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Atef Eraky
E mail:atef_eraky@yahoo.com
Wafaa Metwally
E mail:wafaa@zu.edu.eg

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(2) drafting the article or revising it critically for important intellectual content
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Contents

Significance of Serum Hepatocyte Growth Factor Level in Diagnosis of Hepatocellular Carcinoma

Maha Z. Omar[1], **Eman G. Behiry**[2]

[1]*Hepatology, Gastroenterology and Infectious Diseases Department, Faculty of Medicine, Benha University,Egypt.*

[2]*Clinical and Chemical Pathology Department, Faculty of Medicine, Benha University,Egypt.*

Corresponding Author
Maha Z Omar

Mobile:+201223287312

E mail: mahazeinelabedin@yahoo.com

Key words: Diagnosis,HCC, Hepatocyte growth factor

Background and study aim : Hepatocellular carcinoma (HCC) is the most common primary hepatic tumor and one of the most common cancers worldwide. New serum tumor markers are required for diagnosis of HCC as alpha-fetoprotein (AFP), which is the most widely used marker, has poor diagnostic accuracy. Hepatocyte growth factor (HGF) initially identified and molecularly cloned as a potent mitogen of primary cultured hepatocytes, has multiple activities in a variety of tissues during the course of development and also in various disease states. This study was conducted to assess the diagnostic value of serum HGF as a biomarker for diagnosis HCC.

Patients and Methods: This study was conducted on 30 patients with documented HCC and 30 cirrhotic patients with no evidence of HCC; as well as 25 healthy subjects who served as control group. The levels of AFP and HGF were measured for all cases together with full clinical assessment, liver biochemical profile, viral markers, ultrasound, and abdominal triphasic computerized tomography (CT) scan.

Results: The mean value of serum HGF was highly significantly elevated in HCC group compared to the control group ($P<0.001$), and its level was higher in HCC than cirrhotic group (795.8 ± 312.04 Vs 322.7 ± 45.2 pg/ml respectively) with significant difference ($P<0.001$). There was significant correlation between serum HGF level and both ALT, MELD score in HCC group ($P = 0.005$, 0.02 respectively). At cut off level equal or more than 426.1 pg/ml, HGF could diagnose HCC with 96.7% sensitivity and 98.2% specificity and AUROC was 0.99.

Conclusion: Serum level of HGF could be considered useful marker for diagnosis of HCV related HCC.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary malignant tumor of the liver **[1].** Over a decade (1993-2002), there was nearly a two fold increase of the proportion of HCC among chronic liver disease (CLD) patients in Egypt with a significant decline of hepatitis B virus (HBV) and slight increase of hepatitis C virus (HCV) as risk factors **[2].** The incidence and mortality rates for HCC are nearly identical, indicating the overall poor survival of patients with this tumor. Therefore, the most effective treatment relies on the early diagnosis of HCC **[3].** Alpha-fetoprotein (AFP) is a serological marker currently available for the detection of hepatocellular carcinoma. Its poor sensitivity renders it unsatisfactory for this purpose and suggests an urgent need for novel biomarkers for early stage HCC detection **[4].** HGF is a pleiotropic growth factor originally isolated from rat platelets. HGF-like factor known as macrophage-stimulating protein (MSP) is an 82 kDa, 674 amino acid residue hetero dimeric glucoprotein [5]. It has a wide range of effects from embryonic development and liver regeneration to protection and/or repair of various organs including kidneys, lungs, and cardiovascular system **[6].** HGF (scatter factor) is the most potent growth factor for hepatocytes and its receptor, the N-methyl-N′-nitroso-guanidine human osteosarcoma transforming gene trans-membrane tyrosine kinase (c-Met) is implicated in HCC carcinogenesis and progression through activation of multiple signaling pathways that direct cell growth, proliferation, survival and motility **[7]**. Aberrant Met/HGF activation has been observed in many

tumor types **[8].** Met/HGF inhibition has emerged as targeted anticancer therapies **[9]**. Ongoing clinical development with tivantinib, cabozantinib, onartuzumab, crizotinib, rilotumumab and ficlatuzumab has shown encouraging results **[8]**. The aim of this work was to investigate the diagnostic significance of hepatocyte growth factor in HCV related HCC cirrhotic patients and to assess its sensitivity and specificity as compared to AFP.

PATIENTS AND METHODS

Study groups:
This prospective study was conducted on 60 HCV cirrhotic patients admitted to Hepatology, Gastroenterology and Infectious Diseases Department. Benha Faculty of Medicine, Benha University in period between March 2014 and December 2014. In addition to 25 apparently healthy subjects served as control group. They were divided into three groups:

Group I (liver cirrhosis group): included 30 patients with post hepatitic liver cirrhosis without HCC, the majority of them were males [17 patients (56.7%)].

Group II (HCC group): included 30 cirrhotic patients with HCC, the majority of them were males [23 patients (76.7%)].

Group III (control group): included 25 apparently healthy subjects, 13 of them were males [13 patients (52%)].

Patients less than 18 years old, patients with liver cirrhosis due to other causes than HCV infection (HBV, Autoimmune hepatitis, Alcoholic,… etc), patients with hepatic focal lesions other than HCC (Hemangiona, Hepatoblastoma,…etc), and patients with metastatic focal lesions or with vascular invasion were excluded from this study.

Methodology:
Full history taking and clinical examination were done to all patients and the following routine laboratory investigations:

- Complete blood picture.
- Liver profile tests: alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), serum bilirubin, serum albumin, prothromhin time and concentration (PT and PC) and international normalized ratio (INR).
- Serum creatinine.
- Hepatitis markers: hepatitis B surface antigen (HBsAg) and anti-HCV antibody by 3rd generation ELISA [enzyme linked immune-sorbant assay].
- Evaluation of the severity of liver cirrhosis was assessed in each cirrhotic patient with the Modified Child score **[10]** and MELD score **[11]**.

Imaging studies:

- Abdominal ultrasonography was performed for all patients and controls included in the study using LOGIQ P6/P6 PRO QUICK GUIDE machine with a convex-sector probe (PVF-375 MT-2.57 MHz). Liver was assessed for size, smoothness of the surface, texture, portal vein diameter. The presence of focal lesions and their detailed description as regards number, size, site, echogenicity were reported. Doppler ultrasound was used to assess the patency of the portal vein. It was also used to detect any Doppler signals inside and around the lesions as the presence of intra-lesional arterial signals is highly suggestive of malignancy **[12]**. A complete abdominal scanning was done to detect any other abnormality including the presence of ascites, lymph nodes or abdominal masses.
- Triphasic abdominal CT scanning: Spiral tri-phasic CT abdomen was done to all patients in HCC group for the diagnosis of hepatic focal lesions with specific features of HCC as previously described **[13, 14, 15]**.

Tumor Markers :
A 2 ml blood sample was drawn from each subject after being diagnosed. Blood samples were centrifuged and serum aliquoted and stored at (- 20°C) until tested for AFP and HGF.

1- Measurement of serum (AFP) (ng/dl):
Serum AFP was measured by enzyme-linked immunosorbent assay (ELISA) technique using commercially available immunometric assays (by Monobind Inc. Lake Forest, CA92630, USA) with lower limit of detection (1.8 ng/ml) and normal reference range (2 – 10.9 ng/ml) obtained by manufacturer instruction.

2- Measurement of serum (HGF):
Serum HGF was measured by enzyme-linked immunosorbent assay (ELISA) technique (by Sunred bio company- Lot NO.=201411) with lower limit of detection (4.336 pg/ml) and normal reference range (59.7-157.3 pg/ml) obtained by manufacture instructions.

Principle:
An anti-human HGF coating antibody is adsorbed onto micro wells. Human HGF present in the sample or standard binds to antibodies adsorbed to the micro wells; a biotin-conjugated anti-human HGF antibody binds to human HGF captured by the first antibody. Streptavidin-HRP binds to the biotin conjugated anti-human HGF.

Assay protocol-:
The following steps were done as recommended by (Sunred Bio Biomedical Company): All reagents were brought to room temperature before use and each sample, standard, blank and optional control sample was assayed . First each test sample was diluted before use (50 µL of diluted sample was added into pre-designated wells).Then calibrator solutions were prepared as recommended by manufacture.

The Micro titer Plate was incubated at 22°Croom temperature for thirty (30 ± 2) minutes (Plate was kept covered during incubation).The contents of the wells were aspirated and each well was filled completely with appropriately diluted wash solution and aspirate. This step was repeated three times, Then 50 µL of appropriately diluted Enzyme-Antibody was conjugated to each well and incubated at 22°C room temperature for thirty (30± 2) minutes(Plate was kept covered in the dark during incubation).

The wells were washed then 50 µL of tetra methylbenzidine (TMB) Substrate Solution was added to each well.(The plate was incubated in the dark at RT for precisely 10 minutes). After ten minutes, 50 µL of Stop Solution was added to each well.and the absorbance was determined at 450 nm of the contents of each well.

Statistical Analysis:
Median, range, mean, and standard deviation were used for descriptive statistics, as appropriate. Categorical variables were tested with Fisher's exact test or $\chi 2$ test. Continuous variables were tested with Student t-test or analysis of variance (ANOVA). Comparison of plasma HGF levels and clinical characteristics among the three groups of subjects were analyzed using ANOVA test, post hoc tests, and Mann-Whitney U test. Correlation between plasma levels of HGF and AFP were analyzed using Spearman's correlation coefficient. Receiver operating characteristics (ROC) analysis was used to evaluate the diagnostic value of HGF and AFP and to identify the optimal threshold values. Sensitivity and specificity, positive and negative predictive values of HGF and AFP were profiled by curves. Calculations were done with the Statistical Package for the Social Sciences version 22 (IBM, SPSS, Statistics, V.22, 2012, IBM crop., New York. USA).

RESULTS

Demographic criteria of studied groups were shown in table (1). Mean age of patients with HCC was highly significantly elevated than other groups (P<0.001). The number of males within HCC, cirrhosis and control groups were higher than females.

Three patients (10%) in cirrhotic group were Child class A compared to one patient (3.3%) in HCC group. Seventeen patients (56.7%) in cirrhosis group and 10 patients (33.3%) of the HCC group were Child class B, while 10 patients (33.3%) in the cirrhosis group were Child class C compared to 19 patients (63.3%) in the HCC group with no statistically significant difference between both groups (P value = 0.25).

Table (2) showed highly statistically significant difference between studied groups as regard all laboratory parameters with higher values of liver profile tests and low serum albumin level in HCC group when compared to other groups. The mean value of serum AFP and HGF was highly significantly elevated in HCC and cirrhotic groups than control groups, and the mean level of HGF was higher in HCC group (795.8 ± 312.04 pg/ml) than cirrhotic group (322.7 ± 45.2 pg/ml) with highly significant difference (P<0.001) (Table 3).

Correlation studies revealed significant correlation between serum level of HGF and ALT (P value = 0.005) (Table 4 and Fig. 1) and MELD score (P value = 0.02) (Table 5 and Fig. 2) in HCC group. Table (6) showed no statistically significant correlation between HGF level and tumor number, size, site and shape.

Table (7) and Fig. (3) showed performance of biomarkers (HGF and AFP). At cut off level ≥426.1 pg/ml, HGF had higher sensitivity, specificity, positive predictive value, negative predictive value than AFP in diagnosis of HCC (96.7%, 98.2%, 96.7%, 98.2% vs. 83.3%, 92.7%, 86.2%, 91.1%) respectively with AUROC was 0.99 (P<0.001).

The ultrasonographic features of the focal hepatic lesions in HCC patients showed that 22 patients (73.3%) had single focal lesion, 8 patients (26.7%) had multiple focal lesions. Mainly in Rt lobe [17 patients (56.7%)] while in Lt lobe [8

patients (26.7%)] and 5 patients (16.7%) had focal lesions detected in both lobes. 13 patients (43.3%) had focal lesion <3 cm while 17 patients (56.7%) had focal lesion (3-5 Cm). 26 lesions were hypoechoic (86.7%), 3 lesions were hyperechoic (10%) and only one lesion (3.3%) was isoechoic.

As regard CT pattern of HCC in triphasic CT scan confirmed HCC diagnosis as all lesions showed typical enhancement in arterial phase followed by venous washout in the delayed portal/venous phase.

Table (1): Demographic criteria of studied groups

Variables	Cirrhotic group (N=30)	HCC group (N=30)	Control group (N=25)	P- value
Age (years) (Mean ± SD)	47.03±5.2	49.5±8.9	34.9±8.5	<0.001**
Gender N (%)				
Male	17(56.7%)	23(76.7%)	13(52%)	0.12
Female	13(43.3%)	7(23.3%)	12(48%)	

Table (2): Laboratory characteristics among studied groups

Variables	Cirrhotic group (N=30)	HCC group (N=30)	Control group (N=25)	P- value	Post Hoc Test
	(Mean±SD)	(Mean±SD)	(Mean±SD)		
Hb (g/dl)	11.9±1.9	10.6±2.3	13.01±1.8	<0.001**	P1=0.02* P2=<0.001** P3=0.04*
Platelet (cell/cm)×10^3	127.6±65.8	125.2±55.8	290.9±80.1	<0.001**	P1=0.89 P2=<0.001** P3=<0.001**
WBC (cell/cm)×10^3	7.3±3.9	5.5±2.4	5.8±1.7	0.04*	P1=0.02* P2=0.03* P3=0.95
ALT (IU/L)	47.2±35.4	54.3±30.7	29.4±15.1	0.004*	P1=0.79 P2=0.04* P3=0.001*
AST (IU/L)	57.0±29.5	67.5±46.8	27.7±15.03	<0.001**	P1=0.66 P2=<0.001** P3=<0.001**
Total bilirubin (mg/dl)	1.28 ±0.47	2.69±2.3	0.86±0.19	<0.001**	P1=0.007* P2=0.001** P3=0.001**
Albumin (gm /dl)	3.2±0.93	2.9±0.61	4.3±0.79	<0.001**	P1=0.89 P2=0.002* P3=0.004*
PT (seconds)	16.1±5.8	15.6±3.5	12.7±0.77	<0.001**	P1=0.96 P2=0.008* P3=<0.001**
INR	1.4±0.51	1.4±0.73	1.00±0.01	<0.001**	P1=1.00 P2=<0.001** P3=0.01*
ALP (IU/L)	126.5±28.9	160.1±36.1	52.5±19.2	<0.001**	P1=0.001* P2=<0.001** P3=<0.001**
Creatinine (mg / dl)	0.97±0.79	1.1±0.98	0.51±0.16	<0.001**	P1=0.92 P2=0.01* P3=0.008*

P1. Comparison between cirrhotic and HCC groups P2. Comparison between cirrhotic and control groups
P3. Comparison between HCC and control groups

Table (3): Serum levels of AFP and HGF among studied groups

Variables	Cirrhotic group (N=30)	HCC Group (N=30)	Control group (N=25)	P- value	Mann Whitney test
AFP (ng/dl)	25.01±2.6	264.5±589.7	4.2±4.5	<0.001**	P1=<0.001** P2=<0.001** P3=<0.001**
HGF (pg/dl)	322.7±45.2	795.8±312.04	108.5±48.8	<0.001**	P1=<0.001** P2=<0.001** P3=<0.001**

P1. Comparison between cirrhotic and HCC groups
P2. Comparison between cirrhotic and control groups
P3. Comparison between HCC and control groups

Table (4): Spearman's correlation between serum level of HGF and others parameters among cirrhotic and HCC groups

Variables	Serum HGF level			
	Cirrhotic group (N=30)		HCC group (N=30)	
	r	P value	r	P value
Age (years)	0.240	0.20	0.321	0.08
Hb (g/dl)	-0.001	0.99	-0.212	0.26
Platelet (cell/cm)×10^3	-0.160	0.39	-0.250	0.18
WBC (cell/cm)×10^3	-0.016	0.93	-0.004	0.99
ALT (IU/L)	-0.035	0.85	0.497	0.005*
AST (IU/L)	0.256	0.17	-0.121	0.53
Total bilirubin (mg/dl)	0.140	0.46	0.244	0.19
Albumin	-0.161	0.39	0.206	0.27
PT (seconds)	0.270	0.15	0.405	0.06
INR	0.139	0.46	0.418	0.09
ALP (IU/L)	-0.071	0.71	0.103	0.59
Creatinine	-0.117	0.54	-0.081	0.67
AFP (ng/dl)	0.229	0.23	0.179	0.35

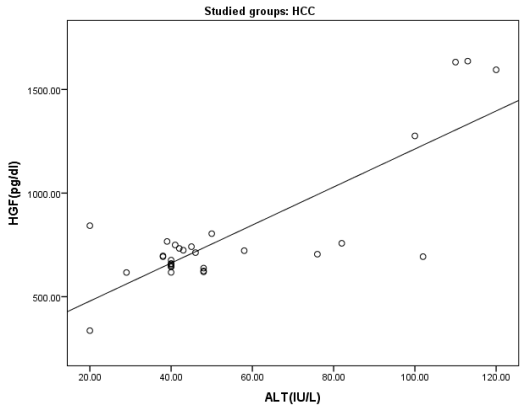

Fig. (1): Scatter Plot curve is showing correlation between serum level of HGF and ALT in HCC Group

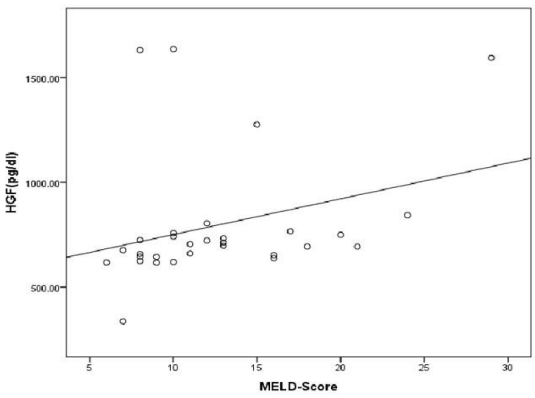

Fig. (2): Scatter Plot curve is showing correlation between serum level of HGF and MELD scores in HCC Group

Table (5): Spearman's correlation between serum level of HGF and severity of liver disease assessed by (Child-Pugh score, MELD scores)

Variables	HGF	
	r	P value
Child score	0.209	0.46
MELD score	0.442	**0.02***

Table (6): Spearman's correlation between serum level of HGF and characters of hepatic focal lesions among HCC group

Variables	HGF	
	r	P- value
Tumor number	-0.141	0.46
Tumor size	0.102	0.59
Tumor site	0.116	0.54
Tumor echogenicity	-0.059	0.76

Table (7): Diagnostic performance of serum level of AFP and serum level of HGF in HCC group

Variables	Cutoff point	Sensitivity	Specificity	PPV	NPV	Accuracy	AUC	P - value
AFP ng/ml	≥ 9.5	83.3%	92.7%	86.2%	91.1%	89.4%	0.900	<0.001
HGF pg/ml	≥ 426.1	96.7%	98.2%	96.7%	98.2%	97.6%	0.995	<0.001

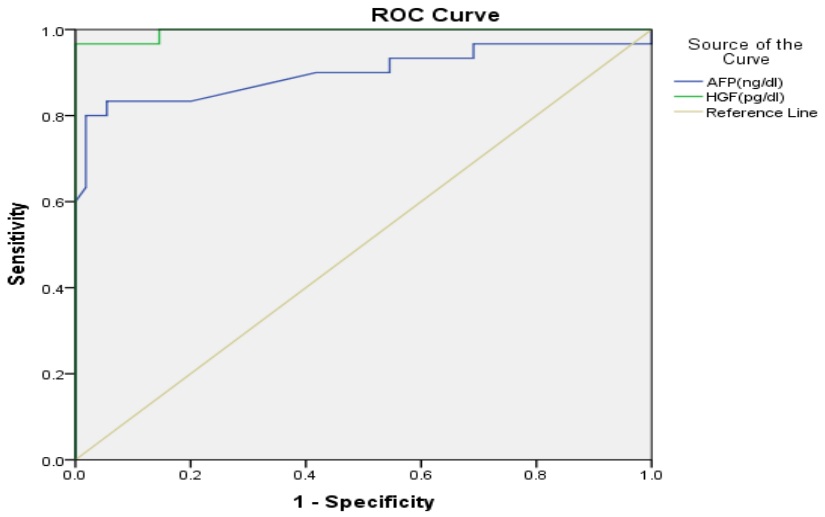

Fig. (3): Receiver Operating Characteristics (ROC) curve which show AFP and HGF sensitivity and specificity in diagnosis of HCC

DISCUSSION

HCC is the primary type of liver cancer, and both the age adjusted incidence and mortality of HCC have steadily increased in recent years **[7].** In Egypt HCC incidence rates have been doubled over the last ten years **[2,16,17].** And that is attributed to the growing HCV incidence **[18,19].** Currently, early diagnosis of HCC is the most important step in HCC management. Most imaging techniques help to discover HCC after considerable time of onset of tumor. In most instances, oncologists depend on AFP as the commonest and feasible marker for assessing HCC in addition to imaging. However, AFP is not completely reliable marker in early HCC diagnosis, prevention or therapy due to its low specifiity and sensitivity. Liver biopsy is always considered as an invasive procedure, so chemical findings are still greatly appreciated **[20].** Despite accumulating data regarding the risk factors for HCC, the mechanisms that contribute to HCC tumorigenesis remain poorly understood. There is vast evidence for protumorigenic growth factor signaling dysregulation in human HCCs affecting different signaling systems such as insulin growth factor, HGF, wingless, TGF-α, epidermal growth factor, and TGF–β signaling. There are many studies about HGF with tissue, cell line, and serum in HCC patients **[21].**

The mitogenic, motogenic, morphogenic, and pro neoangiogenic HGF is produced by non-parenchymal cells such as hepatic stellate cells **[22]**, sinusoidal endothelial cells, and kupffer cells **[23,24].** Increased HGF serum levels have been reported in patients with squamous cell carcinoma of the esophagus **[25].** There is a correlation between HGF serum values and a worsening of hepatic chronic disease **[26].** In the presence of liver cirrhosis, the functions of all these cells deteriorate, as do those of parenchymal cells. The mechanism of the high serum HGF level in liver disease could be a disorder of either production or elimination of HGF itself, but the role of HGF in liver disease is complicated and is not yet completely understood **[8].**

In the present study, we evaluated the diagnostic value of HGF in patients with HCV related HCC. In the present study, it was found that the mean age of patients with HCC was higher than other groups with highly significant difference between them (P<0.001). Also, Liver profile tests and AFP were significantly higher and s.albumin was significantly lower in HCC group than the other groups. In agreement with our findings, the study done by Breuhan et al. **[27]** which concluded that the mean age of patients with HCC was significantly higher than that of the other groups (P<0.001) and they had the highest values for various concurrently measured liver profile tests. The present study revealed that the mean of HGF level was higher in HCC group than the other groups with highly statistically significant difference between studied groups (P<0.001) that in agreement with Funakoshi et al. **[28]** who reported that levels of HGF in serum tend to be higher in patients with liver cirrhosis and HCC than with chronic hepatitis. Also the results of the present work supported by study of Yamagamim et al. **[29]** in which plasma samples were taken from 99 patients with chronic viral hepatitis C, cirrhosis, and HCC. The mean HGF level in HCC group (0.533 ± 0.167 ng/ml) was higher than in either chronic hepatitis group (0.383 ± 0.076 ng/ml) with P value = 0.008 or liver cirrhosis group (0.377 ± 0.054 ng/ml) with P value = 0.0016 and concluded that the serum levels of HGF represents the degree of the carcinogenic state (≥0.3 ng/ml, a high risk of developing cancer) in the liver of patients with viral chronic hepatitis C and cirrhosis.

