathological disorders	- Psoriasis - Peripheral/central neuropathies - Rheumatoid arthritis - Polyarteritis nodosa - Behcet's syndrome - dermatomyositis - Fibromyalgia - Chronic pruritus - Kaposi's pseudosarcoma - Vitiligo - Cardiomyopathies - Mooren corneal ulcer -Necrolytic acral erythema

Mixed cryoglobulinemia (MC)

Is a systemic vasculitis characterized by the deposition of circulating immuno-complexes in small and medium-sized blood vessels resulting in clinical manifestations [10].

Cryoglobulins

Are serum proteins that precipitate at low temperatures and then redissolve during incubation at 37ºC. Different categories have been described that refer to their different immunologic compositions [11].

Table (2): Classification of cryoglobulins [12].

Type	Clonality of immunoglobulins	Associated diseases
I	Monoclonal immunoglobulins (IgM or IgG)	Lymphoproliferative diseases
II (mixed)	Polyclonal immunoglobulins (mainly IgG) plus monoclonal Immunoglobulins (IgM, IgG, IgA)	Mixed cryoglobulinemia
III (mixed)	Polyclonal IgG and polyclonal IgM	Mixed cryoglobulinemia

Prevalence

Although overt symptoms of cryoglobulinemic vasculitis develop in only approximately 5% of chronic HCV infection cases, circulating mixed cryoglobulin complexes are much more common in about 40–50% in chronic HCV-infected patients [10].

Significant geographic diversity appears among patients with HCV-related MC, with greater prevalence in southern Europe compared to northern Europe and North America, with high values (over 90%) in the Mediterranean area [13].

Pathogenesis

The Circulating immune complexes in HCV related MC comprises hepatitis C virions , IgG-IgM-RF antibody complexes and complement [14].

The common hypothesis regarding HCV-related cryoglobulinemia is the chronic antigenic stimulation of the humoral immune system, which facilitates clonal B-lymphocyte expansion [15].

Other hypotheses:

-Chronic HCV infection of B cells and Bcl-2 activation (protoncogene) which increase B cell survival by inhibiting apoptosis [16].

-Interaction of HCV E2 envelope protein with the cell surface glycoprotein CD81 that is present on B cells as well as on hepatocyte reduces the threshold for B-cell activation. HCV-specific proteins also demonstrate molecular mimicry with auto antigens. NS5A and NS core proteins can simulate host auto antigens, possibly resulting in B-lymphocyte activation and auto antibody production which may allow cross-

reaction between a virus-associated epitope and IgG auto antigen [17].

-Cytokine, B-cell activating factor of the tumor necrosis factor family (BAFF) also known as B-lymphocyte stimulators as zTNF4 were found in high levels in patients with MC associated HCV [18].

Correlation with Liver Disease

MC tends to correlate with duration of HCV infection and older age. However, cryoglobulinemia in the serum of HCV patients has been associated with increased risk of advanced fibrosis, the severity of hepatic steatosis on liver biopsy and cirrhosis, irrespective of age or disease duration [19].

Clinical features of MC

More common symptoms are general malaise, arthralgia and weakness. Arthralgia without arthritis is common, typically affecting the proximal interphalangeal joints of the hands, metacarpophalangeal joints, knees, and hips [20].

1- Skin

Commonly involved (95% of cases) with a cutaneous vasculitis ranging from palpable purpura (leukocytoklastic vasculitis) and petechiae in the lower extremities to large necrotic ulcerations. Raynaud's phenomenon occurs in up to 1/3 of cases and involves hands, feet, lips, ears, and the tip of the nose [20]

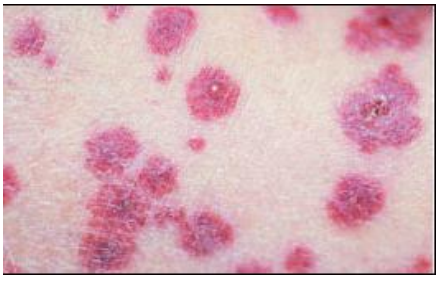

Figure (1): Leukocytoclastic vasculitis [21]

2- Kidney

Frequently involved (35-60%) and Membranous proliferative glomerulonephritis (MPGN) is the prevalent type associated with MC [22]. Anti-HCV-Ab is universal in patients with both cryoglobulinemia and MPGN. HCV-RNA is present in nearly 81% of MC related MPGN versus only 25% of cases of non-cryoglobulinemic MPGN [23].

Less often HCV causes focal segmental glomerular sclerosis or membranous or proliferative glomerulonephritis [24]. The course of renal pathology is variable. A clinical regression is observed in 10-15% of patients with nephritic syndrome. In 30% of cases, the clinical trend is slow and renal function is maintained for many years. In 20% of patients the disease is characterized by recurrent episodes of nephritic syndrome. In less than 15% of MC, dialysis is required [25].

3- Peripheral neuropathy

Mostly sensory and is characterized by numbness, burning, needles and pins sensations most often in the hands and feet [26].

4- Central nervous system (CNS)

CNS involvement in patients with HCV-positive MC is rare [27].

5-Other manifestations

Rarely other organs as lungs, GIT and heart may be involved, secondary to vasculitis has been reported [28].

Diagnosis of MC

Classification criteria for MC diagnosis include clinical and serological data. Some patients with chronic HCV infection may show complete or even incomplete forms of MC. In the latter, a strict follow up of the patient is required [29].

Table (3): Criteria for the diagnosis and classification of patients with MC [29]**.**

Criteria	Serologic	Pathologic	Clinical
Major	Mixed cryoglobulins Low C4	Leukocytoclastic vasculitis	Purpura
Minor	RF HCV+, HBV+	Clonal B-cell infiltrates (liver and/ or bone marrow)	Chronic hepatitis, MPGN, peripheral neuropathy, skin ulcers

"Definite" Mixed Cryoglobulinemia Syndrome

1) Serum mixed cryoglobulins (± low C4) + purpura + leukocytoclastic vasculitis

2) Serum mixed cryoglobulins (± low C4) + 2 minor clinical symptoms + 2 minor serological/pathologic findings [29].

Biopsy of skin lesions shows immune-complexes vasculitis of small vessels with mononuclear infiltration. HCV antigens are detected in skin lesions in 40% of cases [30].

Renal biopsy demonstrates deposits of IgG-IgM-RF activity and C3 in capillary loops. The most characteristic findings are the capillary thrombi consisting of precipitated cryoglobulins at light microscopy [31].

Nerve Biopsy shows show axonal degeneration, differential fascicular loss of axons, demyelinization signs and small-vessel vasculitis with mononuclear cell infiltrates in the perivascular area [32].

