Afro-Egyptian Journal of Infectious and Endemic Diseases

المجلة الافريقية المصرية للامراض المعدية والمتوطنة

ISSN (Online): 2090-7184

ISSN (Print): 2090-7613

An Official Publication of Endemic and Tropical Medicine Department ,Faculty of Medicine ,Zagazig University ,Zagazig 44519 ,Egypt

Published by: Communication and Information Technology Center(CITC),Zagazig University , Zagazig ,Egypt
Atef Eraky
E mail:atef_eraky@yahoo.com
Wafaa Metwally
E mail:wafaa@zu.edu.eg

Scope of the Journal

The Afro-Egyptian Journal of Infectious and Endemic Diseases (AJIED) is a peer-reviewed journal that publishes clinical, parasitological, microbiological, physiological, biochemical, immunological and pathological studies in the field of infectious, endemic and tropical diseases. The scope of the journal includes also articles of endemic

gastroenterology and hepatology. The journal is published quarterly by Endemic and Tropical Medicine Department, Faculty of Medicine, Zagazig University, Zagazig, 44519, Egypt.

Submission Process

The Journal accepts online submissions only. Manuscripts can be submitted at http://mis.zu.edu.eg/ajied/home.aspx. Once the manuscript has been uploaded, our system automatically generates an electronic pdf, which is then used for reviewing. All correspondence, including notification of the Editor's decision and requests for revisions, will be managed through this system. Authors can follow the progress of their paper using this system to final decision. For any problems please contact the Editorial Office at ajied@zu.edu.eg.

Due to editorial policy to accept high quality articles, the journal accept only 50% of received articles.

Authorship

All authors should have made substantial contributions to all of the following:

(1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data
(2) drafting the article or revising it critically for important intellectual content
(3) final approval of the version to be submitted.

Article types

The following types of manuscripts are routinely accepted:

1- **Original Articles:** This should include an abstract, keywords, introduction, patients/material and methods, results, discussion and references. They should be no longer than 5000 words (word count excludes tables, figures and legends).
2- **Reviews:** An abstract and keywords are required. The text should be divided into sections by suitable headings. Tables and figures may be used as appropriate for the text. They should be no longer than 6000 words.
3- **Opinions, Commentaries and Letters to the editor:** These take the same form as a review.
4- **Short Communications:** These should be no more than 2,500 words, with up to 15 references and a maximum of 3 figures or tables.
5- **Case Reports:** Case reports should present only cases of exceptional interest including presentation, diagnosis and management of disease. They should contain short summaries, an introduction, the case report, discussion, a reference list, tables and figure legends.
6- **Images in Infectious and Endemic Diseases:** These consist of interesting cases with high quality images with a short text and no more than 10 references.
7- **Video case**:By invitation.

Preparation of the manuscript

Please ensure that the following are including in your submission: -One author designated as corresponding author: Their E-mail address ,full postal address Telephone and fax numbers -Keywords -Cover letter addressed to the Editor, introducing the manuscript and confirming that it is not being submitted concurrently elsewhere -All figure captions -All tables (including title, description, footnotes) -All necessary files have been uploaded -Manuscript has been spell checked -All text pages have been numbered -References are in the correct format for this journal -All references mentioned in the Reference list are cited in the text and vice versa -Permission has been obtained for use of copyrighted material from other sources (including the Web) -Color figures are clearly marked as being intended for color reproduction or to be reproduced in black-and-white.-Manuscripts :Please type all pages with double spacing and wide margins on one side of the paper. Title page, abstract, tables, legends to figures and reference list should each be provided on separate pages of the manuscript. Use font such as Times New Roman or Arial. The text should be in single-column format. Number the pages. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. In particular, do not use the options to justify text or to hyphenate words. However, do use bold face, italics, subscripts, superscripts etc. Do not embed 'graphically designed' equations or tables, but prepare these using the facility in Word or as a separate file in Excel. When preparing tables, if you are using a table grid, use only one grid for each individual table and not a grid for each row. Do not prepare tables in PowerPoint. To avoid unnecessary errors you are strongly advised to use the spellchecker. The title page should include: the title, the name(s) and affiliation(s) of the author(s), an address for correspondence, and telephone/fax numbers for editorial queries. All articles should include an Abstract of no more than 300 words and 3-6 key words for abstracting and indexing purposes. Please write your text in good English. Use decimal points (not commas); use a space for thousands (10 000 and above).

Provide the following data in your submission (in the order given).

1- **Title page (separate page):** Title should be concise and informative. Avoid abbreviations and formulae where possible. Author names and affiliations. Where the family name may be ambiguous (e.g., a double name), please indicate this clearly. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with an Arabic number immediately after the author's name and in front of the appropriate address. Corresponding author: This should be indicated

after authors affiliations. Clearly indicate who is willing to handle correspondence at all stages of refereeing and publication, also post-publication. . Ensure that telephone and fax numbers (with country and area code) are provided in addition to the e-mail address and the complete postal address.

2- **Abstract**: (separate paper). A concise and informative abstract is required (maximum length 300 words). The abstract should state briefly the purpose of the research, the principal results and major conclusions. Do not cite references in the abstract. Non-standard or uncommon abbreviations should be avoided in the abstract, but if essential they must be defined at their first mention in the abstract itself. The abstract should be divided into: Background and study aims, patients/material and methods, results and conclusion. Keywords Immediately after the abstract, provide a maximum of 6 keywords.

3- **Abbreviations:** Define abbreviations that are not standard in this field at their first occurrence in the article (even if mentioned in the abstract). Ensure consistency of abbreviations throughout the article

4- **Introduction:** State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

5- **Patients/Materials and methods**: Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference. Only relevant modifications should be described. Include in figure legends and table texts, technical details of methods used, while describing the methods themselves in the main text.

6- **Results:** This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate in a Short Communication but not in an Original Article. Ensure that the chapter results stands by itself and explain all results of your work. Note that all tables and figures should be presented in separate papers.

7- **Discussion:** Discuss your results and avoid extensive citations and discussion of published literature.

8- **Acknowledgement:** Collate acknowledgements in a separate section at the end of the article and do not, therefore, include them on the title page, as a footnote to the title or otherwise. When the work included in a paper has been supported by a grant from any source, this must be indicated. A connection of any author with companies producing any substances or apparatus used in the work should be declared in this section. All contributors who do not meet the criteria for authorship as defined above should be listed in an acknowledgements section. Examples of those who might be acknowledged include a person who provided purely technical help, writing assistance, or a department chair who provided only general support. Authors should disclose whether they had any writing assistance and identify the entity that paid for this assistance.

9- **References:** References should be numbered consecutively (with parentheses) as they appear in the text e.g. [5]. Type the reference list with double spacing on a separate sheet. This includes family name and first name initial, up to 6 authors are required and more authors are marked with et al. Examples: 1- Abdel-Wahab M, Esmat G, El-Boraey Y, Ramzy I , Medhat E, Strickland G. The epidemiology of schistosomiasis in Egypt: methods , training, and quality control of clinical and ultrasound examinations . *Am J Trop Med Hyg* 2000 ; 62 (suppl) :17-20. 2- Wright W. Geographical distribution of schistosomes and their intermediate hosts. Ansari N, ed. Epidemiology and control of schistosomiasis (bilharziasis) .*Baltimor* ;*University Park Press* 1973 ;42-48.. Do not include references to personal communications, unpublished data or manuscripts either 'in preparation' or 'submitted for publication'. If essential, such material may be incorporated into the appropriate place in the text. Recheck references in the text against reference list after your manuscript has been revised. All references listed in the text should be included in the reference list and all references in the reference list should be included in the text.

10- **Illustrations:** Photographs should be presented as high quality jpg. Illustrations will not be redrawn by the Publisher: line figures should be suitable for direct reproduction. They should be prepared with black on white background, or be black-and-white images; ; they should be completely and consistently lettered, the size of the lettering being appropriate to that of the illustration, taking into account the necessary reduction in size. Colour figures will be included

11- **Tables:** Number tables consecutively in accordance with their appearance in the text. Place footnotes to tables below the table body and indicate them with superscript lowercase letters. Avoid vertical rules. Be sparing in the use of tables and ensure that the data presented in tables do not duplicate results described elsewhere in the article.

Editorial Review

All manuscripts are subject to peer review. If changes are requested, revisions received later than 2 months after this request will be treated as new submissions. When changes are made, the corresponding author

should go into resubmission under title of submission of revised manuscript, and a word document should be uploaded that indicates changes and modifications done.

Publication charges

No publication charges are needed .

Off prints

The corresponding author, at no cost, will be provided with a PDF file of the article via e-mail. Authors can download the PDF from the journal web page and in the same way the journal cover image can be downloaded.

Policy and Ethics Declarations

Upon submission you will be required to complete this form to declare funding, conflict of interest and to indicate whether ethical approval was sought. This information must also be inserted into your manuscript under the acknowledgements section. If you have no declaration to make please insert the following statements into your manuscript: Funding: None, Competing interests: None declared ,Ethical approval: Not required . Work on human beings that is submitted to AJIED should comply with the principles laid down in the Declaration of Helsinki; Recommendations guiding physicians in biomedical research involving human subjects. Adopted by the 18th World Medical Assembly, Helsinki, Finland, June 1964, amended by the 29th World Medical Assembly, Tokyo, Japan, October 1975, the 35th World Medical Assembly, Venice, Italy, October 1983, and the 41st World Medical Assembly, Hong Kong, September 1989. The manuscript should contain a statement that the work has been approved by the appropriate ethical committees related to the institution(s) in which it was performed and that subjects gave informed consent to the work. Studies involving experiments with animals must state that their care was in accordance with institution guidelines.

Competing interests

All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. Role of the funding source all sources of funding should be declared. Authors should declare the role of study sponsors, if any, in the study design, in the collection, analysis and interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication. If the study sponsors had no such involvement, the authors should so state.

Indexing

1 - Egyptian National Scientific Technical Information Network (ENSTINET): http://derp.sti.sci.eg/details.aspx?Id=Afro-Egyptian%20Journal%20of%20Infectious%20and%20Endemic%20Diseases%20%28Online%29

2- Google Scholar

3- InnoSpace - SJIF Scientific Journal Impact Factor (IF 2012: 2.665): http://www.sjifactor.inno-space.org/passport.php?id=2123

4- Index Copernicus (ICV for 2013: 4.49, ICV for 2014: 51.89 points, Standardized Value: 5.63): http://journals.indexcopernicus.com/masterlist.php?q=Afro-Egyptian+Journal+of+Infectious+and+Endemic+Diseases

5- Global Impact Factor (Impact Factor for year 2015 is =0.544) http://globalimpactfactor.com/afro-egyptian-journal-of-infectious-and-endemic-disease/

6- Universal Impact Factor (Impact Factor for year 2013 is = 1.0599): http://www.uifactor.org/Search.aspx?q=2090-7184

7- CiteFactor: http://www.citefactor.org/search/keywords/journals/Afro-Egyptian+Journal+of+Infectious+and+Endemic+Diseases

8- Pubicon Science Index: http://www.pubicon.org/APUIIR.aspx?cmd=Afro-Egyptian%20Journal%20of%20Infectious%20and%20Endemic%20Diseases

9- Directory of Research Journals Indexing:

10- Polish Ministry of Science and Higher Education (2/10 in 2013 and 2014 journal official report):http://impactfactor.pl/czasopisma/832-afro_egyptian-journal-of-infectious-and-endemic-diseases

11-US National Library of Medicine (NLM) Catalog [NLM ID: 101570965]:http://www.ncbi.nlm.nih.gov/nlmcatalog/101614559

Contents

Monocyte Chemoattractant Protein-1 as a Diagnostic Marker for Detection of Hepatocellular Carcinoma in Egypt

Sherif M. Galal[1] , Nermin Raafat[2]
Departments of Tropical Medicine[1] and Medical Biochemistry[2]
Faculty of Medicine, Zagazig University, Egypt.

Corresponding Author
Sherif M. Galal

Mobile:
+201221053975

E mail:
dsherifgalal@yahoo.com

Key words:
HCC, AFP, MCP-1

Background and study aim: Hepatocellullar carcinoma (HCC) is usually diagnosed at advanced stage resulting in limited therapeutic options and poor prognosis. The role of alpha-fetoprotein (AFP) in the diagnosis of HCC is controversial. Here, we investigated the role of Monocyte Chemoattractant Protein -1 (MCP-1), a serum biomarker, alone or in combination with AFP for detection of HCC.

Patients and Methods: 116 patients with liver cirrhosis were included. The patients were divided into 2 groups: HCC group included 58 patients with HCC and non-HCC patients as a control group included 58 patients with no evidence of hepatic focal masses. Routine laboratory investigations, AFP, MCP-1, pelvi-abdominal ultrasonography (US) and triphasic computed tomography (CT) scan were performed in all patients.

Results: It was found that MCP-1 at a cut-off value >0.390 ng/ml has a sensitivity of 75.8% and specificity of 88.3% with AUROC 0.916; But AFP at a cut-off value >20 ng/ml has a sensitivity of 86.5% and specificity of 96.4% with AUROC 0.924, while combined (AFP+ MCP-1) at a cut-off value >23.390 ng/ml has a higher sensitivity (96.5%) specificity of 100% with AUROC 0.995.

Conclusion: Monocyte Chemoattractant Protein-1 (MCP-1) can be identified as an adjuvant biomarker for HCC detection. Combined (AFP+MCP-1) showed higher diagnostic ability than MCP-1 alone or AFP alone in HCC detection.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the second leading cause of cancer-related deaths worldwide. The incidence of HCC has been increased both in the United States and abroad with approximately 750,000 new cases of liver cancer reported per year **[1,2]**. Egypt has rising rates of HCC and being a unique nature of liver disease that presents questions regarding the etiology of HCC; the currently increasing incidence of HCC in Egyptians may be due to shift of the relative importance of hepatitis C virus (HCV) as primary risk factors **[3]**.

HCC is usually diagnosed at advanced stage resulting in limited therapeutic options and poor prognosis. The identification of biomarkers is an important issue since such markers could facilitate detection of HCC. Furthermore, such biomarkers could display potential therapeutic targets for HCC **[4].**

The most commonly used serum marker of HCC is Alpha-fetoprotein (AFP), which has a reported sensitivity of 49% to 71% and specificity of 49% to 86% in HCCs smaller than 5 cm. Its levels are influenced by tumor size and aggressiveness, as well as by the etiology and activity of the liver disease **[5,6,7]**. Approximately one-third of early-stage HCC patients with small tumors (<5 cm) have normal levels of AFP **[8].** Thus, clinicians are dissatisfied with AFP as a marker due to its high false-positive and false-negative rates. Consequently there is urgent clinical need to identify new biomarkers that will improve new diagnosis of early HCC over the current screening practice of serum AFP measurements. **[9]**.

The Monocyte Chemoattractant Protein-1 (MCP-1), is a small cytokine that belongs to the CC chemokine family. It is a potent chemotactic factor for monocytes. MCP-1is one of the key chemokines that regulate migration and infiltration of monocytes/macrophages It was found that MCP-1was significantly elevated in patients with resectable HCC compared to non-HCC chronic hepatitis B (HBV) carriers **[10].**. Here, we investigated the role of MCP-1, a serum biomarker, alone or in combination with AFP for detection of HCC.

PATIENTS AND METHODS

This comparative case-control study was conducted in Tropical Medicine Department in cooperation with Medical Biochemistry Department, Zagazig University, Egypt, during the period from January 2015 to December 2015 and included 116 cirrhotic patients. The patients were divided into 2 groups:

Group I (HCC group): Included 58 cirrhotic patients with HCC (48 males and 10 females) diagnosed by triphasic computed tomography (CT) scan.

Group II (Non-HCC patients): Included 58 cirrhotic patients (46 males and 12 females) with no evidence of hepatic focal masses as a control group.

Enrollment criteria:
Patients with liver cirrhosis, with or without HCC were included. Diagnosis of liver cirrhosis was based on clinical, laboratory and imaging tests, and liver biopsy if present. Patients with cirrhosis and focal hepatic lesions on ultrasound with normal AFP were subjected to triphasic CT. The diagnosis of HCC by triphasic CT-scan showing was based on typical criteria for HCC (early enhancement during arterial phase followed by washout of contrast in porto-venous and delayed phases).

Exclusion criteria:
Patients with other malignancies elsewhere and patients with Child- Pugh class C were excluded.

Methods:
Patients were subjected to:
1. Detailed history taking.
2. Thorough clinical examination.

Child-Turcotte-Pugh classing was evaluated for each patient (Table I).

Table (I): Child-Pugh-Turcotte criteria

	1 Point	2 Points	3 Points
Albumin (g/dl)	>3.5	2.8-3.5	<2.8
Bilirubin (mg/dL)	<2	2-3	>3
Ascites	None	Minimal	Moderate
Encephalopathy	None	Grade 1-2	Grade 3-4
PT (second prolonged) **INR**	<4 <1.7	4-6 <1.7-2.3	>6 >2.3

PT; prothormbin time INR, International Normalizing Ratio
Class A: 5-6 points; class B: 7-9 points; class C: 10-15 points

3. Routine laboratory investigations (complete blood counts, liver and kidney profiles, coagulation profile).
4. Alpha-feto protein (AFP): It was determined by ELISA Kit provided by RayBiotech, Inc., the catalogue no ELH-AFP.
5. Monocyte Chemoattractent Protein -1 (MCP-1): It was determined by ELISA Kit provided by Biospes Comany, the catalogue no BEK1142.

This kitwasbased on sandwich enzyme-linked immune-sorbentassay technology.Anti-MCP-1 polyclonal antibody was pre-coatedonto 96-well plates. And the biotin conjugated anti-MCP-1 polyclonal antibody was used as detection antibodies. The standards, test samples and biotin conjugated detection antibody were added to the wells subsequently, and wash with wash buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with wash buffer. TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the MCP-1 amount of sample captured in plate. Read the O.D. absorbance at 450nm in a micro-plate reader, and then the concentration of MCP-1 can be calculated **[11].**

6. Pelvi-abdominal ultrasonography (esaote MyLab20Plus).
7. Abdominal triphasic CT scan: Diagnosis of HCC was based on typical criteria; hyper enhancement in the arterial phase with rapid wash out in both portal and delayed phases.

Statistical analysis:
Data were checked, entered and analyzed using SPSS version 19 for data processing and statistic. Data were expressed as mean ± SD for quantitative variable, number and percentage for qualitative one. Chi-squared (X^2) or student "t" test were used when appropriate. P<0.05 was considered significant.

RESULTS

- In this study, no statistical significant differences were observed among both studied groups with respect to the following baseline characteristics: patients' sex and Child-Pugh class. While there was statistically significant difference among both studied groups as regard age (P= 0.003). Most of patients were older in HCC group (57.86 ± 5.73) (Table 1).
- As regard tumor markers, there was statistically significant higher values of both AFP and MCP-1 (374.16 ± 282.77 and 0.703 ± 0.360 respectively) among patients of HCC group (P <0.001) (Table 2).
- Regarding correlation between serum concentrations of AFP, MCP-1 and study parameters in HCC patients, there was no correlation as regard age and laboratory parameters. While, there was statistically significant correlation between tumor size (in cm) and both MCP-1 (P<0.001) and AFP (P= 0.030) (Table 3).
- Concerning diagnostic performance of both serum MCP-1and serum AFP, either alone or in combination, in prediction of HCC, it was found that MCP-1 at a cut-off value >0.390 ng/ml has a sensitivity of 75.8% and specificity of 88.3% with AUROC 0.916; and AFP at a cut-off value >20 ng/ml has a sensitivity of 86.5% and specificity of 96.4% with AUROC 0.924, while combined (AFP+MCP-1) at a cut-off value >23.390 ng/ml has a higher sensitivity (96.5%) and specificity (100%) with AUROC 0.995. Therefore, the diagnostic performance of combined (AFP+MCP-1) is more valuable than MCP-1 alone or AFP alone (Table 4 and Fig. 1).

