# 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**

**Editor-in-Chief:**
Mohamad El-Khashab
E-mail: ajied@zu.edu.eg
elkhashab2005@hotmail.com

**Co-Editor-in-Chief:**
Mohamad Emam
E-mail: ajied@zu.edu.eg
rana4emo90@yahoo.com

**Executive Editor:**
Tarik Zaher
E-mail:ajied@zu.edu.eg
tareqzaher@zu.edu.eg

**Assistant Editors:**
Sahar Elnimr
E-mail: ajied@zu.edu.eg
alnimrsahar@yahoo.com
Mohamad Emara
E-mail:ajied@zu.edu.eg
emara_20007@yahoo.com

**Editorial Board:**

**Zagazig University,Egypt:**
Hamed Suliman,Endemic and Tropical Medicine
Amr Murad,Endemic and Tropical Medicine
Faiza Elgohary ,Endemic and Tropical Medicine
Salama Elghoniemy,Endemic and Tropical Medicine
Ahmad Mahmoud,Endemic and Tropical Medicine
Samy Eisa,Endemic and Tropical Medicine
Ibrahim Hegazy,Endemic and Tropical Medicine
Nahla Elgammal,Endemic and Tropical Medicine
Mohamad Abdel-Tawab,Endemic and Tropical Medicine
Rashed Hasan,Endemic and Tropical Medicine
Mostafa Elshamy,Endemic and Tropical Medicine
El-Said Elbadrawy,Endemic and Tropical Medicine
Amira Suliman,Endemic and Tropical Medicine
Eman Abdel-Aal,Endemic and Tropical Medicine
Maged Bahgat,Endemic and Tropical Medicine
Walid Abdel-Dayem,Endemic and Tropical Medicine
Abeer Nafee,Endemic and Tropical Medicine
Ahmad Sakr,Endemic and Tropical Medicine
Soha Esmat,Endemic and Tropical Medicine
Ghada Salem,Endemic and Tropical Medicine
Hala Ismail,Endemic and Tropical Medicine
Gehan Shawqy,Endemic and Tropical Medicine
Mohamad Refaey,Endemic and Tropical Medicine
Sherief Galal,Endemic and Tropical Medicine
Mohamad Radwan,Endemic and Tropical Medicine
Samah Telep,Endemic and Tropical Medicine
Tagrid Abdallah,Endemic and Tropical Medicine
Nagla Abdel-Monem,Endemic and Tropical Medicine
Noha Shaheen,Endemic and Tropical Medicine
Soha Elhawary,Endemic and Tropical Medicine
Talaat Fathy,Endemic and Tropical Medicine
Mohamad Radwan,Endemic and Tropical Medicine
Reda Lami,Parasitology
Samia Etewa, Parasitology
Mohiddin Abdel-Fattah,Parasitology
Alaa Elgendy,Parasitology
Ahmad Shaheen,Microbiology
Ayman Marii,Microbiology
Shimaa Abdel-Azim,Microbiology
Marwa Abdel-Azim,Microbiology
Rehab El-Sokary,Microbiology
Rehab El-Saiid,Microbiology
Mahmoud Wahid,Pathology
Sahar Zaglol,Internal Medicine
Khaled Talaat,Internal Medicine
Amany Ibrahim,Internal Medicine
Ahmad Refaat,Medical Statistics
Tarek Hamed ,Pediatrics
Mohamad Sand ,Pediatrics
Mohamad Abdel-Raoof, Physiology
Shreen Elaraby,Physiology
Heba Pasha,Biochemistry and Molecular Biology
Randa Hussini ,Biochemistry and Molecular Biology
Rasha Hussini ,Biochemistry and Molecular Biology

**Cairo University,Egypt:**
Ahmad El-Garem,Endemic and Tropical Medicine
Shukry Hunter,Endemic and Tropical Medicine
Sohir Zakaria,Endemic and Tropical Medicine
Laila Ahmad, Endemic and Tropical Medicine
Hosny Salama,Endemic and Tropical Medicine
Ayman Yousry, Endemic and Tropical Medicine

**Ain Shams University,Egypt:**
Fawzy Montasir,Endemic and Tropical Medicine
Ramadan Baddar,Internal medicine
Amr Fateen,Internal Medicine
Mahmoud Osman,Internal Medicine
Reda El-Wakil,Endemic and Tropical Medicine

**Mansura University, Egypt:**
Gamal Sheha,Internal Medicine
Magdy Hamed,Internal Medicine

**Tanta University,Egypt:**
Saber Ismail,Endemic and Tropical Medicine
Abdel-Raoof Abu-Elazm,Endemic and Tropical Medicine
Mohamad Sharaf,Endemic and Tropical Medicine

Nadia Elwan, Endemic and Tropical Medicine

**Assiut University, Egypt:**
Ahmad Nasr,Endemic and Tropical Medicine
Othman Abdel-Hamid Othman , Endemic and Tropical Medicine

**Benha University, Egypt:**
Samir Qabil,Endemic and Tropical Medicine
Magdy Atta,Endemic and Tropical Medicine

**Military Medical Academy,Egypt:**
Mamdouh Elbahnasawy,Endemic and Tropical Medicine

**Sudan:**
Amin A. Elzaki, Radiology
Mustafa Z. Mahmoud, Radiology

**Nigeria:**
Adeolu O. Akinboro, Dermatology

**Greece:**
Angela Revelas, Pathology

**Saudi Arabia**
Osman Elwerwary ,Endemic and Tropical Medicine
Maysaa Saed,Endemic and Tropical Medicine
Mohamed Nasr Eldin Bekhit ,Endemic and Tropical Medicine
Usama Rushdy ,Endemic and Tropical Medicine
Mohamed Hassona, Endemic and Tropical Medicine