Treatment of MC

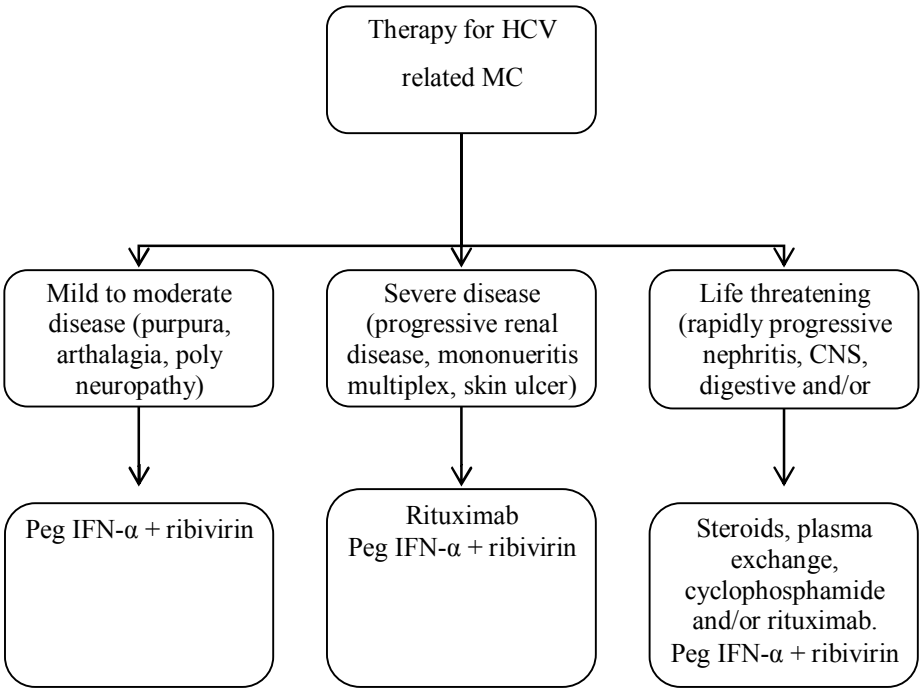

Figure (2): Therapeutic strategies in patients with HCV-associated MC [33].

Therapy should be initiated for patients with symptomatic MC, and is directed to both the virus and the immune-mediated inflammation [34].

Plasma exchange (PE)

PE is the removal of circulating immunocomplexes. Immunosuppressive therapy is usually associated with plasma exchange in order to avoid the rebound increase in cryoglobulinaemia that is commonly seen after discontinuation of exchange [35]. When used in combination with anti-HCV treatment, plasmapheresis did not modify the virological response [36].

Antiviral Therapy

Antiviral therapy is the mainstay of long-term control of both the hepatic and extrahepatic manifestations. It should be started after systemic vasculitis has come under initial control, because the disease can be exacerbated with the initiation of interferon therapy in some patients [37].

IFN alfa monotherapy:

Can reduce viral load and induce clinical improvement in MC. However, relapse within a few months of therapy withdrawal is common [38].

Pegylated IFN-a:

Results showed improving tolerability and giving good outcomes [39].

Ribavirin monotherapy:

Ribavirin may be effective in IFN-α intolerant patient with symptomatic HCV cryoglobulinaemia. Its use in patients with renal involvement should be monitored carefully and the effect is not sustained when therapy is discontinued [38].

Therapy with PEG-IFN and ribavirin:

Has significantly increased sustained virologic response to therapy [40]. Patients with HCV-MC who achieve SVR also achieve prolonged clinical remission. The current treatment duration in HCV–MC is 12 months for all genotypes [19].

Immunosuppressive drugs:

Cyclophosfamid, chlorambucil, and azathioprine

It can be used in life threatening organ involvement when there is no response to steroids. These drugs have severe side effects and can lead to liver disease progression due to their immnuosuppresive effect [41].

Rituximab

Its action includes antibody dependent cellular cytotoxicity, complement dependent cytotoxicity, and apoptosis effective in reducing IgM production [42]. Significant reductions in serum levels of IgM, cryoglobulins, and RF were demonstrated with a rise in C4 levels [43].

Treatment with rituximab at a dose of 375 mg/m2 weekly for 4 consecutive weeks, 80% of patients achieve complete response within 4 months of therapy, with their skin, joint, and neuromuscular symptoms showing strong response to treatment. Therapy with rituximab also allows most patients to discontinue maintenance therapy with corticosteroids [44]. Its combination with antiviral is necessary and duration of its use lasting from 6-12 months according to response. The continued efficacy and safety of repeated therapy in HCV-MC needs further investigations [33].

A low antigen content diets (LAC diet)

Lac-diet consists of a diet with a reduced content of alimentary macromolecules with high antigenic properties, are prescribed in order to help immunocomplexes clearance. It prescribed at the initial stage of disease, to reducing the antigen load to the reticulo–endothelial system, thus allowing a more efficient removal of cryoglobulins. This diet can improve minor manifestations of the MC [9].

The end of therapy criteria

These criteria are needed for patients with cryoglobulinemia.

-Undetectable serum HCV and cryoglobulins,

-Clearance of monoclonal RF producing cells from the blood by B cell clonal expansion analysis, and

-Clearance of HCV and lymphoid aggregates in the liver [45].

It is not clear whether Peg-IFN and ribavirin therapy can produce long-term remission of HCV-MC and whether achieving SVR means that patients are free from the risk of relapse of their HCV-MC symptoms [46]. The optimum length of treatment remains unknown, and severe cases may need long term or even life long therapy [47].

Lymphoproliferative disorders (LPD)

In HCV infected persons, LPD may be as progress of MC in up to 11% of cases (intermediary disorder) or occurred independently in patients without MC [48]. A frequent association reported between HCV infection and non-Hodgkin lymphoma [49].

Prevalence

The prevalence of HCV infection in B-cell NHL has given conflicting results. Several countries data ranges from 9% to 37% [50] and 90% of NHL patients have cryoglobulinemia [51]. Low grade lymphomas are more frequently associated with HCV [52]. The association between HCV and NHL is strongest in geographic areas with the highest prevalence of the viral infection [53].

Pathogenesis

The mechanism may be due to long term HCV infection, resulting in clonal B cell expansion of immunoglobulin (cryoglobulin) secreting lymphocytes, also a combination of a mutation agents like factors (genetic, environmental, immunological) result in activation of oncogenes and resulting in NHL. Another possibility is the inhibition of apoptosis of HCV infected lymphocytes by over-expression of the bcl2, and a second mutation (myc oncogene) may lead to the development of lymphoma [54]. This data suggest that the multi step lymphomagenetic cascade may have points of no-return, making LPD progressively independent from HCV infection [55].