Table (1): Baseline characteristics among both studied groups

	(HCC patients) (n=58)		(Non-HCC patients) (n=58)		t	P-value
Age (Mean ± SD)	57.86 ± 5.73		53.68 ± 4.37		3.116	0.003
	No	**%**	**No**	**%**	**X^2**	**P-value**
Gender					0.112	0.738
Male	48	82.8%	46	79.3%		
Female	10	17.2%	12	20.7%		
Child-Pugh Class					0.293	0.588
Child A	38	65.5%	34	58.6%		
Child B	20	34.5%	24	41.4%		

P< 0.05; significant and P<0.001; highly significant

Table (2): Comparison between both studied groups as regard tumor markers

Tumor markers	HCC patients (N=58)	Non-HCC patients (N=58)	Test	P-value
AFP (ng/ml)			-6.470•	<0.001
Mean ± SD	374.16 ± 282.77	16.39 ± 3.41		
Median (range)	462 (20–761)	16.54 (9.45–23)		
MCP-1 (ng/ml)			-5.440•	<0.001
Mean ± SD	0.703 ± 0.360	0.318 ± 0.064		
Median (range)	0.590 (0.340–1.640)	0.330 (0.180–0.390)		

•Mann Whitney U test. p< 0.05; significant and p<0.001; highly significant

Table (3): Correlation between AFP, MCP-1 and studied parameters in HCC patients

HCC Patients	MCP-1 (ng/mL)		AFP (ng/mL)	
	r	P-value	r	P-value
Age (years)	+0.114	0.557	+0.009	0.961
Tumor size (cm)	+0.737	<0.001	+0.403	0.030
Hemoglobin (gm/dl)	-0.035	0.858	+0.222	0.246
Platelet count ($x10^3/mm^3$)	-0.073	0.707	-0.029	0.882
Total serum bilirubin (mg/dl)	+0.229	0.232	-0.306	0.107
AST (U/L)	+0.370	0.048 (S)	+0.124	0.522
ALT (U/L)	+0.257	0.178 (NS)	+0.066	0.732
Albumin (gm/dl)	-0.166	0.391 (NS)	+0.071	0.716
PC	+0.424	0.022 (S)	-0.181	0.346
Creatinine (mg/dl)	+0.211	0.272 (NS)	-0.331	0.079
Urea (mg/dl)	+0.344	0.068 (NS)	-0.200	0.298
AFP (ng/mL)	+0.173	0.370 (NS)	---	---
(ng/mL)	---	---	+0.173	0.370

r: Spearman's correleation coefficient, P< 0.05; significant and P<0.001;highly significant. PC; Prothrombin concentration, AFP;alpha-fetoprotein; AST; Aspartate transaminase, ALT; Alanine transaminase, MCP-1; Monocyte Chemoattractant Protein-1

Table (4): Diagnostic performance of MCP-1, AFP and both in prediction of HCC; ROC curve Analysis

Cut-off values	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	PPV % (95% CI)	NPV % (95% CI)	Accuracy (95% CI)	AUROC (95% CI)
***MCP-1 > 0.390 ng/ml**	75.8% (56.5-89.7)	88.3% (88.1-100)	100% (84.6-100)	80.6% (64-91.8)	87.9% (72.3-94.9)	0.916 (0.812-0.972)
****AFP > 20 ng/ml**	86.5% (79.2-99.9)	96.4% (88.1-100)	100% (87.7-100)	90.7% (79.8-99.9)	95.3% (82.2-100)	0.924 (0.911-1.000)
*****MCP-1 + AFP > 23.390 ng/ml**	96.5% (82.2-99.9)	100% (88.1-100)	100% (87.7-100)	96.7% (82.8-99.9)	98.3% (85.2-100)	0.995 (0.928-1.000)

* SE= 0.035; Z=11.708; p<0.001
** SE=0.005; Z=85.042; p<0.001
*** SE=0.006; Z=76.779, p<0.001
SE: Standard error
p< 0.05; significant and p<0.001; highly significant
PPV: Positive Predictive Value
NPV: Negative Predictive Value
95%CI: 95% Confidence Interval
AUROC: Area Under Receiver Operating Characteristic curve

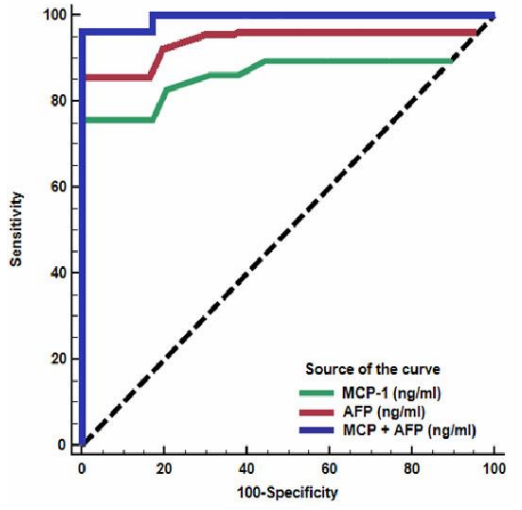


Fig. (1): Receiver operating characteristic (ROC) curve of MCP-1 and AFP as a predictor for HCC.

DISCUSSION

The role of AFP in the diagnosis of HCC is controversial. Most studies used different cut-off values for AFP which gave low sensitivity and specificity especially with small HCC (<5cm) and confirmed the inadequacy of using AFP alone for HCC diagnosis **[12,13].** Thus other biomarkers for better detection of HCC were investigated **[15,16].** In this study, we investigated MCP-1, a serum biomarker, alone or in combination with AFP for detection of HCC. MCP-1 is a potent chemokine which plays a role in the recruitment of monocytes to sites of injury and infection. Association between MCP-1 and liver damage has been implicated due to the findings that hepatic stellate cells are major source of MCP-1 **[18,19].** Different studies showed that MCP-1 expression occurs during severe acute liver injury with significant elevation of its level **[20,21,22].**

Furthermore, studies showed that MCP-1 expression is associated with HCC development and progression**[23].** HCC cells and cancer-associated fibroblasts are prominent contributors of MCP-1, regardless of whether the liver is cirrhotic or **not [24,25].**

According to the demographic data, it was found that most of patients were older in HCC group (57.86±5.73) with statistical significant difference among both studied groups (P= 0.003). These results meet the results of Velazquez et al. which found that cirrhotic patients older than 54 years have four times higher risk for developing HCC than younger ones**[12].** Furthermore, it was found that 82.8% of our HCC patients were males with no statistically significant difference was observed among both studied groups (P= 0.738). This result is consistent with El-Zayadi et al. who found that HCC is more prevalent in males than females which may be explained by differences in exposure to risk factors**[3].** It has been determined that estrogens and androgens could modulate hepato-carcinogenesis and explain the higher incidence of HCC in men **[14,17]**.

Most of our HCC patients were in Child-Pugh class A(65.5%) and the remaining 34.5% were in class B while were excluded patients with Child-Pugh class C because they will not benefit from early diagnosis of HCC **[26].**Our result is in agreement with Wang et al. which stated that the majority of the HCC patients were in Child-Pugh class A (98.4%)**[27]**. Also, our results showed that there was statistically significant higher values of both AFP and MCP-1 (374.16 ± 282.77 and 0.703 ± 0.360 respectively, P <0.001) among patients of HCC group. Furthermore, no correlation were found between serum concentrations of AFP and MCP-1 with laboratory parameters including liver function parameters, while there was statistically significant correlation between tumor size (in cm) and both MCP-1 (P <0.001) and AFP (P= 0.030). This is consistent with Wang and his colleagues which mentioned that no significant correlation between serum MCP-1 level with the other liver function parameters, therefore MCP-1 levels in the HCC patients were likely to be predominantly expressed by HCC tumors and the HCC-associated cells **[27].**

We investigated the diagnostic performance of both serum MCP-1and serum AFP, alone or in combination, by performing ROC analysis and comparing the resulting AUROC. Our results showed that MCP-1 at a cut-off value >0.390 ng/ml has a sensitivity of 75.8% and specificity of 88.3% with AUROC 0.916; But AFP at a cut-off value >20 ng/ml has a sensitivity of 86.5% and specificity of 96.4% with AUROC 0.924, while combined (AFP+MCP-1) at a cut-off value >23.390 ng/ml has a higher sensitivity (96.5%) specificity of 100% with AUROC 0.995

These results agree with the results of Wang et al. which found that AUROC of MCP-1 was 0.823, AUROC of AFP was 0.942 and the AUROC of combined (AFP+MCP-1) was 0.974 [27]. So Combined (AFP+MCP-1) showed higher diagnostic ability than MCP-1 alone or AFP alone.

CONCLUSION

Monocyte Chemoattractant Protein -1 (MCP-1) can be identified as an adjuvant biomarker for HCC detection. Combined (AFP+MCP-1) showed higher diagnostic ability than MCP-1 alone or AFP alone in HCC detection.

Funding: None.
Conflicts of interest: None.
Ethical approval: A written informed consent was taken from all included patients, and the study was approved by the Ethical Committee of Faculty of Medicine, Zagazig University.

REFERENCES

1. Jemal A, Bray F, Center M, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin.* 2011, 61: 69-90.
2. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. *CA Cancer J Clin*. 2012, 62: 10-29.
3. El-Zayadi AR, Badran HM, Barakat EM, Attia Mel-D, Shawky S, Mohamed MK, et al. Hepatocellular carcinoma in Egypt: a single center study over a decade. *World J Gastroenterol*. 2005, 11: 5193-5198.
4. Altekruse SF, McGlynn KA, Reichman ME. Hepatocellular carcinoma incidence, mortality, and survival trends in the United States from 1975 to 2005. *J Clin Oncol*. 2009, 27: 1485-1491.
5. Maringhini A, Cottone M, Sciarrino E, Marceno MP, La Seta F, Fusco G, et al. Ultrasonography and alpha-fetoprotein in diagnosis of hepatocellular carcinoma in cirrhosis. *Dig Dis Sci.* 1988,33: 47-51.
6. Oka H, Saito A, Ito K, Kumada T, Satomura S, Kasugai H, et al. Multicenter prospective analysis of newly diagnosed hepatocellularcarcinoma with respect to the percentage of Lens culinaris agglutinin-reactive alpha-fetoprotein.*J Gastroenterol Hepatol*. 2001, 16: 1378-1383.
7. Ikoma J, Kaito M, Ishihara T, Nakagawa N, Kamei A, Fujita N, et al. Early diagnosis of hepatocellular carcinoma using asensitive assay for serum des-gamma-carboxy prothrombin: aprospective study. *Hepatogastroenterology* 2002, 49: 235-238.
8. Zhao YJ, Ju Q, Li GC. Tumor markers for hepatocellular carcinoma. *Mol Clin Oncol*. 2013, 1: 593-598.
9. Colli A, Fraquelli M, Casazza G, Massironi S, Colucci A, Conte D, et al.Accuracy of ultrasonography, spiral CT, magnetic resonance, and alpha-fetoprotein in diagnosing hepatocellular carcinoma: a systematic review. *Am J Gastroenterol.* 2006, 101: 513–523.
10. Deshmane SL, Kremley S, Amini S, Sawaya BE. Monocyte chemoattractant protein-1 (MCP-1): An overview. *J Interferon Cytokine Res*. 2009, 29: 313-326.
11. Carr MW, Roth SJ, Luther E, Rose SS, Springer T. Monocyte chemoattractant protein 1 acts as a T-lymphocyte chemoattractant. *Proc Natl Acad Sci USA.* 1994, 91:3652–3656.
12. Velazquez RF, Rodriguez M, Navascues CA, Linares A, Perez R, Sotorrios NG, et al. Prospective analysis of risk factors for hepatocellular carcinoma in patient with liver cirrhosis. *Hepatology.* 2003, 37: 520-527.
13. Omata M, Lesmana LA, Tateishi R, Chen PJ, Lin SM, Yoshida H, et al. Asian Pacific Association for the Study of the Liver consensus recommendations on hepatocellular carcinoma. *Hepatol Int*. 2010, 4: 439-474.
14. Soresi M, Magliarisi C, Campagna P, Leto G, Bonfissuto G, Riili A, et al. Usefulness of alpha-fetoprotein in the diagnosis of hepatocellular carcinoma. *Anticancer Res*. 2003, 23: 1747-1753.
15. Marrero JA, Su GL, Wei W, Emick D, Conjeevaram HS, Fontana RJ, et al. Des-gamma carboxy-prothrombin can differentiate hepatocellular carcinoma from non-malignant chronic liver disease in american patients. *Hepatology*. 2003, 37: 1114-1121.
16. Kobayashi M, Kuroiwa T, Suda T, Tamura Y, Kawai H, Igarashi M, Igarashi M, et al. Fucosylated fraction of alpha-fetoprotein, L3, as a useful prognostic factor in patients with hepatocellular carcinoma with special reference to low concentrations of serum alpha-fetoprotein. *Hepatol Res*. 2007, 37: 914-922.
17. Nagasue N, Ogawa Y, Yukaya H, Ohta N, Ito A. Serum levels of estrogens and testosterone in cirrhotic men with and without hepatocellular carcinoma. *Gastroenterology*.1985, 88: 768-772.
18. Friedman SL. Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury. *J Biol Chem*. 2000, 275: 2247-2250.
19. Marra F, DeFranco R, Grappone C, Milani S, Pastacaldi S, Pinzani, M, et al. Increased expression of monocyte chemotactic protein-1 during active hepatic fibrogenesis: correlation with monocyte infiltration. *Am J Pathol*. 1998, 152: 423-430.
20. Fisher NC, Neil DA, Williams A, Adams DH. Serum concentrations and peripheral secretion of the beta chemokines monocyte chemoattractant protein 1 and macrophage inflammatory protein 1alpha in alcoholic liver disease. *Gut.* 1999, 45: 416-420.
21. Antoniades CG, Quaglia A, Taams LS, Mitry RR, Hussain M, Abeles R, et al. Source and characterization of hepatic macrophages in acetaminophen-induced acute liver failure in humans. *Hepatology*. 2012, 56: 735-746.
22. Degre D, Lemmers A, Gustot T, Ouziel R, Trepo E, Demetter P, et al. Hepatic expression of CCL2 in alcoholic liver disease is associated with disease severity and neutrophil infiltrates. *Clin Exp Immunol.* 2012, 169: 302-310.
23. Dagouassat M, Suffee N, Hlawaty H, Haddad O, Charni F, Laguillier, C, et al. Monocyte chemoattractant protein-1 (MCP-1)/CCL2 secreted by hepatic myofibroblasts promotes migration and invasion of human hepatoma cells. *Int J Cancer.* 2010, 126: 1095–1108.
24. Yoong KF, Afford SC, Jones R, Aujla P, Qin S, Price K, et al. Expression and function of CXC and CC chemokines in human malignant liver tumors: a role for human monokine induced by gamma-interferon in lymphocyte recruitment to hepatocellular carcinoma. *Hepatology.* 1999, 30: 100-111.

25. Lin ZY, Chuang YH, Chuang WL. Cancer-associated fibroblasts upregulate CCL2, CCL26, IL6 and LOXL2 genes related to promotion of cancer progression in hepatocellular carcinoma cells. *Biomed Pharmacother*. 2012, 66: 525-529.
26. Bruix J, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, et al. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL Conference. *J Hepatol*. 2001, 35: 421-430.
27. Wang WW, Ang SF, Kumar R, Heah C, Utama A, Tania NP, et al. Identification of serum monocyte chemoattractant protein-1 and prolactin as potential tumor markers in hepatocellular carcinoma. *PLoS One*. 2013, 8: e68904.

Peer reviewer: Tarik Zaher, Professor of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt. **Editors: Sahar El-Nemr, Mohamad Emara,** Assistant Professors of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt.

Comparative Study of MELD Score and Glasgow Coma Scale in Patients with Hepatic Encephalopathy

Samir Mohamed Kabil, Magdy Abdel-Mawgood Gad, Naglaa El-Toukhy Ramadan El-Toukhy, Nahed Samir Abdel-Moteleb
Hepatology, Gastroenterology and Infectious Diseases Department, Faculty of Medicine, Benha University,Benha ,Egypt.

Corresponding Author
Nahed S Abdel-Motelb

***Mobile**:+2010973387 85*

***E mail:**dr_n33@hotmail.com*

Key words:
Hepatic encephalopathy, GCS, MELD score, uMELD score, Child score

Background and study aim: Hepatic encephalopathy occurs in approximately 30-45% of patients with cirrhosis and 10–50% of patients with transjugular intrahepatic Porto systemic shunt, while minimal hepatic encephalopathy affects approximately 20–60% of patients with liver disease**.** There are multiple prognostic scores that predict the mortality from chronic liver disease, of which the Child-Pugh score and the Model for End-stage Liver Disease (MELD) score are the most commonly used**.** The most widely used scale used to evaluate HE is the West-Haven (WH) scale, with scores ranging from 0 to 4. This study is designed to compare Glasgow coma scale to West-Haven scale in prediction of prognosis and survival of patients with hepatic encephalopathy.

Patients and Methods: This study was conducted on 100 patients with liver cirrhosis and overt Hepatic Encephalopathy admitted to The Department of Hepatology, Gastroenterology and Infectious Diseases of Mansoura Health Insurance Hospital, divided into four groups according to the grade of encephalopathy by West-Haven Criteria.

Results: There was no difference in prediction of survival among the studied patients assessed by GCS, MELD score, uMELD score and Child score (all had the same results).

Conclusion: Glasgow Coma Scale can be a prognostic tool for morbidity and mortality, as well as, follow-up in patients with HE and.

INTRODUCTION

Hepatic encephalopathy (HE) is a serious complication of decompensated cirrhosis that manifests as a wide range of neuropsychological clinical findings ranging from minimal HE to coma **[1]**. HE can be classified as either 'overt' or 'minimal'. Overt He (oHE) is a syndrome of neurological and neuronpsychiatric abnormalities that can be detected by bedside clinical tests. By contrast, patients with minimal He (mHE) present with normal mental and neurological status upon clinical examination but specific psychometric tests yield abnormal results **[2]**. Despite the important progresses of neuron imaging methods, clinical scales are commonly considered the best way to assess the degree of impairment and its impact on daily life activities for the majority of neurological diseases. Previous studies recommended using clinical scales for grading hepatic encephalopathy and to report efficacy in therapeutic trials such as the West-Haven criteria and Glasgow Coma Scale to assess the severity of HE **[3]**.

The model for end-stage liver disease (MELD) score was introduced to evaluate hepatic functions in cirrhotic patients. It has the advantage of using three objective and easily measured parameters: creatinine levels, international normalized ratio (INR) and total bilirubin **[4]**. The Model for End-Stage Liver Disease (MELD) score has been adopted as an objective indicator of liver disease severity **[1]**.

Aim of the work: this study aims at assessing the significance of Glasgow coma scale in evaluation of patients with hepatic encephalopathy in comparison

to the standard West-Haven criteria and its ability to predict morbidity and mortality in patients with hepatic encephalopathy in comparison with MELD score.

PATIENTS AND METHODS

This study was carried out on 100 patients with liver cirrhosis and overt Hepatic Encephalopathy. They were 85 males (85%) and15 females (15%), and their ages ranged between 18 and 60 years. All cases were selected from the Department of Hepatology, Gastroenterology and Infectious Diseases, Mansoura Health Insurance Hospital, within the period between January 2014 to June 2014.

The exclusion criteria were severe cardio-pulmonary disease, sepsis, renal disease, hepatocellular carcinoma, diabetes mellitus, patients listed to undergo transplantation.

Patients were subjected to the following:
Full history taking, thorough clinical examination. Routine laboratory investigations, that included: Complete blood picture. Liver profile tests: prothrombin time and concentration S. creatinine, viral markers, arterial blood ammonia.

Samples collection, preparation and handling:
A sample of arterial blood was sampled soon after admission under aseptic condition in preheparinized syringes from indwelling radial or femoral arterial catheters. Admission samples were taken within 24 hours of admission. Ammonia was measured with Ammonia Test Kit II for the PocketChem BA device (Arkay, Inc., Kyoto, Japan).

Abdominal Ultrasonography:
Liver was assessed for: size (span), echogenicity, surface, thickening of portal tracts, portal vein diameter, hepatic veins, inferior vena cava and presence or absence of focal lesions.

Spleen was assessed for: size, echogenicity, splenic vein diameter and presence or absence of collaterals. Other data concerning the gall bladder, both kidneys, pancreas, para aortic region as well as detection of ascites all were fulfilled.

The severity of liver cirrhosis in Hepatic Encephalopathy assessed using: Modified Child score:
Evaluation of the severity of liver cirrhosis was obtained in each cirrhotic patient with modified Child-Pugh score. This system relies on clinical and laboratory evaluation including ascites, grade of encephalopathy, serum albumin, bilirubin and prothrombin time **[5]**.

Parameter	1	2	3
Ascites controlled	None	easily controlled	Poorly controlled
Encephalopathy	none	grades 1-2	grades 3-4
Bilirubin (mg/dl)	< 2.0	2-3	> 3.0
Albumin (g/dL)	> 3.5	2.8-3.5	< 2.8
Prothrombin time (seconds increased)	< 4	4-6	> 6

Points	Class	One year survival	Two year survival
5-6	A	100%	85%
7-9	B	81%	57%
10-15	C	45%	35%

MELD score:
(Model for end stage liver disease) for evaluation of the severity of liver cirrhosis in each cirrhotic patient, and this system relies on laboratory evaluation including serum bilirubin, serum creatinine and INR (international normalized ratio).

MELD score = {9.6 × log (creatinine mg/dL) + 3.8 × log (bilirubin mg/dL)+11.2 × log (INR)+6.4} **[6].**

West Haven Criteria for Grading of mental status in HE **[7]:**

Grade 1	Grade 2	Grade 3	Grade 4
• Trivial lack of awareness • Euphoria or anxiety • Shortened attention span • Impairment of addition or subtraction • Altered sleep rhythm	• Lethargy or apathy • Disorientation for time • Obvious personality change • Inappropriate behavior • Dyspraxia • Asterixis	• Somnolence to semi-stupor • Responsive to stimuli • Confused • Gross disorientation • Bizarre behavior •	Coma with or without painful stimuli response to

Glasgow Coma Scale: **[8]**

Eye Opening Response	Verbal Response	Motor ResponsE
• Spontaneous-open with blinking at base line 4 • To verbal stimuli, command, speech 3 • To pain only (not applied to face) 2 • No response 1 •	• Oriented 5 • Confused conversation, but able to answer questions 4 • Inappropriate words 3 • Incomprehensible speech 2 • No response 1	• Obeys commands for movement 6 • Purposeful movement to painful stimulus 5 • Withdraws in response to pain 4 • Flexion in response to pain (decorticate • posturing) 3 • Extension response in response to pain (decerebrate posturing) 2 • No response 1

Statistical Analysis

Data were tabulated, coded then analyzed using the computer program SPSS (Statistical package for social science) version 21 to obtain Descriptive statistics were calculated in the form of: A- Mean ± Standard deviation (SD) for quantitative parametric data. B- Median and range (Minimum – maximum) for quantitative non-parametric data. C- Frequency (Number-percent) for qualitative data.