**Kuwait**
Mohamad Saria,Endemic and Tropical Medicine
Mohamad Alboray,Internal Medicine

**Yemen**
Abd Elhafez Alsady,Intrernal Medicine
Mostafa Mahmoud,Cardiology

**Morocco**
Zineb Tlamcani ,parasitology

**Secretary:**
Mohamad Radwan,Endemic and Tropical Medicine
Ihab Darwish,Endemic and Tropical Medicine
Ashraf Metwaly,Endemic and Tropical Medicine
Ahmad Behiry, Endemic and Tropical Medicine
Hosam Dawood, Endemic and Tropical Medicine
Sherwet Sahlol, Endemic and Tropical Medicine
Sameh Mahmoud, Endemic and Tropical Medicine
Ahmad Farok, Endemic and Tropical Medicine
Ibrahim Mohamad , Endemic and Tropical Medicine
Amal Abdel-Fattah, Endemic and Tropical Medicine
Said Saad, Endemic and Tropical Medicine
Mohamad Ibrahim, Endemic and Tropical Medicine

**E-Archiving:**
Abeer Hasan
Besheer Helmy
Emad Abdel-Hamid
Ahmad Elgebaly
Nabila Hasan
Kamal Amer
Ahmad Abdel-Razik
Ahmad Attia
Ahmad Saiid
Ahmad Lotfy
Shereif Bahnasawy
Abdel-Monim Elshamy
Ahmad Abulkhir
Dena Mohamad
Sara Refaee
Shimaa Abdel-Fattah
Ramy Elhendawy
Mona Amin
Marwa Attia
Mahmoud Khalil
Marwa Ayesh
Mona Abdelmaksoud
Nada Maher
Mohamad Fouad
Mohamad Abdalla
Shreif Sowilem
Ahmad Khaled
Reham Abdelal

**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 2012: 4.47):
http://journals.indexcopernicus.com/masterlist.php?q=Afro-Egyptian+Journal+of+Infectious+and+Endemic+Diseases

5- **Global Impact Factor**
http://globalimpactfactor.com/journals-list/?snap=A

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

# Contents

ORIGINAL ARTICLES

Percutaneous injection of Acetic Acid and Mitoxantrone Versus Radiofrequency Ablation in Treatment of Hepatocellular Carcinoma
**Moustafa EA, Hegazy IM, Hassan RM,Nawar NE, El-Shamy MH, Elhawari SA** **53**

Study of TUBEX as a Rapid Diagnostic Test of Typhoid Fever
**El-Deeb GS, Seleem HEM,Tawfeek GA, Emara SS** **62**

Antinuclear Antibody Positivity in Chronic Hepatitis C Patients: Effect on Histopathology and Impact on Early Response to Combined Antiviral Therapy
**Younes YS, Shaheen YA, El Feky HM ,Omar MZ, Eldesouky RSH, Amer RZ, Mabrouk RI** **69**

Study of the Relationship between Blood Ammonia Level and Esophageal Varices in Patients with Liver Cirrhosis
**Abo-Alsoud AA, Badawy AM Sonbol AA , Ayad ME** **78**

Study of the Pattern of Brucellosis in Menoufyia Governorate
**Abo EL-Soud A,El-Lehleh AM,Shehab El-Deen SA,El-Hendy AA, Mousa EMM** **86**

Changes in CD4 and CD8 after Interventional Management of Hepatocellular Carcinoma
**El-Sherbiny W,Abousamra NK,Diasty M, Shaltout SW** **94**

Role of Insulin Resistance and Cytokeratin 18 on the Recurrence of Hepatocellular Carcinoma after Radiofrequency Ablation
**Khorshed SE , Fayed A , Kamel LM , Awad SM** **102**

Nurses Knowledge and Practice Regarding Gastrointestinal Endoscopy and Suggested Nursing Guidelines
**Amer WM, Taha NM, Zaton HK** **115**

CASE REPORT

Spontaneous Cryptococcal Peritonitis Successfully Treated with Fluconazole
**Saeed MA, Al Khuwaitir TSA** **131**

# Percutaneous injection of Acetic Acid and Mitoxantrone Versus Radiofrequency Ablation in Treatment of Hepatocellular Carcinoma

**Emad A Moustafa[1], Ibrahim M Hegazy[1], Rashed M Hassan[1], Nashwa E Nawar[2], Mostafa H El-Shamy[1], Soha A Elhawari[1]**

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

[2]*Clinical Oncology and Nuclear Medicine Department, Faculty of Medicine, Zagazig University, Egypt*

*Corresponding Author*
***Emad A Moustafa***

*Mobile:*
*+01003725577*

*E mail:dr.emad777@yahoo.com*

*Key words:*
*Hepatocellular carcinoma, mitoxantrone,* radiofrequency ablation

**Background and study aim:** Hepatocellular carcinoma (HCC) is currently the fifth most common solid tumor worldwide and the third leading cause of cancer-related death. New therapeutic choices have been developed for HCC, including percutaneous ablation therapy, transarterial chemoembolization and molecular target therapy. Percutaneous acetic acid injection (PAI) and radio-frequency ablation (RFA) techniques became well-known procedures for controlling small HCC.The aim of this study was to compare the outcomes of per-cutaneous combined PAI and mitoxantrone injection versus RFA in the treatment of HCC.

**Patients and Methods:** This prospective study was conducted on 120 patients with 120 focal nodular HCCs of 4 cm or less between 2012 and 2014. They were randomly divided into 2 groups, the first group included 60 patients treated with PAI plus percutaneous intratumoral injection of mitoxantrone, and the second group included 60 patients treated with radio-frequency ablation. Clinical assessment, laboratory evaluation and triphasic CT studies were performed to all patients pre-treatment and at 1,3,6 and 12 months post treatment and complications were recorded.

**Results:** The percentage of ablation in both groups at 1, 3, 6 and 12 months were 85%, 83.33%,78.34 and 73.33% in group I versus 88.33%, 88.3%, 85% and 81.66% in group II respectively with no statistical significant difference between the two groups. Percentage of ablation in small tumors was higher than large tumors in both groups. Side effects and complications are statistically higher in group II than group I.

**Conclusion:** Combination of PAI and Mitoxantrone is comparable to radiofrequency ablation in treatments of HCCs with less frequent complications.

## INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common form of cancer worldwide and the third most common cause of cancer-related deaths. HCC often occurs in the background of a cirrhotic liver **[1]**. Early detection strategies have increased the number of small HCC amenable to curative treatment**[2].**

The Barcelona Clinic Liver Cancer classification **[3]** is the most frequently utilized classification for management of HCC **[4]**. With early-stage tumours, potentially curative therapies are used: ablation therapy with (1) percutaneous ethanol injection (PEI) (2) acetic acid injection (PAI) or (3) radiofrequency ablation (RFA), and surgical resection or liver transplantation. These treatments provide better survival rates at 5-years of 40-70% vs <20% for untreated patients; however, they are applicable in only 30-40% of patients with HCC **[5,6].**

Percutaneous ablation under ultrasound guidance is currently the best therapy for early-stage HCC when resection or liver transplantation is not possible **[7]**. RFA is currently considered the most effective local ablative therapy **[8]**. It causes coagulative necrosis of the liver tumor by using electric heating around a probe generating electro-magnetic radiation **[9].**

Acetic acid used as a 50% solution is as cheap as alcohol and in contrast penetrates and destroys intra-nodule septa because of its low pH **[10]** and breaks down lipid and collagen fibres within intra-tumoural septa and capsules which often contain cancer cells. PAI is performed as easily and safely as PEI but requires fewer treatment sessions **[11].**

Mitoxantrone is a cycle specific anthracyclin which induces persistent intracellular DNA damage. It is used as an anticancer agent and has demonstrated clinical activity when administered via multiple routes: intravenous, intraperitoneal, intrapleural, intrapericardial, or intrathecal **[12].** Mitoxantrone was selected for palliative local treatment of malignant liver lesions because of its low tissue toxicity, high intratumoral concentration after intratumoral instillation, since it has a tendency to remain at the application site **[13, 14].** In 1998, Farrés et al**. [15]** concluded that in patient with malignant liver lesions, minimally invasive intratumoral mitoxantrone injection was carried out safely with good tumor delivery of chemotherapy, and tumor necrosis was demonstrated at biopsy, but they advised further investigations .

The aim of this study was to assess the efficacy and safety of combined PAI and intralesional mitoxantrone versus radiofrequency ablation in treatment of HCC.

## PATIENTS AND METHODS

This prospective study was conducted in Tropical Medicine Department in cooperation with Clinical Oncology & Nuclear Medicine Department Faculty of Medicine, Zagazig University, Egypt, during the period from March 2012 to September 2014 and included 120 patients presented with 120 focal hepatocellular carcinoma lesions. Sample size estimation was performed by the Institutional Review Board (IRB). The lesions were randomly divided into 2 groups.

**Group I**: **(acetic acid and mitoxantrone group)** consisted of 60 patients (46 males and 14 females) treated by percutaneous acetic acid injection therapy (PAI) performed at multiple sessions according to the volume estimated, followed by intralesional single injection with mitoxantrone.

**Group II**: **(radiofrequency ablation group)** consisted of 60 patients (51 males and 9 females) treated by percutaneous RFA.

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

All patients met the enrolment criteria: (i) tumor of 4 cm or less in diameter, (ii) liver cirrhosis classified as Child-Pugh class A or B, (iii) platelet counts >50000/mm$^3$, (iv) prothrombin concentration >60% or INR < 1.5, (v) no evident ascites and (vi) Performance status 0-2.

Patients with a Child-Pugh class C, previous history of treatment for HCC, vascular invasion, lymph node or distant metastasis were excluded.

### Pretreatment assessment

Pre-treatment assessment of all patients was done by full history taking, thorough clinical examination, laboratory investigations including CBC, liver function, kidney function, α fetoprotein, and serological markers for HCV and HBV. Radiological examination including ultrasound, triphasic CT study, and ultrasound guided biopsy when indicated.

### Technique of Acetic Acid Ablation

Treatment was performed with the patient under conscious sedation. Injection of acetic acid was performed under real-time US guidance (**esaote MyLab20Plus**) using a 3.5 MHz probe by free hand technique. Sterile 50% acetic acid was injected with a 18-gauge spinal needle.

Typically, one injection at a dose of 5–10 ml acetic acid was given during each treatment session. Acetic acid was slowly injected until the echogenic area appearing immediately after injection covered the entire tumor. After the injection was completed, the needle was left in place for 1–2 min then injection of local anesthetic during withdrawal to minimize the irritant effect of acetic acid reflux on the liver capsule **[16]**.

50% acetic acid was injected at a volume of 1–10 mL per session and total volume was estimated using the modified equation: $V = 1/3\{4/3\pi\ (r + 0.5)^3\}$ where $V$ is the total volume of acetic acid in milliliters (mL) and $r$ is the radius of each tumor in centimeters (cm) and 0.5 cm is added to provide a safety margin of ablation **[10]**.

Four to six sessions were given for lesions. There was no need to give prophylactic antibiotics. Treatment was administered once a week in an outpatient setting.

**Mitoxantrone injection:**
This was done to patients of group I after complete sessions of acetic acid. Ultrasound guided injection of mitoxantrone mixed with lipidol at the time of injection in a single session; the dose of mitoxantrone is 0.5 mg per cubic centimeter of the tumor size. Re-evaluation of the patients was done by laboratory investigations, ultrasound and triphasic CT after treatment and every 3 moths up to one year.

**Technique of RFA :**
The technique was the same with the addition of a small opening is done into the skin using a scalpel (No 11). We used a common, commercially available RFA technique and system (RITA 1500X RF generator and RITA StarBurst XL, RITA Medical Systems, Mountain View, California). Grounding was achieved by attaching 2 pads to the patient's thighs. After administration of analgesia as well as local anesthesia, the electrode needles were introduced into the tumor under ultrasonographic guidance, then a gradual unfolding of the electrodes was obtained, and the generator was activated to achieve RF energy and maintain an average temperature of 105°C. At first, the electrodes were moved by 2 cm, then the electrode needles were pushed forward and unfolded gradually to 3 cm, 4 cm and 5 cm until they reached or crossed the borders of the tumor according to the ablation range, delivering RF energy for 5 minutes for every intermediate step and for 7 to 10 minutes in the final step of the procedure. The ablation area was intended to cover the tumor as well as at least 0.5 to 1.0 cm of the surrounding tissue **[17]**.