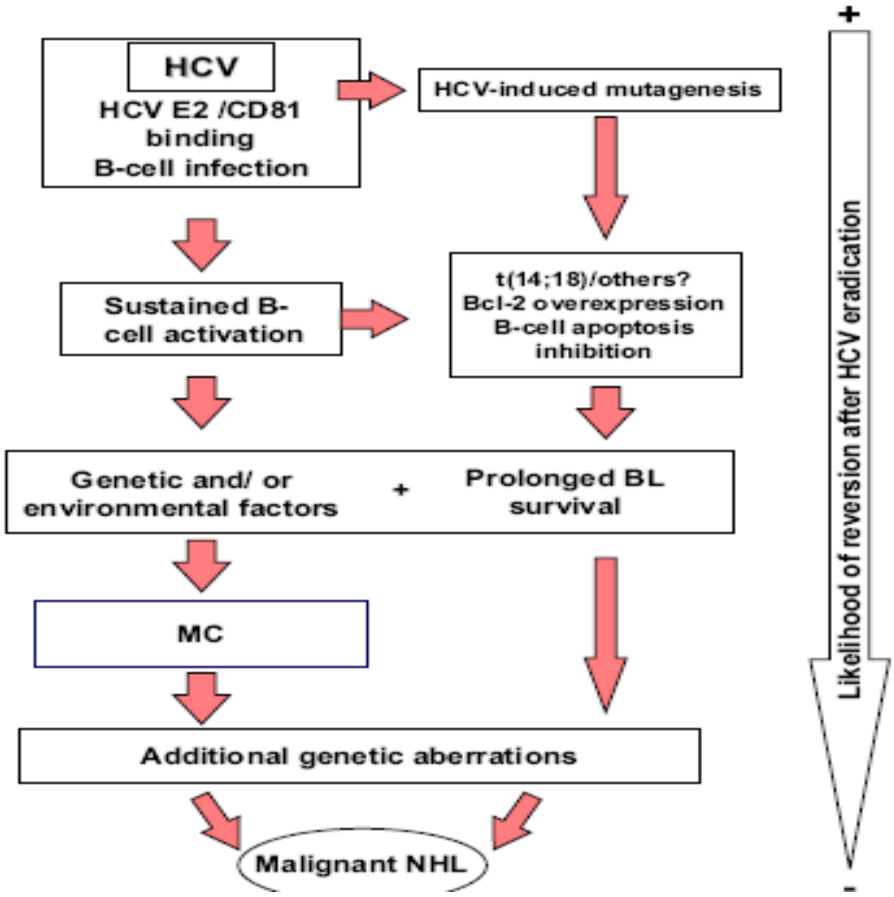

Figure (3): Pathogenesis of hepatitis C virus-related lymphoproliferative disorders [55].

Classification of LPD

All types of lymphoid malignancy can be found in patients with HCV infection but, the strongest association is noticed with B-cell derived NHL [56].

Non- Hodgkin Lymphoma (NHL)

According to the Revised European-American Lymphoma (REAL) classification/World Health Organization (WHO), the most frequent histological subtypes observed is: lympho-plasmacytic (29%), diffuse large B-cell (27%), follicular (16%), marginal zone (10%) and mantle cell (7%) lymphomas [57]. Approximately 65% of HCV-related NHL shows extra nodal involvement [58] . A strong link between HCV infection and mucosa-associated lymphoid tissue (MALT) lymphoma, HCV RNA has been isolated in the gastric mucosa of patients with MALT lymphoma [59].

Splenic marginal zone lymphoma shows a particularly high incidence (35%) of HCV infection, and is related to the hypothesis that B-cell NHL arises selectively from the marginal zone B-cell [60].

Monoclonal Gammopathies:-

Other LPDs reported in the course of HCV infection are monoclonal gammopathies (MG). Usually they are gammopathies IgM/Kappa. In most patients with HCV, MG was classified as monoclonal gammopathies of uncertain significance, which are present in up to 11% patients with HCV infection without cryoglobulins [61], Whereas a few patients who have HCV with MG can be considered as myeloma according to their clinicopathologic characteristics [62].

Soresi et al., [63] found significant relationship between abdominal lymphadenopathy and histological abnormalities of the liver, presence of HCV RNA in the serum and gamma globulin levels indicating a possible interaction between viral antigens and the immune system.

Treatment of LPD

Antiviral Therapy

It is an attractive therapy for low-grade HCV-positive NHL, but in intermediate and high-grade NHL, chemotherapy is necessary while antiviral

treatment possibly could represent a maintenance therapy [64].

Treatment with IFN +/- ribavirin is effective in HCV-associated indolent and marginal zone lymphomas which are mostly occurred with cryoglobulinemia [65]. A complete remission with IFN and ribavirin has been also reported in a patient with HCV-associated mantle cell NHL resistant to chemotherapy and rituximab [66] . In addition, regression of clonal proliferation in response to antiviral treatment was shown to be associated clearly with virological response [67]. Although of SVR, the rearrangement of the monoclonal immunoglobulin genes persistently was detected in the blood even after a complete hematological response [68].

Chemotherapy

Rituximab has become part of the standard treatment regimens used in a variety of B-cell NHL [69]. The use of rituximab in HCV-associated NHL, in monotherapy or in combination with antiviral treatment and/or chemotherapy, appears very promising, particularly in the setting of low-grade NHL, where rituximab monotherapy has been proposed as first-line treatment [70]. However Complications of NHL therapy with rituximab, manifested by increased levels of HCV RNA in blood have been reported [71].

Porphyria cutanea tarda (PCT)

Is a metabolic disease caused by the reduction of hepatic uroporphyrinogen decarboxylase activity, resulting in an over production and deposition of the protein uroporphyrinogen in the blood and urine of patients [72].

Prevalence

HCV infection in patients with porphyria is high, 40-50% depending on the country [73]. A strong association (50-90%) has been demonstrated between sporadic PCT cases and HCV infection in patients from the Mediterranean basin, Japan and the United States [74].

Pathogenesis

HCV does not appear to induce alteration of porphyrin metabolism but it may induce the disease in genetically predisposed individuals [75]. PCT might be related to HCV induced hepatic iron overload. Patients with PCT who are of northern European origin were also found to have increased prevalence of HFE gene mutation, which is responsible of hereditary hemochromatosis [76]. ***Cacoub et al.,*** [3] suggested that cirrhosis may play a role in its development, reporting that the highest rates of PCT were in patients with HCV related liver cirrhosis.

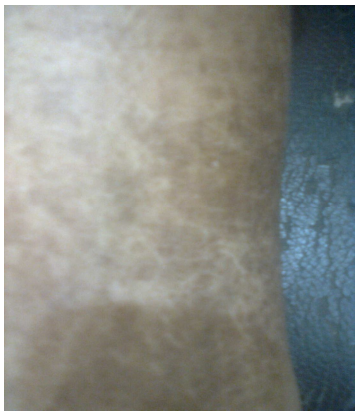

Figure (4): Lower limb pigmentation in HCV cirrhosis

Clinical features

Photosensitivity, skin fragility, bruising and vesicles and bullae that may become hemorrhagic are the main manifestations of PCT. Chronic findings include hypo or hyperpigmentation, alopecia, hirsutism and skin thickening [25].