In the statistical comparison between the different groups, the significance of difference was tested using one of the following tests :

A-Student's *t*-test:-Used to compare between mean of two groups of numerical (parametric) data. B- Mann Whitney U test: Used to compare between two groups of numerical (non-parametric) data. C- Kruskal Wallis test: Used to compare between more than two groups of numerical (non-parametric) data followed by Mann Whitney for multiple comparisons.

Significance level: For all above mentioned statistical tests done, the threshold of significance is fixed at 5% level (p-value). The results were considered: Non-significant when the probability of error is more than 5% ($p > 0.05$). Significant when the probability of error is less than 5% ($p \leq 0.05$). Highly significant when the probability of error is less than 0.1% ($p \leq 0.001$).

RESULTS

The study was conducted on 100 patients (cases group) 85 males (85%), 15 females (15%). the age ranged between 47 and 77 years old in group (1) cases with the mean age being 60.28±7.54 years in comparison with group (4), the age ranged between 50 and 66 years old with mean age 56.50±6.95 years. Table (1) All patients complained from disturbed consciousness.

The mean value of Serum Creatinine was significantly higher in group (3) group than in other groups. No statistical significant difference between the four groups as regards ALT, AST, alkaline phosphatase, total bilirubin, prothrombin concentration, INR and albumin (Table 2).

As shown in table (2) there was statistical significant difference between the four groups as regards the arterial blood ammonia. It was significantly more predominant in group (4) cases (356.50±47.93) than in group (1) cases (90.73±18.42).

As shown in table (3) The most cases of Child A were in group (2), most cases of Child B were in

group (1), and most cases of Child C were in group (3) with statistical significant difference between the four groups. The mean value of Child score was predominant in group (3) cases (10± 1.087) with highly statistical significant difference between the four groups. As regards the severity of liver disease, MELD score was predominantly high in group (3) cases (24.43± 6.45) in comparison with group (1) patients (18.52± 4.62). Also uMELD score was high in group (3) cases with statistically significant difference between the four groups. There was statistical significant difference between the four groups as regards the severity of neurological dysfunction assessed by Glasgow Coma Scale. The GCS was predominantly high in group (1) cases, and less in group (4) cases.

As shown in table (4) average cirrhotic liver was detected in 78.9% of group (1) cases in comparison with group (4) cases (25.0%). Enlarged cirrhotic liver was detected predominant in group (4) cases, while shrunken liver was present predominant in group (3) cases and was statistically significant. Splenomegaly was detected in 100% of group (1-3-4) cases compared to 97.1% of group (2) cases which was statistically not significant. Portal Vein Dilatation was detected predominant in group (4) cases (100%) in comparison with group (1) cases which was present in (63.1%) and was statistically significant. There was no statistically significant difference among the four groups as regards the portal vein dilatation, splenic vein dilatation, collaterals, and gall bladder. There was statistical significant difference between the four groups as regards ascites. Mild ascites was significantly more predominant in group (4) patients (50.0%) than in other groups. Moderate ascites was significantly more predominant in group (1) patients (63.2%) than in other groups. Severe ascites was significantly more predominant in group (3) patients (56.5%) than in other groups.

As shown in table (5) there was statistically significant difference as regards the mortality among the studied patients being more predominant in group (3) patients (87%) in comparison with group (1) cases (34.2%). West Haven Criteria had significant influence on overall survival of patients with hepatic encephalopathy .There was a longest survival time (mean 8.9 months) in the group (1), followed by a longer survival time (mean 4.13 months) in the group (2) and a shorter survival time (mean 2.8 months) in the group (4) and the shortest survival time (mean 2.11months) in the group (3) which was statistically significant. Table (6)

GCS had significant influence on overall survival of patients with hepatic encephalopathy. After one year, survival was predominant in score (11.76±1.93) and death in score (9.24±2.95). MELD score had significant influence on overall survival of patients with HE. After one year, survival was predominantly high when MELD score was early (17.64±4.29) and death occurred when the score was advanced (23.10±6.52). Also uMELD score affected on overall survival of patients with HE. After one year, survival was high when uMELD score was early (3.94±.489) and death occurred when the score (4.57±.737) was advanced with high significant statistically difference. Child score affected on overall survival of patients with HE. Survival was high when score was small (8.38±1.04) and death occurred when the score increased (9.18±1.27) with significant statistically difference between all groups. Table (7)

As shown in table (8) according to Cox regression, there was no difference in prediction of survival among the studied patients assessed by GCS, MELD score, uMELD score and Child score (all had the same results).

Table (1): Demographic features of the studied patients

Items	Group 1 (No=38)		Group 2 (No=35)		Group 3 (No23)		Group 4 (No=4)		Test of sig. p-value
Age									
Mean ± SD	60.28±7.54		59.22±8.24		58.69±9.74		56.50±6.95		F=.366 P=.777
Range	47-77		45-81		41-86		50-66		
Sex									
Male	31	81.6	28	80.0	22	95.7	4	100	X2=3.788 P=.285
Female	7	18.4	7	20.0	1	4.3	0	0	

Table (2): Liver and renal function of the studied patients

Items	Group 1 (n=38)	Group 2 (n=35)	Group 3 (n=23)	Group4 (n=4)	Test of sig. p-value
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	
Creatinine mg/dL	1.17±.659	1.82±1.42	2.36±1.60	1.98±1.55	P1=.030 p2=.001 p3=.222 p4=.108 p5=.805 p6=.574
Albumin gm/dL	2.70±.402	2.69±.444	2.502±.407	2.58±.576	F=1.304 P=.278
Alkaline Phosphatase	178.26±48.41	177.31±79.18	185.61±43.02	196±24.06	F=.197 P=.898
ALT	45.60±21.41	39.91±20.55	56.47±48.45	48.50±23.17	F=1.455 p=.232
AST	47.02±20.37	51.25±21.69	72.17±64.68	41±18.07	F= 2.671 p=.052
Bilirubin mg/dl	4.87±3.51	5.11±3.82	5.81±3.22	3.42±.783	F= .674 p=.570
Prothrombin conc	16.26±3.06	16.42±3.31	17.64±4.09	15.17±.899	F=1.136 P=.338
INR	1.61±.45	1.59±.526	1.66±.494	1.53±.147	F=.131 P=.942
Arterial Blood Ammonia mg/dl	90.73±18.42	123.28±27.53	245.47±60.26	356.50±47.93	**P1=.000**** **p2=.000**** **p3=.000**** **p4=.000**** **p5=.000**** **p6= .000****

P1 comparison between groups 1 – 2. P2 comparison between groups 1 – 3. P3 comparison between groups 1 – 4. P4 comparison between groups 2 – 3. P5 comparison between groups 2 – 4. P6 comparison between groups 3 – 4. * significant, ** highly significant

Table (3): The severity of liver cirrhosis assessed by Child- Pugh classification, MELD and Glasgow Coma Scale

Items	Group 1		Group 2		Group 3		Group 4		Test of sig. p-value
	No=38	%100	No=35	%100	No=23	%100	No=4	%100	
Child grade									
Child A	1	2.6	1	2.9	0	0	0	0	**P1=.985 p2=.000** p3=.857 p4=.001** p5=.883 p6=.273**
Child B	31	81.6	28	80.0	8	34.8	3	75.0	
Child C	6	15.8	6	17.1	15	65.2	1	25.0	
Child score									
Mean ± SD	8.50± 1.059		8.48± 1.14		10± 1.087		9.25± .50		**P1=.955 p2=.000** p3=.192 p4=*.000*** *p5=.185* *p6=.205***
MELD score	18.52± 4.62		21.34± 6.92		24.43± 6.45		21± 6.37		**P1=.048 p2=.000** p3=.435 p4=.058 p5=.913 p6=.293**
uMELD score	4.08± .589		4.34± .785		4.73± .674		4.22± .618		**P1=.103 p2=.001** p3=.691 p4=.039* p5=.739 p6=.176**
GCS	12.84 ± 0.369		10.62 ±0 .91		6.43 ± 0.843		3.75± 1.50		**P1=.000** p2=.000** p3=.000** p4=.000** p5=.000** p6= .000****

P1 comparison between groups 1 – 2. P2 comparison between groups 1 – 3. P3 comparison between groups 1 – 4. P4 comparison between groups 2 – 3. P5 comparison between groups 2 – 4. P6 comparison between groups 3 – 4. * significant, ** highly significant

Table (4): Ultrasonographic features of the studied patients

Items	Group 1 (No=38)		Group 2 (No=35)		Group 3 (No=23)		Group 4 (No=4)		Sig. P
Liver size									
Average	30	78.9	27	77.1	7	30.4	1	25.0	**P1=.983 p2=.001** p3=.032 p4=.002* p5=.045* p6=.346**
Enlarged	1	2.6	1	2.9	1	4.3	1	25.0	
Shrunk	7	18.4	7	20.0	15	65.2	2	50.0	
Splenomegaly									
Remove	0	0	1	2.9	0	0	0	0	X^2=1.876 P=.599
Enlarged (>13cm)	38	100	34	97.1	23	100	4	100	
Collaterals									
No	11	28.9	6	17.1	6	26.1	1	25.0	X^2=1.469 P=.689
Yes	27	71.1	29	82.9	17	73.9	3	75.0	
Ascites									
Mild	2	5.3	3	8.6	1	4.3	2	50.0	**P1=.324 p2=.155 p3=.014 p4=.663 p5=.064 p6=.027***
Moderate	24	63.2	16	45.7	9	39.1	1	25.0	
Severe	12	31.6	16	45.7	13	56.5	1	25.0	
GB									
Thin wall	10	26.3	4	11.4	4	17.4	0	0	X^2=5.553 P=.475
Thick	27	71.1	31	88.6	19	82.6	4	100	
Removed	1	2.6	0	0	0	0	0	0	

P1 comparison between groups 1–2. P2 comparison between groups 1–3. P3 comparison between groups 1–4. P4 comparison between groups 2–3. P5 comparison between groups 2–4. P6 comparison between groups 3–4. * significant, **highly significant

Table (5): One year mortality among the studied patients

Survival	Group 1 (n=38)		Group 2 (n=35)		Group 3 (n=23)		Group 4 (n=4)		Test of sig. p-value
	No	%	No	%	No	%	No	%	
Survived	25	65.8	10	28.6	3	13.0	1	25.0	**P1=.001 p2=.000 p3=.146 p4=.165 p5=1 p6=.495**
Died	13	34.2	25	71.4	20	87.0	3	75.0	

Table (6): Means and Medians for Survival Time of patients with hepatic encephalopathy with reference to West Haven Criteria

West Haven criteria	Median Survival Time				Chi-Square	p-value
	Estimate	Std. Error	95% Confidence Interval			
			Lower Bound	Upper Bound		
1	8.940	0.764	7.444	10.437	32.497	**≤ 001****
2	4.130	0.874	2.418	5.843		
3	2.119	0.851	0.451	3.787		
4	2.808	2.365	0.000	7.443		
Overall	5.454	0.554	4.368	6.541		

** highly significant

Table (7): Overall survival of patients with hepatic encephalopathy with reference to GCS, MELD score, uMELD score and Child score.

	Survived 1 year	Died 1 year	Test of sig. p-value
GCS	11.76±1.93	9.24±2.95	t=4.713 **p=.000****
MELD score	17.64±4.29	23.10±6.52	t= 4.621 **p=.000****
uMELD score	3.94±.489	4.57±.737	t= 4.691 **p=.000****
Child score	8.38±1.04	9.18±1.27	t=3.268 **p=.001****

Table (8): Cox regression for prediction of survival among the studied patients.

Covariates	p-value	Hazard ratio	95% CI for HR	
			Lower	Upper
GCS	.000**	.762	.697	.834
MELD score	.000**	1.106	1.061	1.153
uMELD score	.000**	2.412	1.666	3.491
Child score	.000**	1.513	1.219	1.878

**highly significant

DISCUSSION

Hepatic encephalopathy is a spectrum of neuropsychiatric manifestations ranging from psychomotor difficulties to altered consciousness and even coma **[9]**. Hepatic encephalopathy, a challenging complication of advanced liver disease, occurs in approximately 30–45% of patients with cirrhosis and10–50% of patients with transjugular intrahepatic Porto systemic shunt, while minimal hepatic encephalopathy affects approximately 20–60% of patients with liver disease **[10]**. Minimal hepatic encephalopathy, which is characterized by subtle motor and cognitive deficits, affects approximately 20–60% of patients with liver disease **[11,12]**. There are multiple prognostic scores that predict the mortality from chronic liver disease, of which the Child-Pugh score and the Model for End-stage Liver Disease (MELD) score are the most commonly used **[13]**. The MELD score had discriminative ability for 3-month survival of greater than 80%, regardless of the severity of liver disease, without any significant improvement by adding etiology or complications of cirrhosis **[6]**. MELD is a composite of the patient's laboratory values for serum bilirubin and serum creatinine, and the international normalized ratio (INR) for prothrombin time **[13]**. The most widely used scale used to evaluate HE is the West-Haven (WH) scale, with scores ranging from 0 to 4. This scale is easy to use but not suitable for patients with altered consciousness and is not well known by physicians other than hepatologists who manage these conditions. For deep coma, the validated Glasgow Coma Scale (GCS) has been proposed **[14]**. For all these considerations, the main aim of the present study was to assess comparison of MELD score and Glasgow coma scale in prediction of prognosis and survival of patients with hepatic encephalopathy.

In the current study hepatic encephalopathy commonly presented in males more than females. This was in agreement with Mouri et al. **[14]** and Lehner et al. **[15]** who reported that men are three times higher than women in most regions.

According to the liver biochemical profile in this study, serum creatinine was the only liver biochemical parameter significantly high in most of patients (group (3) patients. This result was in agreement with Gheorghe et al. **[16]** and Botta et al. **[17]** who documented the same results (that in a patient with HE, serum creatinine and INR were the variables significantly associated with six month mortality). In this study, conventional tests of hepatic function did not have statistical significant difference between the four groups, these results were comparable to Botta et al. **[17]** who documented that exactly. Ammonia has been regarded as one of the major pathogenetic factors of cerebral dysfunction in HE, and astrocyts has been the most commonly affected cell **[18,19]**. Ong et al. **[20]** showed that venous ammonia levels correlate with the severity of HE. In this study, arterial blood ammonia had a statistical significant difference between the four groups. It was significantly more predominant in group (4) cases (356.50±47.93) than in group (1) cases (90.73±18.42). This result was in agreement with Bernal et al. **[21]** who documented the same results that arterial ammonia on admission was significantly higher in group 4 patients [median: 113–mol/L (74-164 -mol/L)], and with Gheorghe et al. **[16]** who documented that mean plasma ammonia levels were increase with the severity of HE. Our findings support the final report of the working party at the 11th world congresses of gastroenterology ammonia testing was described as a potential diagnostic tool which, however, correlates poorly with symptoms of HE [9].

In this study, most cases of Child A (2.9%) were in Group (2), most cases of Child B (81.6%) were in Group (1), and most cases of Child C (65.2) were in Group (3) with statistical significant difference between the four groups. These results were in agreement with Stewart et al. **[1]** and Botta et al. **[17]** who documented nearly the same results (63%) of Group (1) cases were Child B. In contrast, these results disagree with Mouri et al. **[14]** who reported that most of the patients with Group (1- 4) cases were Child C (69%) as he collected patients with severe cirrhosis.

The mean value of Child score was predominantly high in most of patients (group (3) cases) (10± 1.087) with highly statistical significant difference between the four groups. These results were in agreement with Wehler et al. **[22]** and Botta et al. **[17]** who documented nearly the same results (10.9 ±1.8) in Group (2-4) cases, Child score was (9-10) in Group (3-4) cases **[1]**, and studies who stated that the mean value of Child score was (9-14) in Group (2-4) cases **[17]**. D'Amico et al. **[23]** found that the Child score, albumin, bilirubin, age, ascites, prothrombin time were the most common predictors of survival in patients with HE.

According to MELD score most of patients (Group (3) patients) (24.43± 6.45) were presented at advanced score comparing to the Group (2) patients (21.34± 6.92) and the Group (4) patients (21± 6.37) which presented at intermediate score, and the early score (18.52± 4.62) were presented in Group (1) patients with statistical significant difference between the four groups. These results in agreement with Stewart et al. **[1]** who stated that the mean value of MELD score in Group (3) patients were (15-25), in Group (2) patients were (13-22), and in Group (1) patients were (7-17), and Laferrière et al. **[24]** who documented nearly the same results with a median of 22 (17-28) in Group (3) patients, and with that who reported that most of the patients with Group (2-4)cases were presented at advanced score (22 ± 9) and Group (1) cases were presented at early score (19± 8) [14].After clinical examination of patients with HE, it was found that as the grades of HE increased, MELD score and Child score were also high **[1]**. The MELD score reflects liver disease severity, with higher values indicating worse disease **[6,25]**. Sanyal et al. **[26]** demonstrated a strong association between MELD score and developing HE as well as HE and mortality. It has recently been suggested that changes in MELD score may be as important as the absolute MELD score in predicting short-term survival **[27]**. Baseline MELD score has been shown also to be an accurate predictor of 3-month mortality on the wait list in patients with end-stage liver disease, and it was suggested that the accuracy may extend to up to 1 year **[28]**. According to uMELD score most of patients (Group 3 patients) (4.73 ± 0.674) were presented at advanced score comparing to the Group (2) patients (4.34± .785) which presented at intermediate score, and the early score (4.08± .589) were presented in Group 1patients with statistical significant difference between the four. These results were in agreement with Craig et al. **[29]** which documented nearly the same results. In this study, the mean value of Glasgow Coma Scale was predominantly high in group 1 cases (12.84 ± 0.369), and less in group 4 cases (3.75± 1.50) with statistical significant difference between the four groups. This result was in agreement with Mouri et al. **[14]** who documented that the mean value of Glasgow Coma Scale was (14.9 ± 0.3) in Group 1 cases.

Abdominal ultrasonography was done to evaluate the liver status in the studied patients and all of the patients (100%) with HE had sonographic evidence of liver cirrhosis. This goes in agreement with Poordad **[30]** who stated that, all cases of HE frequently coexists with cirrhosis and studies who documented nearly the same results **[31]**. This was supported also by Said et al. **[28]** which reported that cirrhosis is present in the vast majority of patients with HE, and Biselli et al. **[32]** which documented that cirrhosis of the liver was present in 100% of patients with HE. Average cirrhotic liver was detected in78.9% of group 1 cases in comparison with group 4 cases (25.0%). Enlarged cirrhotic liver was detected predominant in group 4 cases, while shrunken liver was present predominant in group 3 cases and was statistically significant. This goes in agreement with Stewart et al. **[1]** who documented nearly the same results, and Mouri et al. **[14]** who reported that most of the patients (86%) had average cirrhotic liver. The Homogenous liver was present in 100% in the four groups, compared to heterogeneous liver which is present in 0% in the four groups (as HCC was excluded in this study). This goes in agreement with Stewart et al. **[1]** who documented nearly the same results, and Laferrière et al. **[33]** who reported that 100% in the all groups had homogenous liver. In contrast, these results disagree with Mouri et al. **[14]** who reported that 14% of cases had heterogeneous liver, and Lehner et al. **[15]** who reported that 14.5% of cases had heterogeneous liver. Splenomegaly was detected in 100% of group (1-3-4) cases compared to 97.1% of group (2) cases. These results was in agreement with Stewart et al. **[1]** who reported nearly the same results, and Lehner et al. **[15]** who stated that 100% in the all groups had splenomegaly. Portal Vein Dilatation was detected more in group 4 cases in comparison with group (1) cases which was present in (63.1%) and was statistically significant. These results were in agreement with Stewart et al. **[1]** who documented nearly the same results. There was no statistically significant difference among the four groups as regards the portal vein dilatation, splenic vein dilatation, collaterals, and gall bladder. These results are in agreement with Stewart et al. **[1]** who reported nearly the same results.

After one-year follow up of patients with HE, it was founded that (61%) of patients died. These results in agreement with Fichet et al. **[34]** who reported nearly the same results (54%) [34], and Gildea et al. **[35]** who reported nearly that (69%) of patients died with a median survival of 1 month. Mortality from studies regarding patients with HE ranged from 33% to 91%, depending of severity of the underlying disease **[36,37]**. West

Haven Criteria had significant influence on overall survival of patients with hepatic encephalopathy. The longest survival time (mean 8.9 months) was in the group 1, followed by a longer survival time (mean 4.13 months) in the group 2and a shorter survival time (mean 2.80 months) in the group (4) and the shortest survival time (mean 2.11months) in the group 3category group .These results in agreement with Mouri et al. **[14]** which reported nearly the same results.