The increase of HGF serum levels (≥0.6 ng/ml for the diagnosis) in cirrhotic patients is an indicator of HCC development. Furthermore, serum HGF levels reveal high carcinogenic states in liver cirrhosis type C **[30].** Induction is due to the increased production of HGF, not only in the liver, but also in distant organs, such as the lung. With the progression of liver damage, clearance of HGF in the liver diminishes. In addition, although patients with liver cirrhosis show a marked increase in serum HGF levels as the molecule is processed from a biologically inactive single-chain precursor from of HGF into the two-chain active form. Levels may be significantly disturbed in the damaged liver, and a single-chain precursor form can become a major form in the serum **[28].**

In the present work, correlation study revealed that there was a highly significant positive correlation between HGF level and ALT level (P = 0.005) and significant positive correlation with MELD score (P=0.02) in HCC group but there was insignificant correlation between HGF level and other studied parameters in both cirrhotic

and HCC group such as age, CBC, other liver profile tests, AFP and Child score. This results in agreement with that reported by Karabulut et el. **[8]** who concluded that serum HGF levels were significant higher in patients with elevated serum ALT levels than others with normal ALT levels (P = 0.05) but in the other hand they found that no correlation between serum levels of HGF and MELD score (P = 0.09), which was in contrast to our results. This difference may be related to difference in patients criteria as the last study included HCV, HBV, alcoholic patients. The present study demonstrated that no correlation between serum HGF level and tumor number, site, size and echogenicity in HCC group. This result were in a concordance with that reported by Karabulut et el. **[8]** as they found no correlation between HGF level and tumor number but there was positive correlation with tumor size (P = 0.01). That can be explained by small number of studied patients in the present work (30 patients) in comparison to the last study (54 patients). Analysis of serum HGF level by ROC curve in the present work revealed satisfactory values regarding sensitivity and specificity at a cut off value (≥ 426.1 pg/ml). Serum HGF had higher sensitivity, specificity, PPV and NPV than AFP for diagnosis of HCC (96.7%, 98.2%, 96.7, 98.2 Vs 83.3%, 92.7%, 86.2%, 91.1% respectively) there for, assessment of serum HGF in HCV cirrhotic patients would be beneficial for diagnosis of HCC.

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Conflicts of interest: Non.

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Peer reviewers: Rashed M Hasan ,Professor of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt. **Ehab Darwish** , Lecturer of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt.**Editor: Tarik Zaher**, Professor of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt

Efficacy of Using Neutrophil to Lymphocyte Ratio and Interleukin 6 as Outcome Predictors of Interventional Treatment of Hepatocellular Carcioma

Salama M El-Ghoneimy[1], Amira M Soliman[1], Mervat M Azab[2], Ahmed M El-Gebaly[1], Sameh M Abdel Monem[1]

[1] *Tropical Medicine Department, Faculty of Medicine, Zagazig University, Egypt*

[2] *Clinical Pathology Department, Faculty of Medicine, Zagazig University, Egypt*

Corresponding Author
Ahmed M. El-Gebaly

Mobile:+201225540307

E mail: icegebo2011@yahoo.com

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Background and study aim: Hepatocellular carcinoma (HCC) is currently the fifth most common solid tumor worldwide and the third leading cause of cancer-related death. Percutaneous ethanol injection (PEI) and radiofrequency ablation (RFA) techniques became well-known procedures for controlling small HCC. Inflammation is a major contributing factor to carcinogenesis specially HCC. Inflammatory mediators including interleukin-6 (IL-6) and neutrophil to lymphocyte ratio (NLR) are good candidates to stimulate tumor growth and progression. The aim of this study was to evaluate validity of using NLR and IL-6 as predictors of outcome of interventional treatment for HCC.

Patients and methods: This study was conducted on 136 patients with 145 focal nodular HCCs of 5 cm or less between 2012 and 2015. They were divided into 2 groups, the first group included 72 patients whom had NLR less than 5 and the second group included 64 patients whom had NLR equal or more than 5. Patients underwent PEI or RFA. Clinical assessment, laboratory evaluation and triphasic CT studies were performed to all patients pre-treatment and at 1, 6 and 12 months post treatment. NLR and IL-6 level were performed to all patients pre treatment and at 6 months post treatment.

Results: The percentage of complete ablation is highly significantly higher in group I after 1 month but, there is no significant difference between outcome of treatment after 1 year in both groups one year after treatment. Post-procedural IL-6 showed highly significantly lower levels in patients whose focal lesions remain ablated till 1 year than in those who showed multifocal or local recurrence, while NLR did not show significant difference among these groups. Post-prcedural IL-6 levels were highly significantly lower in survived than died cases, while NLR did not show significant differences between both groups at 1 year follow up. There was no significant difference in the post-procedural level of NLR or IL-6 between the group who was treated with ethanol injection and who was treated with radiofrequency ablation as regard the overall outcome at 1 year follow up.

Conclusions: Pre-procedural NLR is a good indicator of performance status, liver profile, hematological profile and diameter of focal HCC and also is a good predictor for short term outcome (1-6 months) and neither pre-procedural or post-procedural NLR are predictors of long term outcome or survival (1 year). Post-procedural IL-6 could be considered as a good predictor not only for survival, but also for efficacy of treatment.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common form of cancer worldwide and the third most common cause of cancer-related deaths. HCC often occurs in the background of a cirrhotic liver **[1]**. Early detection strategies have increased the number of small HCC amenable to curative treatment **[2].** Percutaneous ablation under ultrasound guidance is currently the best therapy for early-stage HCC when resection or liver transplantation is not possible **[3]**.

Inflammation is a major contributing factor to carcinogenesis **[4]**. Cumulative evidence indicates that inflammatory diseases predispose to the development of different types of tumors **[5]**. HCC represents a classic case of inflammation linked cancer and chemically or genetically induced HCC depends on inflammatory signaling **[4]**. Indeed, HCC almost always develops on a background of chronic liver injury including chronic hepatitis and cirrhosis, conditions regarded as preneoplastic stages **[6]**.

Inflammatory mediators including interleukin-6 (IL-6) and neutrophil to lymphocyte ratio (NLR) are good candidates to stimulate tumor growth and progression **[7-10]**.

NLR is obtained by dividing the absolute neutrophil count by the absolute lymphocyte count. NLR more than or equal to 5 can be considered a valid cutoff **[9,10]**. Patients with elevated NLR have a relative lymphocytopenia and neutrophilic leukocytosis which denote that the balance is tipped in favor of protumor inflammatory response and is associated with poor oncologic outcome **[11].** That fact was observed in many tumors **[12-15]**.

Elevated NLR may correlate with a worse prognosis in patients with HCC who underwent any radiological intervention **[16]**. Preoperative NLR more than or equal to 5 was an adverse predictor of disease free and overall survival for patients undergoing hepatic resection for HCC **[10]**. Increased NLR significantly increases the risk of HCC recurrence and recipient death in patients undergoing transplantation for HCC **[17]**.

IL-6 is essential to trigger hepatocyte proliferation, liver regeneration and survival after partial hepatectomy **[18]**. IL-6 was postulated to correlate with the stage of liver cirrhosis **[19,20]**. IL-6 has both differentiation and growth promoting effects for target cells. It has also been hypothesized that activation of IL-6 gene might trigger initial events leading to oncogenic transformation. IL-6 concentrations are also increased in patients with HCC relative to normal subjects **[19,20]**. There was a significant increase in serum IL-6 concentration of HCC patients as compared to cirrhotic patients**.** Serum IL-6 level was positively correlated with serum α-fetoprotein **[20]**.

The aim of this study was to evaluate validity of using NLR and IL-6 as predictors of outcome of interventional treatment for HCC.

PATIENTS AND METHODS

This study was conducted in the Tropical Medicine in cooperation with Radiotherapy and Clinical Pathology Departments, Faculty of Medicine, Zagazig University, Egypt, during period from December 2012 to February 2015 and included 136 patients presented with 145 focal HCC lesions who underwent Percutaneous Ethanol Injection or Radio Frequency Ablation therapy. Sample size estimation was performed by the Institutional Review Board (IRB). The patients were divided into 2 groups:

Group I: Contained 72 patients with 75 focal lesions (69 patients had single lesion and 3 patients each had 2 lesions). These patients had NLR less than 5.

Group II: Contained 64 patients with 70 focal lesions (58 patients had single lesion and 6 patients each had 2 lesions). These patients had NLR equal or more than 5.

The diagnosis of HCC in a patient with hepatic focal lesion was based on triphasic CT scan showing typical criteria for HCC (early enhancement during arterial phase followed by washout of contrast in porto-venous and delayed phases).

All patients met the enrolment criteria: (i) patients with single focal lesion ≤5 cm or three lesions each ≤3 cm, (ii) liver cirrhosis of Child-Pugh class A or B or non cirrhotic, (iii) platelet counts >50000/mm^3, (iv) prothrombin concentration >60% or INR <1.5, (v) adequate kidney functions (serum creatinine ≤ 2mg/dl). (vi) absence or controlable ascites, (vii) performance status 0-2, (viii) no history of hepatic encephalopathy, (ix) no evidence of extrahepatic metastasis, (x) no evidence of portal vein thrombosis and (xi) no history of operation, chemotherapy, and ablative therapy for the lesions.

Patients with Child-Pugh class C, vascular invasion, extrahepatic metastasis and the patients with coexistent haematological disorders or known active infection (affecting total or differential leucocytic count) were excluded.

A written informed consent was taken from all included patients, and the ethical committee of the university has accepted the study.

Pretreatment assessment

Pre treatment assessment of all patients was done by full history taking, thorough clinical examination, laboratory investigations including liver function,

kidney function, Complete blood count including total and differential WBC counts, α fetoprotein, serological markers for HCV and HBV, NLR (was calculated from the differential count by dividing the absolute neutrophil count by the absolute lymphocyte count) and IL-6 measurement. Radiological examination including ultrasound and triphasic CT study.

RESULTS

Table (1): Comparison between studied groups as regard outcome of treatment after 1 month

Outcome of procedure after 1 month	Group I (n=72)		Group II (n=64)		χ^2	p	Sig.
	No	%	No	%			
Complete ablation	46	63.9 %	21	32.8 %	13.091	<0.001	(HS)
Partial ablation	26	36.1 %	43	67.2 %			

In our study the percentage of complete ablation is highly significantly higher in group I while, the percentage of partial ablation is highly significantly higher in group II.

In our study the percentage of stationary outcome is significantly higher in group I. Otherwise; other outcomes do not show significant differences between both groups as shown in table (2).

Table (2): Follow up of the patients with complete ablation in both groups 6 months after procedures

Outcome of procedures after 6 months	Group I (n=46)		Group II (n=21)		χ^2	p	Sig.
	No	%	No	%			
Stationary	33	71.7	9	42.9	5.142	0.023	(S)
Multifocal	5	10.9	4	19	0.829	0.362	(NS)
Local recurrence	6	13	5	23.8	1.218	0.270	(NS)
Died	2	4.3	3	14.3	2.062	0.151	(NS)

Table (3): Correlation between preprocedural NLR and selected study parameters

Parameters	Preprocedural NLR	
	r	p
Bilirubin (mg/dL)	+ 0.214	0.012 (S)
PT (sec)	+ 0.197	0.022 (S)
Hemoglobin (g/dL)	- 0.452	<0.001 (HS)
Platelet count ($x10^3$/mm3)	- 0.455	<0.001 (HS)
Age (years)	+ 0.519	<0.001 (HS)
Diameter of focal lesion (cm)	+ 0.277	0.001 (HS)

There is a significant direct correlation between the mean pre-procedural NLR with bilirubin and prothrombin time and a highly significant direct correlation with age and diameter of focal lesion and a highly significant indirect correlation with hemoglobin and platelet count.

Table (4): Relation between post-procedural IL-6 and outcome after 1 year

	Stationary (n=35)	Multifocal (n=11)	Local recurrence (n=10)	Test	p	Sig.
IL-6				KW		
Mean	83.29	192.64	141.5	13.341	0.001	(HS)
SD	90.21	153.30	135.19			

There is high significant difference between stationary, multifocal and local recurrence groups as outcome after one year as regard post-procedural IL-6.

Table (5): Relation between postprocedural IL-6 and survival after 1 year

	Survive (n=116)	Died (n=20)	MW	p	Sig.
IL-6					
Mean ± SD	102.91 ± 106.12	237.5 ± 121.22	-4.727	<0.001	**(HS)**
Median (Range)	70 (3 – 437)	215.5 (65 – 437)			

There is high significant difference between survived and died groups after one year as regard post-procedural IL-6.

DISCUSSION

Till the time this study is planned for in October 2012, many studies had been published to evaluate validity of using either NLR or IL-6 as predictors of outcome of interventional treatment for HCC but - to our knowledge- no studies including both NLR and IL6 in the same study.

Jang and collegues showed that IL-6 was the most significant cytokine predictive of HCC survival. A high serum IL-6 level appeared to reflect the tumor burden, because it was significantly associated with tumor size, stage and aggressiveness such as portal vein invasion and metastasis **[21]**. Kim and collegues measured the level of 13 member of cytokine family in HCC patients and found that Among the 13 cytokines tested in this study, IL-6 was most strongly related to tumor characteristics including tumor size, number, and the presence of metastasis suggesting a significant role of IL-6 in HCC progression **[22]**.

In this study, the patients in group II (NLR ≥5) are highly significantly older than group I (NLR <5). Also there is a highly significant direct correlation between the mean preprocedural NLR and age and this is not in agreement with Huang et al. **[16]** who found no difference as regard age while, there is no significant difference as regard sex between both groups in agreement with Fu et al**. [31]**. There is no significant correlation between pre-procedural IL-6 and age in agreement with many researchers **[24,25,30].**

As regard viral hepatitis profile, the mean pre-procedural NLR was significantly higher in patients who are infected by both hepatitis B and C viruses. Between both groups there was no significant difference in the percentage distribution of viral markers in agreement with Liao et al. **[23]** but not in agreement with Huang et al.**[16]** who studied 145 patients, most of them was HBs Ag +ve. IL-6 did not show any significant differences between groups as regard viral hepatitis profile in agreement with Chau et al. and Pang et al. **[24,25]**.

As regard clinical presentation right hypochondrial pain was the most frequent symptom in both groups while, lower limb edema and history of ascites were present in group II only with significant difference in agreement with Gomez et al. **[10]** but not in agreement with McNally et al**[27]** who found no significant difference as regard presence of ascites between both groups. There was no significant difference as regard ultrasonographic findings between both groups in agreement with Pinato et al. **[28].**

The mean diameters of focal lesions were highly significantly larger in group II than group I. There was a highly significant direct correlation between the mean preprocedural NLR and diameter of focal lesion in agreement with many researchers **[23,31,36]** but not in agreement with Huang et al and Motomuraet al. **[16,29]** because they studied 158 patients, 101 cases of them had received previous lines of treatment for HCC before the beginning of the study.

The explanation of that association is unclear. One possible explanation is that patients with a high NLR usually have an enhanced neutrophil response. Circulating neutrophils could produce and secrete pro-angiogenetic factors, including VEGF **[37]**, interleukin- 8 (IL-8) **[38]** and MMP **[39]** which may promote tumor growth. Another explanation is that the host's immune response to tumors depends on lymphocytes, and patients with a high NLR have relative lymphocytopenia and neutrocytosis. Lymphocyte-mediated antitumor immune reaction is attenuated in these patients. On the other hand, neutrocytosis can inhibit the cytolytic activity of lymphocytes, natural killer cells, and activated T cells. Thus, both lymphocytopenia and neutrocytosis may be important factors in tumor progression.

There was no significant correlation between the mean preprocedural IL-6 and diameter of focal lesions. Also IL-6 did not show significant difference in its level between large and small focal lesions in agreement with Parasole et al **[30]** but not in agreement with Jang et al. and

Pang et al. **[21,25]** who proved that high levels of serum IL-6 were associated with larger tumor diameters in HCC patients and they considered the high serum IL-6 levels as a novel tumor marker for HCC.

There was no significant difference as regard number of focal lesions in both groups. There were no significant differences in the mean preprocedural NLR and the number of focal lesions in agreement with Motomura et al**. [29]** and not in agreement with McNally et al**. [27]** who studied 103 HCC patients with multifocal lesions and most of them had more than 3 focal lesions. Also there was no significant differences in the mean preprocedural IL-6 and the number of focal lesions in agreement with many researchers **[21, 24,25,32]**.

There was no significant difference in the percentage distribution of Child-Pugh classes between both groups before procedure. After procedure there was highly significant downgrading in Child-Pugh classes in patients of group II than group I. This downgrading could be due to the inflammatory response induced by the locoregional treatment which is more prominent in group II or due to the natural history of cirrhosis itself. There was no significant correlation between the mean pre-procedural NLR and Child-Pugh classes in agreement with some researchers **[28,35]** but not in agreement with Fu et al**. [31]** who studied 282 Chinese patients with HBV-associated HCC after radical hepatectomy. Hence, the etiology of HCC may affect the predictive ability of NLR.

There was no significant correlation between the mean preprocedural IL-6 and Child-Pugh classes in agreement with Parasole et al**. [30]** and not in agreement with Kim et al**[22]** who measured the level of 13 member of cytokine family in Korean HCC patients and found significant association between IL-6 and Child-Pugh class.

The liver profile tests were more deranged in patients with elevated NLR who showed more derangement after locoregional treatment than patients with normal NLR. Patients with elevated NLR showed also elevation in serum creatinine after locoregional treatment which is absent in patients with normal NLR.

Although the impaired synthetic function accompanying chronic liver disease is a main determinant of reduced serum albumin, this reduction also reflects subclinical inflammatory response and malnutrition. These overlapping conditions affect the prognosis of cancer patients. Hepatic albumin biosynthesis is downregulated by proinflammatory stimuli as part of a negative acute phase reaction, in patients with malignancy **[40].** This is obvious in this study where serum albumin significantly reduced after ethanol injection or radiofrequency ablation in both groups. The cell death that occur after these maneuvers leads to production of macromolecules called damage associated molecular patterns (DAMPs) that trigger the production of cytokines and other proteins that regulate and amplify the inflammatory response with its consequences **[4].**

There was a significant direct correlation between the mean preprocedural NLR and bilirubin and prothrombin time. IL-6 did not show any correlation with these laboratory results in agreement with Chau et al. and Pang et al. **[24,25]**.

Hemoglobin and platelet count were highly significantly lower in group II before and after procedure while, after procedure there were highly significant lowering in hemoglobin, platelet count and white blood cells count in both groups. There was a highly significant indirect correlation between the mean preprocedural NLR and hemoglobin concentration and platelet count. That was not in agreement with Fu et al. **[31]**.

In this study alpha fetoprotein showed highly significant decrease in group I and significant decrease in group II after procedure while no significant difference in its level between both groups either before or after procedures. Neither preprocedural NLR or IL-6 showed any correlation with alpha fetoprotein in agreement with Motomura et al. and Parasole et al. **[29,30]** but not in agreement with Wang et al **[36]** who studies 101 patients, most of them had larger focal lesion from 5 up to 8 centimeters and found direct correlation between NLR and alpha fetoprotein**.**

There was a highly significant direct correlation between the mean preprocedural NLR and the mean postprocedural NLR in agreement with Huang et al. **[16]** but not in agreement with McNally et al**. [27]** who studied 103 HCC patients underwent TACE**.** This may be attributed to the effect of TACE as different procedure on NLR.

After procedure, IL-6 increased in group I and decreased in group II insignificantly and there was highly significant difference in the level of postprocedural IL-6 between both groups in agreement with Chau et al.**[24]** but not in

agreement with Erinjeri et al. **[34].** Both studies showed change in the level of IL-6 but no study of them classified patients from the start according to NLR.

The percentage of complete ablation was highly significantly higher in group I while, the percentage of partial ablation was highly significantly higher in group II in agreement with Fu et al.**[31]** but not in agreement with Sullivan et al. **[35]** who studied lower number of cases (75 HCC patients) who underwent different procedures as resection, surgery or transplantation. The mean preprocedural NLR was highly significantly higher in patients with partial ablation than patients with complete ablation in agreement with Liao et al. **[23]** but not in agreement with McNally et al. **[27]** who studied 103 HCC patients underwent TACE and found no statistically significant difference in the mean preprocedural NLR between the partial and complete ablation. IL-6 did not show significant difference between patients with partial ablation or patients with complete ablation in agreement with Chau et al. and Pang et al. **[24,25]**.

The percentage of stationary outcome was significantly higher in group I than group II 6 months after procedure while, one year after procedure there was no significant difference between outcome of complete ablation in both groups. Also follow up of all patients in both groups one year after procedures showed that there was no significant difference as regard outcome of procedures in both groups. The mean preprocedural NLR levels did not show significant difference between groups with different outcomes not in agreement with Liao et al.**[23]** who studied 256 HCC patients underwent surgery with longer duration of follow up. IL-6 levels did not show any significant difference between groups with different outcomes in agreement with Pang et al.**[25].**

There was no significant difference in one year survival between both groups. Also there was no significant difference in the mean preprocedural NLR between the survived and died groups in agreement with Fu et al.**[31]** but not in agreement with Pinato et al.**[28]** who performed a meta analysis of 578 HCC patients underwent different lines of treatment as surgery, interventional radiology, sorafenib or chemotherapy**.** Also, there was no significant difference in the mean preprocedural IL-6 between the survived and died groups in agreement with Chau et al.and Parasole et al. **[24,30]** but not in agreement with Pang et al.**[25]** who studied only 80 patients underwent resection. Also not in agreement with Jang et al.**[21]** who studied 110 patient underwent TACE.

In this study, the measurement of NLR and IL-6 was done after six months after procedure in order to limit the influence of post procedure inflammation on the results. A study made by Erinjeri et al. **[34]** showed that percutaneous thermal ablation in HCC patients invokes an inflammatory response that is characterized by elevation in the plasma levels of specific cytokines. IL-6 and IL-10 show the most robust increases in plasma levels within 48 hours of ablation. Also they showed that in patients undergoing image-guided ablation, heat-based ablation techniques showed 3.5 fold increases in IL-6 levels compared with a 54 fold increase with cryoablation**.** Some researchers showed that after open surgical radiofrequency ablation of hepatic malignancy, IL-6 levels increased 4 fold and after open hepatic cryoablation, IL-6 levels increased more than 100 fold **[41,42].**

The postprocedural IL-6 showed highly significant difference among patients with different outcomes at one year follow up while, postprocedural NLR did not show any significant difference among different outcomes or survived and died groups. So, postprocedural IL-6 could be considered as a good predictor not only for survival, but also for efficacy of treatment in agreement with Wong et al.**[43]** who proved that patients with hepatitis B and a high plasma level of IL-6 have HCC free survival three times shorter than patients with low IL-6 levels, even after controlling for age, gender, cirrhosis, use of antiviral treatments, hepatitis B virus DNA, and peak alanine aminotransferase levels. This is not in agreement with Chau et al. **[24]** who found no significant difference in the outcome between patients with high or low levels of serum IL-6 and with Parasole et al. **[30]** who failed to confirm that serum IL-6 correlates with HCC stage or prognosis.