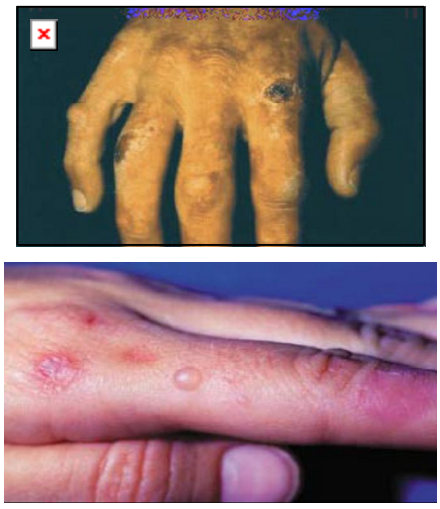

Figure (5): Porphyria Cutanea Tarda [77].

Diagnosis

Gross examination of the urine can provide a valuable clue, because urine of PCT patients is red to brown in natural light and pink to red in fluorescent light. Confirmation requires measurement of porphyrin levels in a 24-hour urine collection [78]. It is recommended that all patients with PCT should be screened for HCV infection [79].

Treatment

Vigorous iron removal by dietary restriction of foods rich in iron, avoidance of alcohol and estrogen use and phlebotomy to remove iron. The next step is the treatment of chronic hepatitis C with interferon and ribavirin. Antimalarial drugs like chloroquine have been used in the treatment of PCT [80].

Lichen planus (LP)

Is a recurrent pruritic eruption characterized by flat-topped violaceous papules that can develop on any skin site (arms, trunk, genitalia, nails and scalp), and mucosal membranes mainly oral mucosa [72]. The presence of HCV RNA in gingival crevicular fluid might have possibly reflected the viral presence in mucosal epithelial cells [81].

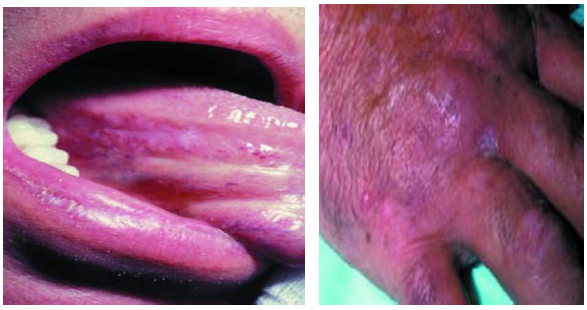

Figure (6): Oral and skin LP [82].

Pathogenesis

HCV infection may induce autoantibodies against the product of a host gene termed GOR which shares several amino acids with the core gene product of HCV [83]. HCV may play a pathogenic role by stimulating LP in genetically succeptible patients [84].

Treatment

LP responds variably to interferon treatment: both improvement and exacerbation of symptoms have been reported [85].

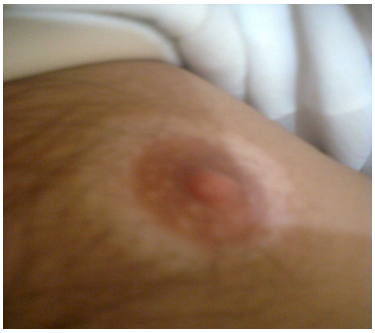

Figure (7): Circum-areola vitiligo in chronic HCV

Diabetes mellitus

DM is found more commonly in patients with chronic HCV infection than in the general population. HCV alone acts as a risk factor for DM, independent from liver disease [86].

Prevalence

DM in patients with cirrhosis due to HCV is 25%, in alcoholic liver disease is 19% and in patients with cirrhosis due to cholestatic liver disease is 13%**.** It was found that the countries which have high prevalence of HCV infection showed increased risk of type 2 diabetes, from 2 to 10 fold compared with liver disease control subjects [87]**.**

HCV positive patients having liver transplantation, reported from 4 to 8 folds increased prevalence of diabetes as compared with patients with other viral or cholestatic liver disease one year after liver transplantation[88]. HCV infection provided a more than three folds increased risk of developing diabetes in individuals aged more than 40 y and two fold for those aged less than 40 years [89].

Pathogenesis

There is evidence that HCV-positive diabetic patients have both peripheral insulin resistance and B-cell dysfunction [90]. TNF-α has been shown to inhibit insulin-stimulated tyrosine phosphorylation of insulin receptor and insulin receptor substrate 1 in adiopocytes, stimulate lipolysis, and increase serum FFA leading to insulin resistance and down regulate of genes in adipocytes encoding proteins such as insulin receptor substrate 1, glucose transporter-4, peroxisome proliferators-activated receptors, and adiponectin. In addition, TNF- α may reduce B-cell function by direct toxic effects. TNF- α receptors were found in higher levels in diabetic HCV patients than non diabetic HCV patients [91]. Also, postmortem studies expose that HCV replicates in the pancreas [92].

HCV genotypes and diabetes

In chronic HCV genotype 1 patients, insulin resistance and overt diabetes are major determinants of advanced fibrosis, regardless of the degree of steatosis [93]**.** ***Chehadeh et al.,*** [94] made two observations that support direct pathogenic role of HCV genotype 4. First, in presence of HCV infection, diabetes occurs at a significantly lower median age with less prevalence of obesity than those diabetic HCV-

negative patients. Second, follow up of HCV patients who had received antiviral drugs revealed a significant decrease of glucose level among diabetic patients who achieved SVR.

Treatment

The role of antiviral therapy is debated due to association between interferon and the induction of anti-pancreas auto anti bodies in some patients [95]. But clinical trials report improvement in measures of glucose metabolism after antiviral treatment [90].

Thyroid dysfunction

The direct link between HCV infection and thyroid diseases is unclear, but thyroid disease usually hypothyroidism is more commonly seen in people with HCV than in the general population [95]. Antiviral therapy can also induce thyroid disease or may unmask autoimmune disease as Graves disease**.** In about 50% of people who develop therapy related hypothyroidism, thyroid function will return to normal when treatment is stopped [97].

The prevalence

About 13% of HCV infected patients have hypothyroidism and up to 25% have thyroid antibodies. Papillary thyroid cancer was reported in patients with HCV infection [98].

Pathogenesis

It was suggested through molecular mimicry between viral antigens and self-antigens [99].

Treatment

The principal risk factor for developing thyroid disease in the course of antiviral therapy is the previous positivity for anti-thyroid antibodies (anti-peroxidase) especially in older women [100] and patients who may be genetically susceptible [101].