In the present study, MELD score had significant influence on overall survival of patients with HE. The score was advanced in the most of patients group 3 category group with a shortest survival time, intermediate in the group (2-4) category group with a longer survival time, early in the group 1 with a longest survival time. These results in agreement with Stewart et al. **[1]** and Laferrière et al. **[24]** who documented nearly the same results.

In the present study, Glasgow Coma Scale had a significant influence on overall survival of patients with HE, overall survival was shorter in patients with lower score and longer with higher score. This result was in agreement with Mouri et al. **[14]** who documented the same results.

CONCLUSION

Glasgow coma scale can help assess patients with hepatic encephalopathy and can with great accuracy assess risk of one year mortality.

Funding: None.
Conflicts of interest: None.

Ethical approval: A written informed consent was taken from all included patients, and the study was approved by the Ethical Committee of our insitution.

REFERENCES

1. Stewart CI, Malinchoc M, Kim WR, Kamath PS. Hepatic encephalopathy as a predictor of survival in patients with end stage liver disease. *Liver transplantation* 2007; 13:1366-1371.
2. Prakash R , Mullen KD. Mechanisms, diagnosis and management of HE. *Nat Rev Gastroenterol Hepatol* 2010;7 (9): 515-25.
3. Ortiz M, Jacas C, Cordoba J: Minimal hepatic encephalopathy. diagnosis, clinical significance and recommendations. *J Hepatol* 2005;42: S45–S53.
4. Ham J, Gish RG , Mullen K, Model for End-Stage Liver Disease (MELD). Liver transplantation, 2006;S102-S104.
5. Pugh RN, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R.Transection of the oesophagus for bleeding oesophageal varices. *Br J Surg* 1973; 60: 646 – 649.
6. Kamath PS, Wiesner RH, Malinchoc M. A model to predict survival in patients with end-stage liver disease. *Hepatology* 2001;33: 464-470.
7. Mullen KD, Ong JP, Aggarwal A, Krieger D, Easley KA, Karafa MT, Van Lente F, Arroliga AC.Correlation between ammonia levels and the Severity of HE. *The American journal of medicine* 2003;114: 188 –193.
8. Teasdale G , Jennett B.Assessment of coma and impaired consciousness. *Lancet* 2003; 81-84.
9. Ferenci P, Lockwood A, Mullen K, Tarter R, Weissenborn K, Blei AT.Hepatic encephalopathy-definition, nomenclature, diagnosis, and quantification: final report of the working party at the 11h World Congresses of Gastroenterology, Vienna. *Hepatology* 2002;35: 716–721.
10. Poordad FF. Review article: the burden of hepatic encephalopathy. *Aliment Pharmacol Ther*. 2007; 25 Suppl 1: 3-9.
11. Romero-Gomez M, Cordoba J, Jover R, Olmo JA, Ramirez M, Rey R, et al.Value of the critical flicker frequency in patients with minimal hepatic encephalopathy. *Hepatology* 2007;45:879–885.
12. Das A, Dhiman RK, Saraswat VA, Verma M, Naik SR. Prevalence and natural history of subclinical hepatic encephalopathy in cirrhosis. *J Gastroenterol Hepatol* 2001; 16(5):531-5.
13. Freeman R.B, Wiesner RH, Edwards E, Harper A, Merion R, Wolfe R.Results of the first year of the new liver allocation plan. *Liver Transpl* 2004; 10: 7–15.
14. Mouri S, Tripon S, Rudler M, Mallet M, Mayaux J, Thabut D, Weiss N.FOUR Score, a reliable score for assessing overt hepatic encephalopathy in cirrhotic patients. *Neurocritical Care* 2014; 1541- 6933.
15. Lehner S, Stemmler H, Mück A, Braess J, Parhofer KG. Prognostic parameters and risk stratification in intensive care patients with severe liver diseases. *J Gastrointestinal Liver Dis* 2014;19(4): 399 -404.
16. Gheorghel L, Iacob S, Iacob R, Gheorghe1C, Popescu I.Variation of the MELD Score as a predictor of death on the waiting list for liver transplantation. *J Gastrointestinal Liver Dis* 2007; 16 (3) 267-272.
17. Botta F, Giannini E, Romagnoli P, Fasoli A, Malfatti F, Chiarbonello B, et al. MELD scoring system is useful for predicting prognosis in patients with liver cirrhosis and is correlated with residual liver function: a European study. *Gut* 2003;52:134–139.
18. Bajaj JS. The modern management of hepatic encephalopathy. *Aliment Pharmacol Ther* 2009; 31:537 – 47.

19. Bhatia V, Singh R, Acharya SK. Predictive value of arterial ammonia for complications and outcome in acute liver failure. *Gut* 2006; 55:98-104.
20. Ong JP, Aggarwal A, Krieger D, Easley KA, Karafa MT, Van Lente F, et al.Correlation between ammonia levels and the severity of hepatic encephalopathy. *Am J Med* 2003; 114: 188-193.
21. Bernal W, Hall C, Karvellas C J, Auzinger G, Sizer E, and Wendon J. Arterial Ammonia and Clinical Risk Factors for Encephalopathy and Intracranial Hypertension in Acute Liver Failure. *Hepatology* 2007;46:1844-1852.
22. Wehler M, Kokoska J, Reulbach U, Hahn EJ and Strauss R: Short-Term Prognosis in Critically Ill Patients with Cirrhosis Assessed by Prognostic Scoring Systems. *Hepatology* 2001; 34:255-261.
23. D'Amico G, Garcýa-Tsao G, Pagliaro L. Natural history and prognostic indicators of survival in cirrhosis: a systematic review of 118 studies. *J Hepatol* 2006; 44: 217-231.
24. Laferrière VM, Homberger C, Bichoupan K, Dieterich DT. MELD score and antibiotics use are predictors of length of stay in patients hospitalized with hepatic encephalopathy. *BMC Gastroenterology* 2014; 14:185.
25. Wiesner R, Edwards E, Freeman R, Harper A, Kim R, Kamath P, et al. Model for end-stage liver disease (MELD) and allocation of donor livers. *Gastroenterology* 2003; 124: 91–96.
26. Sanyal AJ, Banas C, Sargeant C, Luketic VA, Sterling RK, Stravitz RT, et al. Similarities and differences in outcomes of cirrhosis due to nonalcoholic steatohepatitis and hepatitis C. *Hepatology* 2006;43:682-689.
27. Saab S, Landaverde C, Ibrahim A, Durazo F, Han S, Yersiz H, Farmer D, Ghobrial R, Goldstein L, Tong M, et al.The MELD Score in Advanced Liver Disease: Association with Clinical Portal Hypertension and Mortality. *Experimental and clinical transplantation* 2004; 4(1): 395-399.
28. Said A, Williams J, Holden J, Remington P, Gangnon R, Musat A, et al. Model for end stage liver diseases core predicts mortality across abroad spectrum of liver disease. *J Hepatol* 2004; 40: 897–903.
29. Craig DG, Reid TW, Wright EC, Martin KG, Davidson JS, Hayes PC, Simpson KJ: The sequential organ failure assessment (SOFA) score is prognostically superior to the model for end-stage liver disease (MELD) and MELD variants following paracetamol (acetaminophen) overdose. *Aliment Pharmacol Ther* 2012; 35: 705–713.
30. Poordad FF. Review article: the burden of hepatic encephalopathy. *Aliment Pharmacol Ther*.2007; 25 Suppl 1: 3-9.
31. Biggins SW, Rodriguez HJ, Bacchetti P, Bass NM, Roberts JP, Terrault NA. Serum sodium predicts mortality in patients listed for liver transplantation. *Hepatology* 2005,41: 32-39.
32. Biselli M, Gitto S., Gramenzi A. Six score systems to evaluate candidates with advanced cirrhosis for orthotopic liver transplant: which is the winner? .*Liver Transpl* 2010;16: 964-973.
33. Laferrière VM, Homberger C, Bichoupan K, Dieterich DT. MELD score and antibiotics use are predictors of length of stay in patients hospitalized with hepatic encephalopathy. *BMC Gastroenterology* 2014; 14:185.
34. Fichet J, Mercier E, Genée O, Garot D, Legras A, Dequin F, Perrotin D. Prognosis and 1-year mortality of intensive care unit patients with severe hepatic encephalopathy. *Journal of Critical Care* 2009; 24, 364–370.
35. Gildea TR, Cook WC, Nelson DR. Predictors of long-term mortality in patients with cirrhosis of the liver admitted to a medical ICU. *Chest* 2004; 126: 598-603.
36. Aggarwal A, Ong JP, Younossi ZM. Predictors of mortality and resource utilization in cirrhotic patients admitted to the medical ICU. *Chest* 2001; 119:1489-97.
37. Benhaddouch Z, Abidi K, Naoufel M. Mortality and prognostic factors of the cirrhotic patients with hepatic encephalopathy admitted to medical intensive care unit. *Ann Fr Anesth Reanim* 2007; 26: 490-5.

Peer reviewers: Rashed M Hasan, Professor of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt. **Ashraf M Abdelfatah**, Assistant Professor of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt. **Editor: Mohamad Emara,** Assistant Professor of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt.

Dermatological Manifestations of Pegylated Interferon alfa2a and Ribavirin Combination Therapy in Chronic HCV Patients

Mohamed H. Khater[1], Fathia M Khattab[1], Mohamed M. Refaey[2]
[1]*Department of Dermatology and Andrology, Faculty of Medicine, Zagazig University, Egypt*
[2] *Tropical Medicine Department, Faculty of Medicine, Zagazig University, Egypt*

Corresponding Author
Mohamad Refaey

***Mobile**:+2010000891 59*

E mail:
rifae2007@yahoo.com

Key words:
Hepatitis C, pegylated interferon alfa2a, ribavirin, cutaneous manifestations, trichomegaly, tongue pigmentation.

Background and study aim: Dermatological adverse events are an existing concern during treatment of chronic hepatitis C virus infection. Pegylated Interferon (peg –IFN- alfa2a) and ribavirin combination therapy is associated with well-characterized dermatological lesions tending towards a uniform entity of dermatitis. A prospective observational study was designed to evaluate the frequency and clinical pattern of cutaneous side effects in a cohort of patients receiving combination therapy of (pegylated interferon alfa2a) and ribavirin for chronic hepatitis C infection.

Patients and Methods: This study was carried out at Alahrar Center for treating chronic hepatitis C patients which is one of the centers of the national committee for treating chronic hepatitis C patients (HCV), Zagazig, Sharkia governorate, Egypt over a period of one year starting from January, 2014 to December 2014. A cohort of 116 consecutive, HCV-positive patients to be treated with pegylated interferon alfa2a and ribavirin with standard doses, were prospectively enrolled. After taking an informed consent, detailed history and cutaneous examination, before treatment and then monthly follow up for one year(during the course of treatment) were performed and recorded .All patients were subjected to throughout, routine laboratory investigations before enrollment including, CBC, random blood sugar, complete liver and renal function tests, TSH, Alfa fetoprotein, antibilharzial Ab titre, ANA, P.T, INR, quantitative PCR for HCV-RNA, pregnancy test was performed for the ladies.

Results: 113/116 patients (97%) experienced 1 or more cutaneous side effects. The most frequent was hair loss and occurred in 69 cases (61%). Pigmented Oral lichen planus was noted in 50 cases (43%) and generalized pigmentation in 32 (27%). Hypertrichosis of eyelashes (trichomegaly) and eyebrows (synophyrs) was observed in 42 (36%) and 40 (34%) cases respectively. Pruritus occurred in 50 cases (43%), aphthous stomatitis was observed in 33 cases (38%), 19 patients (22%) either developed or had worsening of melasma and 23 (27%) developed urticaria. Brittle nails (10 cases)**,** cheilitis (8 cases), glossitis (3 cases), actinic lichen planus (9 cases), greying of hair (3 cases), discoloration of moustache hair (1 case), and photo-sensitivity (3 cases) were also observed. Preexisting psoriasis (8 cases), and lichen planus (5 cases) aggravated. Eruptive seborrhoeic keratosis was reported in 1 case.

Conclusion: 113/116 patients (97%) experienced 1 or more cutaneous side effects. The most frequent was hair loss and occurred in 69 cases (61%). Pigmented Oral lichen planus was noted in 50 cases (43%) and generalized pigmentation in 32 (27%). Hypertrichosis of eyelashes (trichomegaly) and eyebrows (synophyrs) was observed in 42 (36%) and 40 (34%) cases respectively. Pruritus occurred in 50 cases (43%), aphthous stomatitis was observed in 33 cases (38%), 19 patients (22%) either developed or had worsening of melasma and 23 (27%) developed urticaria. Brittle nails (10 cases)**,** cheilitis (8 cases), glossitis (3 cases), actinic lichen planus (9 cases), greying of hair (3 cases), discoloration of moustache hair (1 case), and photosensitivity (3 cases) were also observed. Preexisting psoriasis (8 cases), and lichen planus (5 case) aggravated. Eruptive seborrhoeic keratosis was reported in (1 case).

INTRODUCTION

The incidence of HCV on a global scale as many as 2 to 3millions persons may be chronically infected in the United States, 5 to 10 millions in Europe, and about 12 millions in India, and most do not know they are infected. About 150 000 new cases occur annually in the US and in Western Europe, and about 350 000 in Japan. Of these, about 25% are symptomatic, but 60 to 80% may progress to chronic liver disease, and 20% of these develop cirrhosis. About 5%-7% of patients may ultimately die from the sequalae of the infection **[1].**

Most European countries report a prevalence of HCV in the general population of between 0.5 and 2%.WHO estimates that about 3% of the world's population has been infected with HCV and that there are more than 170 million chronic carriers who are at risk of developing liver cirrhosis and/or liver cancer. Egypt has the highest prevalence of hepatitis C virus (HCV) in the world, estimated nationally at 14.7% **[2]**.

Pegylated Interferon alfa 2a (peg-IFN-α2a), a biological medication used to treat viral hepatitis, has considerable clinical potential to cause different effects on different organs such as the skin. The response of the skin to peg-IFN–α2a therapy is unpredictable **[3]** and the role of IFN in post-treatment persistence of skin manifestations needs to be assessed. A number of skin disorders are autoimmune in nature and immunomodulatory activity of peg-IFN- α2a may exacerbate these dermatologic disorders **[4,5]**.

The purpose of this study was to assess the frequency and clinical pattern of cutaneous side effects in patients receiving combination therapy (peg-INF-α2a) and ribavirin for chronic hepatitis C.

PATIENTS AND METHODS

One hundred and sixteen HCV positive patients were enrolled in this study.

Diagnosis of HCV was based on detection of serum HCV-RNA by quantitative polymerase chain reaction (PCR). All patients received combination therapy of pegylated interferon alfa2a and oral ribavirin, both were administered at standard doses, for one year **[3]**. The standard combination dose regimen was (peg-IFN- alfa2a, 180 mcg/ once/week) and ribavirin (1,000 - 1,200 mg/day depending on whether body weight was below or above 75 kgs.). Dose modifications of INF and/or ribavirin were performed, as indicated by the presence of adverse effects or hematological abnormalities.

Skin, mucous membranes, hair and nails were examined before starting treatment and there after, monthly till the treatment was completed. Preexisting lesions were documented on starting treatment and were observed subsequently on each visit.

Laboratory assessment included Hb%, WBC, platelet count, TSH, billirubin, ALT, AST and alkaline phosphatase on starting therapy and then monthly.

Dermatohistopathalogical examination was performed.

Exclusion Criteria :

1. Presence of any other etiology of chronic liver disease: positive HAV IgMAb, serum cerulo-plasmin and α 1 antitrypsin concentrations consistent with increased risk of metabolic liver disease.
2. Seropositivity for HIV antibody (anti HIV).
3. Patients with a history of hemorrhage from esophageal varices or evidence of decompensated liver disease (Child B-C class).
4. Presence of Hb % less than 12gm/dl, a white blood cell count lower than 3000/mm^3 or platelet count lower than 75000/mm^3 in complete blood count.
5. Patients who received therapy for hepatitis B in the past six months were excluded.
6. Pregnant female
7. Patients with serum ANA positivity.
8. Patients with thyrotoxicosis.

RESULTS

A total of 116 patients, 70 females and 46 males were observed over a period of one year (whole course of therapy).

Age of the patients ranged from 21 to 53 years (mean age 35 yrs). The type and frequency of skin manifestations are shown in Tables 1 and 2. The most frequently observed cutaneous manifestations involved hair and the oral cavity.

Effect of this therapeutic combination on hair was rather interesting. On one hand there was diffuse thinning of scalp hair and on the other, significant eyelash and eyebrow hypertrichosis was noted. Loss of hair was also noted at the site of subcutaneous injections of pegylated INF-α2a (Fig. 1).

Asymptomatic tongue pigmentation of a peculiar type was noted in a significant number of patients. Hyperpigmentation was in the form of streaks on each side of the tongue in most patients

Oral biopsy was performed to rule out lichen planus in patients who complained of burning in the oral cavity along with pigmentation. Pigment abnormality as generalized darkening of complexion, appearance or darkening of preexisting melasma was a common complaint. Nail pigmentation involving either the whole nail or only lunulae was observed. The number of nails involved also varied. One patient developed white bands on nails (Mee's lines) which are frequently seen in patients with chronic disease. Lichen planus developed in 5 patients towards the end of treatment.

Apart from effects on skin and adenexa a number of extracutaneous effects were also noted (Table 3). Flu-like symptoms and generalized aches and pains lasted from 12 to 48 hours following interferon injection. The intensity of these symptoms was most pronounced following the initiation of therapy. During the treatment period patients complained of feeling of being unwell and loss of interest in daily activities. Forgetfulness, irritability, anger/ hostility and depression were some of the other complaints. A single patient developed psychosis preceded by depression after start of therapy.

None of the cutaneous effects were severe enough to warrant discontinuation of treatment.

Table (1): Type and frequency of cutaneous manifestations in 116 HCV-positive patients receiving pegylated interferon - α2a and ribavirin

Cutaneous manifestations	N (%)
Alopecia	69 (61)
Pigmented Oral lichen planus	50 (43)
Trichomegaly	42 (35.8)
Synophyrs	40 (34.4)
Generalized pigmentation	32 (27.6)
Generalized pruritus	50 (43)
Aphthous ulcers	33 (29)
Melasma	19 (22)
Urticaria	23 (27)
Full nail pigmentation	9 (10.3)
Brittle nails	10 (9.2)
Cheilitis	8 (6.9)
Greying of hair	3 (4.6)
Pigmentation of lunulae	5 (5.7)
Glossitis	3 (4.6)
Photosensitivity Vitiliginous lesions Psoriasis patches Actinic lichen planus	3 (4.6) 14 (12.3) 11 (9.4) 9 (7.9)

Table (2): Effect on preexisting dermatoses in 116 HCV-positive patients receiving pegylated interferon - α2a and ribavirin

Preexisting skin disease	No.	Effect
Psoriasis	8	Aggravated
Lichen planus	5	Aggravated
Seborrhoeic keratosis	1	Aggravated
Dermatitis	4	Aggravated
Vitiligo	7	No effect
Nonspecific rash	1	Resolved

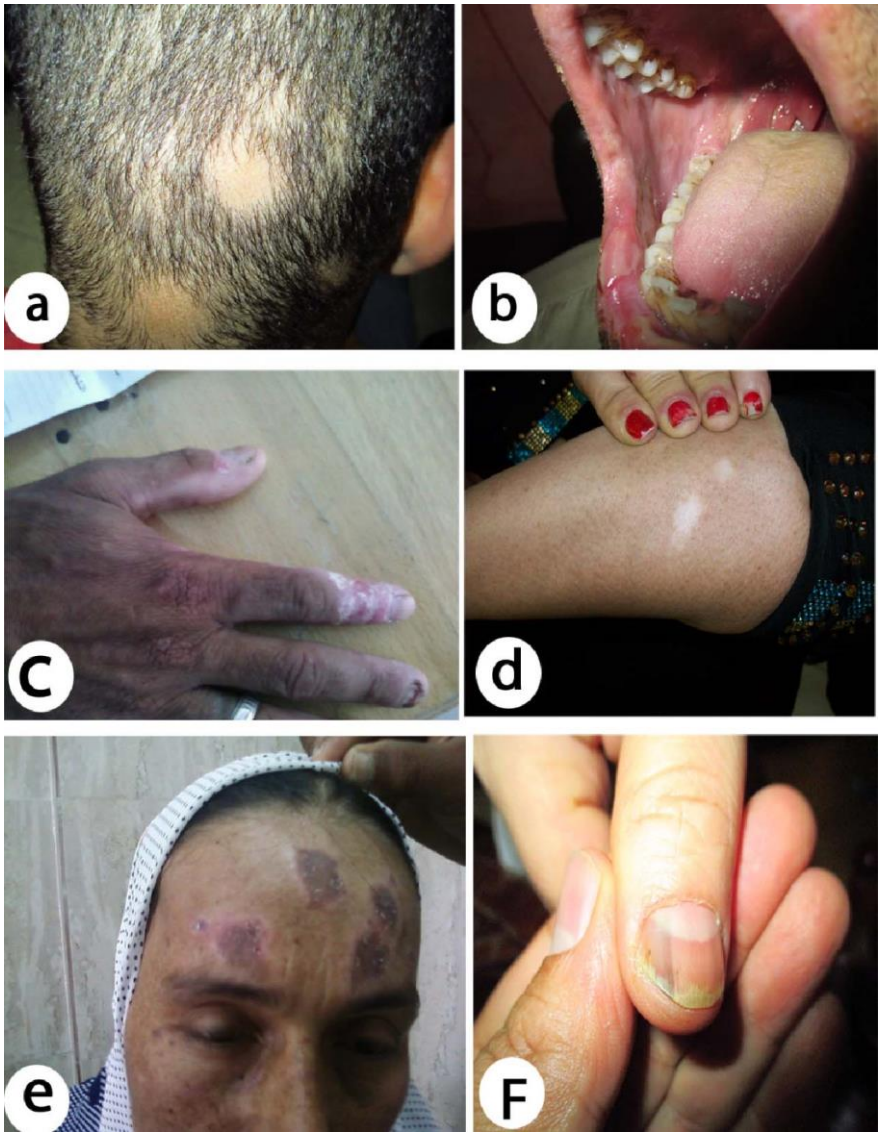


Figure (1): (a) Alopecia in the scalp **(b)** Oral lichen planus **(c)** Psoriasis patches **(d)** Vitiliginous lesions **(e)** Actinic lichen planus **(f)** Nail pigmentation

Table (3) : Extracutaneuos effects in 116 HCV positive patients receiving pegylated interferon alpha 2a and ribavirin

Symptoms	N (%)
Flu-like symptoms	82 (94.5)
Malaise	67 (77.0)
Xerostomia	55 (63.2)
Loss of appetite	32 (36.8)
Burning mouth	28 (32.2)
Loss of weight	27 (31.0)
Altered taste sensation	22 (25.3)
Burning hands and feet	21 (24.1)
Headache	18 (20.7)
Low mood/depression	16 (18.4)
Psychosis	1 (0.8)

DISCUSSION

Hepatitis C virus (HCV) has infected over 170 millions people worldwide and therefore, creates a huge disease burden due to chronic, progressive liver disease **[1]**. Infections with HCV have become a major cause of liver cancer and one of the most common indications for liver transplantation **[2-4]**. The fact that chronic infection with HCV can lead to cirrhosis and hepatocellular carcinoma creates the need to develop drugs that effectively eradicate the infection **[5]** and a prophylactic vaccine that prevents its dissemination. Unfortunately, to date there is no effective vaccine available **[6]**. Currently, the standard of care therapy involves pegylated interferon α2a (peg- INF-α2a) and ribavirin (RBV) **[7]**.