Theoretically, NLR and IL-6 were expected to decrease after procedure because necrotic tumor cells can generate a permanent immunogenic source of tumor antigens (in situ tumor vaccine) for the induction of antitumor immunity. Previous reports described spontaneous regression of pulmonary metastases from renal cell carcinoma after thermal ablation of primary tumor Sanchez-

Ortiz et al. **[44]** or regression of multiple pulmonary metastases after ablation of single metastasis **[45].**

Induced immune response after interventional procedure is mostly weak, and not sufficient for the complete eradication of established tumors or durable prevention of disease progression, but it could produce a favorable effect on the outcome and survival Dan et al. **[46]** but in some patients, the balance between host inflammatory response and immune response is tipped in favor of pro-tumor inflammatory response where IL-6 remain high or increased after interventional treatment and predicts poor oncologic outcome. In this group of patients, it is reasonable to manipulate the systemic inflammatory response through targeted anti-inflammatory mediators such as IL-6 blocking antibodies Chua et al.**[26]**. There was no significant difference in the postprocedural level of NLR or IL-6 between the group who was treated with ethanol injection and who was treated with radiofrequency ablation as regard the overall outcome at 1 year follow up.

The conflict between results of different studies of NLR or IL-6 may be attributed to differences in age, performance status, Child-Pugh classes as some studies included Child C, different races and population, different criteria of selection of HCC patients as presence of metastasis, portal vein invasion, larger diameter of focal lesions or different alpha fetoprotein levels, different predisposing factors of HCC including different serology of viral hepatitis, also use of different lines of treatment as percutaneous ablation, transarterial chemo-embolization, systemic chemotherapy, hepatic resection, sorafenib, liver transplantation or mixed lines of treatment.

Conflict of Interest: None

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

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Insulin Resistance as Predictor for Esophageal Varices in Hepatitis C Virus Cirrhosis

Fatma Mohamed Abd El-Salam[1], Hatem Samir Abd El-Raouf[1], Salama Mohamed El Shinawy[2] and Mohamed Shawky Mostafa[3]

[1] *Hepatology, Gastroenterology and Infectious Diseases Department, Faculty of Medicine, Benha University,Benha,Egypt.*
[2] *Clinical pathology Department, Shebein EL-Kom Teaching hospital, Shebein EL-Kom, Egypt.*
[3] *Hepatology, Gastroenterology and Infectious Diseases Department , Shebein El-Kom Teaching Hospital, Shebein El-Kom,Egypt*

Corresponding Author
Mohammed Shawky Mostafa

Mobile:
+201228135224

E mail:
Dr.shawky2008@gmail.com

Key words:
Oesophageal varices, , Insulin resistance, Upper gastrointestinal endoscopy.

Background and study aim : Portal hypertension is one of the most important complications of liver cirrhosis. Endoscopic screening of all patients with liver cirrhosis would result in a large number of unnecessary additional burden to endoscopic units**.** This study is designed to assess insulin resistance in cirrhotic patients due to hepatitis C infection as non invasive parameter for esophageal varices.

Patients and Methods :This study was conducted on 50 cirrhotic patients (Child A) post hepatitis C who attended the Hepatology Department and outpatient clinic at Shebein El Kom Teaching Hospital. All the patients were evaluated by thorough history ,clinical examination, biochemical parameters ,metabolic features, including insulin resistance by the homeostasis model assessment (HOMA), ultrasonongraphy , liver biopsy and upper gastrointestinal endoscopy to search for esophageal varices.

Results : EVs (esophageal varices) were detected in 18 of 50 patients. By multivariate analysis ,the presence of EVs was independently associated with a low platelet count/spleen diameter ratio (562.75 $\pm$ 99.16) , a high HOMA-IR score (5.49 $\pm$ 0.754), high body mass index , low hemoglobin, low albumin, high alanine aminotransferase and high aspartate aminotransferase.

Conclusion: Insulin resistance in patients with Hepatitis C virus cirrhosis (Child A) measured by HOMA-IR score significantly predicts the presence esophageal varices in this patients and can be used as non-invasive parameter for predicting esophageal varices.

INTRODUCTION

Hepatitis C virus infection is a serious worldwide problem. It has been estimated that there are 130–150 million HCV infection worldwide, with 350 000 to 500 000 people die each year from hepatitis C-related liver diseases **[1]**.

Portal hypertension which is considered as one of the most important complications of liver cirrhosis is associated with development of a hyper dynamic circulation and complications such as ascites, hepatic encephalopathy and oesophago-gastric varices. Patients with cirrhosis and gastro-oesophageal varices have a hepatic venous pressure gradient during hemodynamic catheterization of at least 10-12 mmHg **[2]**.

Estimated prevalence of esophageal varices is approximately 50%.The risk of bleeding from varices is 25%-35% with majority of the initial bleeding occurring within 1 year from varices detection **[3]**.

Variceal bleeding is associated with a high morbidity and mortality The mortality associated with each episode of variceal bleeding ranges from 17% to 57% **[4]**.

The incidence of bleeding can be reduced with nonselective beta-blockers **[5]**.

It is also suggested that prophylactic endoscopic variceal band ligation can decrease the incidence of variceal bleeding and mortality in patients with liver cirrhosis who have large varices **[6]**.

The prevalence of oesophageal varices among cirrhotic patients is variable, ranging from 24% to 80%. Therefore, endoscopic screening of all patients with liver cirrhosis would result in a large number of unnecessary additional burden to endoscopic units **[7]**.

Several studies have examined how to identify patients with varices using non-invasive or minimally invasive methods to avoid endoscopy in patients with a low risk of varices. These studies include biochemical, clinical and ultrasound parameters, transient elastography, CT scanning and video capsule endoscopy **[8]**.

Insulin resistance (IR) is exceedingly common in patients with hepatitis C virus (HCV)-related chronic liver disease **[9]**.

IR has been systematically associated with advanced fibrosis and fibrosis progression in several reports **[10]**.

Camma and his colleagues identify a high HOMA-IR score as a new independent predictor of the presence of esophageal varices **[11]**.

PATIENTS AND METHODS

Study design: Cross-sectional study**.**

Patients

We enrolled in the study 50 newly diagnosed patients with Child A HCV cirrhosis, consecutively observed at Hepatology department at Shebein El Kom Teaching Hospital in the period between May to December 2014, fulfilling all criteria detailed below.

Inclusion criteria:

Patients were included if they had a diagnosis of HCV cirrhosis based on liver biopsy.

Exclusion criteria :

(1) Advanced cirrhosis (Child-Pugh classes B and C).
(2) Other causes of liver disease.
(3) Human immunodeficiency virus infection.
(4) Current or previous history of ascites or hepatic encephalopathy or portal hypertensive bleeding.
(5) Hepatocellular carcinoma.
(6) Portal vein thrombosis.
(7) Current treatment with any dosage of insulin.
(8) Previous or current treatment beta-blockers, diuretics, or other vasoactive drugs.
(9) Parenteral drug addiction or alcohol abuse in the last year.
(10) Type 2 diabetes.

The study was performed after written informed consent from all patients.

Clinical and Laboratory Assessment

The following data were collected at the time of recruitment: age, sex, weight, and height. Body mass index (BMI) was calculated as weight in kilograms/height in square meters. Patients with a BMI of 18.5 to 24.9 kg/m2 were classified as normal, those with a BMI of 25 to 29.9 as overweight, those with a BMI of 30 or more as obese. Clinical examination for Signs of chronic liver disease.

A 12-hour overnight fasting blood sample was drawn at the time of recruitment to determine the serum levels of (AST-ALT-PT-INR-Urea-Creatinine-CBC-Albumin-Bilirubin-HCV RNA and blood glucose level) Serum insulin was determined by enzyme-linked immunosorbent assay (Monobid Inc. Lake forest, USA).IR was determined by the homeostasis model assessment (HOMA) method by using the following equation: Insulin resistance (HOMA-IR=Fasting insulin (µU/mL)×fasting glucose (mg/dl)/405 [12]. Cut off value for diagnosis of IR was ≥1.775 according to the international diabetes federation (IDF) (left) and Adult Treatment Panel III (ATP III) in non-diabetic individuals.

Instrumental Assessment

After an overnight fast, all patients underwent an ultrasound examination with single viewer operator in supine position to detect the presence of liver cirrhosis (irregular surface, Irregular liver margins, coarse texture, attenuated hepatic veins, Relative enlargement of caudate lobe, signs of portal hypertension (presence of abdominal collaterals or splenomegally), ascites, portal vein diameter, splenic vein diameter, the span of the right lobe in the mid clavicular line on oblique view and classified as shrunken (<11 cm), average (11-15 cm) or enlarged (>15 cm) and to exclude hepatic focal lesion.

Upper gastrointestinal endoscopy

Was done by the same endoscopist after fasting for at least 6 hours in left lateral position with examining esophagus for varices occurrence, size and risk signs of bleeding (red wales&cherry red spots and duodenum till second part and stomach for gastropathy and fundal varices.

Esophageal varices was graded on the basis of their size classification and according to AASLD guidelines as follows: Grade 1: Small straight cords of varices confined to the lower third of esophagus. Grade 2: Enlarged and tortuous varices occupying less than one third of the lumen. Grade 3: Large coil shaped esophageal varices occupying more than one third of the lumen **[13,14]**.

Statistical analysis

Statistical presentation and analysis of the present study was conducted SPSS V.20. Data was expressed into two phases:

I- Descriptive 1- Mean value (X) and Standard Deviation [SD]: for quantitative data. 2- Frequency and percenatage for qualitative data.

II- Analytic by t-student test and Chi-square test. P value> 0.05 was considered statistically non significant P value ≤0.05 was considered statistically significant. P value ≤0.001 was considered statistically highly significant. Variables found to be associated with the dependent variable at univariate logistic regression at a probability threshold of less than 0.10 were entered into multivariate logistic regression models. To avoid the effect of co linearity, HOMA-IR score, blood glucose levels and insulin levels as well as platelet count, spleen diameter, and platelet count/spleen diameter ratio, were not included in the same multivariate model.

RESULTS

Study was conducted on 50 patients,30(60%) were males and 20(40%) were females. Mean age was 42.32± 10.13, mean BMI was 33.8± 2.73 and mean HOMA-IR was 31.43± 3.23. Baseline features of studied cases are present in table (1).

In the present study varices was present in 18 (36%) of cases divided 11 patients was grade I and 7 patients was grade II. Factors associated with esophageal varices are shown in table (2).

In the present study high HOMA score and low platelets/splenic ratio were associated with varices presence. HOMA Cut off value was ≥3.6, specificity 74%, sensitivity 67%, positive predictive value 77 and negative predictive value 63.

ROC curves analysis (Fig. 1) identified HOMA-IR score of ≥3.6 (AUC, 0.621; SE, 0.061; 95% CI, sensitivity, 67%; specificity, 74%; positive likelihood ratio, 2.82; negative likelihood ratio, 0.60) as the best cutoff for predicting the presence of EVs.

Figure (1): ROC curve for evaluating role of HOMA in prediction of varices

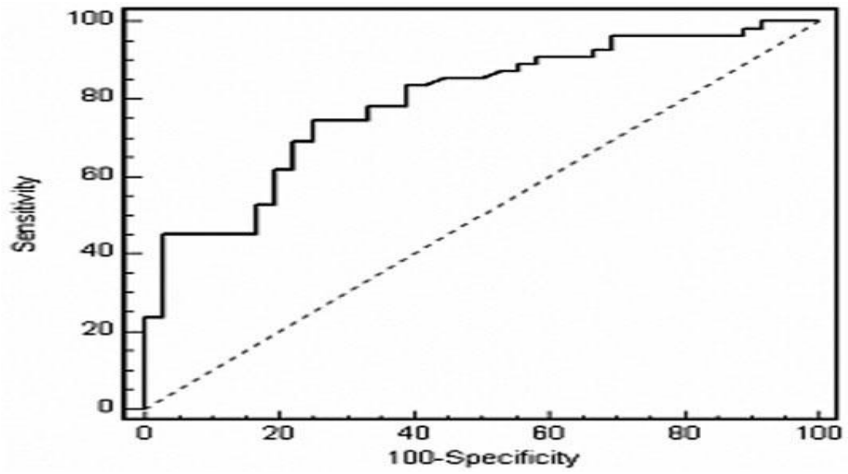

Table (1): Baseline features of studied cases (n=50)

Variable	Mean	Standard Deviation
Age (years)	42.32	± 10.13
BMI(body mass index) kg/m^2	31.43	± 3.23
Waist circumference (cm)	107	± 3.1
Hemoglobin (gm/dl)	12.55	± 1.37
White blood cells (cell/mm3)	6582.0	± 2032.15
Platelet /mm^3	135260	± 38644.19
Albumin (gm/dl)	4.0040	± 0.451
Total Bilirubin (mg/dl)	0.78	± 0.284
Direct Bilirubin (mg/dl)	0.24	± 0.222
Prothrombin time (seconds)	11.81	± 1.320
INR	.997	± 0.183
ALT (IU/L)	86.78	± 14.152
AST (IU/L)	101.68	± 16.531
Spleen Diameter (mm)	131.96	± 15.359
Splenic Vein (mm)	9.31	± 0.946
Platelet/splenic Index	1059.77	± 418.707
Urea (mg/dl)	31.28	± 6.259
Creatinine (mg/dl)	0.82	± 0.217
Fasting Sugar (mg/dl)	94.92	± 16.457
Fasting Insulin (µU/ml)	13.95	± 4.421
HOMA-IR	3.45	±1.39
Right liver lobe diameter (cm)	13.37	± 1.597
Portal vein diameter (mm)	13.25	± 2.460
Child-Pugh A5 45 (90%) A6 5 (10%) **Esophageal varices** Present 18 (36%) Absent 32 (64%)		

Table (2): Correlation between Data of the study and esophageal varices by univariate and multivariate analysis

Variable	With varices n=18	Without varices n=32	Univariate Analysis *P* Value	Multivariate Analysis OR (95% CI) *P* Value
Age (years)	41.0 ± 9.35	43.06 ± 10.63	0.496	-
BMI(body mass index) kg/m^2	33.8 ± 2.73	30.11 ± 2.72	0.002	0.624 (0.015)
Hemoglobin (gm/dl)	11.52 ± 1.01	13.14 ± 1.21	0.001	2.931 (0.024)
White blood cells (cell/mm3)	5727.77 ± 1772.94	7062.5 ± 2035.13	0.024	1.003 (0.987)
Platelet×10^3 (cell/mm3)	71.0 ± 16.28	164.94 ± 23.1	0.001	-
Albumin (gm/dl)	3.59 ± 0.255	4.23 ± 0.366	0.001	1.405 (0.012)
Total Bilirubin (mg/dl)	0.989 ± 0.291	0.663 ± 0.204	0.001	0.005 (0.061)
Direct Bilirubin (mg/dl)	0.407 ± 0.201	0.148± 0.033	0.001	0.015 (0.066)
Prothrombin time (seconds)	12.88 ± 1.27	11.20 ± 0.906	0.001	0.251 (0.071)
INR	1.15 ± 0.185	0.909 ± 0.112	0.001	0.00 (0.091)
ALT (IU/L)	98.77 ± 10.07	80.03 ± 11.42	0.001	0.855 (0.001)
AST (IU/L)	117.33 ± 11.63	92.87 ± 11.64	0.001	0.817 (0.001)
Spleen Diameter (mm)	147.22 ± 14.84	123.37 ± 6.39	0.001	-
Splenic Vein (mm)	10.25 ± 0.289	8.78 ± 0.755	0.001	0.00 (0.989)
Platelets/splenic Index	562.75 ± 99.16	1339.35 ± 218.17	0.001	0.855 (0.001)
Urea (mg/dl)	29.83 ± 6.62	32.09 ± 6.0	0.224	-
Creatinine (mg/dl)	0.783 ± 0.217	0.853 ± 0.217	0.281	-
Fasting Sugar (mg/dl)	114.61 ± 8.49	83.84 ± 6.06	0.001	-
Fasting Insulin (µU/ml)	19.33 ± 1.51	10.93 ± 1.85	0.001	-
HOMA-IR	5.49 ± 0.754	2.29 ± 0.518	0.001	1.0 (0.020)
Right liver lobe diameter (cm)	11.36 ± 0.564	14.51± 0.412	0.001	0.00 (0.996)
Portal vein diameter (mm)	16.25 ± 1.14	11.56± 0.830	0.001	0.00 (0.995)

DISCUSSION

Portal hypertension is one of the main consequences of cirrhosis. It can result in severe complications, including bleeding of esophago-gastric varices as well as spontaneous bacterial peritonitis or hepatorenal syndrome(HRS) as complications of ascites **[15]**.

American Association for the Study of Liver Diseases 2007 single-topic symposium on portal hypertension **[2]** have recommended endoscopic screening for EV in patients with cirrhosis, regardless of Child class and cause. This policy is expensive, and it would be useful to have noninvasive predictors of EV in compensated Child A patients, at low risk of portal hypertension, as a pre-endoscopy screening tool. This goal was reasonably attained in the current study.

Several studies in chronic liver diseases have shown a strong and independent pathogenic link between IR (insulin resistance) and HCV infection **[10,16]** and between IR and the severity of hepatic fibrosis **[9.10.16]**. Although our work was not designed to clarify the pathogenic interaction between IR and presence of EVs, a few hypotheses can be put forward. Insulin is able to modulate the endothelial synthesis of nitric oxide and endothelin **[17,18]**, to induce the production of tumor necrosis factor alpha and connective growth factor, and to stimulate hepatic stellate cells,[19,20] the effectors in the pathogenesis of liver fibrosis and PH **[21]**. Therefore, insulin could contribute to the pathogenesis of PH by interfering with both mechanical and dynamic mechanisms leading to collagen deposition, vasoconstriction, and regulation of sinusoidal structure.

The study was conducted on 50 cirrhotic patients (Child A) post hepatitis C who attended the Hepatology department and outpatient clinic at Shebein El Kom Teaching Hospital.

In this study we aimed to identify HOMA-IR score, an easy biochemical marker, predicting the presence of EVs.

Several studies have shown that splenomegally **[22,23]**, Child score **[24]**, low serum HB level **[24]**, low platelets **[25,26]**, low albumin **[27,28]** ,liver enzymes **[29]**, prothrombin time **[24,28]**, high portal vein diameter **[28]**, high splenic vein diameter **[30]**, right liver lobe diameter **[31]** and Platelets count/spleen diameter ratio **[11,32-35]** could serve as predictors of EVs presence. However, all of these studies were quite heterogeneous, enrolling patients with cirrhosis of different causes (viral, alcoholic, and mixed) and different disease severity (Child B or end stage liver disease).

In our study HOMA-IR score for measuring insulin resistance was found higher in patients with varices (mean= 5.49 $\pm$ 0.754) than patients without varices (mean= 2.29 $\pm$ 0.518) as patients with varices were obese with central type. Statistically there was significance between high HOMA-IR score and esophageal varices presence (p=0.001) .Cut off value was ≥3.6 ,specificity 74%, sensitivity 67% ,positive predictive value 77 and negative predictive value 63.As reported by Camm`a and his collogues **[11]** that there was relation between HOMA-IR score and esophageal varices with cut off value >3.5, specificity 76%, sensitivity 61%.

The small number of patients included in our study and differences among studies in terms of demographic features, baseline severity, and cause of disease may explain the conflicting results, as well as differences in the statistical methods.

On performing multivariate logistic regression analysis, it was found that high body mass index, low hemoglobin level, low albumin level, high ALT, high AST, low PLT/Splenic ratio and high HOMA-IR score are significant predictors of esophageal varices presence.

The study has limitations. First, the analysis was carried out in a small number of patients, and it will be interesting to determine whether this association holds true also in larger groups of patients with HCV cirrhosis and in patients with liver disease of other origins. Lack of data on other variables, such as direct measurement of portal hypertension by HVPG, also could affect the interpretation of our findings. Finally, we cannot exclude the possibility that hidden abuse of alcohol may be responsible for the presence of EV in a few subjects.

In conclusion, insulin resistance in patients with Hepatitis C virus cirrhosis (Child A) measured by HOMA-IR score significantly predicts the presence esophageal varices in this patients and can be used as non-invasive parameter for predicting esophageal varices, and it is cheaper than endoscopy but not sufficient to be good negative test or surrogate marker alone, still endoscopy is the gold standard for diagnosis of varices.

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Study of Serum Golgi Protein 73 Level as a Marker for Diagnosis of Hepatocellular Carcinoma

Hany Said Sabry[1], Hossam Ibrahim Mohamed[1], Wafaa Mahmoud Radwan[2], Naglaa Said Mohamed El-Abd[1]

[1]*Tropical Medicine Department, Faculty of Medicine, Menoufia University, Menoufia, Egypt*
[2]*Clinical Pathology Department, Faculty of Medicine, Menoufia University, Menoufia, Egypt*

Corresponding Author
Naglaa Said Mohamed El-Abd

Mobile: +201092304322

E mail:
Naglaa_elabd@yahoo.com

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Golgi protein 73, Hepatocellular carcinoma, Alpha-fetoprotein

Background and study aim: Hepatocellular carcinoma (HCC) is the fifth most common neoplasm in the world, and the third most common cause of cancer-related death. Golgi protein 73 is normally expressed in epithelial cells of many human tissues. GP73 expression is upregulated in hepatocytes, and in serum from patients with hepatitis and liver cirrhosis regardless the etiology. This work aimed to study the diagnostic role of serum Golgi protein 73level as a marker for HCC.

Patients and methods: This study was conducted on 48 patients with HCC on top of liver cirrhosis (GI), 20 patients with liver cirrhosis (GII), and 20 healthy controls (GIII). Patient and controls were subjected to careful medical history, full clinical examination and laboratory investigations including CBC, ESR, liver function tests, renal function tests, viral markers, serum AFP and Serum Golgi protein 73 by ELISA.

Results: Serum GP73 showed highly significant increase (with P value <0.001) in HCC group X±SD (1765.92±747.99) in comparison with cirrhoticX±SD (772.45±73.84) and control X±SD (458.30±103.03) groups, also significantly increased in cirrhotic group in comparison with control group. There was significant increase in mean values of serum GP73 in patients with HCC associated with portal vein thrombosis or lymph node enlargement also there was significant positive correlationbetween GP73 and tumor size. In diagnosis of HCC, at cut off point 55 ng/ml, AFP had sensitivity 81.3% and specificity 70.0%, and Gp73 at cut off point 847.5 ng/l, the sensitivity was 93.8% and specificity 90.0%. With combined use of AFP and Gp73: the sensitivity of diagnosis of HCC increased to 95.8%.