Antiviral therapy is contraindicated in patients with thyroid disease not controlled but the presence of autoantibodies against thyroid without clinical manifestations is a relative contraindication to antiviral therapy. In the case of a good therapeutical control of a preexistent thyroid disease, antiviral therapy can be continued. During treatment, frequent controlled tests for thyroid functionality should be performed [25].

Lung involvement

Idiopathic pulmonary fibrosis, diffuse alveolar damage, desquamative interstitial pneumonia, bronchiolitis obliterans organizing pneumonia, pulmonary vascuilitis and acute respiratory distress syndrome have been described in only anecdotal case reports [102].

Idiopathic pulmonary fibrosis

Is a chronic inflammatory interstitial lung disease characterized by an accumulation of alveolar macrophages and neutrophils in the lower respiratory tract, parenchymal injury, and interstitial fibrosis [103].

Pathogenesis

HCV may trigger a subclinical lymphocyte alveolitis [104]. Age, liver cirrhosis and smoking enhance the development of IPF in patients with chronic hepatitis C infection [105].

Treatment

Treatment with corticosteroid and antiviral therapy in most cases of lung involvement associated HCV mainly have no good results [103].

Noncryoglobulinemic nephropathies

HCV associated renal disease including membranous, membranoproliferative and acute proliferative glomerular disease are well documented [106]**.** HCV related glomerulonephritis, must be considered before the onset of therapy with antiviral and/or immunosuppressive agents by the histological demonstration and classification of inflammatory glomerular damage in the renal biopsy [26]. About 30% of patients have complete or partial remission of their renal disease, 30% suffer from intermittent exacerbations and remissions, 30% have an indolent course and 10% may develop chronic renal failure [107].

Erectile Dysfunction

Ferri et al., [15] diagnosed erectile dysfunction in 39% HCV-positive patients and in 14% control subjects. Erectile dysfunction was more common in patients with cryoglobulinemic vasculitis than in those with chronic HCV infection. Plasma levels of total and free testosterone were generally lower in HCV-positive patients, but they were significantly lower in patients with erectile dysfunction versus those without. However, it is also possible, that

antiviral treatment may improve erectile function in some patients.

Psychopathological disorders

Neuropsychiatric symptoms as malaise, fatigue and depressive symptoms have been reported during both acute and chronic stages of hepatitis C and IFN-α treatment [108]. Patients, have a low quality of life and decreased cognitive ability [109].

Prevalence

Depression was reported in 2%–30% of hepatitis C patients [110].

Pathogenesis

It has been supposed that the virus may cause direct cerebral dysfunction by an unknown mechanism [111]. Plasma tryptophan and kynurenine content in blood, together with indoleamine 2, 3-dioxygenase activity in macrophages, was evaluated in whom had mild HCV related chronic liver disease. Serum tryptophan concentrations were lower than those of healthy subjects or patients who had chronic HBV infection, and were associated with high levels of anxiety and depression [112,113].

Treatment

Antidepressants can help in the reduction of depression associated with hepatitis C treatment which should be under supervision [109].

Peripheral neuropathy (PN)

Up to 15.3% of the HCV population has PN. The exact cause of HCV related PN is not completely understood. Some theories suggest that HCV related PN is by HCV RNA deposits in blood vessels that supply oxygen to the nerves, HCV infection of the nerves, an inflammation process in the nerves, and/or HCV related immune disorder [114].

The best initial treatment option in patients with slight to moderate neuropathy is corticosteroids and/or IFN-α monotherapy [115]. However, treatment with interferon has produced mixed results and there is a chance that interferon could exacerbate PN. In patients who do not respond, combined antiviral therapy or intravenous immunoglobulins should be considered. The best option in severe or refractory cases is plasmapheresis [26].

Psoriasis

HCV is suggested to be one of the triggering factors of psoriasis [116]. The management of patients with psoriasis and concomitant HCV is often difficult because treatments for hepatitis C may trigger or exacerbate psoriasis. In addition, most systemic therapies for psoriasis, including immunosuppressants are relatively contraindicated in HCV infection [117]. Etanercept (TNF inhibitor) has an excellent safety profile for the treatment of severe psoriasis with psoriatic arthritis and concomitant hepatitis C virus [118].

Arthralgia

Arthralgia is common in patients with chronic HCV infection and is reported in 19% of HCV patients [3].

HCV-related Arthritis

This includes arthritis associated with or without the presence of MC [119]. Overt arthritis occurs less frequently than arthralgia, with prevalence of less than 5% in patients with chronic HCV infection [26].

Clinical Manifestations

It commonly presented as rheumatoid like symmetrical inflammatory polyarthritis involving mainly small joints or less commonly as mono or oligoarthritis of large joints [21]. In about 2/3 of the affected individuals, morning stiffness may be severe, resolving after more than an hour [120]. The presence of MC in patients with HCV infection consists of an intermittent, mono or oligoarticular, nondestructive arthritis affecting large and medium size joints [119].

Differences between true RA disease and HCV related arthritis.

Differentiation may be difficult. HCV related arthritis usually runs a relatively benign course that is typically non deforming [21]. Furthermore, unlike classic RA, ESR is elevated only in about half of the patients, articular bony erosions and subcutaneous nodules are absent [120].

Patients with HCV related arthritis are seropositive for RF. Therefore; anti keratin antibodies (AKA) are a useful marker to differentiate patients them. In a study AKA were detected in 69% of patients with RA compared to only 8% with HCV associated arthritis [121].

Positive HCV antibody and HCV RNA may be useful in distinguishing between HCV related arthritis and RA. Anti-cyclic citrullinated peptide antibodies were rarely present in HCV infected patients and were a reliable serological marker to discriminate between patients with HCV associated rheumatological manifestations and patients with rheumatoid arthritis [122].

Pathogenesis

HCV arthritis may be a part of MC or it may be directly or indirectly mediated by HCV infection. Direct invasion of synovial cells by the virus, causes local inflammatory response, cytokine induced disease or immune complex disease, particularly in genetically susceptible individuals [123]. HLA-DR4 histocompatibility antigen is significantly elevated in HCV infected patients with autoimmune diseases, including RA [124].

Fibromyalgia (FM)

Rivera et al., found that 15% of patients with FM have an HCV infection. IFN-α therapy can trigger FM symptoms in some patients [125].

Pruritus (Itching)

Pruritus is a presenting symptom in 20% of HCV infected patients and is associated with nonspecific lesions [126].

Pathogenesis

The pathogenesis is uncertain, but it may be caused by a portion of the hepatocyte cell membrane in association with a non-bile pruritogen acting as an opioid agonist [127]. However, subclinical cholestasis may also be a factor. Others causes may contribute in the pathogenesis of pruritus as accumulation of toxins as bilirubin, autoimmune conditions associated HCV, side effects of interferon and ribavirin which causing dry skin [128].