Interferon was originally described as a protein capable of inducing antiviral activity in the cells. It is a leukocyte derived cytokine that is used in the treatment of viral, inflammatory and auto-immune diseases **[11]**. It has established antiviral, antiproliferative and immune-modulatory properties. It is used for treatment of HCV, hepatitis B viral infection (HBV), Kaposi sarcoma, CTCL, hairy cell leukemia, chronic myeloid leukemia, low-grade non- Hodgkin's lymphoma, Walden storm acroglobulinemia, multiple myeloma and cryoglobulinemia **[8]**.

Ribavirin is a nucleoside analogue of guanosine. Its broad spectrum antiviral activity was first reported in 1972 and was initially used for respiratory syncytial virus infection in children **[9]**. The ribavirin/IFN combination was approved in 1983 and since has been used successfully for chronic HCV. The clinical efficacy of this combination is superior to the individual monotherapies. Both these drugs act synergistically to enhance host T cell-mediated immunity against viral infection by switching the T-cell phenotype from type 2 to type 1 **[10]**.

Many dermatological side effects have been reported including immune and non-immune mediated **[12]**. The non-immunological mediated side effects include lichen planus, dry skin, excessive sweating, acne, nail disorders, epidermal necrolysis, and skin discoloration **[13]** and the immune mediated include psoriasis, pemphigus, vitiligo, and alopecia **[14]**. It is not clear, however, whether IFN- α2a dosage is correlated with the development or exacerbation of psoriasis or vitiligo **[15]**.

Guillot et al. reported hair loss in 48.4% of 33 patients with melanoma who were treated with IFN- α2a **[16]**. Fattovich et al. reported 14 cases of cutaneous side effects induced by IFN including nine patients with lichen planus, three with psoriasis and two with vitiligo **[17]**. Kontorinis et al. reported 13 of 81 patients experienced dermatological side effects who were treated with peg-IFN-α2a for HCV including seven patients with nonspecific rash, five with psoriasis and one with eczema. **[18]**. Tinio et al. reported that after treatment of chronic HCV patients with peg-IFN-α2a and ribavirin, one patient developed hair curling and vitiligo **[19]**. Tomasiewicz et al. have described a case of vitiligo that occurred during the third month of treatment with Peg-IFN- α 2a and RBV **[20]**.

Skin manifestations observed in our patients are also most likely due to the Synergistic immuno-modulatory effect of this combination **[19]**.

Hair physiology seemed to be most affected by peg-IFN-α2a /ribavirin.Studies have shown that peg-IFN-α2a treatment can cause hair loss which may occur all over the body, not just on the head **[18]**.

The side effect of peg-IFN- α2a therapy is usually noticed in up to 36% of treated patients in pivotal clinical trials **[5]** and it seems that the incidence of alopecia increases with the duration of treatment **[13]**. It is possible that Peg-IFN-α2a induces immunologic modulation (shift from a Th2 immune-driven response to a Th1) and stimulates the synthesis of Th1-cytokines such as IL-1, IL-2, and IFN. In addition Peg-IFN-α2a increases cytotoxic T cell activity **[14]**. These are in accordance with the findings of Hoffmann who has described increased mRNA and protein expression of Th1-c ytokines (IFN-, IL-2), and IL-1 in skin biopsies from patients with alopecia **[21]**.

Alopecia, eyelash and eyebrow hypertrichosis, greying and lightening of hair was observed in our study. Loss of hair started within the first month of the treatment and continued throughout the therapy. Hair disorders have been frequently described with peg- IFN-α2a therapy. In our study, diffuse thinning of scalp hair and eyelash and eyebrow hypertrichosis was observed in a large proportion of patients. While thinning of scalp hair was commoner in females, eyelash and eyebrow hypertrichosis was more frequent in males. Eyelid and eyebrow trichomegaly has

been reported earlier, with this combination in only a few case reports **[20]**.

The development of pigmentation during treatment with peg-interferon α2a and ribavirin is not associated with any specific genotype of hepatitis virus C, dose or duration of interferon or ribavirin treatment, or response to treatment **[22]**. Pigmentation usually increases up to the end of treatment and tends to partially resolve after discontinuation of treatment. However, there are no reports of complete resolution of lesions in the long-term. Although oral lesions may cause subjective discomfort, this is not severe and it is not recommended to discontinue treatment **[27]**.

Hyperpigmentation of oral mucosa associated with peg- interferon-α2a and ribavirin combination therapy for hepatitis C was first described by Willems et al. in 2003. Since then, 20 cases of patients with pigmentation of oral mucosa associated with peg-IFN-α2a and ribavirin therapy have been reported **[28]**.

A large number of our patients had pigmentary disturbance. Similar lingual**[5]** and generalized pigmentation **[32]** has been noticed earlier. All the reports to date however, are predominantly in the dark skin individuals. Peg-IFN- α 2a increases the expression of alpha-melanocyte stimulating hormone (MSH) surface receptors. The high incidence of pigmentary disturbances i.e. oral pigmentation, darkening of complexion, melasma and nail pigmentation in our patients could be related to the predominant Fitzpatrick skin type IV and V in this part of the world. However, the reason for the peculiar linear distribution of hyperpigmentation like the opposite effect of hair loss at one place and growth at other, pigment deposition increases in skin but seems to have quite the opposite effect on hair, producing greying of hair and discoloration of moustaches **[28]**.

Nail discoloration in our patients followed different patterns. Mostly discoloration of all nails was noted. In some patients one or two nails, toe nails or only the lunulae were involved. Pigmentation of nails was also observed as linear or horizontal streaks.

Occurrence of vitiligo in patients with hepatitis on peg- IFN-α2a therapy has been reported to occur as early as one month. The longest time interval between starting treatment and appearance of vitiligo is between 18 and 35 months. However, in our series the shortest duration was one week, while the longest is 8 months. It occurs in the fourth to the sixth decade of life and affects both genders equally as clearly noticed in our series.

The exact mechanism responsible for this autoimmune phenomenon is still unknown, but it most likely related to the biological features of peg- IFN-α 2a **[29]**. It is possible that peg-IFN-α2a causes vitiligo via the induction of anti-melanocyte autoantibodies or by activation of cytotoxic T cells, which can be integrated in the understanding of the autoimmune nature of the diseases **[30,31]**. Several investigators believe that the presence of autoantibodies prior to peg-IFN- α 2a therapy poses a risk for developing autoimmune disorders once peg- IFN- α2a is started. It exerts various effects on the immune system, including modulation of immunoglobulins production, inhibition of T-suppressor cell function and stimulation of T-cell cytotoxicity, monocyte/ macrophage functions and natural-killer cell activity **[32]**. It enhances expression of class I major histocompatibility (MHC) antigens and can increase the frequency of blood leukocytes that express class II MHC antigens **[33]**.

Lichen planus may be induced **[59],** aggravated **[50]** with IFN and ribavirin combination. It is difficult to say if LP was due to the drug combination or HCV itself, as this process may be triggered by circulatory antigens, which may be either viral or pharmacologic. HCV and LP is assossiated. However, some studies have reported this association to be limited to erosive forms, or only an incidental finding especially in areas where HCV is endemic.

Known patients of psoriasis and eczema in our group also complained of exacerbation of their lesions. The patient with vitiligo did not observe any change. However, 1 patient with a nonspecific rash, reported resolution of his symptoms after the start of therapy.

Psoriasis associated with peg-IFN- α2a treatment for chronic hepatitis C was first reported in the English-language medical literature in 1993. To our knowledge, nine case reports have been published in the English literature, linking the development or the exacerbation of psoriasis to treatment of HCV infection with IFNα, either pegylated or non-pegylated and either as monotherapy or combined with ribavirin **[34]**.

Aggravation of psoriasis and eczema could again be explained on the basis of several mechanisms

have been proposed to clarify the close relationship between interferon treatment and the induction of psoriasis. Interferon stimulates Th1 mediated inflammatory response, which has been reported in psoriatic T-cell infiltrates, and could thus be responsible for psoriasis exacerbation. Alternatively, because interferon acts to increase the lymphocytotoxic activity of natural killer lymphocytes and induces keratinocytes to produce interleukin-1, it may trigger and initiate the psoriatic process. On the other hand, not all psoriasis patients develop peg-interferon-alfa2a induced exacerbation, which probably reflects the heterogeneity in the pathogenesis **[35]**.

Injection site reaction included erythema, itching, induration and epilation, none of our patients experienced necrosis or ulceration as described by Sparsa et al. **[36]** or granuloma formation as described by Sanders et al. **[37]** peg-IFN-α2a can trigger granuloma formation and sarcoidosis **[38]**, this effect may be exaggerated in patients who have undergone aesthetic procedures, such as intradermal permanent fillers, in which adequate response depends on weak granulomatous reaction, leading to permanent disfiguring **[39]**. This new contraindication should be borne in mind while inducting patients for this combination.

Depression has been observed with this therapeutic combination **[40]**. peg-IFN-α2a treatment modulates the serotoninergic system through cytokine production. It has been shown to decrease tryptophan availability for serotonin synthesis and also modify central serotoninergic receptors **[41]**. It is recommended that it should be taken into account and a close eye be kept on psychological issues.

CONCLUSION

A number of cutaneous manifestations were noted in the patients receiving combination therapy of peg-INF-α2a plus ribavirin for chronic hepatitis C. The most frequent and distressing for the patients were the effects on hair. However, none of the cutaneous effects were severe enough to warrant discontinuation of therapy. Awareness of these cutaneous side effects may be useful for the dermatologist to counsel the patients receiving this treatment. A prolonged follow up is required to see when, or if at all, any of these adverse effects settles down after discontinuation of treatment.

Funding: None.
Conflicts of interest: None.

Ethical approval: A written informed consent was taken from all included patients, and the study was approved by the Ethical Committee of our insitution.

REFERENCES

1. Mohamoud YA, Mumtaz GR, Riome S Miller D, Abu-Raddad LJ . The epidemiology of hepatitis C virus in Egypt: a systematic review and data synthesis. *BMC Infect Dis*. 2013; 24:13-288.
2. Cuadros DF, Branscum AJ, Miller. FD, Abu-Raddad LJ .Spatial epidemiology of hepatitis C virus infection in Egypt: analyses and implications. *Hepatology*, 2014; 60(4):1150-9.
3. Hadziyannis SJ. Skin diseases associated with hepatitis C virus infection. J EurAcad *Dermatol Venereol*. 1998; 10: 12 – 21.
4. Edwards L. The interferons. *DermatolClin*. 2001; 19: 139 – 46, ix.
5. JadaliZ. Dermatolo -gic manifestations of hepatitis C infection and the effect of interferon therapy: a literature review. *Arch Iran Med*. 2012; 15(1):43-8.
6. Echeverría N, Moratorio G, Cristina J,Moreno P. Hepatitis C virus genetic variability and evolution. *World J Hepatol.* 2015; 28;7(6):831-45.
7. Milara J, Outeda-Macias M, Aumente-Rubio MD, Más-Serrano P, Aldaz A, Calvo MV et al. PEG-Interferon-α ribavirin-induced HCV viral clearance: a pharmacogenetic multicenter Spanish study. *Farm Hosp*. 2015; 1, 39(1) : 29-43.
8. Aamir S, Ullah Z, Iqbal Z.Cutaneous manifestations of interferon alfa and ribavirin for hepatitis C. *Journal of Pakistan Association of Dermatologists*. 2008; 18: 14-20.
9. Davis GL.Current therapy for chronic hepatitis C.skin and mucous membranes, and pruritus. , *Gastroenterology* 2000; 118: S104–S114.
10. Nakamoto S, Kanda T, Shirasawa H , Yokosuka A. Antiviral therapies for chronic hepatitis C virus infection with cirrhosis. *World J Hepatol.* 2015; 18;7 (8): 1133-41.
11. Maddrey W.C. Safety of combination interferon alfa-2b/ribavirin skin lesions that began to manifest on average 3.2 months therapy in chronic hepatitis C: relapsed and treatment-naïve after beginning treatment, with a range of 0.5–8 months (the patients. *Semin Liver Dis*.1999; 19: 67–75.
12. Moore MM, Elpern DJ, Carter DJ. Severe, generalized nummular eczema secondary to interferon alfa-2b plus ribavirin combination therapy in a patient with chronic hepatitis C virus infection. *Arch Dermatol* 2004; 140: 215–217.
13. Raanani P and Ben Bassat I. Immune-mediated complications during interferon therapy in

hematological patients. *Acta Haematol* 2002; 107: 133–144.
14. Seckin D, Durusoy C , Sahin S. Concomit -ant vitiligo and psoriasis in a patient treated with interferon alfa-2a for chronic hepatitis B infection. *Pediatr Dermatol* 2004; 21: 577–579.
15. Ketikoglou I, Karatapanis S, Elefsiniotis I , Kafiri G, Moulakakis A. Extensive psoriasis induced bypegylated interferon alpha-2b treatment for chronic hepatitis B. *Eur J Dermatol* 2005; 15: 107-9.
16. Guillot B, Blazquez L, Bessis D , Dereure O, Guilhou JJ. A prospective study of cutaneous adverse events induced by low-dose alphainterferon treatment for malignant melanoma. *Dermatology* 2004 ; 208: 49–54.
17. Fattovich G, Giustina G, Favarato S , Ruol A. A survey of adverse events in 11,241 patients with chronic viral hepatitis treated with alfa interferon. *J Hepatol.* 1996; 24: 38–47.
18. Kontorinis N, Garas G, Young J, Speers D, Chester BP, MacQuillan GC et al . Outcome, tolerability and compliance of compassionate use interferon and ribavirin for hepatitis C infection in a shared care hospital clinic. *Intern Med J*. 2004; 34: 519.
19. Tinio P, Hadi A, Al-Ghaithi K, Al-Qari H, Rudikoff D . Segmental vitiligo and hair curling after interferon alpha and ribavirin treatment for hepatitis C. Skinmed *Eur J Dermatol*. 2006 ; 5: 50–51.
20. Tomasiewicz K, Modrzewska R, Semczuk G Vitiligo associated with pegylated interferon and ribavirin treatment of patients with chronic hepatitis C: a case report. *AdvTher*. 2006 ; 23: 139 – 142.
21. Hoffmann TW, Duverlie G, Bengrine A. Micro RNAs and hepatitis C virus: toward the end of miR-122 supremacy. *Virol J.* 2012; 12; 9:109
22. McHutchison JG, Gordon SC, Schiff ER, Shiffman ML, Lee WM, Rustgi VK et al . Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. *N Engl J Med* . 1998; 339: 1485–1492.
23. Hoffmann R. The potential role of cytokines and T cells in alopecia areata. *J Investig Dermatol Symp Proc.* 1999; 4: 235 – 238.
24. Yu ML, Dai CY, Huang JF Hou NJ, Lee LP, Hsieh MY et al . A randomized study of peginterferon and ribavirin for 16 versus 24 weeks in patients with genotype 2 chronic hepatitis C. *Gut.* 2007; 56: 553 – 559.
25. Trapero M, García-Buey L, Muñoz C, Vitón M. Moreno-Monteagudo JA, Borque MJ et al. Maintenance of T1 response as induced during PEG-IFNalpha plus ribavirin therapy controls viral replication in genotype- 1 patients with chronic hepatitis C. *Rev Esp Enferm Dig*. 2005; 97: 481 – 490.
26. Ghosh S, Duseja A, Dhiman RK, Chawla YK. Tongue hyperpigmentation resulting from peginterferon alfa-2b and ribavirin treatment in a patient with chronic hepatitis C. *Dig Dis Sci.* 2012; 57:820-1.
27. Gurguta C, Kauer C, Bergholz U, Formann E, Steindl-Munda P, Ferenci P. Tongue and skin hyperpigmentation during PEG-interferon-alpha/ ribavirin therapy in dark-skinned non-Caucasian patients with chronic hepatitis C. *Am J Gastroenterol.* 2006; 101:197-8.
28. Willems M, Munte K, Vrolijk JM Den Hollander JC, Böhm M, Kemmeren MH et al. Hyper-pigmentation during interferon-alpha therapy for chronic hepatitis C virus infection. *Br. J. Dermatol*. 2003; 149:390 - 4.
29. Oiso N, Sato M and Kawada A. Vitiligo after combination therapy of pegylated interferon-α-2a, ribavirin and vitamin D in a patient with chronic hepatitis C. J Dermatol. 2013 ;40(9):772-3
30. Popescu C, Popescu GA and Arama V. Type 1 diabetes mellitus with dual autoimmune mechanism related to pegylated interferon and ribavirin treatment for chronic HCV hepatitis. *J Gastrointestin Liver Dis.* 2013; 22(1):101-4
31. Jadali Z. Dermatologic manifestations of hepatitis C infection and the effect of interferon therapy: a literature review *Arch Iran Med.* (2012);15(1):43-8.
32. Arya V, Bansal M, Girard L, Arya S, Valluri A. Vitiligo at Injection Site of PEG-IFN-α 2a in Two Patients with Chronic Hepatitis C: Case Report and Literature Review.Case Rep *Dermatol.* (2010); 27; 2(2): 156-164.
33. Hamadah I, Binamer Y, Sanai FM , Abdo AA, Alajlan A. Interferon-induced vitiligo in hepatitis C patients: a case series. 2010; *Int J Dermatol*. ;49(7):829-33.
34. Afshar M , Martinez AD, Gallo RL,Hata TR. Induction and exacerbation of psoriasis with Interferon-alpha therapy for hepatitis C: a review and analysis of 36 cases. *J Eur Acad Dermatol Venereol*. 2013; 27(6):771-8.
35. Horev A, Halevy S. New-onset psoriasis following treatment with pegylated interferon-alpha 2b and ribavirin for chronic hepatitis C. *Isr Med Assoc J.* 2009;11(12):760-1.
36. Sparsa A, Loustaud-Ratti V, Liozon E Denes E, Soria P, Bouyssou-Gauthier ML et al. Cutaneous reactions or necrosis from interferon alpha: can interferon be reintroduced after healing? Six case reports. *Rev Med Interne*. 2000; 21: 756-63.
37. Sanders S, Busam K, Tahan SA, Johnson RA, Sachs D . Granulomatous and suppurative dermatitis at interferon alfa injection sites: reports of 2 cases. *J Am Acad Dermatol*. 2002; 46: 611-6.

38. Cogrel O, Doutre MS, Marliere V , Beylot-Barry M, Couzigou P, Beylot C. Cutaneous sarcoidosis during interferon plus ribavirin treatment of hepatitis C virus: two cases. *Br J Dermatol* 2002; 146: 320-4.
39. Fischer J, Metzler G, Schaller M. Cosmetic permanent fillers for soft tissue augmentation. A new contraindication for interferon therapy. *Arch Dermatol* 2007; 143: 507-10.
40. Chen WC, Lai HC, Su WP , Palani M, Su KP. Bupropion for interferon-alpha-induced depression in patients with hepatitis C viral infection: an open-label study *Psychiatry Investig*. 2015; 12(1):142-5
41. Oxenkrug G, Turski W, Zgrajka W, Weinstock J, Ruthazer R, Summergrad P. Disturbances of Tryptophan Metabolism and Risk of Depression in HCV Patients Treated with IFN-Alpha. *J Infect Dis Ther*. 2014; 25; 2(2).