Following therapy, patients were put under observation for 6 hours where vital signs were checked every half-hour.

**Assessment of therapeutic response and follow-up**
Included all the investigations that were done before procedure. AFP and triphasic spiral CT were done after one month and every three months up to one year. The response to treatment was rated as complete when dynamic CT scans showed no contrast enhancement inside the lesion in the arterial phase. The response was rated as partial when dynamic CT showed areas of enhancement within the boundaries of the original lesion in the arterial phase **[18]**.

***Follow up of the patients*** of the two groups was done for one year with special emphasis on recurrence of HCC, any remote complications related to both procedure, development of liver decompansation (ascites, jaundice, encephalopathy, bleeding tendency), haematemesis, or death.

**Statistical Analysis :**
Data were checked, entered and analyzed using SPSS 15 for Windows. Data were expressed as mean ± SD for quantitative variable, number and percentage for qualitative one. Chi-squared (X2) or fisher exact, t test and paired t test were used when appropriate. $P<0.05$ was considered significant.

## RESULTS

In our study, no significant differences were observed between both groups with respect to the following baseline characteristics: patient age and sex; Child Pugh class; proportions of patients positive for hepatitis C virus antibody and positive for hepatitis B surface antigen (Table 1).

The biochemical profile in our study (performed before and one month after the end of sessions) showed no statistically significant difference as regard all parameters in PAI and mitoxantron group, while in RFA group, αFP and ALT show statistically significant improvement in those patients after the procedure (P=0.042 and 0.001 respectively).

Concerning the complications encountered in our study as shown in table (2). The most frequent complication was intolerable pain (needs analgesics) which was significantly higher in group II (45%) than in group I (16.6%). Other complications; vomiting, fever and pleural effusion were comparable in both groups. All these complications were controlled by conservative management.

Regarding primary success (complete ablation): After one month, there was no statistically significant difference between both groups regarding procedure success (Table 3).

Regarding endpoint of our study: there was no statistically significant difference among patients of studied groups as regards stationary ablation (cancer free survival), local recurrence rate and overall survival. By the end point of the study; in group I; 7(11.67%) patients died due to terminal hepatic failure as a result of multifocal hepatoma (4 patients), and hepatorenal syndrome after spontaneous bacterial peritonitis (3 patients). While 5 patients were discontinued due to develop of new focal lesion (4 patients) and develop of decompensation (1 patient). On the other hand,

in group II; 5 (8.33%) patients died due to terminal hepatic failure as a result of multifocal hepatoma (3 patients), and repeated attacks of bleeding (2 patients). While 5 patients were discontinued due to develop of new focal lesion (4 patients) and develop of decompensation (1 patient) (Table 4).

As regard to the lesion size, although the difference was statistically not significant but, Most of the ablated focal HCC lesions of both groups were less than 3 cm (Table 5).

**Table (1)** General features of the two groups:

| | Group I (n=60) | | Group II (n=60) | | Total (N=120) | | P value |
|---|---|---|---|---|---|---|---|
| **Age** Mean ± SD | 57.18 ± 5.24 | | 54.60 ± 3.98 | | | | 0.071 |
| **Gender** | **No** | **%** | **No** | **%** | **No** | **%** | |
| Male | 46 | 76.67 | 51 | 85 | 97 | 80.8 | 0.782 |
| Female | 14 | 23.33 | 9 | 15 | 23 | 19.2 | |
| **Viral markers** | | | | | | | |
| +ve HCV | 52 | 86.67 | 54 | 90 | 106 | 88.4 | 0.762 |
| +ve HBV | 7 | 11.67 | 6 | 10 | 13 | 10.8 | |
| HCV & HBV | 1 | 1.66 | 0 | 0.0 | 1 | 0.8 | |
| **Child-Pugh** | | | | | | | |
| Child A | 34 | 56.67 | 31 | 51.67 | 65 | 54.17 | 0.456 |
| Child B | 26 | 43.33 | 29 | 48.33 | 55 | 45.83 | |

**Table (2):** Complications related to both techniques among both studied groups.

| Complication | Group I (n=60) | | Group II (n=60) | | Total (N=120) | | P value |
|---|---|---|---|---|---|---|---|
| | **No** | **%** | **No** | **%** | **No** | **%** | |
| Intolerable pain | 10 | 16.67 | 27 | 45.0 | 37 | 30.8 | 0.001 |
| Vomiting | 4 | 6.67 | 7 | 11.67 | 11 | 9.2 | 0.526 |
| Fever | 9 | 15.0 | 9 | 15.0 | 18 | 15.0 | 0.798 |
| Pleural effusion | 2 | 3.33 | 3 | 5.0 | 5 | 4.2 | 1.000 |
| Haematemesis | 0 | 0.0 | 1 | 1.67 | 1 | 0.8 | 1.000 |
| Ascites (controllable) | 6 | 10.0 | 2 | 3.33 | 8 | 6.7 | 0.272 |
| Decompensation (Child C) | 2 | 3.33 | 2 | 3.33 | 4 | 3.3 | 0.611 |
| No complication | 27 | 45.0 | 9 | 15.0 | 36 | 30 | <0.001 |
| **P value** | 0.004 | | | | | | |

**Table (3):** Follow up the success rate of both procedures after one month.

| According to spiral CT | Group I (n=60) | | Group II (n=60) | | P value |
|---|---|---|---|---|---|
| | **No** | **%** | **No** | **%** | |
| Complete ablation | 51 | 85 | 53 | 88.33 | 0.432 |
| Partial ablation | 9 | 15 | 7 | 11.67 | |

**Table (4):** End point of both studied groups after one year follow up.

| | Group I (n=60) | | Group II (n=60) | | P value |
|---|---|---|---|---|---|
| | No | % | No | % | |
| Stationary ablation (cancer free survival) | 44 | 73.33 | 49 | 81.66 | 0.381 |
| Local recurrence | 4 | 6.67 | 1 | 1.67 | 0.360 |
| Discontinued cases | 5 | 8.33 | 5 | 8.33 | 0.741 |
| Total deaths | 7 | 11.67 | 5 | 8.33 | 0.760 |
| Overall survival | 53 | 88.33 | 55 | 91.67 | 0.760 |