Necrolytic Acral Erythema

Necrolytic acral erythema is a rare, but pathognomonic manifestation of HCV. All cases are associated with HCV. Patients develop annular, hyperkeratotic, and violaceous plaques with raised scaly borders, although some lesions may be vesiculobullous. Lesions are acral in distribution. The pathogenesis of the disorder is unknown and the response to treatment is highly variable. Suggested treatments include amino acid and zinc, interferon-alpha, and ribavirin [129].

Mooren corneal ulcer

Chronic HCV virus infection is associated with Mooren type peripheral ulcerative keratitis. The cause appears to be due to cross reactivity between the HCV envelope protein and corneal antigen. All patients with Mooren type ulcers should be tested for HCV infection. Even when improvement is obtained with interferon alfa-2b treatment, however, continued follow up is important because relapse is common and repeated treatment may be effective [130].

Cardiomyopathy

Multiple studies have recorded relationship between HCV infection and the development of hypertrophic and dilated cardiomyopathy [131].

Prevalence

In a research project for the Study of Idiopathic Cardiomyopathy, HCV antibody was found in 10.6% with hypertrophic cardiomyopathy and 6.3% with dilated cardiomyopathy patients [131]. The association between chronic hepatitis C in various types of cardiomyopathy was originally reported in Japan up to 15%. A study was done in Italy reported a prevalence of hepatitis C antibodies in patients with cardiomyopathy to be 3.9%, [132]. A study from Brazil reported a prevalence of hepatitis C carrier state of 2.9% [133].

Pathogenesis

HCV induced cardiomyopathy is still controversial. The mechanisms by which this virus damages the myocardium have not been known. The development of HCV associated cardiomyopathy may take place in genetically susceptible individuals in whom viral, immunologic, and apoptotic mechanisms may act to produce myocardial damage. However, HCV may promote the development of cardiomyopathy by inducing continuous myocarditis, similar to other virus infections [134]. Some studies have proposed that hepatitis C virus (HCV) generates a tissue lesion mechanism similar to that caused by enterovirus and Coxsackie-B-virus, which are common in cases of myocarditis [135].

The physiopathology involves complex processes characterized by three phases:

- Infection of myocytes, and immunologically mediated cytotoxicity [136].

- Changing the entire heart anatomical and functional structure.

- Activation of an adaptive mechanism known as heart remodeling, which involves heart dilation and ventricular dysfunction in patients with CHF [137].

Okabe et al., [138] have reported strands of HCV RNA in cardiac tissue from patients with chronic active myocarditis.

Treatment

The understanding of cardiomyopathy as an extrahepatic manifestation of HCV infection is of great importance because the treatments available for chronic hepatitis C at present are considered relative contraindicated in patients with myocardial dysfunction. However, if the cause is HCV associated cardiomyopathy may benefit from therapeutic managment that may result in eradication of the virus and reversal of myocardial dysfunction [139].

HCV related Thrombocytopenia

HCV antibodies were identified in 30% of patients with chronic idiopathic thrombocytopenia purpura [140].

Pathogenesis

Thrombocytopenia associated HCV may be present even in the absence of clinically evident liver disease or splenomegaly and may be wrongly diagnosed as ITP [140]. The detection of HCV in platelet and megakaryocytes make HCV related thrombocytopenia is probable cause. High affinity binding of HCV to platelet membrane with subsequent binding of anti-HCV antibody might lead to phagocytosis of platelets [141]**.** High rate of HCV RNA in HCV related thrombocytopenia than non thrombocytopenic patients was detected. Furthermore, HCV may be causative factor for the production of platelate associated immunoglobulin G inducing thrombocytopenia in mechanism similar to idiopathic thrombocytopenia purpura (ITP) [142].

Treatment

Classical therapeutic approaches such as corticosteroid, antiviral therapy and intravenous immunoglobulin and splenectomy can be used. Disappearance of HCV RNA after IFN α associated with improvement of thrombocytopenia. Caution is recommended in thrombocytopenic patients treated with PEG-IFN α and ribavirin when platelet count less than 50,000/µl as significant aggravation of thrombocytopenia may occur [143]**.** Platelet count can be decrease from 30-50% in patient who administrates interferon or peginterferon, so reduction of the dose must be if the platelet counts reach 50.000/mm and discontinuation of the antiviral therapy if the counts reach 25.000/mm. Peg interferon alpha 2a can reduce the weekly dose from 180µg to 135 or even to 90µg, and peg interferon alpha 2b can reduce from 1.5µg/kg to 1µg/kg or even to 0.5µg/kg [40].

Human Recombinant Interleukin (IL)-II (Oprelvekin)

Oprelvekin promoting proliferation and maturation of megakerocytes which can be used to stimulate increasing number of platelet count at dose of 5 µg/kg/day S.C for 7 days initially and if necessary during antiviral therapy maintainance by taking 1-3 doses per week [144].

Elthrombopag

Active thrombopoietin receptor agonist (Elthrombopag) may be applied before and during antiviral therapy in HCV related thrombocytopenia at dose 30, 50 and 75mg lead to sustained increase of platelate count and it allows initiation and/or continuation of antiviral therapy [145].

Rituximib has promising therapeutic approach, especially in refractory cases or aggravating thrombocytopenia during the course of antiviral therapy [146].

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Ethical Approval: Not needed.

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Video Case :Stenting of Periampullary Carcinoma

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Comment

A 45 years old female Egyptian patient from Fakus , Sharkia , Egypt was presented by upper abdominal pain and jaundice .Abdominal ultrasonography revealed dilated common bile duct (CBD). ERCP showed apparently healthy major duodenal papilla with mild swelling , X ray showed dilated CBD . Sphincterotomy was done with introduction of both basket and balloon in many attempts without extraction of stones. The patient pain and jaundice were relieved .2 months later the patient complained of pain and jaundice again. Another ERCP was performed and showed infiltration of the major papilla. 6.5 cm , 10 F plastic stent was introduced in the CBD with good drainage of bile (video) . Microscopic examination of the biopsies revealed malignant cells. The patient was operated radically later on.

Image Case: Ascaris Worm Extraction During Upper Gastrointestinal Endoscopy

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Nematode infection including Ascariais is a worldwide infection [1,2]. Nematode infection in particular ancylostomiasis and ascariasis have long been prevalent in Egypt and trials to trace this infection goes back to Lord Kitehenere in 1913 [3]. Although the decline in the incidence of this form of infection in Egypt, due to application of good hygienic techniques, mass and effective treatment ……etc, we reported a 55 years old male patient with chronic hepatitis C virus infection, non-responder to pegylated interferon therapy complained from persistent epigastric pain and was non-responder to multiple courses of proton pump inhibitors. We decided to examine this patient by upper gastrointestinal endoscopy, where this long white worm was seen in the duodenum ; extraction by biopsy forceps was done.