Conclusion: Significant increase in sensitivity, accuracy and negative predictive value with combined use of AFP and Gp73 than AFP alone in diagnosis of HCC.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common neoplasm in theworld, and the third most common cause of cancer-related death. The burden of this devastating cancer is expected to increase further in coming years **[1]**.As most patients with HCC are diagnosed at an advanced stage with underlying liver dysfunction, the mortality rate of HCC is similar to the incidence rate. Early detection of HCC is therefore extremely important in improving the survival of patients **[2]**.

Approximately 70%-90% of patients with HCC have an established back-ground of chronic liver disease and cirrhosis, with major risk factors for developing cirrhosis including chronic infection with hepatitis B virus (HBV),hepatitis C virus (HCV), alcoholic liverdisease, and nonalcoholic steatohepatitis(NASH). Patients with cirrhosis are thus usually included in surveillance plans **[3]**.

AFP is the most widely tested biomarker in HCC. But the clinical value of AFP is challenged due to low sensitivity and specificity. AFP is not elevated in all patients with HCC. Some patients with cirrhosis and/or hepatic inflammation can have an elevated AFP, even withoutthe presence of a tumor **[4]**. Alternative serum biomarkers are being actively sought include prothrombin induced byvitamin K absence (PIVKA), glypican-3,

and squamous cell carcinoma antigen-1; however, none of these have been adequately investigated to be recommended as a screening test **[5]**.

Golgi protein 73 (GP73) also named Golgi phosphorprotein 2 (GOLPH2), is a type II Golgi localized integral membrane protein that is normallyexpressed in epithelial cells of many human tissues. GP73 was first identified in a genetic screen for proteins with differential expression in adult giant-cell hepatitis (GCH) **[6]**. It is consistently present in biliary epithelial cells in normal livers, and hepatocytes show little or no signal. However, GP73 expression is upregulated in hepatocytes, and in serum samples from patients with acute & chronic hepatitis and liver cirrhosis regardless the etiology **[7]**. The aim of the present work was to study the diagnostic role of serum Golgi protein 73level as a marker for HCC in comparison with serum Alpha-fetoprotein.

PATIENTS AND METHODS

This study was conducted on 68 patients with liver cirrhosis with or without HCC and 20 healthy subjects of matched age and sex as controls. Patients and controls were selected from inpatients and outpatients clinic of TropicalMedicineDepartmentMenoufiaUniversityHospital in the period between June 2012 and December 2013. Patients were 52 (76.5%) males and 16 (23.5%) females. Their ages were ranging between 31-76 years old. An informed consent was obtained before patients entered the study. Diagnosis of cirrhosis was done by clinical examination, ultra-sonographic finding and laboratory investigations; while diagnosis of HCC was done by their characteristic features in 2 imaging methods (abdominal ultrasonography and triphasic CT).

Patients and controls were classified into the following groups:

Group I: Comprised 48 patients with HCC on top of liver cirrhosis. All patients did not receive prior treatment for HCC.

Group II: Comprised 20 patients with liver cirrhosis.

Group III: Comprised 20 healthy subjects as a control group.

Exclusion criteria:

1. Focal hepatic lesions other than HCC (cholangiocarcinoma, hemangioma, hepato-blastoma, metastatic focal lesions…etc).
2. Malignancy elsewhere

Patients and controls were subjected to the following:

1- Careful medical history
2- Full clinical examination
3- Laboratory investigations including complete blood picture, ESR, liver function tests, renalfunction tests and viral markers
4- Serum AFP level by ELISA
5- Serum Golgi protein 73 was quantitatively determined by ELISA GP73 provided by Glory science catalog number 11598. The range of the kit was: 50ng/L→1500ng/L. Principle of test; the kit used a double-antibody sandwich ELISA to assay the level of Human Golgi protein-73(GP-73) in samples. Add (GP-73) to monoclonal antibody Enzyme well which is pre-coated with Human (GP-73)monoclonal antibody, incubation; then, add (GP-73) antibodies labeled with biotin, and combined with Streptavidin-HRP to form immune complex; then carry out incubation and washing again to remove the uncombined enzyme. Then add Chromogen Solution A, B, the color of the liquid changes into the blue, and at the effect of acid, the color finally becomes yellow. The chroma of color of the Human Substance (GP-73) of sample was positively correlated.
6- Imaging studies (abdominal ultrasonography, triphasic CT).

Statistical Analysis:

The data were collected, tabulated, and analyzed by SPSS (statistical package for social science) version 17.0 on IBM compatible computer. Significance of results: Non-significant difference if $P > 0.05$, Significant difference if $P < 0.05$ and highly significant difference if $P < 0.01$.

RESULTS

Demographics of the studied groups

There was no significant difference between the three studied groups as regards age and sex.Patients were 52 (76.5%) males and 16 (23.5%) females. Their ages were ranging between 31-76 years old as well as 20 healthy subjects of matched age and sex as controls. Themean of age in HCC group was 57.85±7.23, in cirrhotic group was 48.25±7.99 and in control group was 54.70±8.12. In GI, there were 38 (79.2%) males and 10 (20.8%) females, in GII there were14 (70%) males, and 6 (30%) females and in GIII there were14 (70%) males, and 6 (30%) females.Manifestations of cirrhosis were

present in various proportions in all GI and GII patients. Some manifestations consistent with HCC (anorexia and loss of weight) were present in various proportions in HCC patients.

Biochemistry of the studied groups

There was highly significant decrease in mean values of hemoglobin concentration and platelet count in HCC and cirrhotic groups in comparison with control group. While there was no significant difference between GI & GII as regards platelets count and Hb concentration. There was no significant difference between studied groups as regards total WBCs.

Statistical analysis revealed that there was highly significant increase in mean values of AST, ALT and serum bilirubin as well as highly significant decrease in mean values of serum albumin and prothrombin concentration in HCC and cirrhotic groups in comparison with control group.

Chronic HCV was the commonest etiology of cirrhosis in GI (85.4%) and GII patients (80.0%) while chronic HBV was far less common etiology. There was non-significant difference between HCC group and cirrhotic group as regards the Child classification.

Triphasic CT revealed evidence of cirrhosis in all GI & GII patients as well as imaging evidence consistent with HCC in in all GI patients (Table 1).

There was highly significant increase in mean values of serum AFP & GP73 in HCC group in comparison with other groups and in cirrhotic group in comparison with control group (Table 2).

There was no significant difference in mean values of AFP as regard tumor size in the HCC group, While there was statistical significant increase in the mean values of Gp73 with increasing the tumor size (more than 5 cm) (Table 3 and Figure 1).

Statistical analysis revealed significant increase in mean values of serum GP73 in patients with HCC complicated by portal vein thrombosis in comparison with patients without portal vein thrombosis, while there was no significant difference between patients with HCC with or without portal vein thrombosis as regards mean values of AFP. Also there was significant increase in mean values of serum GP73 in patients with HCC associated with lymph node enlargement in comparison with patients without, while there was no significant difference between patients with HCC with or without lymph node enlargement as regards mean values of AFP (Table 4).

Statistical analysis revealed no significant difference in mean values of AFP as regard Child-Pugh grades (A, B, C) in the HCC group, while there was significant increase in the mean values of Gp73 with increasing Child grades in HCC.There was no significant difference in mean values of both AFP and GP73 as regard Child-Pugh grades (A,B,C) in the cirrhotic group (Table 5).

Receiver operating characteristic curve (ROC curve) of AFP and GP73 for diagnosis of HCC versus cirrhotic cases showed that the cutoff point was 55ng/ml for AFP and 847.5ng/l for GP73 (Figure 2).

At cut off point 55 ng/ml, AFP had sensitivity 81.3%, specificity 70.0%, and accuracy 77.9%, while for Gp73 at cut off point 847.5 ng/l, the sensitivity was 93.8%, specificity 90.0% and accuracy 92.6% in diagnosis of HCC. With combined use of AFP and Gp73: the sensitivity increased to 95.8% (Table 6).

Table (1): Triphasic CT findings among the studied patients

Triphasic CT findings	GI (N = 48)		GII (N = 20)	
	No	%	No	%
Liver size				
Average	3	6.25	1	5.0
Hepatomegaly	18	37.5	1	5.0
Shrunken	27	56.25	18	90.0
Wavy or lobulated Outline	48	100	20	100
Size of the focal lesion(s)				
X ± SD	2.30 ± 2.36			
Range	1.7 – 12		-	-
Number of the focal lesion(s)				
One	32	66.7		
Two	4	8.3	-	-
Multicentric	12	25.0		
Ascitis	38	79.2	17	85.0
Spleen				
- Splenomegaly	47	97.9	19	95.0
- Splenectomy	1	2.1	1	5.0
- Dilated collaterals	10	20.8	5	25.0
Portal vein (PV)				
Normal	6	12.5	2	10.0
Dilated	42	87.5	18	90.0
PV Thrombosis	10	20.8	1	5.0
Lymph nodes enlargement	6	12.5	0	0.0

Table (2): Comparison between studied groups as regards AFP and Gp73

	The studied groups			Mann Whitney U test	P value
	Group I N = 48	Group II N = 20	Group III N = 20		
AFP(ng/ml)					
X ± SD	771.89±1002.99	30.0±27.17	1.15±0.94	4.86 6.44 5.31	<0.001[1] <0.001[2] <0.001[3]
Range	19 – 2850	1 – 90	0.8 – 5		
Gp73(ng/l)					
X ± SD	1765.92±747.99	772.45±73.84	458.30±103.03	6.23 6.41 4.92	<0.001[1] <0.001[2] <0.001[3]
Range	810 – 3515	635-890	350 - 665		

1 = Comparison between HCC cases and cirrhotic cases
2 = Comparison between HCC cases and control
3 = Comparison between cirrhotic cases and control

Table (3): The relation between tumor size and AFP & GP73 levels in GI

Variable	Tumor size		Kruskal Wallis Test	P value
	< 5 cm (n= 26)	≥ 5 cm (n= 22)		
AFP (ng/ml) X±SD Range	705.38±967.58 7 – 2850	850.5±1060.69 19 – 2800	0.17	0.87
Gp73 (ng/l) X±SD Range	1485.85±575.02 815 – 2900	2106.0±787.99 895 – 3515	2.76	0.006

Correlation between GP73 and tumor size

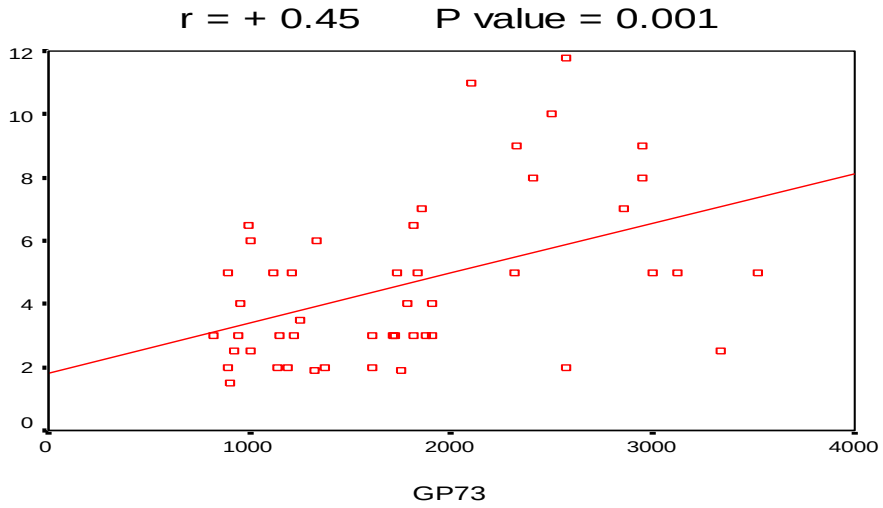

Figure 1 Showed significant positive correlation between GP73 and tumor size

Table (4): The relation between AFP & GP73 levels and PV thrombosis or LN enlargement in GI

Variable	Portal vein		Mann Whitney U	P value
	Patent(n=38)	Thrombosis(n= 10)		
AFP (ng/ml) X±SD Range	743.84±1014.92 19 – 2800	878.5±1001.46 10 – 2850	0.56	0.58
Gp73 (ng/l) X±SD Range	1660.63±726.35 815 – 3515	2186.0±681.76 895 – 2915	2.07	0.04
	Lymph nodes enlargement			
	LN enlargement (n=6)	**No LN enlargement (n= 42)**		
AFP (ng/ml) X±SD Range	1455.0±1438.95 49 – 2850	674.31±907.01 19 – 2800	1.28	0.20
Gp73 (ng/l) X±SD Range	2487.67±455.41 1781 - 3515	1662.81±727.29 810 – 2995	2.56	0.01

Table (5): The relation between Child classification and AFP & GP73 in GI & GII

Variable	Child-Pugh classification in GI			Kruskal Wallis Test	P value
	A (n= 5)	B (n=11)	C (n= 32)		
AFP(ng/ml) X±SD Range	1510.80±1355.54 13 – 2750	536.45±790.44 7 – 2750	737.37±988.75 19 – 2850	0.64	0.72
Gp73 (ng/l) X±SD Range	986.0±132.64 895 – 1220	1661.45±785.76 895 – 3515	1929.94±709.9 815 – 2915	9.92	0.007
	Child-Pugh classification in GII				
	A (n= 3)	**B (n= 6)**	**C (n= 11)**		
AFP (ng/ml) X±SD Range	26.33±29.16 9 – 60	53.17±33.82 13 – 90	18.36±13.65 1 – 44	3.93	0.14
Gp73 (ng/l) X±SD Range	703.0±96.16 645 – 814	776.5±44.13 699 – 815	789.18±75.96 655 – 890	2.21	0.33

ROC curve of AFP and GP73 for diagnosis of HCC versus cirrhotic cases

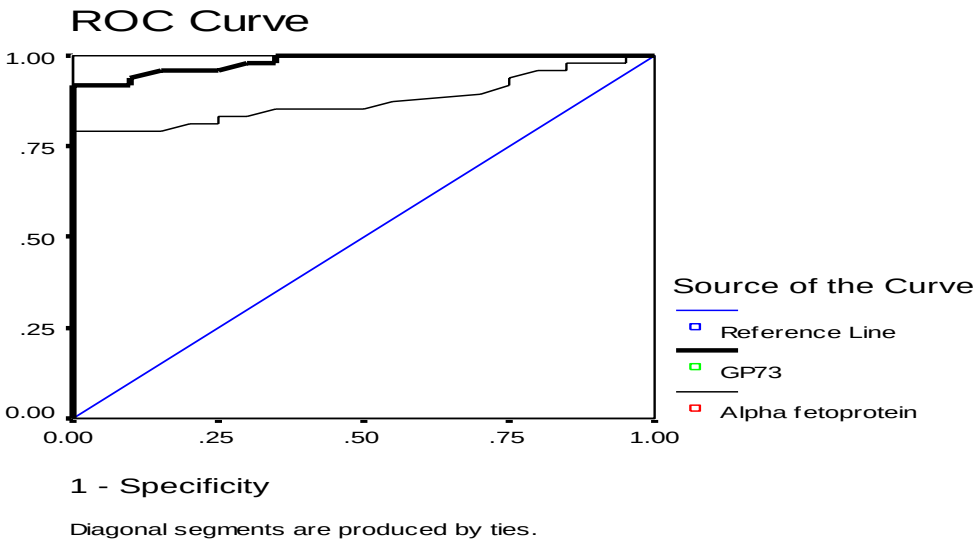

Figure (2) : ROC curve of AFP and GP73 for diagnosis of HCC versus cirrhotic cases.

Table (6): Diagnostic performance of AFP and gp73 in diagnosis of HCC

Variable	AFP	GP73	Both
AUC	0.88	0.98	---
P Value	<0.001	<0.001	---
Cutoff	55	847.5	----
Sensitivity (%)	81.3	93.8	95.8
Specificity (%)	70.0	90.0	70.0
Positive predictive value (%)	86.7	95.7	88.5
Negative predictive value (%)	60.9	85.7	87.5
Accuracy (%)	77.9	92.6	88.2

DISCUSSION

Regarding age distribution in HCC group, the present study showed that age was ranging from 43 to 76 years with mean age of the patients 57.85±7.23. This was in agreement with Amer et al. **[8]** who reported thatmean age of the patients with HCC was 54±8.6 years, while Yang and Roberts **[9]** found HCC to be more frequent in individuals of an average age of about 64 years andEl-Serag**[10]** reported that HCC is rare before 40 and peaks around age 70. The endemicity of HCV infection in Egypt, in addition, other contributing environmental factors such as the presence of aflatoxin in many food stuff, and contamination with insecticides may explain the occurrence of HCC at younger age groups **[11]**.

Regarding the gender distribution, the present study showed that HCC is more prevalent in men than in women with 3.8 times higher in men, these results were in agreement with El-Serag**[10]** who reported that there was a striking male HCC predominance, with the highest male: female ratios (averaging between 2:1 and 4:1) in high HCC incidence areas. Male sex predominance of HCC may be attributed to sex hormones and sex-specific differences in exposure to risk factors. Men are more likely to be infected with HBV and HCV, consume alcohol, smoke cigarettes, and have increased iron stores. However, experiments show a 2 - 8 fold increase in HCC development in male mice. These data support the hypothesis that androgens influence HCC progression rather than sex-specific exposure to risk factors **[10]**. Several studies conducted in Taiwan reported a positive association between increased circulating testosterone levels and HCC in HBV-infected men **[12]**.

In the present study, Chronic HCV was the commonest etiology of cirrhosis in HCC group (85.4%), HBV was less frequent (8.3%) and coinfection with both viruses in (6.3%), this was in agreement with Amer et al. **[8]** who found that the major risk factor for cirrhosis and the subsequent development of HCC was chronic hepatitis C (67.7%) and to a lesser extent chronic hepatitis B (8.8%) and coinfection with both viruses (4.8%). The major factors increasing the risk of HCC are chronic hepatitis B and C as well as cirrhosis, irrespective of its etiology. In North America, Europe, and other areas of low prevalence of HCC, most patients have underlying cirrhosis unrelated to HBV or HCV infection **[13]**. The etiological contribution of viral hepatitis toward the risk of HCC differs in different countries according to the prevalence of viral infection and other causes of liver cirrhosis. Kumar et al. **[14]** found that chronic HBV infection is the major factor for the development of HCC in China.

As regards the relation between HCC and the severity of cirrhosis, the present study revealed that about two thirds of HCC patients (66.7%) were Child C cirrhosis, 22.9% of the patients were Child B and 10.4% were Child A liver cirrhosis). These results were in agreement with Amer et al. **[8]** who found that 54.65% of HCC patients were Child C cirrhosis and HCC occurred least frequently in patients with Child A liver cirrhosis (15.4%).

The present study revealed a higher level of AFP in both cirrhotic and HCC groups than the control group and it is significantly higher in HCC group when compared to cirrhotic and control groups. AFP levels in cirrhotic group ranged from 1 to 90ng/ml and in HCC group it was 1.9–2850ng/ml. The value of AFP in diagnosis of HCC was variable in different studies. Chan et al. **[15]** found elevated serum AFP levels not more than 200ng/ml in patients with benign liver conditions such as hepatitis and

cirrhosis. Also Yoshida et al. **[16]** reported normal AFP levels in approximately one-third of patients with HCC a large number of HCC patients have AFP values <400 ng/mL, making them very difficult to undergo detection and prognosis of HCC. Similarly, Tsai et al. **[17]** observed that an AFP level less than 400ng/ml was noted in 51% of HCC patients, furthermore, at least one third of small HCC and up to 30% of advanced HCC will be missed unless other diagnostic tools are used. In addition, AFP may be elevated in non-malignant liver diseases, so, it is obvious that AFP alone is not a reliable indicator for detection and prognosis of HCC.

In our study, statistical analysis revealed that there was highly significant increase in mean values of serum GP73 in HCC group in comparison with other groups and in cirrhotic group in comparison with control group. GP73 in HCC group ranged 810 – 3515 ng/l and in cirrhotic group ranged 635-890 ng/l while in control group it was 350 - 665. These results were in agreement with Mao et al. **[18]** have found that the elevation of serum GP73 is mildest in chronic viral hepatitis, moderate in patients with cirrhosis and dramatic increased in patients with HCC. Similarly Tian et al. **[19]**, El Shafie et al. **[20]** and Wang et al. **[21]** reported that, serum GP73 in HCC was higher than in liver cirrhosis and chronic hepatitis and in all patients were higher than those in healthy individuals. On the other hand Ozkan et al. **[22]** and Shi et al. **[23]** failed to find significant elevation of serum GP73 in HCC groups compared with that in liver cirrhosis groups and that the potential clinical value of GP73 as a better serum biomarker than AFP remains controversial.

The relation between elevation of serum GP73 in patients with HCC and the etiology of liver cirrhosis couldn't be assessed in the present study because of the small number of patients with etiology other than HCV (only 4 patients with HCC have chronic HBV infection), although it was found that serum GP73 was increased in HCC that developed on top of cirrhosis caused by either HCV or HBV. Previous study reported that the most profound elevation of serum levels of GP73 was detected in patients who had developed an HCC on the background of HCV infection **[24]**. Similarly Riener et al. **[25]** found significant (3- to 5-fold) increases the serum GP73 levels were seen in HCC patients with underlying HCV infection (especially HCV genotype 1b) when compared with patients with HCC unrelated to HCV. However Mao et al. **[26]** showed that serum GP73 levels in HCC patients with underlying HBV infection were significantly higher than those of the HBV carriers, patients with liver cirrhosis, and healthy controls. They reported that serum GP73 had a higher sensitivity and specificity in diagnosis of hepatitis B-related HCC than AFP, and that it could be as an effective HCC tumor marker in Chinese Patients.

In the current study there was no significant difference in mean values of AFP as regard tumor size however Ba et al. **[27]** repotted that serum AFP levels have been shown to correlate with tumor size. There was significant increase in mean values of serum GP73 in patients with HCC with increased tumor size (more than 5cm), this was in agreement with Sun et al. **[28]**. In contrast,Ozkan et al. **[22]** and Mao et al. **[18]** reported that serum levels of GP73 were not correlated with tumor sizes.

In the current study there was significant increase in mean value of serum GP73 in patients with HCC complicated by portal vein thrombosis while there was no significant difference as regards mean value of AFP. These results were similar to those obtained by El Shafie et al. **[20]** who reported that the level of GP73 correlated with more aggressive tumor characters including vascular invasion with no significant difference as regards mean value of AFP, On the other hand Ozkan et al. **[22]** reported that there was no correlationbetween GP73 levelsportal vein thrombosis. In the current study there was significant increase in mean value of serum GP73 in patients with HCC associated with lymph nodes, this was in agreement withFimmel and Wright **[29]** who recorded that, the degree of GP73 expression correlated with lymph nodes invasion while Ozkan et al. **[22]** did not find significant correlation.