**Table (5):** Outcome of the study in relation to HCC lesion diameter in both studied groups one year after treatment.

| | Group I | | | | Group II | | | |
|---|---|---|---|---|---|---|---|---|
| | Size <3cm (n=27) | | Size ≥ 3cm (n=33) | | Size <3cm (n=38) | | Size ≥ 3cm (n=22) | |
| | No | % | No | % | No | % | No | % |
| Stationary ablation (cancer free period) | 22 | 81.5 | 22 | 66.67 | 33 | 86.84 | 16 | 72.72 |
| Local recurrence | 1 | 3.7 | 3 | 9.09 | 1 | 2.63 | 0 | 0.0 |
| Total deaths | 3 | 11.11 | 4 | 12.12 | 2 | 5.26 | 3 | 13.64 |
| Overall survival | 24 | 88.89 | 29 | 87.87 | 36 | 94.73 | 19 | 86.3 |
| P value | 0.554 | | | | 0.154 | | | |

## DISCUSSION

Percutaneous Ablation is the best treatment option for patients with early stage HCC who are not suitable for surgical resection (SR) or transplantation **[19]**.

The advantages of PAI are that it is easy to perform and have greater safety and tolerance than RFA. However, RFA has the advantage of requiring fewer treatment sessions and yielding a higher rate of complete tumour necrosis and local recurrence free survival at the risk of a higher rate of major complications **[20,21].**

Previous literature reporting the therapeutic efficacy of PAI is rather limited, and few studies have specifically compared the therapeutic efficacy between RFA and PAI for HCC **[22].**

Till the time this study is planned for in March 2012, few studies had been published to evaluate effect of percutaneous radiofrequency ablation and injection of acetic acid in treatment of HCC, but only one study evaluating percutanous injection of mitoxantrone in treatment of HCC was published by Farre et al. **[15]** and only two studies- to our knowledge-evaluated the effect of percutanous injection of combined ethanol and mitoxantrone in treatment of HCC **[23,24].**

RFA is generally considered a relatively low risk procedure **[25].** In this study, although the incidence of complications was significantly higher in the RFA group, no major complications apart from single case of haematemesis (0.8%) and no RFTA related deaths occurred and most complications were minor and mainly transient.

This was not in agreement with Curley et al. **[26],** Poon et al. **[27]** and Huo et al. **[28]** who rate major complications of 13.1%, 17% and 9.2% respectively. The difference in major complication is attributed to selection of patients and experience of the operator. The occurrence of major complication as haemothorax and haemoperitoneum in subcapsular tumors where injury of the pleura and capsule of the liver is due to a technical error and bad selection of subcapsular lesions.

Here, we must point out that some of the complications observed could be due to the effect of the learning curve **[27],** and professionals' differing degree of experience in RFA **[29].**

PAI causes ablation of HCC in 89% of cases selected by Ohnishi et al. **[16].** PAI followed by local injection of mitoxantrone resulted in 85% ablation rate in our study. On the other hand El-Kady et al. **[30]** ablated 75% of HCC cases using acetic acid injection **[30].** The difference in these results may be attributed to patient's selection criteria and tumor size in each study.

The complete ablation rate of combined PAI plus mitoxantrone was comparable with RFA results 85% versus 88.3% respectively with no statistically significant difference between both groups in our study. However El-Kady et al. **[30]** found statistically significant difference between PAI and RFA in his study. This difference between both studies could be attributed to the size of the lesion or to the additive DNA damaging effect of mitoxantrone on HCC after completion of PAI. The initial injection of acetic acid leads to blockage of the blood vessels of the tumors which in turn leads to persistence of mitoxantron in the tumor in high concentration and prevent its systemic effect.

Ohishi and his colleagues **[31]** stated that Intratumoral instillation of mitoxantrone results in a 1000-fold higher concentration in the tumor compared with intravenous administration, moreover ; lipidol have high affinity to malignant hepatocyts so it increase the duration and efficacy of mitoxantrone. After that Farre et al. **[15]** selected mitoxantrone for local treatment of malignant liver lesions because of its low tissue toxicity, high intratumoral concentration after intratumoral instillation, and long time in the tumor, since it has a tendency to remain at the application site **[14].** The histological effects of locoregional mitoxantrone treatment were evaluated by Hoffmann et al. **[32]** and characterized by complete tumor necrosis in which dead tumor cells are surrounded by an inflammatory infiltrate and a fibrotic organization of liver tissue around the tumor.

Also we can't neglect the effect of acetic acid on the tumor tissue as it has a strong ability to penetrate cells and can dissolve lipids and extract collagen from intra-tumoral septa and capsules that frequently contain viable cancer cells **[11]**, leading to more localization and hence more effect of mitoxantrone on malignant tissue.

Our results also showed that overall survival was not significantly different but higher in the RFTA group (91.67%) than in the PAI and mitoxantrone group (88.33%). This finding was in agreement with that of Lin et al. **[22]** in which the one year survival was 93% and 90% in RFA group and PAI group respectively. The cause of death was HCC progression in most cases. Therefore, a more effective local treatment such as RFA can achieve lower HCC recurrence and consequently contributes to better survival.

Our results were close to the study done by Guglielmi et al**. [33]**. They had found the survival rate of patients after treatment were 87% after 1 year. Survival was significantly related to Child Pugh class After 3 years survival was 83% in Child Pugh A cirrhotic patients and 31% in Child Pugh B patients.

Cancer free survival reflects local recurrence and new tumour formation elsewhere in the liver. Because lower recurrence was lower in the RFA group therefore the cancer free survival rate was also higher in RFTA group than in the other group in this study.

The local recurrence rate was higher in patients with HCCs larger than 3 cm. The independent factors related to local recurrence were large tumour size (>3 cm). This result was consistent with that of Komorizono and colleagues **[34]**. A larger tumour usually has a higher rate of local recurrence because it frequently requires multiple overlapping ablations, and targeting of its viable foci is difficult because of lack of clarity of the image obtained between the ablated and non-ablated tumour after repeated ablation is performed under sonography **[35]**.