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Image Case : Pyloric Stenosis in Female Infant

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Pyloric stenosis also known as Infantile Hypertrophic Pyloric Stenosis (IHPS), is the most common cause of intestinal obstruction in infancy. IHPS occurs secondary to hypertrophy and hyperplasia of the muscular layers of the pylorus, causing a functional gastric outlet obstruction. IHPS can be diagnosed by radiography and/or ultrasonography. An Endoscopy-Guided Balloon Dilatation (EGBD) is a new non-operative technique which can be used as a choice in treating IHPS.

Hypertrophic Pyloric Stenosis (HPS) is commonly encountered in pediatric practice. The typical infant presents with non bilious projectile vomiting and dehydration (with hypochloremic metabolic alkalosis) if the diagnosis is delayed. Premature infants tend to present at 3-6 weeks from birth—not at 3-6 weeks from the due date—and these infants may have borderline normal muscle thickness, because they are comparatively smaller. Hypertrophic pyloric stenosis is rarely seen in children older than 6 months [1].

The etiology of IHPS is obscure but probably is multifactorial, involving genetic predisposition and environmental factors [2].

This condition accounts for one third of non bilious vomiting occurrences in infants and is the most common reason for laparotomy before age 1 year. A striking male preponderance is seen, with a male-to-female ratio of 4-6:1 [1]. Nuala et al. revealed in their study of 99 infants who underwent pyloromyotomy, that female developed the symptoms of pyloric stenosis later than males with a 6:1 male-female ratio [3].

We reported a 2 months female infant with a projectile vomiting in type referred to the x-ray department. When a longitudinal abdominal ultrasound was performed a hypertrophic pyloric stenosis was seen (Image 1). Since the degree of confidence in a negative examination can be false in a patient who is seen early in the disease [1], a Barium meal was performed to confirm the diagnosis. The lateral barium contrast media showed a string sign indicating a IHPS (Image 2).

IHPS is treated by surgical intervention. An Endoscopy-Guided Balloon Dilatation (EGBD) can be used as a new method of non-operative treatment for (IHPS) [4].

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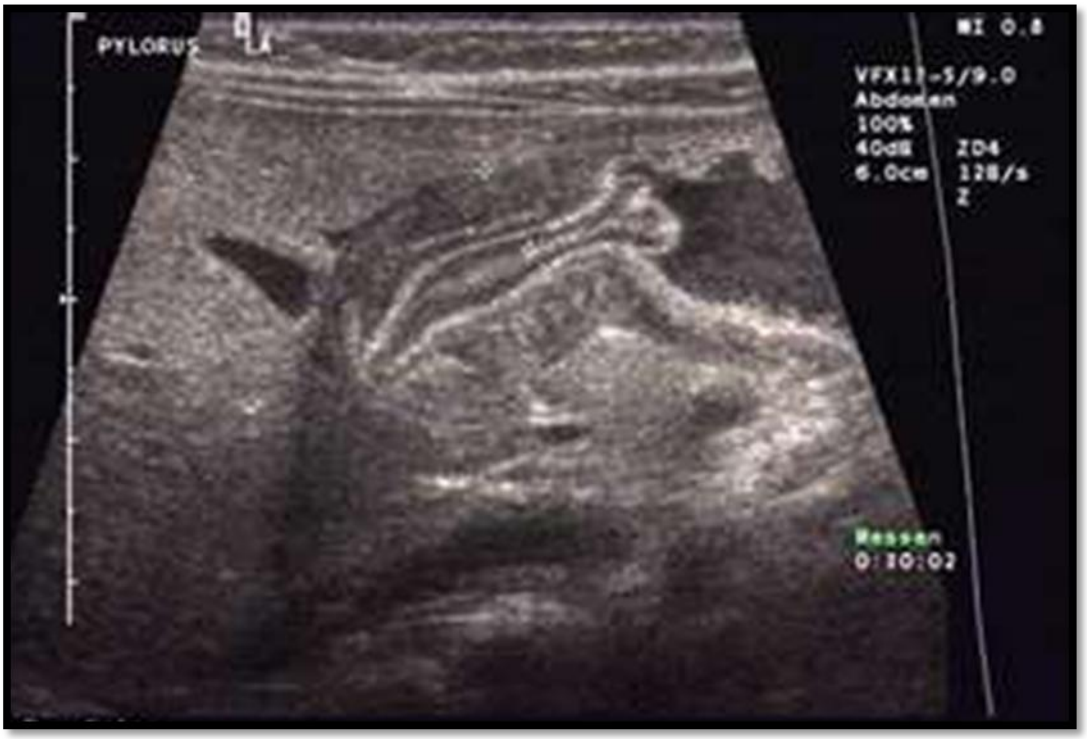

Image (1): Longitudinal ultrasound through a 2 months female infant showing an IHPS

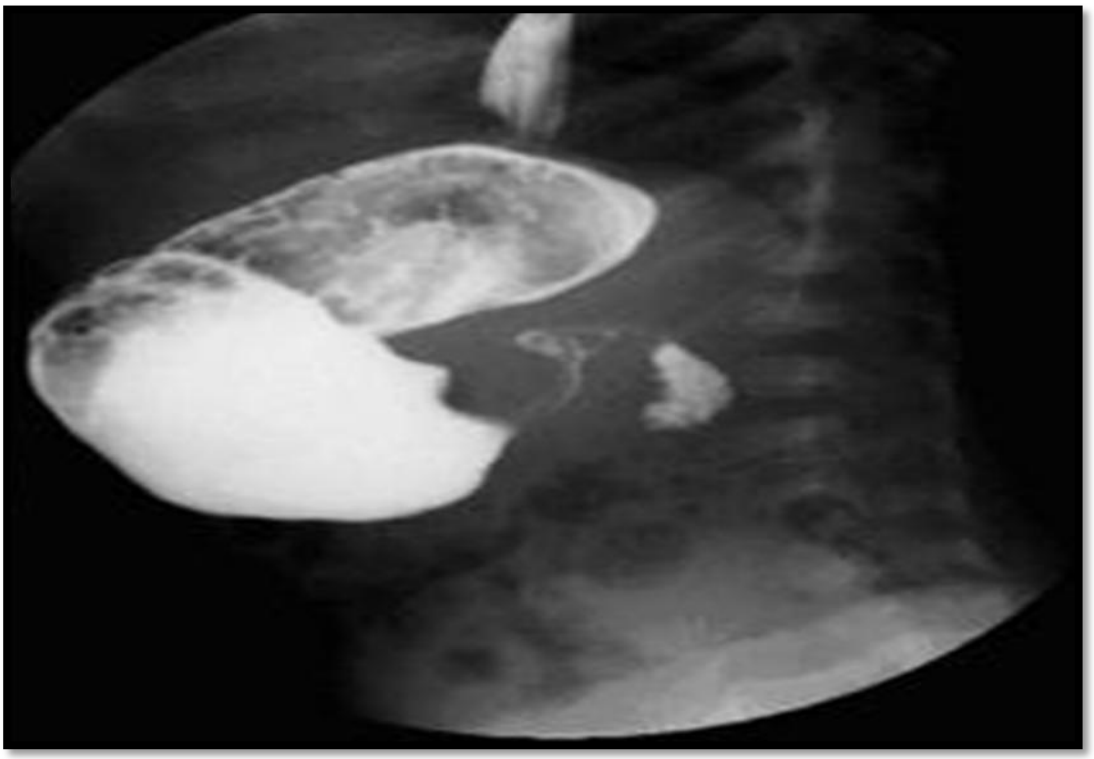

Image (2): A lateral barium meal for a 2 months female infant showing string sign indicating IHPS