Peer reviewers: Tarik Zaher, Professor of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt.
Editor: Mohamad Emara, Assistant Professor of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt.

Interferon-Gamma Inducible Protein-10(IP10) as a Predictor of Early Virologic Response in Chronic Hepatitis C Infected Egyptian Patients Stratified for the Interleukin-28B rs12979860 Genotype

Mohamed AM Bahgat[1] , Walid A Abdeldayem[1], Heba F Pasha[2], Hala IM Hussein[1], Ibrahim M Ibrahim[1], Ahmed A Abdelmoaty[1]

Departments of Tropical Medicine[1] and Biochemistry[2], Faculty of Medicine, Zagazig University

Corresponding Author
Ahmed Abdelmoaty

Mobile:
+01001941058

Email:*drahmedatya33 @gmail.com*

Key words: *IP10 ;HCV; IL28B rs12979860*

Background and study aim: Single nucleotide polymorphisms near Interleukin 28B are strongly associated with favourable treatment response of chronic hepatitis C such as, the homozygous CC at markers12979860. Interferon-Gamma Inducible Protein-10 (IP-10) can be produced by a variety of cells, including hepatocytes. Pre-treatment plasma levels of IP10 are elevated in patients chronically infected with hepatitis C virus of genotypes 1 or 4 who do not achieve early virological response(EVR)to treatment. The aim of this study was to evaluate the rule of adding IP-10 to IL28B rs12979860 Genotype in predicting EVR .

Patients and methods: The study enrolled 78 naïve chronic HCV patients who have criteria that met the pegylated interferon plus ribavirin (pegIFN-RBV) treatment for chronic hepatitis C virus (HCV) .IP-10 assay and single nucleotide polymorphisms of the IL28B genotype were performed.

Results: Patients with EVR was younger than those without EVR with statistically significant different. Patients with EVR had less elevated liver enzyme ,low viral load and fasting blood sugar than those without EVR with statistically significant difference. 100% patients with out early virological response had A2 activity while 31.7% only of patients with EVR had A2 activity with statistically significant difference .Patients with EVR showed lower level of IP10 and 81.7% of them had CC allele genotype with statistically significant difference when compared to patients without EVR. Patients with CC genotype were associated with lower level of IP10, ALT, AST and also low viral load. Patients with low level of IP10 had lower levels of liver enzyme. Cut off level of IP 10 was<605 pico gram/ml; at this cut off value sensitivity was=100%, specificity was= 96.7% and area under the curve (AUC)= 0.99.

Conclusion: IP10 level was lower among responder group. IL28 genotype CC was significantly higher in responders when compared with non responders. .Patients with CC genotype were associated with lower level of IP10 and liver enzymes. Patients with CC genotype were associated also with low viral load.

INTRODUCTION

Hepatitis C virus (HCV) infects more than 175 million people worldwide **[1]** and is the leading cause of end-stage liver disease as well as the primary indication for liver transplantation in Western countries worldwide **[2]**. Egypt has the highest prevalence of HCV worldwide with 9% countrywide and up to 50 % in certain rural areas **[3]** and the highest prevalence of HCV of genotype 4 which is responsible for almost 90% of infections and is considered a major cause of chronic hepatitis, liver cirrhosis, hepatocellular carcinoma (HCC) and liver transplantation in the country **[4]**.

The treatment based on the combination of peginterferon alpha and ribavirin leads to a sustained virological response (SVR) in 40–50% in patients with HCV genotype 1 and 4 and in 80% of those with genotype 2 and 3 **[5]**. Several baseline and on-treatment variables affect the likelihood of achieving SVR. Older age, advanced stage of fibrosis, African-American ethnicity and HCV-related factors including HCV

genotype 1 and high viral load at baseline predict poor response to antiviral therapy [6]. Furthermore, metabolic factors such as high body mass index (BMI), presence and severity of liver steatosis have been reported as negative predictors of response **[7]**. On the other hand, early on-treatment kinetics of HCV RNA e.g. undetectable HCV RNA at week 4, 12 have a high positive predictive value of SVR **[8]**.

Among the baseline predictors of response, the pre-treatment activation of IFN-stimulated genes (ISG) and the host genetic polymorphisms have been the subject of recent and major studies. Regarding ISG, it has been shown that low levels of intrahepatic and systemic CXC chemokine Interferon-gamma inducible protein 10 kDa (IP-10, or CXCL10)**[9]**, a valid surrogate marker of ISG activation, predict a more pronounced first phase decline of HCV RNA during anti-viral therapy and increased SVR rates **[10]**. On the other hand, several independent studies have consistently shown that single nucleotide polymorphisms (SNPs) near Interleukin28B (IL28B) gene, which encodes the type III interferon are strongly associated with response to treatment of chronic hepatitis C. In particular, the homozygous genotypes TT at marker rs8099917, CC at marker rs12979860 and AA at marker rs12980275 are all associated with favourable treatment outcomes **[11]**.

Interferon-gamma inducible protein 10 kDa is a chemotactic CXC chemokine of 77 AA in its mature form**[12]**. Unlike other CXC chemokines, IP-10 lacks chemotactic activity for neutrophils. Rather, it appears to target T lymphocytes, NK cells, and monocytes through its receptor **[13]**. IP-10 can be produced by a variety of cells, including hepatocytes **[14]**, and it has been implicated in the pathophysiological progression of multiple sclerosis**[15]**, diabetes mellitus **[16]** and HIV **[17]**.In the setting of HCV infection, IP-10 mRNA expression in the liver has been reported to be associated with the presence of lobular necroinflammatory activity in liver-biopsy samples**[14]**.

Recently, baseline pre-treatment plasma levels of CXCL10 are elevated in patients chronically infected with hepatitis C virus (HCV) of genotypes 1 or 4 who do not achieve a sustained viral response (SVR) after completion of antiviral therapy **[10]**. CXCL10 in plasma is mirrored by intrahepatic CXCL10 mRNA and both strikingly predict the first days of elimination of HCV RNA "first phase decline" during interferon/ribavirin therapy for all HCV genotypes **[9]**.

PATIENTS AND METHODS

This prospective cohort study was carried out in Tropical Medicine Department, Faculty of Medicine, Zagazig University Hospital Egypt during the period from June, 2013 to May, 2015.The study enrolled 78 naïve chronic HCV patients who have criteria that met the pegylated interferon plus ribavirin (pegIFN-RBV) treatment for chronic hepatitis C virus (HCV) infection according to the Egyptian national program for prevention of HCV infection. Patients were selected as systematic random sample.

All subjects were chronic HCV patients of genotype 4 submitted to the standard of care therapy (SOC) of pegylated interferon-alfa 2a (180 ug/week) (Pegasys-Roch-Switzerland) or pegylated interferon-alfa 2b (1.5ug/kg/ per week) (PegIntron-Scheringcorporation-USA) in combination with ribavirin according to weight based criteria (less than 70 kg given 1000 mg daily and more than 70 kg given 1200 mg daily). Patients included met the criteria of the Egyptian national program for prevention of HCV infection. Male or Female patients between 18-60 years old with Positive anti-HCV and HCV RNA (by PCR test) were included with the following criteria: white blood cell (WBC) >4.000/mm^3, neutrophil count >2.000/mm^3, platelets >100.000 mm^3, Hb >12gm for females and 13gm for males, prothrombin Time <2 seconds above upper limit of normal (ULN), fasting blood sugar not more than115 mg/dl or within 20% ULN (140 mg/dl) and If diabetic, HBA1C <8.5%, albumin >3.5 gm/dl, serum creatinine within normal limit, TSH within normal limit, negative markers for HBV, ANA <1/160, alpha fetoprotein <100 IU /ml, If alpha fetoprotein is >100 IU/ml, C.T is recommended and it should be free from any radiological signs of HCC or advanced cirrhosis. Female patients should be practicing adequate contraception and male patients should have their wives practicing adequate contraception. Patients younger than 18 years and older than 60 years, pregnant and breast-feeding females, decompensated liver cirrhosis patients, alcoholics, addicts, patients under immunosuppressant drugs or corticosteroids, patients with history of organ

transplantation, those with active epileptic seizures, ischemic heart disease within the last 6 months, chronic renal failure, autoimmune diseases, retinal abnormalities, severe psychiatric conditions, BMI >35, uncontrolled diabetes, hypersensitivity to PEG-INF or RBV were excluded from the study.

An informed written consent was taken from each patient before inclusion in this study. All patients with positive HCV RNA PCR were subjected to the following**:** Complete history taking, full clinical examination, routine laboratory investigations including CBC, LFT, KFT, PT, INR, FBS and special laboratory investigations including HBs Ag, ANA, AFP, TSH, antibilharzial antibodies, pregnancy test for females in the child-bearing period, IP-10 assay and single nucleotide polymorphisms of the IL28B genotype. Abdominal ultrasonography was performed for all patients to asses liver, spleen, portal vein diameter, ascites, and both kidneys.

IL28B rs12979860 polymorphism genotyping was performed using polymerase chain reaction with confronting two-pair primers (PCR-CTPP) which was developed by Hamajima et al. **[18]** with a purpose to be simpler than the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). For the detection of serum IP-10, the human cytokine multiplex immunoassay kit (MPXHCYTO- 60K- 06) was used according to the manufacturer's instructions from Millipore (Merck Millipore, Bill-erica, MA,USA) **[19]**.

Patients were followed up at weeks 1, 2, 4 and 8 by the following laboratory tests: CBC, LFT and KFT, TSH and quantitative PCR for HCV RNA at week 12 were further performed.

Patients were divided according to response to SOC therapy into two groups: Group I (Early virological Responders) which included patients who achieved 2 log or greater decline in HCV RNA by week 12 and Group II (Non Early virological Responders) which included patients who failed to achieve at least 2 log decline in HCV RNA by week 12 and their treatment was stopped.

All data were analyzed using SPSS 15.0 for windows (SPSS Inc., Chicago, IL, USA) & MedCalc 13 for windows (MedCalc Software bvba). Continuous data are expressed as the mean ± SD, and the categorical data are expressed as a number (percentage). Continuous data were checked for normality by using Shapiro-Wilk test. Independent Student t-test was used to compare two groups of normally distributed data, Mann-Whitney test to compare non-parametric distributed data between two groups. Categorical data were compared using the Chi-square (χ^2) test**.** Receiver Operating Characteristic (ROC) curve was obtained to calculate the cutoff point for IP10to reach the best compromise in prediction of EVR. The sensitivity, which describes the probability of patients with EVR to have a low level of IPI0 and IL28B (1.0 in binary test) was calculated. The specificity, which describes the probability of patients without EVR to have a high level of IPI0 and other IL28B genotype (0.0 in binary test) was calculated. The positive predictive value (PPV), which describes the probability of that patient with a low level of IPI0 and IL28B has EVR was calculated. The negative predictive value (NPV), which describes the probability of that patient with a high level of IPI0 and other IL28B genotype has no EVR was calculated.

P value <0.05 was considered statistically significant (S), p value< 0.005 was considered highly statistically significant (HS) and p value >0.05 was considered non statistically significant (NS). The results were analyzed by the suitable statistical methods.

RESULTS

Table (1): Demographic data of patients with early virologic response (EVR) versus those without EVR

Parameter		No EVR N = 18	EVR N= 60	Test	P
Age		42.67 ± 2.3	38.700 ± 6.50	2.5	0.014S
Sex	Female	4 (22.2%)	26 (43.3%)	1.06	0.3
	Male	14 (77.8%)	34 (56.7%)		

Patients with EVR were younger than those without EVR with a statistically significant difference.

Table (2): Laboratory data of patients with early virological response (EVR) versus those without EVR

Parameter	No EVR N = 18	EVR N= 60	Test	P
WbCs(4_10x10^9/l)	6.44 ± 0.62	6.38 ± 0.52	0.42	0.67
Hemoglobin 13-17 g/dl(men) 12-15(women)	13.2±.4	12.8±.7	3.5	.6
Platelets (150-400$10^9$/l)	246.22 ± 25.4	239.70 ± 23.08	1.02	0.30
ALT(N=5-35)	81.33 ± 7.34	49.40 ± 9.18	13.5	< 0.001 HS
AST (N=5-35)	72.33 ± 6.51	41.33 ± 8.32	14.4	< 0.001 HS
Albumin(N=5-35)	4.72 ± 0.35	4.76 ± 0.38	0.43	0.65
Prothrombin concentration%	89.78 ± 4.89	89.57 ± 4.80	0.16	0.87
Alkaline phoshatase(50-100 Iu/ml)	195.78 ± 8.70	134.27 ± 6.58	0.79	0.43
Alphafeto protein(up to 11Iu/ml)	3.04 ± 0.34	2.88 ± 0.45	1.36	0.17
PCR (Iu/ml)	(328091-8769543) 876543*	(5432-765432) 7865*	4.99	<0.001 HS
Fasting blood sugar(70-110mg/dl)	92.11 ± 18.4	82.81 ± 7.00	3.24	0.002S
Serum Creatinine (0.6-1.2mg/dl)	0.71 ± 0.09	0.69 ± 0.07	0.99	0.32

Patients with EVR had less elevated liver enzyme, low viral load and fasting blood sugar than those without EVR with a statistically significant difference. where * is the median.

Table (3): Histopathologic data of patients with early virologic response (EVR) versus those without EVR

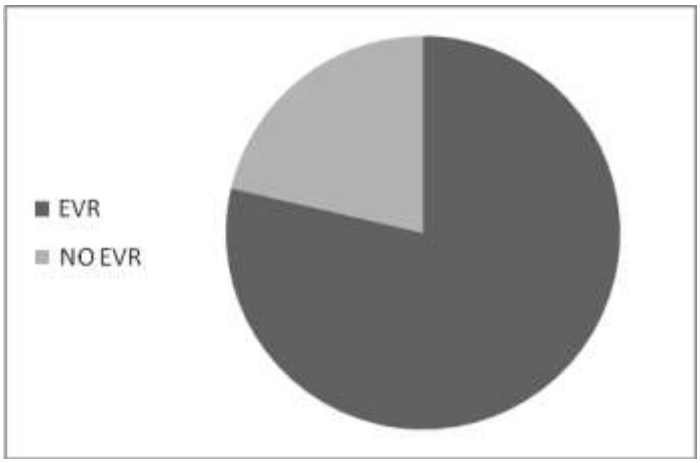
Parameter	No EVR N = 18		EVR N= 60	Test	P
Activity	A_1	0 (0%)	41 (68%)	5.8	0.15
	A_2	18 (100%)	19 (31.7%)	8.06	<0.005 s
Fibrosis	F_1	2 (11.1%)	1 (1.7%)	0.33	0.56
	F2	8 (44.4%)	41 (68.3%)	3.3	0.065
	F_3	8 (44.4%)	18 (30%)	3.8	0.05

100% of patients with out early virological response had A2 activity while 31.7% of patients with EVR had A2 activity with a statistically significant difference.

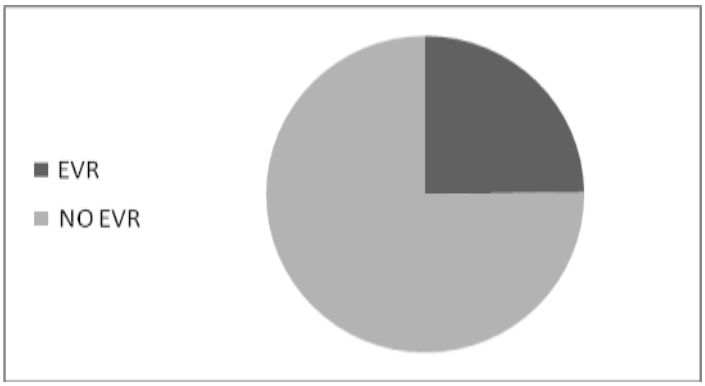
Table (4): IP10 and IL28B in the patients with early virological response versus those without EVR

Parameter	No EVR N = 18	EVR N= 60z	Test	P
IP 10	866.67 ± 105.49	467.00 ± 90.71	15.78	<0.001 HS
IL 28				
CC	4 (22.2%)	49 (81.7%)	22.17	< 0.001
TT	4 (22.2%)	0(0%)	24.06	< 0.001
CT	10 (55.6%)	11 (18.3%)	7.95	0.004

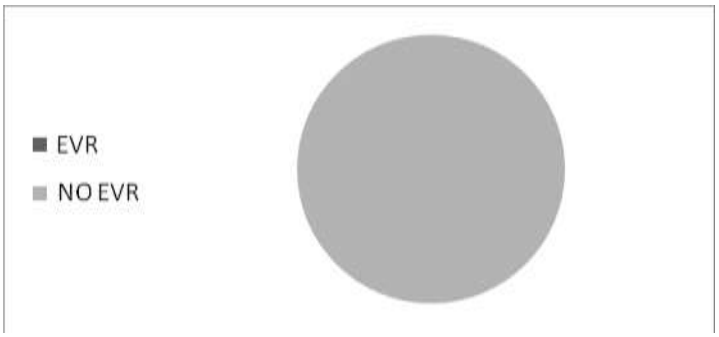
Patients with EVR showed lower level of IP10 and 81.7% of them had CC allele genotype with a statistically significant difference compared to patients without EVR.



A) IL28CC genotype



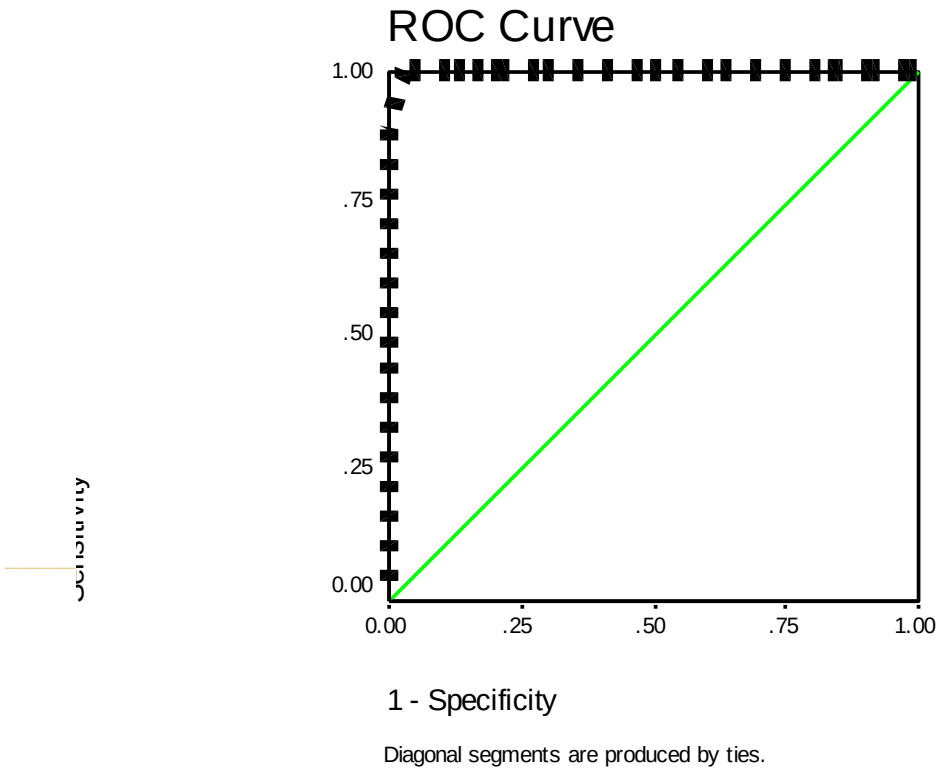
B) IL28CT genotype



C) IL28TT genotype

Table (5): Predictive value for treatment response according to IP10 and IL28B

Marker	Sensitivity	Specificity	PPV	NPV
IL 28 CC	81.7 %	77.8%	92.5%	56.0%
Ip 10 Value <605pico gram/ml	100%	96.7%	98.3	100%
Combination of both IP10 and IL2B CC	96.7%	72.22%	92.1%	86.7%



D) I.P 10 area under the curve 0.99 (0.99 – 1.003)

Table (6): Different studied parameters in relation to IP10 and IL28B

IL 28B	CC (53)	CT (21)	TT (4)	F	P
Ip 10	472.45± 139.9	704.76±146.3	945.0 ± 17.3	37.29	<0.001
ALT	48.92± 10.9	71.66 ± 12.5	82.5± 2.8	42.01	<0.001
AST	40.77± 9.6	62.14± 11.2	79.0 ± 1.15	55.26	<0.001
PCR	(5432-8769543) 8765*	(65432-1234543) 87654*	(687651-845876) 766763.5	0.11	0.84

Patients with CC genotype were associated with lower level of IP10, ALT, AST and also lower level of HCV RNA level.

Table (7): Correlation of IP 10 and some studied data

Parameter	r	P
ALT	0.81	< 0.001
AST	0.85	< 0.001
Alphafeto protein	0.03	0.799
Prothrombin concentration%	0.12	0.27
Albumin	0.017	0.88
PCR	0.46	.001

Patients with low levels of IP10 had low levels of ALT and AST. also lower level of HCV RNA level.