Despite the wider range (1 cm safety margin) of injections of acetic acid herein, the distribution of acetic acid might be unpredictable both within the tumour and outside due to interference of the fibrous septum **[10]** and the presence of satellite nodules around the target tumour **[36]** respectively.

Therefore, a 1 cm safety margin can be achieved in patients treated with RFA but not in patients treated with PEI or PAI. This limitation of the homogenous distribution of ethanol or acetic acid around the safety margin of the target tumour may explain the benefit of lower local recurrence favouring RFA than PEI or PAI in treating HCCs larger than 3 cm in the present study or in other investigations **[10,34,22].** The rates of new HCC recurrence were also similar among the two groups, perhaps because of the similar baseline parameters.

PAI required fewer treatment sessions and smaller volume of injection materials to achieve complete tumour necrosis than PEI and provided better survival after long-term follow-up **[37].** An additional advantage of PAI therapy over PEI is its ability to destroy more effectively, in small time and not limited by a formation. In contrast, fewer injection sessions are required in PAI, because acetic acid injected into one nodule will penetrate through septa largely because of its low pH, which induces swelling of the fibers and promotes dissociation of intermolecular cross-links containing aldimine bonds of collagen in the septa **[38,39,10].**

Despite of all this advantages of PAI over PEI, yet mitoxantrone fill this gap as concluded by a study done by Helmy et al **[24]** in which the cancer free survival after one year was 71.9% in consistent with our results in which the cancer free survival after one year was 73.33%.

From this study and its results, we can observe that PAI and mitoxantrone is a very effective method for ablating HCC, with high power to penetrate the septa and the capsule. It is simple and applicable technique, this is particularly important in emerging economies where HCC is prevalent. Compared to RFA; acetic acid is cheap, readily available, besides being equally effective and safe. All these criteria enables acetic acid to be the first choice ablative procedure especially in low economic levels where facilities are minimum or lacking or when the lesions are not candidate for RFA.

## CONCLUSION

PAI followed by mitoxantrone seems to be comparable to radiofrequency ablation in the treatment of HCC.

**Funding:** None.
**Conflicts of interest:** None.
**Ethical approval:** A written informed consent was taken from all included patients, and the ethical committee of the university has accepted the study under the number of 213/12-5-2012.

## REFERENCES

1. El-Serag HB. Epidemiology of viral hepatitis and hepatocellular carcinoma. *Gastroenterology 2012*, 142: 1264-1273.
2. Bosch FX, Ribes J, Diaz M, Cleries R. Primary liver cancer: worldwide incidence and trends. *Gastroenterology* 2004, 127: S5-S16.
3. Llovet JM, Fuster J, Bruix J. The Barcelona approach: diagnosis, staging, and treatment of hepatocellular carcinoma. *Liver Transpl* 2004,10: S115-120.
4. Llovet JM, Bruix J. Novel advancements in the management of hepatocellular carcinoma in 2008. *J Hepatol* 2008, 48: S20-S37.
5. Llovet JM, Bustamante J, Castells A, Vilana R, Ayuso Mdel C, Sala M, et al. Natural history of untreated nonsurgical hepatocellular carcinoma: rationale for the design and evaluation of therapeutic trials. *Hepatology* 1999, 29: 62-67.
6. Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. *Lancet* 2003, 362: 1907-1917.
7. Germani G, Pleguezuelo M, Gurusamy K, Meyer T, Isgro G, Burroughs AK. Clinical outcomes of radiofrequency ablation, percutaneous alcohol and acetic acid injection for hepatocellular carcinoma: a meta-analysis. *J Hepatol* 2010, 52: 380-388.
8. Raza A, Sood GK. Hepatocellular carcinoma review: Current treatment, and evidence-based medicine. *World J Gastroenterol* 2014, 20(15): 4115-4127.
9. Kudo, M. Radiofrequency ablation for hepatocellular carcinoma: updated review in 2010. *Oncology* 2010, 78 (Suppl 1): 113-124.
10. Ohnishi K, Yoshioka H, Ito S, Fujiwara K. Prospective randomized controlled trial comparing percutaneous acetic acid injection and per-cutaneous ethanol injection for small hepato-cellular carcinoma. *Hepatology* 1998, 27: 67-72.
11. Huo TI, Huang YS, Wu JC. Percutaneous ablation therapy for hepatocellular carcinoma: current practice and future perspectives. *J Chin Med Assoc* 2005, 68: 155-159.
12. Zamboglou N, Wurm R, Pape H. Simultaneous radiotherapy and intratumoral instillation of mitoxantrone in locoregional recurrence of head and neck carcinoma. *Reg Cancer Treat* 1991, 4: 79-84.
13. Fox ME, Smith PJ. Subcellular localisation of the antitumour drug mitoxantrone and the induction of DNA damage in resistant and sensitive human colon carcinoma cells. *Cancer Chemother Pharmacol* 1995, 35: 403-410.
14. 14-Markman M, Alberts D, Rubin S, Hakes T, Lewis JL, Reichma B, et al. Evidence for persistence of mitoxantrone within the peritoneal cavity following intraperitoneal delivery. *Gynecol Oncol* 1993, 48: 185-188.