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Case 1,2012: A 65 Years Old Man with Tetanus

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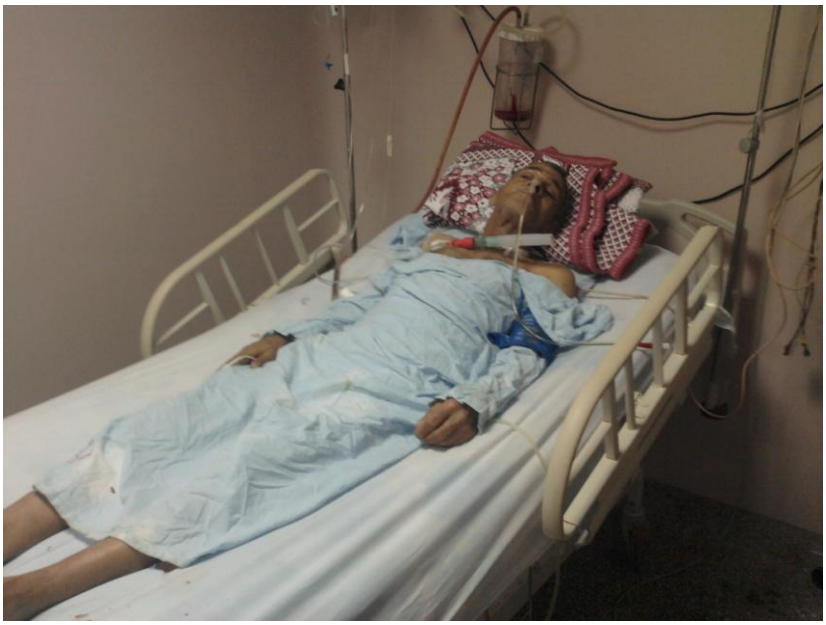

Presentation of the case:

A 65 years old man from Dyrab Nejm, Sharkia Governorate, Egypt with wound in the foot due to penetration by a nail was presented 2 weeks later by dysphagia, trismus (lock jaw), rigidity of the neck, limbs and abdominal muscles. There was no history of bite. Attacks of spasms (convulsions) with occasional cyanosis were noticed. The patients was admitted initially to Zagazig Fever Hospital, then the patient was referred to Dr Atef Radwan Intensive Care Unit Surgical Building , Zagazig University Hospitals, CSF examination was normal, tracheostomy was performed with aspiration of chest secretions and oxygen supply through it. Nasogastric tube (Ryle) was also difficulty introduced. The feeding was enteral .100,000 U equine antitetanic serum was administered intramuscularly in the first days of hospitalization after test for hypersensitivity . The patient was conscious with inability to talk. He wrote what he wanted. The patient was put in dark silent room. Diazepam in a dose of 20 mg/8 hrs infusion was administered for 3 weeks then tapered gradually. Intravenous muscle relaxants was administered for 4 weeks also chlorpromazin in a dose of 50 mg/8 hrs through the Ryle was administered in the first 2 weeks. Metronidazol 500 I.V. every 8 hrs for 10 days was administered as well as prophylactic macrolid antibiotic. By the end of 4 th week of illness the spasms ceased and the patient could talk but neck , abdominal and limbs rigidity were still present .

Differential diagnosis[1,2]:

1. Local causes of trismus as dental abscess, mumps and quinzy.
2. Meningitis and encephalitis.
3. Tetany.
4. Strychnine poisoning.
5. Peritonitis.

6. Rabies.

7. Epilepsy.

8. Dystonic reaction to phenothiazin.

Discussion:

Tetanus still continues to constitute a serious health problem in Egypt and other developing countries. The caustive organism is clostridium tetani which is Gram-positive rod shaped bacteria. Spores are present in soil ,street dust, human and animal feces. Two exotoxins are liberated by the organisms namely tetanospasmin and tetanolysin. Tetanospasmin increases the excitability of the spinal cord and has also a local action on the muscles[1].

The above case is a typical case of tetanus due to presence of lock jaw, rigidity of muscles, tonic spasms and absence of disturbance of consciousness (conscious till the end).

Local causes of trismus are excluded due to absence of neck lymph nodes and presence of other features of tetanus as generalized muscle rigidity and spasms.

Meningitis and encephalitis are excluded due to normal CSF examination and presence of generalized muscle rigidity rather than only neck rigidity of meningitis.

In tetany the spasms are confined to the hands and feet, so it is excluded.

No history of ingestion of strychnine, so it is excluded.

In peritonitis the rigidity is confined to the abdomen.

Early stages of rabies (Hydrophobia) may be confused with dysphagia of tetanus. However no rigidity of muscles are present in rabies.

Epilepsy is associated with clonic convulsions rather than tonic spasms of tetanus.

The patient survived due to early introduction of antitetanic serum, good intensive care and tracheostomy with aspiration of pulmonary secretions and introduction of prophylactic antibiotics.

Tetanus has a 40-60% mortality due to pneumonia, respiratory failure, circulatory disturbance and septicemia[2].

Puncture wounds like this case, animal and human bites, wounds contaminated with soil or feces and burns should not be sutured and cleaned. A booster dose of tetanus toxid should be given if the last dose was given 5-10 years ago. If no active immunization was given or given more than 10 years ago, a full dose of tetanus toxid is given with passive immunization by 250 units intramuscularly of human tetanus immunoglobulines (HTIG) or 1500 units equine antitetanic serum. The dose should be doubled in high risk or delayed wounds [2].

Conclusion:

Tetanus is still a health problem in developing countries as Egypt. Intensive care of tetanus patient and early antitetanic serum as well as tracheostomy and mechanical ventilation if needed decrease the mortality .

References:

1. Saif El Din S and Abdel Wahab MF. A Guide Book of Tropical and Infectious Diseases. Tropical Medicine Department , Ain Shams University , Cairo 1995;244-246.

2. Smith MD. Tetanus .Cook GC ,ed .Manson's Tropical Diseases .Saunders, 20th edition 1996; 48:906-913.