The present study revealed significant increase in the mean values of GP73 in HCC group in relation to the severity of liver cirrhosis (the highest value was in patients with Child-Pugh grade C. This was in agreement with El Shafie et al. **[20]** who found significant correlation between serum GP73 level and child score in HCC patients on the other hand Mao et al. **[18]** reported that serum levels of GP73 in patients with HCC were not correlated with Child-Pugh grades (A, B,C). our study revealed no significant difference in mean values of GP73 as regard Child-Pugh grades (A, B, C) in the cirrhotic group, on the

other hand Tian et al. **[19]** reported that, serum GP73 in patients with Child-Pugh class A was lower than in class B and C in liver cirrhosis.

In the current study the area under AUROC for AFP was 0.88 and at cut off point 55 ng/ml, the sensitivity of AFP was 81.3% and specificity was 70.0%. Trevisani et al. **[30]** reported that specificity of AFP varied from about 76% to 96% and increased with elevated cutoff value, also El Shafie et al. **[20]** found that AFP had a sensitivity of 77.4% and a specificity of 60% at a cut-off 28.51 ng/ml.

In our study the area under AUROC for GP73 was 0.98 with sensitivity 93.8% and specificity 90.0% at cut off point 847.5ng/l. Marrero and Lok**[31]** postulated that, GP73 is up-regulated in HCC and measurement of serum GP73 revealed a sensitivity and specificity of 69% and 75%, respectively. Also El Shafie et al. **[20]** postulated that GP73 had a sensitivity of 87% and a specificity of 95% at the optimal cut-off value of7.62 ng/ml. These results were disappointing with Gu et al. **[32]** who found that GP73 elevated in patients with liver disease but did not distinguish between HCC, cirrhosis, and chronic hepatitis. Riener et al. **[25]** reported GP73 was surprisingly found to be decreased in HCC patients and doubt on the diagnostic utility of GP73 as a serum marker of HCC.

In our study we found that with combined use of AFP and GP73 there was significant increase in sensitivity of detection of HCC up to 95.8% than using either of them alone, these results were in agreement with Wang et al. **[33]** and Mao et al. **[18]** who reported that the combined measurement of GP73 and AFP can further increase the sensitivity for the detection of HCC, also Omran et al. **[34]** reported that by combining serum GP73 and AFP for the diagnosis of HCC, it was found that sensitivity rises to 93% and El Shafie et al. **[20]** reported that when GP73 used in combination with AFP, they lead to an enhanced sensitivity of detection of HCC up to 90.3%.

CONCLUSION

The sensitivity, accuracy and negative predictive value for diagnosis of HCC in cirrhotic patients increased to 95.8%, 88.2% and 87.5% respectively with combined assay of serum GP73 and serum AFP. Serum GP73 levels correlated positively with tumor size, portal vein thrombosis and lymph node involvement in cirrhotic patients with HCC.

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Ethical approval: Was granted by the Institutional Review Board and informed consent was obtained from each patient prior to inclusion in the study.

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Peer reviewers: Dr. Amal Fawzy; Assistant Professor of Clinical and Chemical Pathology, National Cancer Institute, Cairo University, Egypt.**Dr. Salem Yousef Mohamed,** Department of Internal Medicine, Faculty of Medicine, Zagazig University, Egypt.**Editor: Mohamed H Emara;** Lecturer of Tropical Medicine, Faculty of Medicine ,Zagazig University, Egypt.

Radiofrequency Thermal Ablation versus Microwave Ablation for Small Hepatocellular Carcinoma

Mohamed Abd El-Hamid Mohamed[1], Hatem Samir Abd El-Raouf[1], Waleed Ahmed El Agawy[2], Mohamed Elsayed Elshewi[1], Esam Elsaid Hamed[2]

[1]*Hepatology, Gastroenterology and Infectious Diseases Department, Faculty of Medicine, Benha University,Benha,Egypt*

[2]*Hepatology, Gastroenterology and Infectious Diseases Department, Mahalla Hepatology Teaching Hospital, Egypt.*

Corresponding Author ***Esam Elsaid Hamed***

Mobile: 01063809608

E mail: esam.hamed84@gmail.com

Key words: Hepatocellular carcinoma, Radiofrequency ablation, Microwave ablation

Background and Study Aim : Hepatocellular carcinoma (HCC) is the fifth most common form of cancer worldwide and the third most common cause of cancer-related deaths. This study was designed to investigate the therapeutic efficacy of percutaneous Radiofrequency ablation versus Microwave ablation for small HCC measuring ≤3 cm in diameter.

Patients and methods : This study was carried out in Al-Mahalla Hepatology Teaching Hospital on 30 patients with cirrhosis and small HCC. All the patients were evaluated by thorough history, clinical examination, laboratory investigations, abdominal ultrasound and spiral triphasic CT.

Results: The mean age was 56.2±5.8, 70% (21) were males and 30% (9) were females. There was highly statistical significant increase in liver function in MW ablation as regard AST, ALT and bilirubin, and decrease in α FP level of both groups after treatment. There was no significant difference between two groups in the response to treatment as regarding Triphasic CT and complications.

Conclusion: Microwave (MW) and Radiofrequency (RF) ablation are similar in pathologic appearance and imaging characteristics, but RFA has many limitations and many complications. MW ablation offers many of the advantages of RF ablation while overcoming some of its limitations and the heat-sink effect.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common form of cancer worldwide and the third most common cause of cancer-related deaths. HCC often occurs in the background of a cirrhotic liver **[1]**.

Hepatectomy offers the best outcomes for patients with HCC **[2.3]**. Unfortunately, there are less than 30% of cases are amenable to hepatectomy at the time of diagnosis due to advanced tumor stage and underlying liver cirrhosis **[4]**.

When hepatectomy options are contraindicated, image-guided tumor ablation therapy is recommended as the most appropriate therapeutic choice and is considered a potentially curative treatment in properly selected candidates **[5]**.

In the past two decades, thermal ablation therapy by using energy sources has been increasingly accepted due to the advantages of greater capacity to ablate HCC with fewer treatment sessions. Among the thermal ablation therapy Radiofrequency (RF) ablation and Microwave (MW) ablation are the most commonly used modalities **[6.7]**.

Radiofrequency (RF) ablation has been considered to be the most common thermal ablation modality worldwide for early stage HCC, with 80–95% complete tumor necrosis and 33–57% 5-year survival **[8]**.

Microwave (MW) ablation, which generates an electromagnetic field in the tissue and causes rapid and homogeneous rotation of molecules in order to damage the tumor thermally. Microwave ablation (MWA) offers many of the advantages of RF ablation.

In addition, a larger zone of ablation, faster treatment time, more complete tumor kill And overcome heat sink effect of adjacent blood vessel **[9]**.

Microwave ablation results in significantly larger zones of coagulation than does RF ablation this is due to longer lengths of the coagulated zone, as there is no significant difference between MW and RF ablation for short-axis diameter or maximum diameter **[10]**.

PATIENTS AND METHODS

Type of the study: Prospective (cohort) study.

Patients :
We enrolled in the study 30 diagnosed patients with cirrhosis and small hepatocellular carcinoma ≤3cm at Hepatology Department at Al Mahalla Hepatology Teaching Hospital in the period between May 2014 to March 2015, fulfilling all criteria detailed below.

Inclusion criteria:
1- Solitary tumour 3 cm or less in diameter.
2- Patients with Child-Pugh class A or B, not candidate for hepatic resection or no possibility of surgical intervention.

Exclusion criteria :
1- Tumours larger than 3cm in diameter.
2- Hepatic metastasis or abdominal lymph node infiltration.
3- Portal vein thrombosis.
4- Tumours located within 1cm of the liver hilum, gall bladder or common bile duct.
5- Tumours in the dome of the liver may be unreachable percuaneously.
6- Platelet count less than 60,000/ml or pro-thrombin concentration less than 60%.
7- Patients with Child-Pugh class C.

The patients were randomized into two group:
Group 1: included 15 patients treated by RF thermal ablation.
Group 2: included 15 patients treated by MW ablation.

Clinical and Laboratory Assessment:
All patients were subjected to History taking, Thorough clinical examination General: orientation, jaundice, lower limb oedema or other manifestation suggesting hepatic encephalopathy. Local: hepatomegaly, splenomegaly or ascites. Laboratory investigations: viral markers: anti-HCV antibodies and HBsAg. Complete blood count including (Hb%, WBC and platelets). Serum creatinine, liver function tests including (ALT, AST, alkaline phosphatase, serum albumin, serum bilirubin and prothrombin concentration. Serum alpha fetoprotein before and one month after ablation. Imaging: Abdominal ultrasound for assessment of **:** Hepatic focal lesion**:** site, size and echo pattern. Size of spleen and ascites. Spiral triphasic CT: for enhanced criteria of the hepatic lesion which represents the backbone in the diagnosis of HCC in cirrhotic patients. Liver biopsy or fine needle aspiration (FNA) cytology (if result of triphasic CT is non conclusive)**.** Patients were evaluated for liver function reservoir according Child-Pugh classification **[11]**.

Percutaneous Ablation Procedures :
Treatment was performed with the patient under conscious sedation and analgesia induced by the administration of diazepam 10-20 mg (Neuril; Nile) or propofol (Deprivan) intravenously. All ablation procedures were performed under local anesthesia with 1% lidocaine. Real-time ultrasound was used for the guidance and monitoring of ablation procedures. The aim of the treatment was to completely destroy the tumor with a safety margin of 0.5–1.0 cm normal liver tissue. At the end of the procedure, needle track can be done to prevent any tumor cell dissemination.

Percutaneous RF ablation.
In this study we used both RITA Medical System and Boston Scientific :

- **RITA Medical System:**
  This device relies on direct temperature measurement. Five of the electrodes are hollow and contain thermocouples in their tips to measure the tissue temperature. Probe-tip temperatures, tissue impedance and wattage are displayed on the RF generator and are recorded by dedicated software. Maximum power output of the RF generator, amount of electrode array deployment from the trocar and duration of the effective time of the ablation depend on the desired volume of ablation. The needle is expanded up to 4 cm to achieve safety margin (0.5-1.0 cm) **[12.13]**.

- **Boston Scientific:**
  Another manufacturer (Boston Scientific, Natick, MA) device that relies on electrical measurement of tissue impedance rather than on tissue temperature. The electrode is made by an insulated 14-gauge outer needle that

houses retractable curved electrodes **[14]**. The needle used is up to 4 cm which is expanded in the tumor for ablation. The ablation is performed in two phases (phase I & phase II).

Percutaneous MW ablation.

- **H.S System:**
  This microwave delivery system consisted of a mw generator named AMICA Gen., operating at frequency of 2450 MHz and a power output up to 100 W, and a 14 gauge (14g × 150 mm and 14g × 200 mm) cooled shaft electrode, with real-time us guidance, the needle is percutaneous introduced through the guiding needle into the tumor and the active tip is placed in the deepest part of the tumor to completely eradicate the tumor, for small tumors (≤ 3cm), single application MWA was performed, to prevent possible tumor seeding, the needle track is cauterized for 10 seconds when the antenna is withdrawn **[15]**.

Statistical analysis :

Statistical presentation and analysis of the present study was conducted SPSS V.20. Data was expressed into two phases :

I. Descriptive 1- Mean value (X) and Standard Deviation [SD]: for quantitative data. 2- Frequency and percenatage for qualitative data.
II. Analytic by t-student test and Chi-square test. P value >0.05 was considered statistically non significant P value ≤0.05 was considered statistically significant. P value ≤0.001 was considered statistically highly significant.

RESULTS

Baseline features of studied cases are present in (Tables 1,2).

Study was conducted on 30 patients with mean age 56.2±5.8, 70% (21) were males and 30% (9) were females.

In the present study The total bilirubin (TB) levels, the AST and ALT levels were significantly elevated at 48 h in patients after both RF ablation and MW ablation compared with the baseline levels (P,<0.001). The increase in the AST, ALT and total bilirubin (TB) levels was highly significantly larger in MW ablation group than in RF ablation. There were no significant changes of albumin (ALB) after treatment (Table 3).

There was highly significant decrease in α FP level of both groups after treatment shown in table (4).

There was no significant difference between two groups in the response to treatment as regarding Triphasic CT. (Table 5).

There was no statistical significant difference between MWA and RFA as regarding complications (pain, hematoma, abscess and fever). A low-grade Fever was observed after treatment in 4 patients in MWA group (26.7%) and in 2 patients of RFA group (13.3%), it resolved with antipyretic. Pain in the upper abdomen was observed in 3 patients of MWA group (20%) and in 5 patients of RF group (33.3%) during the course of sessions, this patient required the administration of analgesics with prescription of Nimesulide (0.1 g/d) for 3–4 days. Hematoma occurred in one patient of MWA group (6.7%). Abscess occurred in one patient of RF group (6.7%), it resolved with repeated aspiration. There were no major complications observed in the studied groups. There were no skin burn and tumor seeding in the study (Table 6).

Table (1): Sex distribution among studied groups (n=30)

Males		Females	
No	**%**	**No**	**%**
21	70	9	30

Table (2): Age distribution among studied groups (n=30)

Variable	Mean ± SD
Age	56.2±5.8

Table (3): Biochemical profile of both groups (MW& RFA) before & one month after the end of treatment

Test	*MW group*			RF group		
	Before mean ± DS	After mean ± DS	P value	Before mean ± DS	After mean ± DS	P value
AST	73.3±49.9	81.2±51.7	<0.001	57.6±44.7	54.2±44.5	.0277
ALT	67.3±48	73.7±48.5	<0.001	52.3±43.5	52.4±43.2	0.860
Bilirubin (total)	1.7±0.7	1.6±0.8	<0.001	1.2±0.4	1.3±0.4	0.167
Albumin	3.8±0.5	3.7±0.5	0.074	3.9±0.4	3.9±0.5	0.171

Table (4): α feto protein levels before and one month after treatment in both groups, paired comparison of Both groups (MWA & RFA group)

Group	α F.P pre	α F.P post	P value
	Mean± S D	Mean± S D	
MW (n.15)	210± 195.3	63.6±65.4	<0.001
RFA (n.15)	169.5±177.9	45.5±55.8	<0.001

Table (5): Criteria of response after treatment in both groups (RFA &MWA)

Criteria of response	MW group (n = 15)		RF group (n = 15)		P value
	No	%	No	%	
Triphasic CT					
Non enhancing	14	93.3	13	86.7	1.0
Enhancing	1	6.7	2	13.3	

Table (6): Complications of both Groups (RFA &MWA)

Complications	MW group (n = 15)		RF group (n = 15)		P value
	No	%	No	%	
Pain	3	20	5	33.3	0.28
Hematoma	1	6.7	0	-	1.0
Abscess	-	-	1	6.7	0.37
Fever	4	26.7	2	13.3	1.0

DISCUSSION

Hepatocellular Carcinoma (HCC) has an increasing incidence worldwide, and it is the leading cause of death in patients with cirrhosis. It is the fifth most common cancer and the third most common cause of cancer-related death **[16.17]**.

Microwaves have higher heating efficiency than RF which renders them unaffected by "heat sink" effect resulting in larger ablation volumes achieved in less time **[18]**.

MW ablation offers many of the advantages of RF ablation and overcoming some of the limitations as conduction of electricity into tissue, a larger zone of ablation, faster treatment time and more complete tumor kill. MW ablation has a much broader power field than does RF ablation up to 2 cm **[9]**.

The mean age of the study group was 56.2±5.8 years.

The earlier age in Egyptians could be explained by the early age of acquisition of viral hepatitis

due to the high incidence of HCV and HBV in some areas, so although the patients in Egypt are relatively young they may still have had 30-40 years of continuous inflammation and necrosis which might be sufficient to develop cirrhosis and eventual HCC at early ages.

Regarding the sex distribution of the studied patients, nearly all the two groups were males.This was in agreement with Omata et al., study who had four females (9.3%) in 43 HCC patients **[19]** and in agreement with El-Kady et al. who had 8 females (13.8%) in a study of 58 patients **[20]** and Livraghi et al. where out of 158 patients 23 were females **[21]**.

The liver biochemical profile in this study (performed before and one month after the end of sessions) showed slight changes after the RF procedure, this was in agreement with Chen et al. who included 110 patients with HCC in their study on RFA **[22]** and El-Kady et al. who included 58 patients with HCC in their study on RFA using the multiple array (Le Veen) needles, they found slight transient increase in transaminase levels above the pre-ablation level in 77% of patients **[21]**. In MWA group there was highly significant change (significant increase) in the liver function tests namely (AST, ALT, bilirubin) after treatment, this was in agreement with Qian et al. and Yamashiki et al. who studied 19 patients with HCC and observed reversible liver dysfunction with elevation of serum level of transaminases in 12 patients after treatement with MW **[23.24]**.

Complete response was observed in 27 patient (90%), partial response in 3 patients (10%) who were treated with another sessions. The results of this study were in agreement with the results of Zhang et al., in which the complete ablation rate was achieved in 83.4% (78/93) of the treated tumors with RF ablation and 86.7% (91/105) in those treated with MW ablation, with no significant difference between RF and MW ablation **[25]**.

In conclusion, MW and RF ablation are means of thermo ablations which are similar in pathologic appearance and imaging characteristics, but RF has many limitations and many complications as it is effective for small and favorably situated tumors, but local progression rates are substantially higher for large tumors (≥3 cm). MW ablation offers many of the advantages of RF ablation while possibly overcoming some of its limitations. MW ablation may be less affected by the heat-sink effect that is thought to contribute to local recurrence after RF ablation.

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Study of the Impact of Liver Cirrhosis on Health-Related Quality of Life in Chronic Hepatic Patients, Menoufia Governorate

Mohamed Alaa Eldin Nouh[1], Amira Maher Badawy[1], Hala Marwan Mohamed[2], Sherien Abd El-Aziz El-Kashash[1]

[1] *Tropical Medicine Department, Faculty of Medicine, Menoufiya University,Egypt.*
[2] *Public Health Department, Faculty of Medicine, Menoufiya University,Egypt.*

Corresponding Author
Sherien Abd El-Aziz El-Kashash

Mobile: 01006660886

E mail: dr.Shosho3@yahoo.com

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Background and study aim: cirrhosis is associated with morbidity and mortality and complications of cirrhosis. Health-related quality of life should be considered an important outcome measure in the treatment of cirrhotic patients. This study is designed to assess the impact of liver cirrhosis on a weighted score of health – related quality of life in those patients.

Patients and Methods: This study was conducted on four groups of patients and control subjects. Group I included 50 Child A liver cirrhosis. Group II included 50 Child B cirrhosis. Group III included 50 Child C liver cirrhosis. Group IV as healthy control subjects. All patients and control subjects completed a Medical Outcomes Study Short Form 36 (SF-36) questionnaires used for measuring the health related quality of life.

Results: There was a highly significant decrease in the short form-36 questionnaire score of health related quality of life with the Child score progression in comparison to other healthy persons, There was statistical significant positive correlation between The SF-36 scores (vitality (energy/fatigue) scale and physical functioning scale) and occupation and education level.

Conclusions: cirrhosis reduced the quality of life of the patients; advanced Child score and development of complications have a negative effect in their quality of life.

INTRODUCTION

Cirrhosis is a serious disease that is associated with significant morbidity and mortality. Patients may suffer from specific complications of cirrhosis such as (hepatic encephalopathy, ascites and variceal bleedings. Furthermore, fatigue, joint pain, pruritis, loss of appetite, depression, abdominal pain, worries about complications of the disease, decreased sexual interest and activity, loneliness, hopelessness, problems with social interaction and problems with memory and concen-tration) have been associated with Chronic liver disease **[1]**.

Quality of life has an inherent meaning to most people. It is comprised of broad concepts that affect global life satisfaction, including good health, adequate housing, employment, personal and family safety, interrelationships, education, and leisure pursuits. For matters related to health care, quality of life has been applied specifically to those life concerns that are most affected by health or illness, hence the term "health-related quality of life" **[2]**.

Health-related quality of life refers to the subjective assessment of patients regarding the physical, mental and social dimensions of well-being. It has become an important measure in clinical and epidemiological studies in gastroenterology and hepatology **[3]**.

Health-related quality of life refers to a patient's /the patient's perception of his/her state of health the assessment is not limited to medical interventions. The perception of quality of life varies between individuals and is dynamic. Individuals who present with the same clinical picture have different expectations and report different qualities of life **[4]**.

The quality of life of the patients were evaluating from the point of view of the patients, their family, and their care takers to find appropriate interventions, and training and counselling programme to support patients **[5]**.

PATIENTS AND METHODS

This study was carried out on patients attending the outpatient clinic and inpatient of Shebin El-Kom Fever Hospital and patient admitted at Endemic Medicine Department, Menoufia University Hospitals in the period between April to December 2014, from which 150 cirrhotic patients were selected, in addition to 50 healthy persons of matched age and sex as controls. The patients were further subdivided into three groups. Group I included 50 Child A patients, Group II included 50 Child B patients , group III included 50 Child C patients and group IV included 50 healthy persons as controls.

After have an informed consent, all patients and controls were subjected to full history taking, full clinical examination, abdominal ultrasonography, and laboratory investigations including: CBC, ESR, RBS, liver function tests, Alfa feto protein, urine ,kidney function, Prothrombin time and concentration, bilirubin, HCV Ab & RNA. HBs Ag and core antibodies, HBV-DNA, International normalized ratio, TSH, Iron, Transferritin, Alkaline phosphatise, Gamma glutamyl transferase, Fasting total cholesterol, ceruloplasmine and the medical study short form (SF-36).

Exclusion criteria

- Patients with hepatic encephalopathy.
- Patients with unrelated psychological disturbance.
- Chronic medical or inflammatory disease.

Sample collection and measurement of quality of life

The questionnaire was translated into Arabic, Patients in outpatient clinic took a copy to home to complete it and brought it in the next visit while inpatients completed it inside the hospital and for each patient. The SF-36 questionnaire consists of 36 questions (items) measuring physical and mental health status in relation to eight health concepts (physical functioning ,role limitations due to physical health, bodily pain, social functioning, role limitations due to emotional health, general health scale, physical functioning, vitality (energy/fatigue), general mental health (psychological distress/wellbeing) **[6]**.

Statistical analysis :

Data were collected, tabulated, statistically analyzed by computer using SPSS version 22, two types of statistics were done:

1- Descriptive statistics:

Quantitative data expressed to measure the central tendency of data and diversion around the, mean (x) and standard deviation (SD). Qualitative data expressed in number and percentage.

2- Analytic statistics:

- Student test was used for comparison of two groups of normally distributed variables, chi-square (x2) was used to compare categorical outcomes.
- ANOVA test was used for comparison of more than two groups of normally distributed variables, post hoc (Scheffee test) was used to test significance between individual groups.
- Spearman correlation (r) was used to detect association between quantitative variable and ordinal qualitative variable.

The level of significance: $p<0.05$, $P<0.001$.