15. Farres MT, de Baere T, Lagrange C, Ramirez L, Rougier R, Munck JN, et al . Percutaneous Mito-xantrone Injection for Primary and Seconrary Liver Tumors. *Cardiovascular and Interventional Radiology* 1998, 21(5): 399-403.
16. Ohnishi K, Yoshioka H, Ito S, Fujiwara K. Treatment of nodular hepatocellular carcinoma larger than 3 cm with ultrasound-guided per-cutaneous acetic acid injection. *Hepatology* 1996, 24(6): 1379-1385.
17. Tanaka S, Shimada M, Shirabe K, Taketomi A, Maehara S, Tsujita E, et al. Surgical radio-frequency ablation for treatment of hepatocellular carcinoma: an endoscopic or open approach. *Hepatogastroenterology* 2009, 56(93):1169-1173.
18. Chen MH, Yang W, Yan K, Zou MW, Solbiati L, Liu JB, et al. Large Liver Tumors: Protocol for Radiofrequency Ablation and Its Clinical Application in 110 Patients – Mathematic Model, Overlapping Mode, and Electrode Placement Process. *Radiology* 2004, 232(1): 260–271.
19. Bruix J, Sherman M. AASLD practice guidlines: Management of Hepatocellular Carcinoma. *Hepatology* 2005, 42(5): 1208-1236.
20. Livraghi T, Goldberg SN, Lazzaroni S, Meloni F, Solbiati L, Gazelle GS. Small hepatocellular carcinoma: treatment with radio-frequency ablation versus ethanol injection. *Radiology* 1999, 210(3): 655-661.
21. Lencioni RA, Aligaier HP, Cioni D, Olschewski M, Deibert P, Crocetti L, et al. Small hepatocellular carcinoma in cirrhosis: randomized comparison of radiofrequency thermal ablation versus percutaenous ethanol injection. *Radiology* 2003, 228(1): 235-240.
22. Lin SM, Lin CJ, Lin CC, Hsu CW, Chen YC. Randomised controlled trial comparing per-cutaneous radiofrequency thermal ablation, per-cutaneous ethanol injection, and percutaneous acetic acid injection to treat hepatocellular carcinoma of 3 cm or less. *Gut* 2005, 54(8): 1151-1156.
23. Bihery AS, El-Shamy MH, El-Mokadem TE, Zaher TI, Abd El Dayem WA, Fathy T, et al. Percutaneous Local Injection of Ethanol and Mitoxantrone in Treatment of Hepatocellular Carcinoma. *Afro-Egypt J Infect Endem Dis* 2011, 1 (2): 28-36.
24. Helmy BM. Percutaneous Local Injection of Ethanol and Mitoxantrone versus radiofrequency ablation in the Treatment of Hepatocellular Carcinoma. MD Thesis. Tropical Medicine Department, Faculty of Medicine, Zagazig University, Egypt. 2015, P.: 129-43.
25. Livraghi T, Solbiati L, Meloni MF, Gazelle GS, Halpern EF, Goldberg SN. Treatment of focal liver tumors with percutaneous radio-frequency ablation: complications encountered in a multicenter study. *Radiology* 2003, 226(2): 441-451.
26. Curley SA, Izzo F, Ellis LM, Nicolas Vauthey J, Vallone P. Radiofrequency ablation of hepatocellular cancer in 110 patients with cirrhosis. *Ann Surg*. 2000, 232(3): 381-391.
27. Poon RT, Ng KK, Lam CM, Ai V, Yuen J, Fan ST. Effectiveness of radiofrequency ablation for hepatocellular carcinomas larger than 3 cm in diameter. *Arch Surg*. 2004,139(3): 281-287.
28. Huo TI, Huang YH, Huang HC, Wu JC, Lee PC, Chang FY, et al. Fever and Infectious Complications After Percutaneous Acetic Acid Injection Therapy for Hepatocellular Carcinoma: Incidence and Risk Factor Analysis. *J Clin Gastroenterol* 2006, 40(7): 639-642.
29. Hildebrand P, Leibecke T, Kleemann M, Mirow L, Birth M, Brusch HP, et al. Influence of operator experience in radiofrequency ablation of malignant liver tumours on treatment outcome. *Eur J Surg Oncol* 2006, 32(4): 430-434.
30. El-Kady N, El-Bendary M, El-Sherbeeny W, El-Diasty M. Medium and large sized hepatocellular carcinoma: acetic acid versus radiofrequency ablation. *MMJ* 2011, 24(1): 21-30.
31. Ohishi H, Uchida H, Yoshimura H, Ohue S, Ueda J, Katsuragi M, et al. Hepatocellular carcinoma detected by iodized oil: Use of anticancer agents. *Radiology* 1985, 154(1): 25-29.
32. Hoffmann W, Reichel H, Schiebe M, Btiltmann B, Bamberg M. Intrapericardial instillation of mitoxantrone in malignant pericarditis: Histo-pathological appearance. *Reg Cancer Treat* 1993, 2: 91-99.
33. Guglielmi A, Ruzzenente A, Battocchia A, Tonon A, Fracastoro G, Cordiano C. Radiofrequency ablation of hepatocellular carcinoma in cirrhotic patients. *Hepatogastroenterology* 2003, 50(50): 480-484.
34. Komorizono Y, Oketani M, Sako K, Yamasaki N, Shibatou T, Maeda M, et al. Risk factors for local recurrence of small hepatocellular carcinoma tumors after a single session, single application of percutaneous radiofrequency ablation. *Cancer* 2003, 97(5): 1253-1262.

35. Cha CH, Lee FT Jr, Gurney JM, Markhardt BK, Warner TF, Kelcz F, et al. CT versus sonography for monitoring radiofrequency ablation *in a porcine liver. Am J Roentgenol* 2000, 175(3): 705-711.
36. Okusaka T, Okada S, Ueno H, Ikeda M, Shimada K, Yamamoto J, et al. Satellite lesions in patients with small hepatocellular carcinoma with reference to clinicopathologic features. *Cancer* 2002, 95 (9):1931-1937.
37. Tsai WL, Cheng JS, Lai KH, Lin CP, Lo GH, Hsu PI, et al. Clinical trial: percutaneous acetic acid injection vs. percutaneous ethanol injection for small hepatocellular carcinoma– a long– term follow–up study. Aliment Pharmacol Ther 2008, 28(3): 304-311.
38. Timpl R, Wiedemann H, Delden VV, Furthmayr H, Kuhn K. A network model for the organization of type IV collagen molecules in basement membranes. *Eur J Biochem* 1981, 120(2): 203-211.
39. Miller E, Rhodes RK. Preparation and characterization of the different types of collagen. In: Cunningham LW, Frederiksen DW, eds. Methods in enzymology. Volume 82. Structural and contractile proteins. Part A. Extracellular matrix. *New York: Academic*, 1982:23-64.