DISCUSSION

This study was designed as Egypt has the highest prevalence of HCV worldwide. HCV is a major cause of chronic hepatitis, liver cirrhosis, hepatocellular carcinoma and liver transplantation in the country. Several baseline and on-treatment variables affect the virologic response to treatment of chronic HCV patients as age, stage of fibrosis, HCV genotype and viral load at baseline. Assessment of new markers as IP10 and IL28B will help in prediction of early virologic response. This may be useful in encouraging patients who are difficult-to-treat to initiate therapy.

This study included 78 patients; 76.9% (60 patients) had early virologic response (EVR) while the rest of the patients (23.1 %) had no early virologic response.

We found in our study that patients with EVR were younger than those without EVR with a statistically significant difference and this was in agreement with **[20] [21]**. Also, the efficacy and safety of antiviral therapy in the older population is not clear and are limited to small, single-center studies. Immunological suppression, chronic disease and other medication use in the elderly age group can significantly impair the drug response. However, in patients above 50 years with positive prognostic factors including patients with low HCV RNA levels and those without advanced fibrosis, the SVR rates are comparable with younger patients below 50 years highlighting the importance of other factors in addition to age **[22]**.

In our study we found that patients with low level of ALT and AST has good response to treatment and this result was in agreement with Derbala et al. **[23]**. It was reported that patients with normal liver enzyme have significantly lower inflammation and fibrosis scores on liver biopsy than patients with elevated ALT and the spectrum of liver fibrosis tends to be more severe in patients with elevated ALT than in those with normal ALT. This necroinflammatory activity may affect the response of treatment **[24]**.

Patient with EVR showed lower level of IP10 and 81.7% of them had CC allele genotype with statistically significant different when compared to those without EVR. CC genotype was associated with lower level of proinflammatory mediators in the form of IP10, ALT andASTand patients with low IP10 had low level of enzymes.

We also found high level of IP10 in patients with high level of enzymes and this was in agreement with Zeremski et al. **[25]** who found that the serum IP-10 levels at the time of liver biopsy were predictive of the development of fibrosis 3–5 years later.

Upon HCV infection, IP-10 and other ISGs are produced by hepatocytes and many other cell types. Some ISGs, like IP-10, are produced directly by viral infection without the need for interferon production**.** The relation between ISG expression in response to infection is unknown but clearly relates to the IL28B genotype **[26]**. In chronic HCV, patients with the favourable IL28B genotype tend to have low levels of ISG expression allowing for strong gene induction with therapeutic interferon, lastly leading to clearance. In contrast, those with the unfavourable IL28B genotypes tend to have pre-activation of ISGs with near maximal expression before treatment, resulting in no further gene induction with interferon therapy and thus non-response **[27]**.

If ISG induction is required for clearance, one might have anticipated that in acute HCV infection, patients with higher ISG expression would be more likely to spontaneously clear infection. If plasma IP-10 levels are a reflection of ISG expression, the opposite pattern was seen. However, the relationship between IP-10 and HCV RNA levels may help clarify this apparent paradox. In patients with the favourable IL28B genotype, IP-10 levels tended to be lower but correlated well with the level of HCV RNA suggesting that in this setting, IP-10 production and possibly production of other ISGs is driven by and thus proportionate to the amount of virus present. However, in patients with the unfavourable genotypes, IP-10 levels were on average higher and appeared to be entirely independent of the HCV RNA level. This might suggest that the fundamental problem in those with the unfavourable genotypes is a loss of regulation of ISG induction such that ISGs are produced independent of the stimulus and therefore lead to a less coordinated viral response **[28]**.

Also other studies had shown that IL28B could inhibit HCV replication in a dose- and time-dependent manner and through the JAK-STAT pathway **[29].**So, it had been found that IL28B genotype is associated with differential expression of intrahepatic interferon-stimulated

genes in patients with chronic hepatitis C . All these evidences indicate both anti-viral effect and immune-mediated effect of IL28B, which could be affected by these polymorphisms **[30]**.

Funding: None.
Conflicts of interest: None.
Ethical approval: A written informed consent was taken from all included patients, and the study was approved by the Ethical Committee of our insitution.

REFERENCES

1. Lauer, G., Walker, B. Hepatitis C virus infection. *N Eng J Med* 2001; 345:41–52.
2. Shepard, C., Finelli,L., Alter M. Global epidemiology of hepatitis C virus infection. *Lancet Infect Dis* 2005; 5: 558–67.
3. Kamal, S., Nasser, I. Hepatitis C Genotype 4: What We Know and What We Don't Yet Know. *Hepatology* 2008; 47:1371-83.
4. Abdel-Hamid M, El-Daly M, Molnegren V, El-Kafrawy S, Abdel-Latif S, Esmat G., et al. Genetic diversity in hepatitis C virus in Egypt and possible association with hepatocellular carcinoma. *J. Gen. Virol.* 2007; 88: 1526-1531.
5. Ghany MG, Strader DB, Thomas DL, Seeff LB. Diagnosis and management, and treatment of hepatitis C: An update. *Hepatology* 2009; 49(4):1335-74.
6. Zeuzem, S. Heterogeneous virologic response rates to interferon-based therapy in patients with chronic hepatitis C: who respond less well? *Ann Intern Med* 2004; 140: 370–81.
7. Bressler BL, Guindi M, Tomlinson G, Heathcote J. High body mass index is an independent risk factor for nonresponse to antiviral treatment in chronic hepatitis C. *Hepatology* 2003; 38: 639–44.
8. Poordad, F. Review article: the role of rapid virological response in determining treatment duration for chronic hepatitis C. *Aliment Pharmacol Ther* 2010; 31: 1251–67.
9. Askarieh G, Alsiö A, Pugnale P, Negro F, Ferrari C, Neumann AU, et al. Systemic and intrahepatic interferon-gamma-inducible protein 10 kDa predicts the first-phase decline in hepatitis C virus RNA and overall viral response to therapy in chronic hepatitis C. *Hepatology* 2010; 51 (5): 1523–30.
10. Romero AI, Lagging M, Westin J, Dhillon AP, Dustin LB, Pawlotsky JM, et al. Interferon (IFN)-gamma inducible protein-10: association with histological results, viral kinetics, and outcome during treatment with pegylated IFN-alpha 2a and ribavirin for chronic hepatitis C virus infection. *J Infect Dis* 2006; 194: 895–903.
11. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009; 461: 399–40.
12. Neville, L., Mathiak, G., Bagasra, O. The immunobiology of interferon gamma inducible protein 10 kD (IP-10): a novel, pleiotropic member of the C-X-C chemokine superfamily. *Cytokine Growth Factor Rev* 1997; 8:207–19.
13. Taub DD, Lloyd AR, Conlon K, Wang JM, Ortaldo JR, Harada A, et al. Recombinant human interferon inducible protein 10 is a chemoattractant for human monocytes and T lymphocytes and promotes T cell adhesion to endothelial cells. *J Exp Med* 1993; 177:1809–14.
14. Harvey CE, Post JJ, Palladinetti P, Freeman AJ, Ffrench RA, Kumar RK., et al. Expression of the chemokine IP-10 (CXCL10) by hepatocytes in chronic hepatitis C virus infection correlates withhistological severity and lobular inflammation. *Journal of Leukocyte Biology* 2003; 74(3): 360-9.
15. Liu, M., Keirstead, H., Lane, T. Neutralization of the chemokine CXCL10 reduces inflammatory cell invasion and demyelination and improves neurological function in a viral model of multiple sclerosis. *J. Immunol*. 2001; 167: 4091.
16. Christen, U., Von Herrath, M. IP-10 and type 1 diabetes: a question of time and location. *Autoimmunity* 2004; 37:273–82.
17. Kutsch O1, Oh J, Nath A, Benveniste EN. Induction of the chemokines interleukin-8 and IP-10 by human immunodeficienc virus type 1 tat in astrocytes. *J Virol* 2000; 74:9214–21.
18. Hamajima N, Saito T, Matsuo K, Kozaki K, Takahashi T, Tajima K. Polymerase chain reaction with confronting two-pair primers for polymorphism genotyping. *Jpn. J. Cancer Res.* 2000; 91: 865–868.
19. Loetscher M, Gerber B, Loetscher P, Jones SA, Piali L, Clark-Lewis I, et al. Chemokine receptor specific for IP10 and MIG: Structure, function, and expression in activated T lymphocytes. *J. Exp. Med* 1996; 184: 963-969
20. Koyama R, Arase Y, Ikeda K, Suzuki F, Suzuki Y, Saitoh S, et al. Efficacy of interferon therapy in elderly patients with chronic hepatitis C. *Intervirology* 2006; 49: 121-126.
21. Terranova R, Luca S. Preliminary results of beta-interferon treatment in the elderly. *Control Clin Trials* 1996; 17: 123-129.
22. Reddy KR, Messinger D, Popescu M, Hadziyannis SJ. Treatment with Peginterferon-alpha 2a and ribavarin in older HCV genotype 1 patients with positive prognostic factors leads to high rates of sustained virological response. *J Hepatol* 2008; 48(Suppl. 2): 310A.

23. Derbala M, Nasser M, Rizk MN, Al-Kaabi S. The predictive value of IL28B rs12979860, rs11881222 and rs8099917 polymorphisms and IP-10 in the therapeutic response of Egyptian genotype 4 patients .*Virology* 2013; 444; 292–300.
24. Shiffman ML, Diago M, Tran A, Pockros P, Reindollar R, Prati D, et al. Chronic hepatitis C in patients with persistently normal alanine transaminase levels. *Clin Gastroenterol Hepatol* 2006; 4: 645–652.
25. Zeremski M, Dimova R, Astemborski J, Thomas DL, Talal AH. CXCL9 and CXCL10 chemokines as predictors of liver fibrosis in a cohort of primarily African-American injection drug users with chronic hepatitis C. *J Infect Dis* 2011; 204: 832-836.
26. McGilvray I, Feld JJ, Chen L, Pattullo V, Guindi M, Fischer S, et al. Hepatic cell-type specific gene expression better predicts HCV treatment outcome than IL28B genotype. *Gastroenterology* 2012; 142: 1122-1131.
27. Feld JJ, Nanda S, Huang Y, Chen W, Cam M, Pusek SN, et al. Hepatic gene expression during treatment with peginterferon and ribavirin: Identifying molecular pathways for treatment response. *Hepatology* 2007; 46:1548-1563.
28. Sarasin-Filipowicz M, Oakeley EJ, Duong FH, Christen V, Terracciano L, Filipowicz W, et al. Interferon signaling and treatment outcome in chronic hepatitis C. *Proc Natl Acad Sci USA* 2008; 105:7034-7039.
29. Zhang L, Miao L, Han F, Dou XG. Cytokine levels in serum of patients with chronic hepatitis C and its significance. *J. Cell. Mol. Immunol.* 2011; 27, 301–303.
30. Urban TJ, Thompson AJ, Bradrick SS, Fellay J, Schuppan D, Cronin KD, et al. IL28B genotype is associated with differential expression of intrahepatic interferon-stimulated genes in patients with chronic hepatitis C. *Hepatology*. 2010; 52 : 1888-1896.

Peer reviewer: Nahla Elgammal, Professor of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt. **Editor: Mohamad Emara**, Assistant Professor of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt.

Gut Microbiota and Type 2 Diabetes Mellitus : What is The Link ?

Nesma A Ibrahim
Internal Medicine Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt

Corresponding Author
Ibrahim NA

Mobile: *+01007587891*

Email:
dr_nesmali@yahoo. com

Key words:
Type 2 Diabetes Mellitus, Gut Microbiota

Abstract:

The rapid increase of cases of type 2 diabetes mellitus (T2DM) in the past decades has made it a widespread metabolic disorder. In addition to well-established risk factors for T2DM, including genetic predisposition, poor physical activity, fetal programming and obesity, an altered configuration of the microbial community in our gut – the microbiota – has emerged as a new candidate that may be linked to T2DM. The aim of this review is to focus the light on the role of gut microbiota as a novel key organ involved in metabolism, and discussing the putative mechanisms linking gut microbiota and T2DM, as well as the therapeutic perspective of intestinal microbiota modulation for T2DM.

INTRODUCTION

Microorganisms which colonize all surfaces of the human body that are exposed to the environment (that live on and inside humans) are collectively called the human microbiota **[1]**. The human microbiota consists of as many as 10 to 100 trillion microorganisms, representing at least 10-fold more cells than exist in the human body. The main mass of microorganisms associated with humans resides in the gastrointestinal tract. The weight of the bacteria living in a human intestine is about 1.5 kg and make up about 50% of the fecal matter **[2]**. Apart from the intestinal microbiota, also the skin, oral, nasal and vaginal microbiota had been studied extensively. The human microbiota is not restricted to these sites but do also reside in for example the lungs, the blood and atherosclerotic plaques **[3]**.

MICROBIOTA COMPOSITION

In adults, *Bacteroidetes* (eg, *Bacteroides, Prevotella*) and *Firmicutes* (eg, *Clostridium, Enterococcus, Lactobacillus, Ruminococcus*) usually dominate the intestinal microbiota, whereas *Actinobacteria* (eg, *Bifidobacterium*), *Proteobacteria* (eg, *Helicobacter, Escherichia*) and *Verrucomicrobia* are in considerably minor proportion. Methanogenic archaea (represented by *Methanobrevibacter smithii*), eukaryotes (mainly yeast) and viruses (mainly bacteriophages) are also components of this microbiota **[4]**.

MICROBIOTA ESTABLISHMENT

The microbial colonization of the gut begins in infants immediately after birth. Facultative anaerobes, such as enterobacteria, enterococci and lactobacilli are the first colonizers. Anaerobic microorganisms, including *Bifidobacterium*, *Bacteroides* and *Clostridium* establish gradually, and contribute to a progressive decrease of the facultative anaerobes to strict anaerobes ratio in time **[5]**. At about 3 years of age, the gut microbiota reaches a composition and diversity similar to adults and remains more or less stable over time in adulthood. New changes appear in the senescence, the microbiota of elderly people differing from the core microbiota and diversity levels of younger adults **[6]**.

FACTORS AFFECTING MICROBIOTA

Changes are produced in our microbiota from birth to adulthood. The composition and function of the

gut microbiota are influenced by several factors. The mode of delivery (vaginal versus Caesarian section) as well as the method of feeding (breast versus formula feeding) are influencing the gut microbiota. Both host genotype and lifestyle factors such as diet, physical activity, antibiotics, age and probably several additional but yet unidentified factors may simultaneously influence the gut microbiota **[7]**.

GUT MICROBIOTA AND METABOLISM

The gut microbiota has gone from being considered an accompanying commensal to a «metabolic organ» **[8]**, with functions in nutrition, immunity regulation, and systemic inflammation. The microbiota regulates, via different mechanisms, some important physiological functions of the host, such as those related to energy expenditure, satiety and glucose homeostasis. It can also act as a barrier against the establishment of food borne pathogens **[9]**. Growing evidence in clinical studies suggested that obese people with insulin resistance were characterized by an altered composition of gut microbiota, particularly an elevated *Firmicutes*/*Bacteroidetes* ratio compared with healthy people **[10]**. Furthermore, trans-plantation of the obese gut microbiota in animals greatly affected the energy harvest of hosts **[11]**.

THE ROLE OF GUT MICROBIOTA IN THE PATHOGENESIS OF T2DM

(I) Storage hypothesis

Gut microbiota is involved in several intestinal biological functions such as defense against pathogens, immunity, the development of the intestinal microvilli and the degradation of non digestible polysaccharides (fermentation of resistant starch, oligosaccharides, inulin). Hence, the gut microbiota harvests energy for the host from dietary compounds ingested but not digested by the host **[12]**. It was found that gut microbiota conventionalisation results in a doubling of the density of capillaries in the small intestinal villus epithelium, thereby helping to promote intestinal monosaccharide absorption **[13]**.

Backhed, *et al*. found that the mice raised in the absence of microorganisms (germ free) had about 40% less total body fat than mice with a normal gut microbiota. The mechanisms of the apparent weight gain implied an increase in the intestinal glucose absorption, energy extraction from non-digestible food component and concomitant higher glycemia and insulinemia, two key metabolic factors regulating lipogenesis **[14]**. Moreover, glucose and insulin are also known to promote hepatic *de novo* lipogenesis through the expression of several key enzymes such as aceyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) **[15]**.

Interestingly, a role for adipocyte lipoprotein lipase (LPL) activity was proposed. Consistent with this hypothesis, it was suggested that gut microbiota promote fat storage through a mechanism linking circulating triglycerides with suppression of the intestinal expression of an LPL inhibitor (FIAF, fasting-induced adipose factor). FIAF inhibits LPL activity, thereby reducing fatty acid release from circulating triacylglycerols. Hence, upon gut colonisation with microbiota, FIAF expression is reduced, leading to higher LPL activity and greater fat storage **[14]**.

Also, microbiota impacts muscle metabolism and consequently influences the regulation of insulin resistance. AMP-activated kinase is an enzyme expressed in the muscle that activates glucose utilization during hypoxia and exercise **[16]**. It was found that germ-free mice are characterized by a very high concentration of AMP-activated kinase and its downstream targets that are involved in fatty acid oxidation in skeletal muscle favoring muscle glucose utilization and allowing the mice to resist high-fat, sugar-rich diet induced diabetes. More precisely, the gut microbiota was found to suppress AMPK-driven fatty acid oxidation in the liver and in skeletal muscle **[17]**.

Moreover, a pathway involving short chain fatty acids (SCFAs) has been proposed. SCFAs act as signaling molecules and are specific ligands for at least two G protein-coupled receptors, GPR41 and GPR43 **[18]**. Samuel et al. have demonstrated that G-protein coupled receptor 41 (GPR41) knockout mice colonized with a specific fermentative microbial community resist fat mass gain compared to their wild-type littermates **[19]**. Therefore, this supports the idea that specific metabolites coming from the gut (i.e., SCFAs) act in a variety of ways (e.g., as energy substrates and as metabolic regulators) **[20]**.

(II) The inflammatory hypothesis

Metabolic diseases are linked to disruption of both the innate and the adaptive immune systems. One of the mechanisms proposed to explain the crosstalk between gut microbiota, regulation of fat storage and development of obesity-related diseases is metabolic endotoxemia (i.e., increased plasma lipopolysaccharides levels) **[21]**. Bacterial

lipopolysaccharides (LPS) are a component of the cell wall of gram-negative bacteria capable of triggering an inflammatory state, augment adipose inflammation and reduce insulin sensitivity, which are present in metabolic disorders **[22]**.

It was suggested that diet may play an important role in gut permeability since LPS absorption from the gut was found to be associated with the ingestion of dietary fat **[23]**. A study had raised the possibility that LPS could be associated with chylomicrons within the enterocyte, being LPS then secreted from cell-associated pools in a chylomicron-dependent manner **[24]**. Another possibility is that dietary fat leads to paracellular leakage of LPS across the intestinal epithelium. This is supported by the observation that intestinal tight-junction integrity is impaired in obese mice and by studies in which intestinal luminal exposure to oleic acid can cause intestinal epithelial damage **[25]**.

Gut microbiota may have a critical function in the regulation of gut permeability, contributing to endotoxemia, through the endocannabinoid system (eCB) and LPS regulatory loop **[26]**. On the other hand, LPS regulates the synthesis of eCB in macrophages **[27]**. Moreover, intestinal alkaline phosphatase (IAP) is known to be involved in the breakdown of dietary lipids, it also plays an important role in LPS detoxification by dephosphorylating the lipid portion of the LPS, thus acting as a host defense factor against LPS. IAP expression is not only modulated by dietary components, including fat, but also controlled by gut microbiota **[28]**. Interestingly, Everard and colleagues had defined the protective role of the bacterium *Akkermansia (A.) muciniphila* against the development of metabolic diseases **[29]**. The normalization of *A. muciniphila* abundance through prebiotic administration is correlated with an improved metabolic profile, reduced fat-mass, metabolic endotoxemia, adipose tissue inflammation and insulin resistance **[30]**.

These results indicate the involvement of the gut microbiota in the inception of gut barrier alterations and thus increased intestinal permeability and increased absorption of LPS, a state of metabolic endotoxemia is initiated, characterized by elevated serum LPS concentration, leading to increased activation of inflammatory pathways and impairment of the insulin signaling **[31]**.

Metabolic Endotoxemia and Insulin Resistance

Toll-like receptors (TLRs) play an important role in the activation of innate immune responses in mammals by recognizing conserved pathogen-associated molecular patterns **[32]**. TLR4 is a subclass of TLRs that can be activated by LPS and by nonbacterial agonists, such as saturated fatty acids **[18]**. The activation of TLR4 signaling induces up-regulation of inflammatory pathways related to the induction of insulin resistance **[33]**. Activation of TLR4 by LPS in pre-adipocytes increases the expression of several cytokines, mainly TNF-α and IL-6, impairing the insulin signaling in adipocytes. Moreover, LPS can promote the expression of inducible nitric oxide synthase (iNOS), which is also known as capable of interfering with the insulin signaling **[34]**. Likewise, TLR2 has been shown as an important modulator of insulin resistance. It was reported that short-term inhibition of TLR2 expression using TLR2 oligonucleotide antisense in diet-induced obese mice leads to increased insulin sensitivity and signaling **[35]**. Thus it was suggested that a signalling cascade initiated by an LPS/ TLR-4/CD14-dependent mechanism in turn activates TLR-2 expression to support innate immune system inflammatory responses **[36]**.