RESULTS

There was a high statistically significant past history of hematemesis ,jaundice and anti bilharzial treatment in hepatic patients in comparison with control group as shown in table (1) ($P<0.001$).

There was a high statistically significant difference on the short form 36 (SF-36) questionnaires as regarding to physical functioning, role of limitation due to physical functioning, bodily pain scale, general health scale of quality of life among studied groups that were collected from them as shown in table (2).

There was no statistical significance between group 1 (Child A) and group 4 (controls) in all scores of the short form 36 (SF-36) questionnaires (physical functioning, role of limitation due to physical functioning, bodily pain scale, general health scale, vitality scale, social functioning scale, mental health scale) of quality of life with highly significance in emotional problems limitation scale .

On the other hand, there was a high statistical significance on the short form 36 (SF-36) questionnaires as regarding vitality scale, social functioning scale, role of limitation due to emotional problems scale, mental health scale of quality of life among studied groups that were

collected from them(p value <0.001) as shown in figure (1).

There was statistical significant positive correlation between the education and the parameters of the SF-36 scores as regard to the vitality (energy/ fatigue) scale and physical functioning scale (p value <0.05) as shown in table (3).

There was statistical significant positive correlation between the occupation and the parameters of the SF-36 scores as regard to the vitality (energy/ fatigue) scale and physical functioning scale(p value <0.05) as shown in table (4).

Table (1): Shows the results of Risk factor and related past history in the studied groups

Studied past history variables	Controls (50)	Patients (150)	X^2 test	P value
Anti bilharzial Treatment injections				
Yes	0	67	9.21	< 0.001**
No	50	83		
Jaundice : Yes	0	97	20.43	< 0.001**
No	50	53		
Haematemesis: Yes	0	62	10.76	< 0.001**
No	50	88		

** Highly significant difference

Table (2): SF-36 scores in the studied groups of patients regarding to physical functioning, role of limitation due to physical function, bodily pain and general health scale of quality of life among studied groups

	Group 1 Child A (50)	Group 2 Child B (50)	Group 3 Child C (50)	Group 4 Controls (50)	ANOVA	P value	Post hoc test
Physical functioning	91.23 ±3.75	76.85 ±1.53	53.65 ±8.74	93,60 ±4.03	76.98	<0.001**	P1>0.05 P2<0.001** P3<0.001** P4<0.001** P5<0.001** P6<0.001**
Role of limitation due to physical function	92.20 ±2.37	76.80 ±1.39	52.90 ±2.22	93.25 ±2.87	756.00	<0.001**	P1>0.05 P2<0.001** P3<0.001** P4<0.001** P5<0.001** P6<0.001**
Bodily pain scale	90.40 ±2.64	77.10 ±1.62	60.65 ±4.69	92.20 ±3.51	231.39	<0.001**	P1>0.05 P2<0.001** P3<0.001** P4<0.001** P5<0.001** P6<0.001**
General health scale	90.60 ±1.90	76.85 ±1.31	62.05 ±4.77	94.21 ±4.12	321.43	<0.001**	P1>0.05 P2<0.001** P3<0.001** P4<0.001** P5<0.001** P6<0.001**

** Highly significant difference

P1: Probability for comparison between G1 and G4
P2: Probability for comparison between G2and G4
P3 Probability for comparison between G3and G4
P4 Probability for comparison between G1 and G2
P5 Probability for comparison between G1 and G3
P6 Probability for comparison between G2nd G3

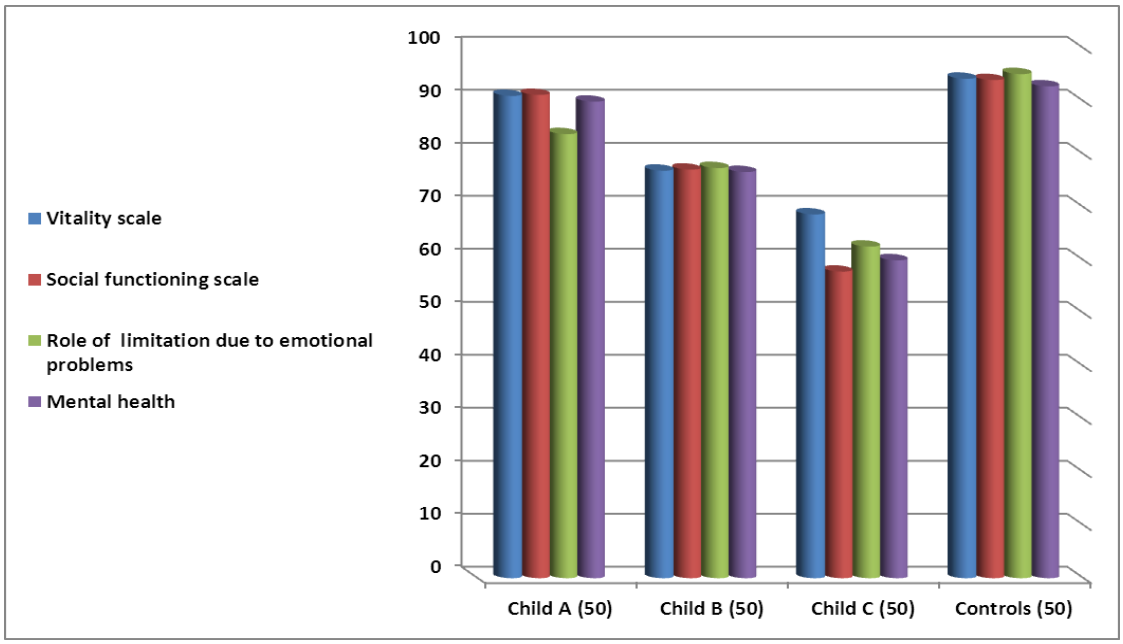

Figure (1)

SF-36 scores in the studied groups of patients regarding to vitality scale, social functioning scale, role of limitation due to emotional problems and mental health scale of quality of life among studied groups:

Table (3): SF-36 scores in correlation to education in the studied patients

Educational level	Spearman Correlation(r)	P value
Physical functioning	0.432	<0.05*
Role physical scale	0.175-	>0.05
Bodily pain scale	0.203-	>0.05
General health scale	0.165	>0.05
Vitality scale	0.415	<0.05*
Social functioning scale	0.175	>0.05
Role emotional scale	0.123	>0.05
Mental health	0.136-	>0.05

* Significant difference

Table (4): SF-36 scores in correlation to the occupation in the studied patients

Occupation	Spearman Correlation (r)	P value
Physical functioning	0.495	<0.05*
Role physical scale	0.143-	>0.05
Bodily pain scale	0.214	>0.05
General health scale	0.178	>0.05
Vitality scale	0.483	<0.05*
Social functioning scale	0.183	>0.05
Role emotional scale	0.145	>0.05
Mental health	0.162-	>0.05

* Significant difference

DISCUSSION

Cirrhosis represents a serious public health problem worldwide and they cause physical and psychological morbidity and mortality and also significant social costs **[7]**.

The importance of patients' health related quality of life (HRQL) in medical practice is nowadays beyond dispute. The assessment of the physical, psychological and social functioning of the patient in terms of the impact of disease is an essential part of clinical diagnosis, a major determinant of therapeutic choices, a measure of their efficacy, and a guide in planning long-term care **[8]**.

The study was conducted to assess the impact of cirrhosis on a weighted score of health–related quality of life in those patients. This study found that, there was high statistical significance between the cirrhotic patients and control groups as regard past history of hematemesis and jaundice (P<0.001). Past history of anti bilharzial treatment was statistically significantly present in the cirrhotic patients in comparison with control groups (P<0.05%).

There was a high statistically significant difference of the short form 36 (SF-36) questionnaires as regarding physical functioning, role of limitation due to physical functioning, bodily pain scale, general health scale of quality of life between group 1 (Child A) , group 2 (Child B) , group 3 (Child C) and in group 4 (Controls). This agreed with other study done by Gao et al. **[9]** who selected a total of 392 Chinese patients with CLD and 91 healthy controls and showed that mean ± SD regarding physical functioning in controls, Child A,B,C was 91.7±9.3, 85.2±17.8, 72.4±21.5, 55.7±25.9 respectively and regarding physical functioning limitation the mean ± SD in controls, Child A ,B,C was 88.2 ± 13.2 , 73.3 ± 22.2, 61.6 ± 21.5, 48.7 ± 25.8 respectively (P<0.001). In a study done by Marchesini et al. **[10]** on 544 patients with cirrhosis ;all domains of health-related quality of life, except pain, were altered in cirrhosis (by 9%-42%). The significance in our study in bodily pain scale may be explained to the occurrence of sever ascites and malnutrition and neuritis in group 3 Child C than the other groups.

There was no statistical significance between group 1 (child A) and group 4 (controls) in all scores of the short form 36 (SF-36) questionnaires (physical functioning, role of limitation due to physical functioning, bodily pain scale, general health scale, vitality scale, social functioning scale, mental health scale) of quality of life with high significance in emotional problems limitation scale. This may be due to that child A patients has better clinical and laboratory investigations than child B & C.This agreed with other study done by Sobhonslidsuk et al. **[11]** on total of 200 subjects with 150 CLD and 50 normal subjects. The ratio of cirrhotic patients classified as Child A:B:C was 37(50%): 26(35%): 11(15%). The highest scores of CLDQ domains were in the normal group, scores were lower in the compensated group and lowest in the decompensated group.

There was high statistically significant difference on the short form 36 (SF-36) questionnaires as regarding to vitality scale, social functioning scale, role of limitation due to emotional problems scale, mental health scale of quality of life between Child A, Child B , Child C and Controls with P value <0.001. This agreed with other study done by Gao et al. **[9]** that selected a total of 392 Chinese patients with CLD and 91 healthy controls and showed that mean ± SD regarding vitality scale in controls, Child A,B, C was 79.0±9.5, 74.3±13.1, 70.8±13.1, 59.3±20.1 respectively and as regard emotional problems was 92.4±9.8, 86.2±15.8, 84.6 ±17.9, 74.2±26.8 respectively with P<0.001.Also this agreed with the study done by Younossi et al. **[12]** using social functioning scales and found that there was a gradient correlates between patients without cirrhosis, Child's A cirrhosis, and those with Child's B or C cirrhosis and CLDQ. Nguyen et al. **[13]** showed that Primary caregivers of patients with advanced liver disease have significantly lower SF-36 mental health scores compared with the general population.

There was statistically significant positive correlation between the education and the parameters of the SF-36 scores as regard to the vitality (energy/ fatigue) scale and physical functioning scale (p value<0.05) as shown in table (3). Zandi and his colleagues **[14]** found that the educational and self care programs had positive effects on the quality of life of cirrhotic patients. Extensive educational and self-care programs along with long-term follow up such as the program conducted in their study are suggested. This significance may be explained by that low education cause ignorance of the nature of the chronic liver diseases and lack of follow up.

There was statistical significant positive correlation between the occupation and the parameters of the SF-36 scores as regard to the vitality (energy/ fatigue) scale and physical functioning scale(p value <0.05) as shown in table (4).This agreed with the reported results by Sobhonslidsuk **[15]**, his study conducted on two-hundred and fifty patients with CLD and fifty normal subjects and the numbers of low educated, unemployed and poor health perception had deteriorated HRQL and increased significantly from chronic hepatitis to Child's Classes A , B and C.

CONCLUSION

Finally, Cirrhotic Patients had a poor baseline quality of life as indicated by the deterioration in SF-36 questionnaire which was related to increasing disease severity (Child scoring), educational level and occupation. So, we recommend that increasing the educational level and proper occupation could increase the vitality and physical functioning of quality of life of those patients.

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Study of the Role of Serum Procalcitonin Level in Differentiation between Bacterial and Viral meningitis

Mohamed A. Nouh[1], Gamal S. Eldeeb[1], Mohamed S. Rizk[2], Mohamed S. Abdelhakam [3]

[1] *Tropical Medicine Department , Faculty of Medicine, Menoufia University, Egypt*
[2] *Biochemistry Department ,Faculty of Medicine - Menoufia University, Egypt.*
[3] *Menouf Fever Hospital, Egypt*

Corresponding Author
Mohamed Salama Abdelhakam

Mobile:
+201061814831

E mail:
mohammed.salama.abdel.hakam@Gmail.com

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Serum PCT. Septic meningitis .Aseptic meningitis

Background and Study aim: Serum procalcitonin (PCT) is specific for the diagnosis of bacterial infection. The aim of this study is to evaluate the role of serum PCT in diagnosis of septic meningitis in adults and its efficacy in differential diagnosis.

Patients and Methods: The study included 30 adults of septic meningitis and 30 adults with aseptic meningitis admitted in Menouf Fever Hospital and Tropical Medicine Department with fever, headache, vomiting and seizure. The diagnosis of septic meningitis was based on clinical features; physical examination, blood and cerebrospinal fluid (CSF) cytochemical findings, Gram stain and bacterial culture. Thirty cases of aseptic meningitis admitted during same period were also included in the study, and 20 subjects of matched age and sex, free from any CNS diseases undergoing spinal anaesthesia for non CNS surgical causes as control. Serum PCT was measured by Gloryscience ELISA Kit.

Results: Serum PCT level was significantly higher in patients with septic meningitis than those with aseptic meningitis ($P<0.001$). In culture and Gram stain positive 23 and 20 cases respectively, serum PCT was significantly elevated (23.123 ± 9.894 pg) than aseptic meningitis (8.652± 1.777pg) ($P<0.001$). At optimum cut off value of ≥10.36 pg/mL, based on area under receiver operating characteristic (ROC) curve, PCT showed sensitivity, specificity of 100 % and 86.6% respectively for the differentiation of septic from aseptic meningitis.

Conclusions: Serum PCT may be used as diagnostic marker for septic meningitis and its differentiation from aseptic meningitis.

INTRODUCTION

Despite the advances in diagnosis and treatment of infectious diseases, meningitis and encephalitis are still considered as important causes of mortality and morbidity **[1]**. To reduce the morbidity and mortality related to bacterial meningitis, it is important to discriminate bacterial meningitis from aseptic meningitis during the acute phase of the disease, when the clinical symptoms are often similar **[2]**. An ideal marker for bacterial infections should allow early diagnosis, inform about the course and prognosis of the disease, and facilitate therapy **[3]**. Clinical criteria, Gram staining, and bacterial antigen testing of CSF as well as the classic biological markers in the blood (C-reactive protein [CRP] level, white blood cell count [WBC], and neutrophil count) or CSF (protein level, glucose level, WBC count, and neutrophil count) used alone do not offer 100% sensitivity with high specificity for distinguishing between bacterial and aseptic meningitis **[3]**. Waiting for at least 2 days was recommended to identify bacterial growth in CSF cultures, whereas this period is 3-8 days for viral cultures **[4]**. Moreover, identifying the frequently encountered viral agents via polymerase chain reaction is not always possible in every institution. Therefore, intensive research has been carried out to find new and rapid diagnostic methods for differential diagnosis of bacterial and viral meningitis **[5]**. PCT, which is a calcitonin

propeptide, is supposed to be synthesized in C cells of the thyroid gland and secreted from leukocytes of the peripheral blood **[6]**. Serum PCT is more specific for the diagnosis of bacterial infection **[7]**. PCT levels do not or only slightly increase in non-bacterial inflammatory syndromes. PCT also provides prognostic information and risk stratification assessment in the emergency unit **[8]**. It was previously shown that serum PCT levels increase during the course of bacterial, parasitic, or fungal infections, but remain normal or slightly increase in viral infections and inflammatory reactions that are not infectious **[9]**.

PATIENTS AND METHODS

For this purpose, adult patients with clinical presentations of meningitis (41 males and 19 females) were included in the study who presented to Menouf Fever Hospital in the period between April 2014 and March 2015. These patients were classified according to the results of CSF findings into 30 patients of septic meningitis as group I (GI) and 30 patients of aseptic meningitis as group II (GII). In addition to 20 subjects of matched age and sex, free from any CNS diseases undergoing spinal anaesthesia for non CNS surgical causes were included in the study as GIII (control group).

Clinical manifestations; laboratory examination of CSF (glucose, protein, leukocyte count, Gram stain and bacterial cultures); and serum inflammatory markers (peripheral blood leukocyte count and CRP) were evaluated for their ability to differentiate bacterial from aseptic meningitis.

All patients and control were examined using Gloryscience PCT kits for serum PCT level.

Statistical analysis:

Statistical presentation and analysis of the present study was conducted, using the mean, standard deviation, student t-test, Paired t-test, Chi-square and Mann-Whitney by SPSS V17.

The results were tabulated and statistical analysis was done and the results were considered significant at P value < 0.05.

Diagnostic validity test:

a. The diagnostic sensitivity: It is the percentage of diseased cases truly diagnosed among total diseased cases or the ability of the screening test to discover the truly positive cases.
b. The diagnostic specificity: It is the percentage of non-diseased truly excluded by the test among total non-diseased cases or the ability of the screening test to discover the truly negative cases.
c. The predictive value for a positive test: It is the percentage of cases truly diagnosed among total positive cases.
d. The predictive value for a negative test: It is the percentage of cases truly negative among total negative cases.
e. The efficacy or the diagnostic accuracy of the test: It is the percentage of cases truly diseased plus truly non-diseased among total cases.

RESULTS

Clinical symptoms and signs were of little assistance in differentiating bacterial from aseptic meningitis except for meningeal irritation signs which were found to be statistically different between septic and aseptic groups.

Examination of CSF revealed:

- A statistically significant difference in aspect of CSF between bacterial meningitis group compared to the aseptic meningitis group ($p<0.001$) (Table 1).
- Significantly higher CSF leukocyte count with marked increase in the polymorphnuclear leukocyte count, CSF protein level and low CSF glucose in the bacterial meningitis group compared to the aseptic meningitis group ($p<0.001$) (Table 2).
- CSF culture was positive in 23 patients of GI (78 %) while it was negative in 7 patients of the same group (22%) and The most common detected organisms were *St. pneumoniae* (Gram positive cocci) in 11 patients out of 23 positive cultures (47.8%), *N. meningitidis* (Gram negative diplococci) in 7 patients (30.4%), *H. influenza* (Gram negative pleomorphic rods) in 3 patients (13.04%). *Staph. aureus* and *E. coli* were the least common organisms with the incidence of (4.33%)for both (Table 3).
- High statistically significant differences in ESR and CRP between patients with septic meningitis and those with aseptic meningitis.
- CRP results were positive in 80% of patients with bacterial meningitis, and 20% of patients with aseptic meningitis (Table 4).

- The mean values of peripheral blood WBCs were 13830 in GI and 8543 in GII respectively, and these results were statistically highly significant (p <0.001).
- High level of serum PCT in septic (23.123 ± 9.894 pg/dl) when compared with aseptic meningitis (8.652 ± 1.777 pg/dl) and control group (6.045 ± 0.908 pg/dl) (Table 5).
- Cut off PCT level >7.5 pg/dL clearly distinguished patients with meningitis from control group (all patients with bacterial and viral meningitis had a serum PCT level above this level).
- Cut off PCT level >10.36 pg/dL differentiate patients with bacterial meningitis from those with aseptic meningitis with 100% sensitivity and 86.6 % specificity (Table 6).

Table (1): Physical findings of the CSF of group I in comparison to group II

Variable		Group I N = 30		Group II N = 30		Total N = 60		X^2	P-value
		N	%	N	%	N	%		
Colour	Colorless	20	66.67	24	80.00	44	73.33	2.010	0.366
	Whitish	7	23.33	3	10.00	10	16.67		
	Bloody	3	10.00	3	10.00	6	10.00		
Aspect	Turbid	19	63.33	5	16.67	24	40.00	17.076	<0.001*
	Clear	4	13.33	18	60.00	22	36.67		
	Hazy	7	23.33	7	23.33	14	23.33		

Table (2): Cytological and chemical findings of the CSF the studied groups

Variable		Group I	Group II	t	P-value
CSF glucose	Range	10 - 58	16 - 168	- 6.568	<0.001*
	Mean ±SD	28.700 ± 14.613	74.333 ± 35.139		
CSF protein	Range	25 - 396	12 - 116	4.484	<0.001*
	Mean ±SD	143.633 ± 115.927	45.000 ± 32.768		
CSF WBCs	Range	550 - 45000	15 - 315	3.518	0.001*
	Mean ±SD	6224.000 ± 9497.920	123.967 ± 87.344		
CSF neutrophils	Range	45 - 90	10 - 50	13.700	<0.001*
	Mean ±SD	69.833 ± 11.633	30.667 ± 10.483		

Table (3): Organisms isolated from CSF of septic meningitis patients

Organisms	CSF culture		Gram stain	
	N	%	N	%
St. pneumoniae	11	47.8	10	50
Meningocci	7	30.4	5	25
H. influenza	3	13.04	3	12.5
Staph. aureus	1	4.34	1	4.16
E. coli	1	4.34	1	4.16

Table (4): CRP of group I in comparison to group II

CRP	Group I		Group II		Total		X^2	P-value
	N	%	N	%	N	%		
Negative	6	20.00	24	80.00	30	50.00	21.600	<0.001*
Positive	24	80.00	6	20.00	30	50.00		
Total	30	100.00	30	100.00	60	100.00		

Table (5): Values of mean & SD of serum PCT among the three studied groups

Groups	PCT		F	P-value
	Range	Mean ± SD		
Group I	10.5 - 42.8	23.123 ± 9.894	60.041	<0.001*
Group II	5.8 - 11.88	8.652 ± 1.777		
Group III	5.2 - 8.4	6.045 ± 0.908		
TUKEY'S Test				
I&II	**I&III**		**II&III**	
<0.001*	<0.001*		0.316	

Table (6): Accuracy of serum PCT level in differentiation between bacterial meningitis and aseptic meningitis

PCT ROC curve					
Cutoff	**Sens.**	**Spec.**	**PPV**	**NPV**	**Accuracy**
> 10.36 *	100.0%	86.6%	88.2 %	100.0%	98.2%

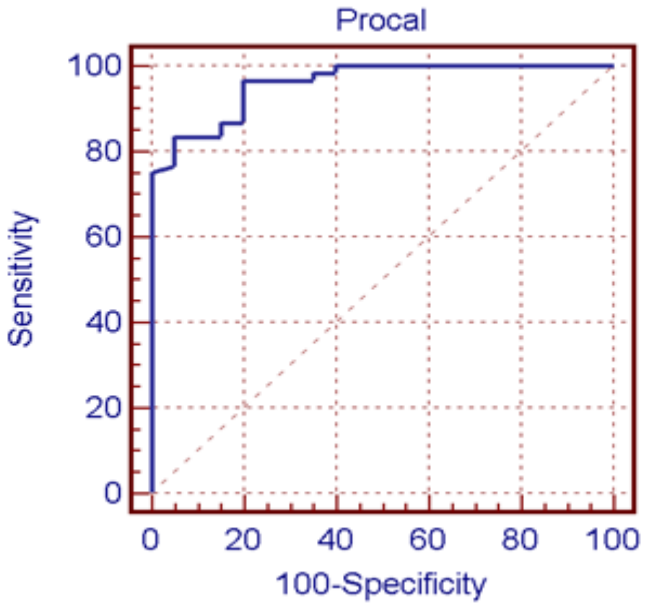

Figure (1): ROC curve for sensitivity and specificity of serum PCT level in diagnosis of meningitis.