Like TLRs, nucleotide oligomerization domain (NOD)-1 and -2 proteins are intracellular pattern recognition receptors that sense bacterial cell wall peptidoglycan (PGN) moieties, which induce stress and inflammation pathways **[37]**. Several studies have associated NOD1- and NOD2-activating bacterial motifs with insulin resistance **[38]**. On the other hand, SCFAs were shown to affect pancreatic beta-cell function (promoting β-cell development, proliferation, and differentiation) or by indirectly increasing glucagon-like peptide-1 (GLP-1) secretion from enteroendocrine L-cells. Furthermore, SCFAs reduce the release of pro-inflammatory cytokines by adipose tissue and weaken leukocyte activation. These anti-inflammatory effects improve insulin resistance, tissue glucose uptake, and blood glucose levels **[39]**.

THERAPEUTIC PERSPECTIVE OF GUT MICROBIOTA MODULATION FOR TYPE 2 DIABETES MELLITUS

Understanding the metabolic impact of the complex interaction between gut microbiota and the host has driven interest in manipulating microbiota in order to develop new therapeutic targets for the metabolic diseases **[40]**.

Diet and Physical activity

Weight loss promoted by calories restricted diet and increased physical activity is associated with

significant changes in the composition of gut microflora **[30]**. Recent studies in humans and rodents have demonstrated a remarkable ability of the metabolic capacity of the gut microbiota to be altered by long-term dietary patterns, indicating that the potential benefits of dietary intervention on host metabolism might very well be significant **[41]**. Moreover, evidence for the existence of a modulating effect of physical activity on the gut microbiota is accumulating, although mainly from rodent models. Physical activity has been shown to affect gut microbiota composition and diversity in healthy rats **[42]**, with some results indicating that the demonstrated effects are independent of diet **[43]**.

Antibiotics

Antibiotic treatment is another method of gut microbiota modulation. Mouse studies have shown improved glucose tolerance that was independent of weight changes, and also lower levels of circulating LPS and a lower bacterial count following antibiotic treatment, with an improved metabolic state **[44]**. However, the administration of antimicrobial agents, including broad spectrum antibiotics, has been proposed as a possible contributor to the obesity epidemic and the shrinking gut microbiome richness in the western world **[45]**, it was shown that even prenatal exposure to antibiotics increases the risk of childhood obesity, perhaps through an effect on the maternal gut microbiota **[46]**. On the other hand, treatment with vancomycin has been shown to reduce peripheral insulin sensitivity in individuals with metabolic syndrome, whereas treatment with amoxicillin does not, reflecting the preferential targeting of Gram positive butyrate-producing bacteria by the former **[47]**. Although some studies indicate that antibiotics have not only short- but also long-term effects **[48]** on the diversity and/or configuration of the gut microbiota, further studies should be performed to investigate the effects of different antibiotics and administration routes on metabolism and T2DM **[49]**.

Bariatric surgery

It was suggest that the more long-term health benefits of bariatric surgery may in part be due to alterations in the gut microbiota **[50]**. Some studies conducted on subjects submitted to surgical Roux-en-Y gastric by-pass (RYGB) reported a profound change of gut microbiota composition, related to the surgically reverted anatomy of alimentary tube **[30]**. The direct transit of carbohydrates to the small intestine, without the prior exposure to gastric acids, promotes the growth of *Proteobacteria* and *Enterobacteria* fermenting complex carbohydrates **[51]**. The increased production of metabolites deriving from oligosaccharides fermentation is well known to contribute to increased GLP-1 and peptide YY production, which contribute to reduce appetite and to improve beta-pancreatic cell function and insulin secretion **[12]**.

Manipulating the Gut Microbiota by Probiotics

Probiotics are defined as 'live microorganisms that, when administered in adequate amounts, confer a health benefits on the host' **[52]**. Probiotics are live microorganisms administered in attempt to reconstitute the gut microbiota, namely *Bifidobacterium*, *Lactobacillus*, *Saccharomyces*, *Streptococcus*, and *Enterococcus* **[53]**.

Several studies have demonstrated that probiotic strains, in particular those of the *Lactobacillus* and *Bifidobacterium* genera, exert multiple beneficial effects in subjects affected by metabolic syndrome. Indeed, they seem to promote weight loss and the reduction of visceral adiposity, to improve glucose tolerance, and to modulate intestinal low grade inflammation **[30]**. Molecular mechanisms involving the anti-diabetic effects of probiotics are not fully elucidated, but may be related to reduction of oxidative stress, immuno-modulation, attenuation of inflammation and modification of the intestinal microbiota **[54]**.

Manipulating the Gut Microbiota by Prebiotics

Prebiotics are defined as 'the selective stimulation of growth and/or activity(ies) of one or a limited number of microbial genus(era)/species in the gut microbiota that confer(s) health benefits to the host' **[55]**. The prebiotic approach dictates that non-viable food components are specifically fermented in the colon by indigenous bacteria thought to be of positive value, e.g., bifidobacteria, lactobacilli. Any food ingredient that enters the large intestine is a candidate prebiotic **[56]**. Prebiotics contribute to modify gut microbial composition, enhancing the growth of *Bifidobacteria* **[57]**, *Bacteroides*, *Prevotella* and *Roseburia* **[58]** and promoting the relative decrease of *Firmicutes* **[57]**.

Substrates that are widely accepted prebiotics include the fructans inulin and fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), and lactulose **[59]**. Some non-digestible carbohydrates **(**NDCs) are recognised as prebiotics

after they were preferentially fermented by specific types of bacteria generally considered beneficial for the host **[55]**.

The mechanisms of action remain unclear but could be related to the regulation of intestinal mucosal biology where the intestinal mucosa was characterized by higher villi, deeper crypts, increased number of goblet cells and a thicker mucus layer on the colonic epithelium **[60]**. The reduction of intestinal low grade inflammation promoted by the improvement of gut barrier integrity **[58]** and the decrease of pro-inflammatory cytokines release, lead to an improvement of glucose tolerance and insulin sensitivity **[61]**.

Manipulating the Gut Microbiota by Fecal Transplants

Fecal microbiota transplantation (FMT) has been utilized for over 50 years, the main principle of fecal transplant is the possibility of this procedure replacing pathogenic microbes by beneficial communities, thus restoring the gut microbiota balance and enabling the cure of the disease **[40]**.

Vrieze et al. evaluated the effects of FMT on insulin sensitivity in individuals with metabolic syndrome. Subjects with metabolic syndrome were randomly assigned to groups set to receive small intestinal infusions from lean donors or autologous microbiota. Subjects who received infusions from lean donors were noted to have an increase in insulin sensitivity and an increase in butyrate producing intestinal microbiota 6 weeks post transfusion. This led authors to conclude that intestinal microbiota might be developed as a therapeutic agent to increase insulin sensitivity in patients with metabolic syndrome and insulin resistance [**62**].

CONCLUSION

Gut microbiota may play an important role in the pathogenesis of T2DM by influencing body weight, proinflammatory activity and insulin resistance. Modulating the gut microbiota through the use of probiotics, prebiotics, antibiotics, and fecal microbiota transplantation may have benefits in improving glucose metabolism and insulin resistance in the host. Although ground-breaking research in recent years has enormously expanded our understanding of the microbiota-host interplay, the numerous cellular and molecular mechanisms involved in the pathophysiological interactions await further discovery and characterization. Future studies are required to increase our understanding of the complex interplay between intestinal microbiota and the host to enable the development of new effective treatments for T2DM.

REFERENCES

1. Karlsson F, Tremaroli V, Nielsen J , Bäckhed F. Assessing the Human Gut Microbiota in Metabolic Diseases. *Diabetes* 2013; 62:3341–3349.
2. Zhao L. The gut microbiota and obesity: from correlation to causality. *Nat Rev Microbiol* 2013;11, 639-647.
3. Amar J, Lange C, Payros G, Garret C, Chabo C, Lantieri O, et al. Blood microbiota dysbiosis is associated with the onset of cardiovascular events in a large general population: the D.E.S.I.R. study. *PLoS One* 2013; 8, e54461.
4. Kim BS, Jeon YS , Chun J. Current status and future promise of the human microbiome. *Pediatr Gastroenterol Hepatol Nutr* 2013; 16: 71-79.
5. Arboleya S, Solís G, Fernández N, de los Reyes-Gavilán CG and Gueimonde M. Facultative to strict anaerobes ratio in the preterm infant microbiota: a target for intervention? *Gut Microbes* 2012; 3: 583-588.
6. Salazar N, López P, Valdés L, Margolles A, Suárez A, Patterson AM, et al. Microbial targets for the development of functional foods accordingly with nutritional and immune parameters altered in the elderly. *J Am Coll Nutr* 2013; 32: 399-406.
7. Allin KH, Nielsen T , Pedersen O. Gut microbiota in patients with type 2 diabetes mellitus. *European Journal of Endocrinology* 2015; 172, R167–R177.
8. Frazier TH, DiBaise JK and McClain CJ. Gut microbiota, intestinal permeability, obesity-induced inflammation and liver injury. *JPEN J Parenter Enteral Nutr.* 2011;35 5 Suppl:14S---20S.
9. Reigstad CS , Kashyap PC. Beyond phylotyping: understanding the impact of gut microbiota on host biology. *Neurogastroenterol Motil* 2013; 25: 358-372.
10. Tilg H , Kaser A. Gut microbiome, obesity, and metabolic dysfunction. *Journal of Clinical Investigation* 2011;121 : 2126–2132.
11. Tremaroli V , Bäckhed F. Functional interactions between the gut microbiota and host metabolism. *Nature* 2012;489 : 242–249.
12. Cani PD , Delzenne NM. The Role of the Gut Microbiota in Energy Metabolism and Metabolic Disease. *Current Pharmaceutical Design* 2009; *15*, 1546-1558.
13. Stappenbeck TS, Hooper LV, Gordon JI. Developmental regulation of intestinal angiogenesis by indigenous microbes via Paneth cells. *Proc Natl acad Sci USA* 2002; 99:15451-15455.

14. Ba¨ckhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, et al. The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci U S A* 2004;101: 15718–15723.
15. Denechaud PD, Dentin R, Girard J , Postic C. Role of ChREBP in hepatic steatosis and insulin resistance. *FEBS Lett* 2008; 582: 68- 73.
16. Serino M, Fernandez-Real JM, García-Fuentes E, Queipo-Ortuño M, Moreno-Navarrete JM, Sánchez A, et al. The gut microbiota profile is associated with insulin action in humans. *Acta Diabetol* 2012; DOI:10.1007/s00592-012-0410-5.
17. Ba¨ckhed F, Manchester JK, Semenkovich CF, Gordon JI. Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proc Natl Acad Sci USA* 2007; 104:979–84.
18. Samuel B S, Shaito A, Motoike T, Rey FE, Backhed F, Manchester JK, et al. Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. *Proc. Natl Acad. Sci. USA* 2008;. 105, 16767−16772.
19. Lee YH, Giraud J, Davis RJ , White MF. c-jun *N*-terminal kinase (JNK) mediates feedback inhibition of the insulin signaling cascade. *J. Biol. Chem.* 2003; *278*, 2896–2902.
20. Bjursell M, Admyre T, Göransson M, Marley AE, Smith DM, Oscarsson J, et al. Improved glucose control and reduced body fat mass in free fatty acid receptor 2 (Ffar2) deficient mice fed a high fat diet. *Am. J. Physiol. Endocrinol. Metab* 2011; 300, E211−E220.
21. Geurts L, Lazarevic V, Derrien M, Everard A, Van Roye M, Knauf C, et al.. Altered gut microbiota and endocannabinoid system tone in obese and diabetic leptin-resistant mice: impact on apelin regulation in adipose tissue. *Frontiers in Microbiology* 2011; 2: 149.
22. Feuerer M, Herrero L, Cipolletta D, Naaz A, Wong J, Nayer A, et al. Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. *Nat Med* 2009; 15: 930–939.
23. Erridge C, Attina T, Spickett CM, Webb DJ. A high-fat meal induces low-grade endotoxemia: evidence of a novel mechanism of postprandial inflammation. *Am J Clin Nutr*. 2007 Nov; 86(5): 1286-92.
24. Ghoshal S, Witta J, Zhong J, de Villiers W, Eckhardt E. Chylomicrons promote intestinal absorption of lipopolysaccharides. *J. Lipid Res.* 2009; *50*, 90–97.
25. Brun P, Castagliuolo I, di Leo V, Buda A, Pinzani M, Palù G, et al. Increased intestinal permeability in obese mice: New evidence in the pathogenesis of nonalcoholic steatohepatitis. *Am. J. Physiol. Gastrointest. Liver Physiol.* 2007; *292*, G518–G525.
26. Silvestri C and Di Marzo V. The endocannabinoid system in energy homeostasis and the etiopathology of metabolic disorders. *Cell Metab* 2013; 17: 475-490.
27. Côté M, Matias I, Lemieux I, Petrosino S, Alméras N, Després JP, et al. Circulating endocannabinoid levels, abdominal adiposity and related cardio-metabolic risk factors in obese men. *Int J Obes* 2007; 31: 692-699.
28. de La Serre CB, Ellis CL, Lee J, Hartman A L, Rutledge J C , Raybould H. E. Propensity to high-fat diet induced obesity in rats is associated with changes in the gut microbiota and gut inflammation. *Am J Physiol* 2010; 299: G440-448.
29. Everard A, Belzer C, Geurts L, Ouwerkerk JP, Druart C, Bindels LB, et al. Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. *Proc Natl Acad Sci USA* 2013; 110: 9066-9071.
30. Festi D, Schiumerini R, Eusebi LH, Marasco G, Taddia M , Colecchia A. Gut microbiota and metabolic syndrome. *World J Gastroenterol* 2014; 20(43): 16079-16094.
31. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, et al. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* 2007; 56(7):1761–1772.
32. Akira S, Uematsu S , Takeuchi O. Pathogen recognition and innate immunity. *Cell* 2006, *124*, 783–801.
33. Tsukumo DM, Carvalho-Filho MA, Carvalheira JB, Prada PO, Hirabara SM, Schenka AA, et al. Loss-of-function mutation in Toll-like receptor 4 prevents diet-induced obesity and insulin resistance. *Diabetes* 2007; *56*, 1986–1998.
34. Carvalho-Filho MA, Ueno M, Hirabara SM, Seabra AB, Carvalheira JB, de Oliveira MG, et al. *S*-nitrosation of the insulin receptor, insulin receptor substrate 1, and protein kinase B/Akt: A novel mechanism of insulin resistance. *Diabetes* 2005; *54*, 959–967.
35. Caricilli AM, Nascimento PH, Pauli JR, Tsukumo DM, Velloso LA, Carvalheira JB, et al. Inhibition of Toll-like receptor 2 expression improves insulin sensitivity and signaling in muscle and white adipose tissue of mice fed a high-fat diet. *J. Endocrinol.* 2008; *199*, 399–406.
36. Rabot S, Membrez M, Bruneau A, Gérard P, Harach T, Moser M, et al. Germ-free C57BL/6J mice are resistant to high-fat-diet-induced insulin resistance and have altered cholesterol metabolism. *FASEB J.* 2010; *24*, 4948–4959.
37. Schertzer JD and Klip A. Give a NOD to insulin resistance. *Am. J. Physiol. Endocrinol. Metab.* 2011; *301*, E585–E586.
38. Zhao L, Hu P, Zhou Y, Purohit J and Hwang D. NOD1 activation induces proinflammatory gene expression and insulin resistance in 3T3-L1 adipocytes. *Am. J. Physiol. Endocrinol. Metab.* 2011; *301*, E587–E598.

39. Puddu A, Sanguineti R, Montecucco F and Viviani GL. Evidence for the Gut Microbiota Short-Chain Fatty Acids as Key Pathophysiological Molecules Improving Diabetes. Hindawi Publishing Corporation. *Mediators of Inflammation* 2014; Article ID 162021, 9.
40. Lau E, Carvalho D, Pina-Vaz C, Barbosa JA , Freitas P. Beyond gut microbiota: understanding obesity and type 2 diabetes. *Hormones* 2015; 14(3):358-369.
41. Koeth RA, Levison BS, Culley MK, Buffa JA, Wang Z, Gregory JC, et al. Gamma-butyrobetaine is a proatherogenic intermediate in gut microbial metabolism of L-carnitine to TMAO. *Cell Metab.* 2014;20:799–812.
42. Queipo-Ortuño MI, Seoane LM, Murri M, Pardo M, Gomez-Zumaquero JM, Cardona F, et al. Gut microbiota composition in male rat models under different nutritional status and physical activity and its association with serum leptin and ghrelin levels. PLoS One. 2013, 28;8(5):e65465.
43. Kang SS, Jeraldo PR, Kurti A, Miller ME, Cook MD, Whitlock K, et al. Diet and exercise orthogonally alter the gut microbiome and reveal independent associations with anxiety and cognition. *Mol Neurodegener*. 2014;9:36.
44. Carvalho BM, Guadagnini D, Tsukumo DM, Schenka AA, Latuf-Filho P, Vassallo J, et al. Modulation of gut microbiota by antibiotics improves insulin signalling in high-fat fed mice. *Diabetologia*. 2012;55:2823–34.
45. Trasande L, Blustein J, Liu M, Corwin E, Cox LM , Blaser MJ. Infant antibiotic exposures and early-life body mass. *Int J Obes* (Lond). 2013; 37:16–23.
46. Mueller NT, Whyatt R, Hoepner L, Oberfield S, Dominguez-Bello MG, Widen EM, et al. Prenatal exposure to antibiotics, cesarean section, and risk of childhood obesity. *Int J Obes* (Lond). 2014.
47. Vrieze A, Out C, Fuentes S, Jonker L, Reuling I, Kootte RS, et al. Impact of oral vancomycin on gut microbiota, bile acid metabolism, and insulin sensitivity. *Journal of Hepatology* 2014; 60 824–831.
48. Jernberg C, Lofmark S, Edlund C , Jansson JK. Long-term ecological impacts of antibiotic administration on the human intestinal microbiota. *ISME J*. 2007;1:56–66.
49. Han JL , Lin HL. Intestinal microbiota and type 2 diabetes: from mechanism insights to therapeutic perspective. *World J Gastroenterol* 2014; 20(47): 17737-17745.
50. Liou AP, Paziuk M, Luevano JM, Machineni S, Turnbaugh PJ and Kaplan LM. Conserved shifts in the gut microbiota due to gastric bypass reduce host weight and adiposity. *Sci Transl Med*. 2013; 5:178ra141.
51. Li JV, Ashrafian H, Bueter M, Kinross J, Sands C, le Roux CW, et al. Metabolic surgery profoundly influences gut microbial-host metabolic cross-talk. *Gut* 2011; 60: 1214-1223.
52. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol* 2014; 11: 506_14.
53. Thygesen JB, Glerup H and Tarp B. *Saccharomyces boular diifungemia* caused by treatment with a probioticum. *BMJ Case Rep* 2012; doi:10.1136/bcr.06. 2011.4412.
54. Ejtahed HS, Mohtadi-Nia J, Homayouni-Rad A, Niafar M, Asghari-Jafarabadi M and Mofid V. Probiotic yogurt improves antioxidant status in type 2 diabetic patients, *Nutrition* 2012;28 : 539–543.
55. Roberfroid M, Gibson GR, Hoyles L, McCartney AL, Rastall R, Rowland I, et al. Prebiotic effects: metabolic and health benefits. *British Journal of Nutrition* 2010;104: S1-S63.
56. Roberfroid M. Prebiotics: the concept revisited. J *Nutr* 2007; 137: 830S_7S.
57. Parnell JA , Reimer RA. Prebiotic fibres dose-dependently increase satiety hormones and alter Bacteroidetes and Firmicutes in lean and obese JCR: LA-cp rats. *Br J Nutr* 2012; 107: 601-613.
58. Neyrinck AM, Possemiers S, Druart C, Van de Wiele T, De Backer F, Cani PD, et al. Prebiotic effects of wheat arabinoxylan related to the increase in bifidobacteria, Roseburia and Bacteroides/ Prevotella in diet-induced obese mice. *PLoS One* 2011; 6: e20944.
59. Macfarlane G, Steed H , Macfarlane S. Bacterial metabolism and health-related effects of galacto-oligosaccharides and other prebiotics. *J Appl Microbiol* 2008; 104: 305_44.
60. Guarner F. Studies with inulin-type fructans on intestinal infections, permeability, and inflammation. *J Nutr* 2007; 137:2568S–2571S.
61. Cani PD, Neyrinck AM, Fava F, Knauf C, Burcelin RG, Tuohy KM, et al. Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. *Diabetologia* 2007; 50: 2374-2383.
62. Vrieze A, Van Nood E, Holleman F, Salojärvi J, Kootte RS, Bartelsman JF, et al. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology* 2012; 143(4):913.

Peer reviewer: Rashed Hasan, Professor 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.

Image Case: Visualization of moving living adult worm (Entrobious) during total colonoscopy

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

Visualization of moving living adult worm(Entrobious) during total colonoscopy for 42 yrs.old male complaining of chronic diarrhea with abdominal pain.