DISCUSSION

In the present study, serum PCT level was found significantly higher in the septic meningitis group than the control group. Further in septic meningitis group, higher level was found than aseptic meningitis group. The diagnostic value of serum PCT was calculated with optimum combination of sensitivity and specificity at optimum cut off level obtained through receiver operating characteristic curve (ROC).

These results agree with Prasad et al. **[10]** who reported that the mean level of serum PCT in patients with septic meningitis and control group was (22,669.21 ± 7,656.45 pg/ml) and (3,943.8 ± 632.27 pg/ml) respectively and showed a highly significant difference among both groups ($p<0.001$) **[10]**.

Kepa et al. **[11]** in another study carried out on17 adult patients with suppurative bacterial meningoencephalitis and 16 patients with lymphocytic meningitis measured the levels of serum and CSF PCT and concluded that using serum PCT is a key element in differentiating bacterial meningitis from viral meningitis.

These results also agree with Knudsen et al. **[12]** who concluded that PCT and CRP had very high diagnostic accuracy for distinguishing between bacterial and non bacterial infection in patients with spinal fluid pleocytosis.

Ray et al. **[13]** in a prospective study carried out on 151 adult patients with meningitis signs concluded that laboratory test results of cerebrospinal fluid are of moderate importance in differentiating bacterial meningitis from the nonbacterial meningitis in cases which Gram staining for bacteria is negative in the beginning however serum PCT is an excellent predictive factor for differentiating acute bacterial meningitis which is similar to our study.

Dubos et al. **[14]** found that a PCT used alone offered the best sensitivity (99%) and specificity (83%) in agreement with our results.

In this study, the level of PCT was significantly higher among patients with neck rigidity.

Andreola et al. **[15]** agreed that CRP and PCT are both valuable markers for detection of severe bacterial infection in children according to the serum PCT characteristics which agree with the results of the present study.

The present study revealed highly significant increase in ESR (1st h) and CRP in GI patients when compared to GII ($p <0.001$).

These results were in agreement with Yetkin et al. **[16]** who stated that, there were high statistically significant differences in ESR ($p<0.007$) and CRP ($p <0.001$) between patients with septic meningitis and those with aseptic meningitis. In contrast to Makoo et al. **[17]** who found that ,there was no significant difference between patients with septic meningitis and those with a septic meningitis ESR ($p = 0.07$) and CRP ($p = 0.35$). This disagreement may explained by the fact that, the peripheral white blood cells, ESR and CRP can be different very early in the disease and in patients insufficiently treated by antibiotics.

In this study, CSF culture was positive in 23 patients of GI (78 %) while it was negative in 7 patients of the same group (22%). Patients with negative culture diagnosed as septic meningitis by CSF parameters (WBCs, glucose level, protein level, aspect of CSF and Gram staining) and other inflammatory markers like CRP, peripheral TLC and ESR.

CONCLUSION

Serum PCT may be used as diagnostic marker for septic meningitis and its differentiation from aseptic meningitis. PCT may be used as prognostic marker as all cases that had bad outcome, had higher level of PCT than cured cases.

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Ethical approval: Was granted by the Institutional Review Board and informed consent was obtained from each patient prior to inclusion in the study

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Peer reviewers: Dr. Ayman Abd El-Sabour Abd El-Azim, MBBCh, MSc, MD, MRCPCH, Lecturer of Pediatrics, Zagazig University, Associate Consultant of Pediatrics, International Medical Center, Jeddah, KSA; **Dr. Ghada Abd El-Ghaffar Salem,** Assistant Professor, Tropical Medicine Gastroentrology and Hepatology, Faculty of Medicine , Zagazig University , Egypt. **Editor: Mohamed H Emara;** Lecturer of Tropical Medicine, Faculty of Medicine, Zagazig University, Egypt.

Dengue and Dengue Hemorrhagic Fever

Maysaa Abdallah Saeed[1] , Tarek Hamed Attia[2]
[1]*Tropical Medicine Department, Faculty of Medicine, Zagazig University, Egypt*
[2]*Pediatric Department, Faculty of Medicine, Zagazig University, Egypt*

Corresponding Author ***Maysaa Abdallah Saeed***

E mail: dr.maysaaabdallah@windowslive.com

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Dengue is endemic in at least 100 countries in Asia, the Pacific, the Americas, Africa, and the Caribbean. The World Health Organization (WHO) estimates that 50 to 100 million infections occur yearly, including 500,000 dengue hemorrhagic fever (DHF) cases and 22,000 deaths, mostly among children. Both epidemic and endemic transmission of dengue viruses are maintained through a human-mosquito-human cycle involving mosquitoes of the genus *Aedes* (*Stegomyia*). Typical clinical manifestations of dengue range from self-limited dengue fever (DF) to dengue hemorrhagic fever with shock syndrome. Most dengue virus infections in adults are symptomatic. In contrast, most infections among children under age 15 years are asymptomatic or minimally symptomatic. Classic dengue fever is an acute febrile illness accompanied by malaise, headache, retro orbital pain, and marked muscle and joint pains, which evoked the term "break-bone fever". DHF is the most serious manifestation of dengue virus infection and can be associated with circulatory failure and shock. Plasma leakage is the most specific and life-threatening feature of DHF.

HISTORY

Because of dengue fever nonspecific clinical features, the interpretation of historical records for evidence of past epidemic is open to speculation. However, Benjamin Rush's description of a 1780 Philadelphia epidemic was the earliest description in English of so called break-bone fever. Subsequently, sporadic outbreaks were reported throughout the tropics and subtropics **[1]**.

In 1903 Mosquito borne transmission of dengue virus infection by *A. aegypti* was demonstrated and its viral etiology in 1906. Sabin demonstrated the failure of two viral strains to cross protect humans, while isolating the virus in 1944, thus establishing the existence of dengue virus serotypes. Hammons characterized two more serotypes in 1956. After World War II, the start of a pandemic with transmission of multiple viral serotypes began in Southeast Asia, leading to outbreaks of dengue hemorrhagic fever **[1]**.

EPIDEMIOLOGY

Dengue is endemic in at least 100 countries in Asia, the Pacific, the Americas, Africa, and the Caribbean. The World Health Organization (WHO) estimates that 50 to 100 million infections occur yearly, including 500,000 DHF cases and 22,000 deaths, mostly among children **[2]**.

Dengue viruses are members of the family Flaviviridae, genus *Flavivirus* **[3,4]**. They are small, enveloped viruses containing a single-strand RNA genome of positive polarity **[3]**. *A.aegypti* breed in or close to houses, laying eggs in both man-made and natural water containers. The typical flight distance is relatively short **[5]**. *A. aegypti* are day time feeders that prefer to bite humans and are frequently unnoticed. For this reason, family members who are at home during the daytime, typically women and young children, are at particularly high risk for infection **[6]**.

TRANSMISSION CYCLE

Both epidemic and endemic transmission of dengue viruses are maintained through a human-mosquito-human cycle involving mosquitoes of the genus *Aedes* (*Stegomyia*) **[7]**.Susceptible humans become infected after being bitten by an infected female *Aedes* mosquito. Viremia in humans begins toward the end of a four- to six-day incubation period and persists until fever abates, which is typically three to seven days **[8,9]**. An uninfected *Aedes* mosquito may acquire the virus after feeding during this viremic period. The mosquito has an incubation period of 8 to 12 days before it is capable of transmitting the virus to susceptible individuals. Once infected, mosquitoes carry the virus for their lifespan and remain infective for humans.

In rare cases dengue can be transmitted in organ transplants or blood transfusions from infected donors, and it can be transmitted by accidental needle stick **[10]**. Vertical transmission from an infected pregnant mother to her fetus has been reported **[11]**. But in the vast majority of infections, a mosquito bite is responsible.

PATTERNS OF TRANSMISSION

Dengue virus transmission follows two general (but not mutually exclusive) patterns

Epidemic dengue

Epidemic dengue transmission occurs when the introduction of dengue virus into a region is an isolated event involving a single virus strain.

Hyper endemic dengue

"Hyper endemic" transmission refers to the continuous circulation of multiple dengue virus serotypes in the same area.

FACTORS INFLUENCING TRANSMISSION

The worldwide incidence of dengue and DHF has been increasing in the past several decades, and the geographic distribution of these diseases has expanded.

The transmission cycle for dengue viruses is dependent upon the interaction between infective mosquitoes and susceptible humans and between susceptible mosquitoes and viremic humans.

Dengue virus transmission is enhanced by the following factors **[7]**:

1- Increased vector density: In many tropical countries, seasonal increases in rainfall contribute to an increased density of mosquitoes. In addition higher humidity lengthens mosquitos' lifespan **[12]**.

2- Shorter mosquito incubation: The extrinsic incubation period is inversely associated with the ambient temperature. Warmer temperatures increase the length of time that a mosquito remains infective.

3- Increased movement of mosquito vectors and viruses: Air, land, and water transportation of mosquitoes or viremic humans facilitate the dissemination of dengue viruses.

4- Increased density of susceptible hosts: Crowded conditions probably increase the potential for virus transmission.

5- Increased duration and magnitude of viremia in humans.

PATHOGENESIS

After an infectious mosquito bite, the virus replicates in local lymph nodes and within 2 to 3 days disseminates via the blood to various tissues. Virus circulates in the blood typically for 5 days in infected monocytes\macrophages and to a lesser degree in B cells and T cells. It also replicates in skin, reactive spleen lymphoid cells, and macrophages **[13,14]**.

Malaise and flue like symptoms that characterize dengue probably reflect patients 'cytokine response; however myalgia, a cardinal features of the illness, may also indicate pathologic changes in the muscle, typified by a moderate perivascular mononuclear infiltrate with lipid accumulation **[15]**. Musculoskeletal pain(break-bone fever) conceivably could reflect viral infection of bone marrow elements, including mobile macrophages and dendritic cells (CD11b\CD18 [MAC-11]-positive) and relatively non motile adventitial reticular cells (nerve growth factor receptor-positive).Local suppression of erythrocytic, myelocytic, and thrombocytic poiesis within 4 to 5 days is reflected in peripheral cytopenias. Histopathological examination of skin from patient with rash discloses a minor degree of

lymphocytic dermal vasculitis and, variably, viral antigen **[13,9]**.

Neurologic complications have attributed chiefly to metabolic alterations and to focal and sometimes massive intracranial hemorrhages, but anecdotal cases and limited case series have indicated the possibility of viral CNS invasion and encephalitis **[16,17]**.

Shock in dengue shock syndrome (DSS) occurs after the sudden extravasation of plasma into extravascular sites, including the pleural and abdominal cavities, usually with the defervescence of fever **[18,19]**. The extensive increase in vascular permeability is associated with immune activation, as manifested by increased levels of plasma-soluble tumor necrosis factor receptor (sTNFR\75), IL-28, interferon, and local endothelial production of IL-28 RANTES with apoptotic endothelial cell death **[20,21]**. In addition immune complex formation activates the complement system, with increase in C3a and C5a **[22]**. Levels of IL-6 and intercellular adhesion molecule-1 are depressed in parallel with hypoalbuminemia and the general loss of serum proteins. Reduced cardiac output may contribute further to shock **[23]**.

The hemorrhagic diathesis is complex and not well understood, reflecting a combination of cytokine action and vascular injury, viral antibodies' binding to platelets or cross reacting with plasminogen and other clotting factors, reduced platelet function and survival, and a mild consumptive coagulopathy **[24,25,26,27]**.

The increased frequency of DHF in secondary dengue virus infection has suggested a role for heterologous antibodies in enhancing viral uptake and replication in fc receptor- bearing cells (antibody mediated immune enhancement) **[28,29]**. Simultaneously, levels of TNF-alpha, soluble CD8, and soluble IL-2 that are higher in patient with DHF than in those with dengue fever indicate an activation of cross reactive memory $CD4^+$ and $CD8^+$ T cells in response to a second infection **[20]**.

CLINICAL MANIFESTATIONS

Typical clinical manifestations of dengue range from self-limited dengue fever (DF) to dengue hemorrhagic fever with shock syndrome **[30]**. With wider availability of laboratory testing, there are increasing reports of unusual clinical manifestations, as discussed below **[31]**. The risk of severe disease is much higher in repeated infection than primary infection **[32]**.

Asymptomatic infection

Most dengue virus infections in adults are symptomatic **[33]**. In contrast, most infections among children under age 15 years are asymptomatic or minimally symptomatic. In one study of schoolchildren in rural Thailand, 53 percent of dengue virus infections were not associated with a recognized febrile illness despite intense active surveillance **[11]**. Dengue virus type 2 and 4 may be more likely to cause inapparent infection in flavivirus naïve person **[34]**.

Classic dengue fever

Classic dengue fever is an acute febrile illness accompanied by malaise, headache, retro orbital pain, and marked muscle and joint pains, which evoked the term "break-bone fever" **[35]**. Symptoms typically develop between 4 and 7 days after the bite of an infected mosquito; the incubation period may range from 3 to 14 days. Dengue can essentially be excluded as the cause of symptoms in a traveler who develops illness more than 14 days after returning from a dengue-endemic country **[36]**.

Fever typically lasts for five to seven days. Some patients have a biphasic ("saddleback") fever curve, with the second febrile phase lasting one to two days; this has been described in approximately 5 percent of patients **[37,38]**. The febrile period may also be followed by a period of marked fatigue that can last for days to weeks, especially in adults.

The frequency and severity of symptoms was influenced by the patient's age and sex and differed in patients with primary versus secondary dengue virus infection. All symptoms were less frequent in patients ≤19 years of age. Joint pain, body aches, and rash were more common in females. Constitutional symptoms and gastro-intestinal symptoms were more common in patients experiencing a second infection, whereas rash was more commonly noted during primary infection **[39]**. Disease severity may be increased among infants and elderly **[40]**.

Vertical transmission of dengue virus to neonates whose mother has an onset of primary or second dengue fever zero to 8 days before delivery has resulted in acute neonatal dengue manifesting as fever, cyanosis, apnea, mottling, hepatomegaly and thrombocytopenia **[11,41]**. The outcome of infection acquired earlier in pregnancy has not been addressed satisfactorily. Previous reports have described spontaneous abortion, variety of

birth defects and, in post epidemic investigation, an increase in neural tube defects **[42]**.

Hemorrhagic manifestations

Hemorrhagic manifestations occur commonly in patients with DF and, in rare cases, can be life threatening. In a large study in Thailand, spontaneous bleeding occurred in 68 percent of children with DF **[43]**. The main bleeding sites were the skin (58 percent) and nose (19 percent); gastrointestinal bleeding was less common (4 percent). In another series of 18 adults who acquired DF during travel, hemorrhagic phenomena were noted in 22 percent; two patients had purpura and two had melena **[37]**.

Other symptoms

Acute dengue virus infection often presents without the full picture of classical DF, especially in children. Gastrointestinal or respiratory tract symptoms may dominate the clinical picture in some patients **[39]**. Hepatitis frequently complicate dengue fever **[44]**. Neurologic symptoms have been reported sporadically and attributed to hemorrhage and cerebral edema in addition to the possibility of primary dengue encephalitis **[16,17,45,46]**. Myositis with rhabdomyolysis has also been reported **[1]**.

Physical examination

Physical examination in patients with DF is generally nonspecific. Conjunctival injection, pharyngeal erythema, lymphadenopathy, and hepatomegaly are observed in 20 to 50 percent of patients **[47]**. The rash is typically macular or maculopapular and may be associated with pruritus.

Laboratory findings

Laboratory findings typical of DF include the following: Leukopenia is common in both adults and children with DF and is a useful diagnostic feature **[47,48,49]**. Thrombocytopenia is noted in most patients with DF **[50]**. In several studies, platelet counts <100,000 cells/mm^3 were observed in 16 to 55 percent of patients. Serum aspartate transaminase (AST) levels are frequently elevated in both adults and children with DF; the elevations are usually modest (2 to 5 times the upper limit of normal values), but marked elevations (5 to 15 times the upper limit of normal) are occasionally noted **[47,48]**.

Dengue hemorrhagic fever

Dengue hemorrhagic fever (DHF) is the most serious manifestation of dengue virus infection and can be associated with circulatory failure and shock. The four cardinal features of DHF, as defined by the World Health Organization (WHO), include **[31,32,51]**: 1-Increased vascular permeability (plasma leakage syndrome) that lead to hemo concentration (20 percent or greater rise in hematocrit above baseline value), pleural effusion, or ascites, 2-Marked thrombocytopenia (100,000 cells/mm^3 or lower), 3-Fever lasting two to seven days, 4-A hemorrhagic tendency (as demonstrated by a positive tourniquet test) or spontaneous bleeding.

The term dengue shock syndrome (DSS) is used when shock is present along with these four criteria. The period of maximum risk for shock is between the third and seventh day of illness. This tends to coincide with resolution of fever **[51]**.

Blood levels of soluble dengue NS1 protein (>600 ng/mL) were predictive of DHF in one study of Thai children with secondary dengue 2 virus infections **[52]**.

Plasma leakage

Plasma leakage is the most specific and life-threatening feature of DHF. The increase in vascular permeability develops over a period of 24 to 48 hours. Shock may develop in patients with marked plasma leakage, especially if supportive treatment is delayed. This clinical presentation is referred to as "dengue shock syndrome" (DSS) and is associated with a case-fatality rate as high as 12 percent in some studies, even with aggressive therapy **[53]**.

Plasma leakage usually occurs between three and seven days after the onset of illness. This coincides with defervescence, severe thrombocytopenia, and elevation of aminotransferases **[48]**. Abdominal pain is also reported to precede the onset of plasma leakage in approximately 60 percent of patients with DHF **[54,55,56]**. The presence of intense abdominal pain, persistent vomiting, and marked restlessness or lethargy, especially coinciding with defervescence, should alert the clinician to possible impending dengue shock syndrome **[57]**.

Chest radiography and chest/abdominal ultrasound are the imaging modalities useful for detection of plasma leakage in DHF. Right lateral decubitus chest radiograph was sensitive for detection of pleural effusion, but ultrasound was useful for detecting larger effusions and also had the advantages of evaluating for presence of peritoneal fluid. Plasma leakage was detected by ultrasound as early as three days after the onset of fever;

pleural effusions were more common than ascites or edema of the gallbladder wall **[58]**.

Hemorrhagic manifestations

The severity of hemorrhagic manifestations is quite variable among patients with DHF. Previous studies reported spontaneous petechiae or ecchymoses in approximately one-half of adults and children with DHF **[54,55]**. Other less-frequent hemorrhagic manifestations reported in these studies included: hematemesis (15 to 30 percent of subjects), menorrhagia (40 percent of adult women), melena (5 to 10 percent), and epistaxis (10 percent).Hemorrhagic manifestations are also common in dengue fever **[43,59]**; this can be severe, requiring hospitalization and transfusion in rare cases **[60]**.

Laboratory testing

Confirmation of acute dengue virus infection is most frequently accomplished using serology **[51,61]**. Tests for detection of viral RNA or NS1 antigen are commercially available and more successful than serology in detecting dengue virus infection in the early stages **[62]**. The following diagnostic approach is recommended, to the patient with suspected dengue if laboratory support is available **[51,61,63]**:

- An acute phase, serum or plasma sample should be obtained. If the acute phase sample is obtained ≥3 days after the onset of illness, the IgM immunoassay (MAC-ELISA or equivalent) is the procedure of choice for rapid confirmation of the diagnosis. The potential for a false-negative result remains elevated within the first six days of illness.
- If the acute phase sample is obtained within the first three days after the onset of illness or if the sample is obtained within the first six days of illness and there is a negative IgM assay result, testing for the presence of the dengue viral RNA or NS1 antigen has the highest diagnostic yield.
- To confirm a positive IgM result or if initial testing is negative in a patient with suspected dengue virus infection, a convalescent phase serum sample should be obtained at least 10 to 14 days after the acute phase serum. The acute and convalescent specimens should be analyzed together by a hemagglutination inhibition (HI) or enzyme immunoassay to provide definitive serologic testing for acute dengue virus infection.

Serologic testing

The most frequently used serologic tests for the diagnosis of acute dengue virus infection are the HI assay and IgG or IgM enzyme immunoassays. Complement fixation and neutralizing antibody assays are more technically demanding and are used in specialized laboratories only.

The HI assay remains the gold standard for serologic testing for dengue virus-specific antibodies. Analysis of paired acute and convalescent serum samples is essential; a fourfold or greater rise in HI antibody titer between acute and convalescent samples defines acute infection.

The antibody response depends on whether the patient has primary or secondary dengue virus infection. In primary infection, HI antibodies develop relatively late (after the fifth day of illness) and reach titers of less than 1:1250 in the convalescent phase. In secondary infection, HI antibodies rise early and reach titers above 1:1250 (often 1:10,240 or higher) in the convalescent phase **[51]**.

Immunoassays for the detection of dengue virus-specific IgG antibodies have demonstrated sensitivity and specificity of approximately 99 percent and 96 percent, respectively, compared with the HI assay **[64]**. Testing of paired acute and convalescent serum samples is required for the diagnosis of acute dengue virus infection using the IgG ELISA.

A component of the antibody response is dengue virus serotype-specific; a substantial portion of the antibody response has cross-reactivity with other dengue virus serotypes and even other flaviviruses. Cross-reactivity is more problematic in secondary dengue virus infection and also in individuals who have been immunized with vaccines against other flaviviruses such as Japanese encephalitis virus **[65]**. Although neutralizing antibody assays have greater specificity than HI or ELISA assays, serologic assays cannot be relied on for identification of the infecting dengue virus serotype **[66]**.

Virus detection

Isolation of dengue virus or detection of dengue viral RNA or protein in an acute phase serum or tissue specimen provides the most definitive confirmation of infection **[61]**. A real-time RT-PCR assay kit developed by the Disease Control and Prevention (CDC) was approved by the FDA in 2012 for diagnostic use in the United States **[67]**. In both prospective and retrospective

testing, the sensitivity and specificity of the test were ≥98 percent compared with a reference method **[68]**. RT-PCR is the only method that can detect virus within a clinically meaningful time frame (one to two days or less) **[70,71,72]** and it has comparable sensitivity to viral isolation **[69,73]**.

Virus isolation is generally performed only for epidemiologic or research purposes. Serum and plasma are the preferred specimens for virus isolation, although virus can occasionally be isolated from liver tissues after clearance of virus from the serum **[74]**. Virus isolation typically requires one to two weeks **[75]**.

Regardless of the specific method used, optimal detection is achieved when specimens are obtained early after the onset of symptoms, during the febrile period. In one study of children in Thailand, dengue viruses could be isolated from all plasma samples obtained at least two days before defervescence but from no samples obtained two or more days after fevers resolved **[9]**.

The dengue viral nonstructural protein 1 (NS1) can be detected in plasma, especially during the first five to six days of illness. In one study, high levels early in infection were associated with DHF **[52]**. Two assays have become commercially available outside the United States. The sensitivity of these assays for diagnosis of acute dengue infection at the time of hospital admission is 50 to 70 percent, with specificity >95 percent **[76,77]**. However, neither assay is formulated to provide either identification of the specific dengue virus serotype or quantitative measurement of soluble NS1 protein levels.

For many resource-limited dengue endemic countries, routine laboratory testing is not readily available. One study of 1250 children aged 2 months to 10 years presenting to a pediatric hospital in southern Vietnam evaluated whether an assessment tool designed for first-level healthcare workers, using only clinical signs, could appropriately classify and guide management of acute illnesses in an endemic area **[58]**.

PREVENTION

Dengue prevention currently relies on public health and community based *A.aegypti* control programs to remove and destroy mosquito-breading sites **[78]**.

Public health approaches for prevention of dengue infection in endemic areas include control of *Aedes* mosquitoes and development of vaccines.

Mosquito control

Mosquito control is the most effective approach for prevention of dengue transmission.

Insecticide spraying in response to dengue outbreaks is not highly effective against *A. aegypti* mosquitoes, which frequently breed inside houses **[79,80]**. Community-based approaches involving education of the population in efforts to reduce breeding sites, such as discarded tires and other containers that accumulate standing water, have shown some promise **[79]**. Indoor insecticidal fogging may be effectual.

Vaccination

Infection with dengue virus provides long-term protection against the particular serotype that caused the disease, supporting the feasibility of a dengue vaccine. However, it provides only short-lived immunity to the other three dengue virus serotypes. In view of the association of dengue hemorrhagic fever (DHF) with previous exposure to dengue viruses and the recognition that all four serotypes are capable of inducing DHF, it is the general consensus in the scientific and public health communities that any candidate vaccine should produce protective immunity against all four serotypes. Since waning immunity might also increase the risk for DHF in vaccine recipients, vaccine-induced protective immunity should also be long lived **[81]**.

No licensed vaccine is available for preventing dengue **[82,83]**.

Travelers

Most travelers from non-endemic countries are at exceedingly low risk for DHF because they lack previous exposure to dengue viruses.

Travelers are well advised to wear clothing that reduces the amount of exposed skin. Also advised to protect themselves by using repellents and insecticidal sprays indoor. Bed netting is of little use since the mosquitoes are most active during the daytime **[80]**.

Treatment

There is no specific therapy available for dengue virus infections, it is important to exclude other treatable diagnoses.

Management of fever

Fever and myalgia can be managed with acetaminophen (maximum 60 mg/kg/day in children or 4 g/day in adults). Aspirin or nonsteroidal anti-inflammatory agents should generally be avoided because of the risk of bleeding complications and in children because of the potential risk of Reye's syndrome.

Patients with dengue fever should maintain oral fluid intake to avoid dehydration. The most important measure to assist the patient with suspected dengue fever is to carefully evaluate the patient for impending complications or early evidence of dengue hemorrhagic fever (DHF) **[1]**.

Management of significant bleeding

Gastrointestinal bleeding, epistaxis, or menorrhagia in patients with DHF (and occasionally in patients with dengue fever) can be severe enough to require blood transfusion. In these circumstances, blood replacement should be performed with 5 mL/kg of packed red blood cells (or 10 mL/kg whole blood). The clinical response and post-transfusion hematocrit should be monitored. Use of a histamine H2 receptor antagonist or proton pump inhibitor is reasonable in patients with gastrointestinal bleeding, although there is no evidence of benefit. Platelet transfusions have not been shown to be effective at preventing or controlling hemorrhage but may be warranted in patients with severe thrombocytopenia (<10,000/mm^3) and active bleeding. Prophylactic platelet transfusions in patients with severe thrombocytopenia but without active bleeding are generally not recommended **[84,85]**. Administration of intravenous vitamin K1 is recommended for patients with severe liver dysfunction or prolonged prothrombin time **[31]**.

Management of plasma leakage

Plasma leakage in DHF is important to manage with intravascular volume repletion to prevent or reverse hypovolemic shock **[86]**. In mild cases, particularly when medical attention is received early, oral rehydration may be sufficient. However, in patients with established intravascular volume loss, intravenous fluid administration is recommended. Blood transfusion is appropriate in patients with significant bleeding or those who have low hematocrit and fail to improve despite fluid resuscitation. Subsequent hematocrit measurements must be interpreted with caution since it is critical to assess the adequacy of both blood and fluid repletion; in complex cases, it can be challenging to distinguish whether a decrease in hematocrit reflects volume repletion or blood loss **[86]**.

Treatment of shock

For patients with shock, initial resuscitation with normal saline or Ringer's lactate (10 mL per kg of body weight for children or 500 mL for adults), preferably with 5 percent dextrose, is recommended, either as an infusion over the first hour or as a bolus (infused over 10 to 15 minutes) for patients in profound shock. A second infusion of an equal volume is recommended in patients who remain in shock **[84]**.

There has been debate as to whether crystalloids or colloids should be used for volume replacement in critically ill patients. Three randomized trials have investigated the effect of different fluid regimens on outcome **[87,88]**. The largest of these studies was a double-blind randomized comparison of three fluids for initial resuscitation of 512 Vietnamese children with dengue shock syndrome **[89]**. Three hundred eighty-three patients with moderate shock were assigned to Ringer's lactate or one of two different colloid solutions: 6 percent dextran 70 or 6 percent hydroxyethyl starch. One hundred twenty-nine patients with severe shock were randomized to receive one of the two colloids. The treatment regimen closely followed the WHO protocol, with 15 mL/kg administered over the first hour and 10 mL/kg over the second hour. Only one patient died. This trial established that Ringer's lactate is a safe, effective, and inexpensive alternative in initial resuscitation of patients with moderate shock. In patients with severe shock, dextran and starch performed similarly, although dextran was associated with more hypersensitivity reactions **[51,90]**.

In patients who remain in shock despite the two initial boluses of crystalloid, it is preferred to switch to a colloid solution (10 mg/kg over the next hour). 10 percent dextran 40 in normal saline is the colloid of choice. Switching to a colloid solution is also appropriate in patients who have signs of fluid overload (eg, puffy eyelids, distended abdomen, tachypnea, or dyspnea). Patients who have persistent hypo perfusion with falling hematocrit require blood transfusion. Other possible complications, such as acidosis, hypoglycemia, or hypocalcemia, should also be investigated and corrected as needed **[90]**.

Once blood pressure has been restored, intravenous fluids should be continued but the infusion rate should be gradually reduced over the next 24 to 36 hours. The patient’s clinical

condition, including vital signs, urine output, and hematocrit, should be checked prior to each reduction in the infusion rate. Close clinical observation is essential, even after normal blood volume is restored, because patients can develop recurrent shock over the 24 hours after the initial resuscitation, which represents the period of increased vascular permeability in DHF. Most patients who present for medical attention before profound shock develops and who receive appropriate fluid therapy will recover quickly.

The fluids that are lost into potential spaces (eg, pleura, peritoneum) during the period of plasma leakage are rapidly reabsorbed. Thus, intravenous fluid supplementation should be discontinued once patients have passed the period of plasma leakage. Usually no more than 48 hours of intravenous fluid therapy are required. Excessive fluid administration after this point can precipitate hypervolemia and pulmonary edema **[90]**.

Adjunctive therapies

The basis of DHF pathogenesis is hypothesized to be immunologic, which has led to interest in immune-modulatory drugs for therapy.

Several trials have demonstrated that cortico-steroids are no more effective than placebo in reducing death, need for blood transfusion, or serious complications **[91,92,93]**.

Other modalities, including intravenous immuno-globulins, pentoxifylline, and activated factor VII, have also been proposed for use **[94,95,96]**. However, no benefits have been established in a controlled evaluation.

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Rhinocerebral Mucormycosis Presented with Cranial Nerves Deficit

Maysaa A Saeed[1], Tarek H Attia[2], Abdullah S Al Ghamdi[3]
[1] *Tropical Medicine Department, Faculty of Medicine, Zagazig University, Egypt.*
[2] *Pediatric Department, Faculty of Medicine, Zagazig University, Egypt.*
[3] *Internal Medicine Department, King Saud Medical City, Kingdom of Saudi Arabia.*

Corresponding Author
Professor Dr. ***Maysaa Abdallah Saeed***

Mobile:
+201275594979

E mail:
dr.maysaaabdallah@windowslive.com

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Mucormycosis is a rare opportunistic fungal infection, associated with high mortality, characterized by infarction and necrosis of host tissue. Rhinocerebral mucormycosis (RCM) is the most common clinical variant often associated with poorly controlled diabetes mellitus. The treatment is complex and involves both antifungal and surgery. There is no formal guideline regarding the duration of antifungal, timing and extent of surgical management. We present a case of RCM in a diabetic girl who presented with a 2 weeks history of left facial and periorbital swelling associated with left facial numbness and deficit of the fifth and seventh cranial nerves. Diagnosis of RCM was confirmed by histopathological examination of tissue biopsy. She was successfully treated with liposomal amphotericin B and posaconazole, as step-down therapy, along with minimal surgical debridement. We present this case because of the rarity of RCM, calling for prompt initiation of treatment in a suspected case and to present data about therapeutic modality.

INTRODUCTION

Mucormycosis is a rare opportunistic fungal infection caused by filamentous fungi of order Mucorale. It is characterized by infection and necrosis of host tissue that is resulted from invasion of vasculature by hyphae. The genera most commonly found in human infection is Rhizopus and Mucor **[1]**. Based on its clinical presentation and anatomic sites invasive mucormycosis is classified into 6 clinical forms: rhino-cerebral, pulmonary, cutaneous, gastro-intestinal, disseminated and uncommon rare form such as endocarditis, peritonitis and renal infection **[2]**. The most important risk factors predisposing to mucormycosis include malignant hematological diseases, prolonged and sever neutropenia, poorly controlled diabetes mellitus with or without ketoacidosis, iron overload, major trauma, prolonged use of corticosteroid and malnutrition **[1,3]**. In most cases the infection is rapidly progressive and results in death unless underlying risk factors are corrected and aggressive treatment, with antifungal agents and surgical excision, is initiated **[3]**.

CASE REPORT

A 14 year-old diabetic girl presented to outpatient clinic, at Infectious Diseases Unit, with a 2 weeks history of left facial and periorbital swelling appearing 2 days after tooth extraction. Also there was history of left facial numbness but there was no fever. The patient has initially visited a dentist because of headache and left sided teeth ache. The dentist decided to extract her left upper molar tooth and prescribed for her cefuroxime but after 2 days the patient condition had worsened and the aforementioned symptoms appeared. She had medical history of diabetes which was poorly controlled. On examination she was conscious, oriented, had well body built and her vital signs were stable. There were left facial and periorbital edema with dark bluish discoloration around left eye. There was left facial hypoesthesia in the area supplied by ophthalmic, maxillary and mandibular branches of the fifth cranial nerve. Also there was left facial palsy. Laboratory results were; white blood cells 7.8 $x10^9$/L with 70% neutrophils; hemoglobin 13g/L; plasma glucose

11.2 mmol/L; hemoglobin A_1C 12%; ESR 90 mm/h; CRP 25; serum sodium 133mmol/L. Other biochemical results were normal. Computed tomography (CT) revealed mucosal thickening of all left paranasal sinuses (Fig. 1). Further imaging with magnetic resonance imaging (MRI) also showed involvement of all left paranasal sinuses with retro-orbital extension and there was abscess formation in the anterior maxillary area and left orbital floor (Fig. 2). There was no involvement of central nervous system. Based on the history, clinical presentation and imaging findings a provisional diagnosis of RCM was considered. Liposomal amphotericin B (5 mg/kg/day) was started immediately and blood glucose level was controlled with regular insulin. Ophthalmology and otolaryngology were consulted. She was taken for endoscopic evaluation which revealed extensive necrosis of the left maxillary sinus. Left maxillary sinus was also full of pus which was drained out and send for fungal culture which did not reveal any growth. Biopsies send for histopathological examination demonstrated broad non septate hyphyae at right angles consistent with mucormycosis. Minimal endoscopic debridement was done because extensive surgery was refused by the patient family. Liposomal amphoterecin B was continued. Over the next 2 months the patient showed continual clinical improvement and follow up MRI revealed partial resolution of the lesion. The dose of liposomal amphoterecin B was increased to 7 mg/kg/day. Follow up MRI revealed regression of the lesions after 2 months. Liposomal amphoterecin B was stopped, oral posaconazole started (400 mg twice daily with fatty meal) and the patient discharged . 4 months after posaconazole there was complete resolution of MRI findings.

DISCUSSION

RCM is the most common and fatal clinical form of mucormycosis which presumed to start with inhalation of spores into paranasal sinuses of susceptible host **[1]**. Dental care may also precede such an infection by creating a post extraction wound which may be susceptible to fungal infection as seen in our case **[4]**. Hyperglycemia, usually with an associated metabolic acidosis, is the most common underlying condition **[1]**. Rhizopus organisms have an enzyme, ketone reductase, which allow them to thrive in high glucose levels, at the same time hyperglycemia may alter the immunologic capability to resist mucormycosis through reduction of leucocytes chemotaxis **[5]**.

RCM usually present as acute sinusitis, headache, sinus pain and purulent nasal discharge with or without fever. All of the sinuses become involved and spread to contiguous structures such as the palate, orbit and brain **[6]**. The hall marks of spread beyond the sinuses are tissue necrosis of the palate resulting in palatal eschar, facial swelling, erythema and cyanosis of the facial skin overlying the involved sinuses **[7]**. Signs of orbital involvement include periorbital edema, proptosis and blindness. Facial numbness is frequent and results from infarction of sensory branches of the fifth cranial nerve **[8]**. Our patient had left facial numbness.

Endoscopic evaluation of the sinuses should be performed to look for tissue necrosis and to obtain specimens to confirm the presence of infection **[6]**. Histopathological examination of surgical specimens confirm the clinical diagnosis with the appearance of right-branching non septate hyphae, which are considered typical for mucor species, along with the evidence of angioinvasion and tissue necrosis. Fungal culture can provide further confirmation however a large number of false negative results have been reported compared to direct histopathological examination **[9]**.

Imaging study are of little help during the early stages of RCM. However CT and MRI scan should be frequently obtained due to the rapidity of disease progression and are indispensable for appropriate planning of surgical intervention **[10]**.

Treatment of RCM is based on reversal of underlying predisposing factor, prompt initiation of antifungal therapy and surgical debridement of involved tissues **[3]**. However there was no any recommendation in the literature on the duration of antifungal, extent and timing of appropriate surgical management. Our case was successfully treated with antifungal, for 8 months, and minimal surgical debridement.

CONCLUSION

Clinician awareness, prompt initiation of antifungal and timely surgical intervention is of paramount while managing a case of RCM. Mucormycosis should be considered in a predisposed patient who presented with cranial nerves deficits or who seems to deteriorate after tooth extraction. Although extensive surgical debridement could not be performed, disease regression could be achieved with medical therapy and minimal debridement.

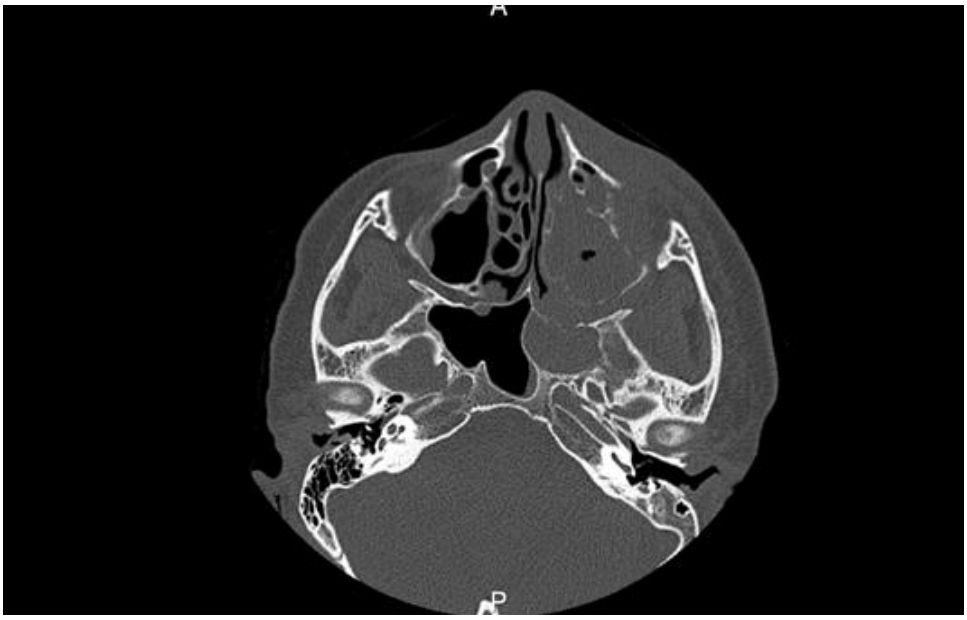

Figure 1
Axial CT of the para nasal sinuses showed extensive mucosal thickening of the left maxillary antrum, sphenoidal sinus and left ethmoidal air cells

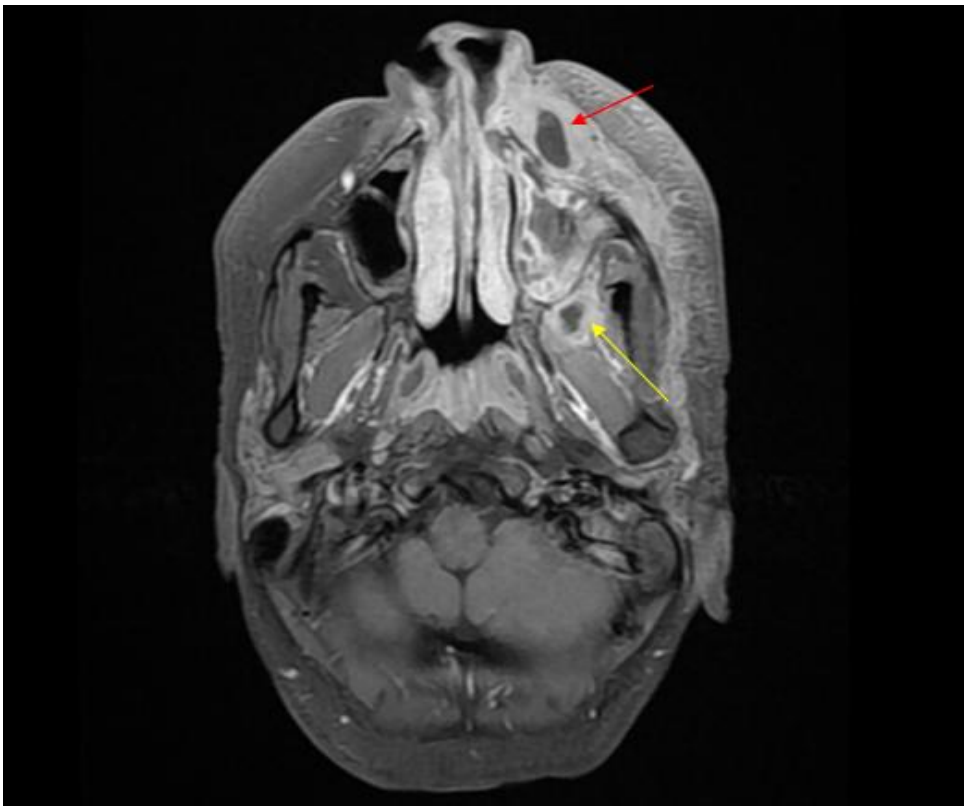

Figure 2
Axial MRI T1 fat saturation post contrast showed mucosal thickening of all left paranasal sinuses. Also it revealed thickened enhanced wall abscess in the left pre maxillary soft tissue (red arrow), retro antral region (yellow arrow) and left orbital floor.

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Video case: Multiple Colonic Polyposis in 28 Years Old Man with Family History of Cancer Colon

Tarik I Zaher
Tropical Medicine Department, Faculty of Medicine ,Zagazig University, Egypt
tareqzaher@gmail.com

28 years old man with family history of cancer colon presented for screening colonoscopy , which revealed 3 sessile ascending colon polyps which were difficult for excision due to looping and jumping of the scope to be followed and excised later on (after 3 monthes). Another pedunculated ascending colon polyp was excised .Also single sigmoid colon and 3 different sizes rectal polyps were excised.

Image Case:Endoscopic Banding of Dieulafoy's Lesion in 9 Years Old Girl

Mohamed Refaey , Tarik Zaher
Tropical Medicine Department, Faculty of Medicine, Zagazig University, Egypt.
tareqzaher@gmail.com

A 9 years old girl presented with first attack of hematemesis and melena .Upper gastrointestinal endoscopy revealed active blood spurting from Dieulafoy's lesion ~ 5 cm away from the cardia .Endoscopic banding was performed ,followed by mucosal tattooing using methylene blue,with excellent hemostasis.

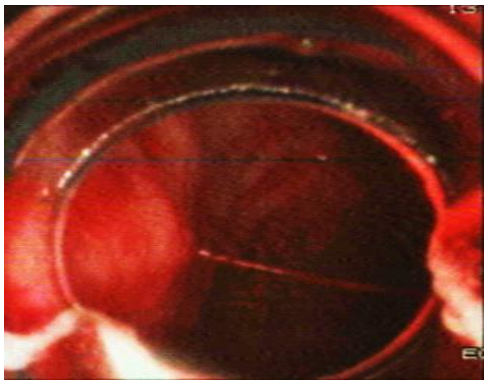

Figure 1: Active blood spurting from Dieulafoy's lesion.

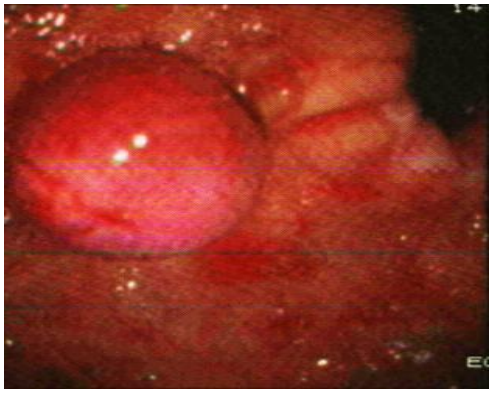

Figure 2: Dieulafoy's lesion after banding.

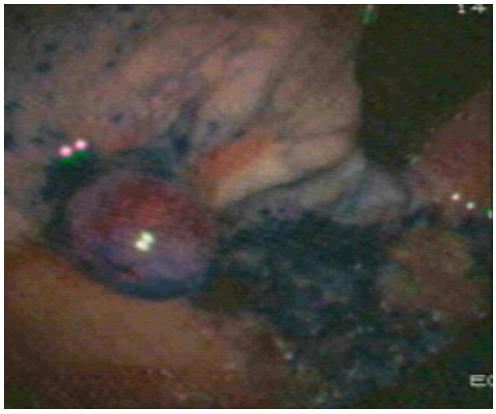

Figure 3: Mucosal tattooing using methylene blue,with excellent hemostasis.

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