**Peer reviewer: Tarik Zaher** ,Professor of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt.
**Editor: Mohamed Emara**, Lecturer of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt

# Study of TUBEX as a Rapid Diagnostic Test of Typhoid Fever

**Gamal Saad El-Deeb[1], Hosam El-Din Mostafa Seleem[2], Gehan Abd El-Fatah Tawfeek[3], Sameh Salah Emara[4]**

*[1]Tropical Medicine Department , Faculty of Medicine , Menoufia University,Egypt*
*[2]Tropical Medicine Department , Faculty of Medicine ,Menoufia University,Egypt*
*[3]Clinical Pathology Department ,Faculty of Medicine ,Menoufia University ,Egypt*
*[4]Shebin El-Kom Fever Hospital ,Shebin El-Kom, Egypt*

*Corresponding Author*
***Sameh Salah Emara***

*Mobile:*
*01067679331*

*E mail:*
*samegp@yahoo.com*

*Key words:*
*Typhoid Fever, TUBEX test, Blood Culture, Widal test*

**Background and study aim :** Several serologic tests for typhoid fever have been introduced which detect IgM or IgG antibodies to various purified antigens of *S. Typhi* as TUBEX test. This study aims to evaluate the performance of TUBEX test as a rapid diagnostic test of typhoid fever.
**Patients and Methods:** The present study involved 44 patients admitted to Shebin El Kom Fever Hospital fulfilling the criteria of typhoid fever by WHO as (suffering from continuous fever at least 2 days, greater than 38.5°C in addition to headache, constipation or diarrhea) without identified cause of fever as pneumonia. Compared with 20 subjects; 10 with non specific fevers and 10 without fever using TUBEX test in correlation to the usual Widal test and blood culture as a gold standard.
**Results:** We revealed sensitivity, specificity, positive predictive value and negative predictive value respectively for Widal; 75%, 60%, 80.5%, 52.2% and for culture; 65.9%, 100%, 100%, 57.1%. In correlation with TUBEX test the results are at cutoff point 5 showing sensitivity, specificity, positive predictive value and negative predictive value respectively; 84.1%, 95%, 97.4% and 73.1%.
**Conclusion:** TUBEX results are superior to Widal test results in specificity and slightly in sensitivity as compared to the blood culture as a reference test.

## INTRODUCTION

In most cases, the cause of a febrile illness is a self limiting and presumed viral disease. However, 5–10% of febrile illnesses have serious bacterial infections such as pneumonia, urinary tract infection, meningitis, bacteraemia or typhoid infection. These bacterial conditions can be difficult to distinguish from viral infections and benefit from early antibiotic therapy. The consequences of a delayed or missed diagnosis can be serious and, occasionally, fatal for the patient in the outbreak setting **[1]**. Typhoid fever remains an important cause of disease in developing countries. In 2010, it caused an estimated 408,837 episodes of illness in Africa **[2]**. The estimated incidence of typhoid fever in Egypt was 59/100,000 persons/year **[3]**. *Salmonella typhi*, the causative agent, is most frequently isolated from blood during the first week of illness but can also be isolated during the second or third week of illness, during the first week of antimicrobial therapy and during clinical relapse **[4]**. Typhoid is transmitted by the fecal-oral route through ingestion of food and water contaminated by urine or feces from infected cases or carriers. The infection is rarely spread by casual contact. Shellfish (particularly oysters) taken from sewage-contaminated beds, raw fruits, vegetables fertilized by night soil (human excrement) and eaten raw, contaminated milk and milk products (usually contaminated by hands of carriers), are important sources of infection. Flies may also infect foods in which the organism can multiply to achieve an infective dose. The infective dose for typhoid is much lower than that of paratyphoid **[5]**. Recently, researchers have used mouse studies to find that a toxic protein complex

produced by the bacterium (even in the absence of the microbe itself) causes most typhoid symptoms such as lethargy, stupor, and weight loss and leads to death **[6]**. Today most of the burden of typhoid fever occurs in the developing world, where sanitary conditions remain poor. Reliable data to estimate the burden of the disease in these areas are difficult to obtain, since many hospitals lack facilities for blood culture, and up to 90% of patients with typhoid are treated as outpatients. Community based studies have consistently shown higher levels of typhoid fever than public health figures suggest. The definitive diagnosis of typhoid fever requires the isolation of *Salmonella enterica* subspecies enterica serovar *Typhi* (*S. Typhi*) from the patient. Cultures of blood, stool, urine, rose spots, blood mononuclear cell-platelet fraction and bone marrow can all be useful for diagnosis **[7]**. Developing an inexpensive and rapid diagnostic test for typhoid fever that is both sensitive and specific has become a public health priority. Several serologic tests for typhoid fever have been introduced which detect IgM or IgG antibodies to various purified antigens of *S. Typhi* as TUBEX test. Studies evaluating TUBEX test revealed marked variation in its results **[8]**.

## PATIENTS AND METHODS

**Study area**

The present study was performed in El Menoufia governorate and all patients were admitted to Shebin El-Kom Fever Hospital.

**Time of the Study**

From December 2013 to October 2014.

They were chosen after taking written consent from out-patient clinic and in-patient department in Shebin El-Kom Fever Hospital.

The subjects are divided into two groups;

**Group 1 ( Patients )**

The present study involved 44 patients admitted to Shebin El-Kom Fever Hospital fulfilling the criteria of typhoid fever by WHO as (suffering from continuous fever at least 2 days, greater than 38.5°C in addition to headache, constipation or diarrhea) without identified cause of fever as pneumonia. The patients were 25 males and 19 females all are over 12 years old.

**Group 2 (Control)**

The control group was 20 subjects; 10 with non specific fevers and 10 without fever. They were 10 males and 10 females and all are over 12 years old.

**Procedure of the study**

All patients and control subjects were subjected to the following :

- Full and complete history with stress on fever, headach, abdominal pain, nausea, vomiting, diarrhea and constipation [9].
- Full clinical examination with stress on fever, rose spot, spleen and liver examination [9].
- Liver function tests including AST, ALT, ALP and Bilirubin [10].
- Complete blood picture [9].
- Widal agglutination test (Widal test is positive only in the second week of typhoid fever) [11].
- Blood culture [9].
- TUBEX TF.

**Sample collection**

Blood samples were collected from patients and controls, centrifuged and sera were stored at -20°C.

**Statistical analysis**

The data collected were tabulated and analyzed by SPSS (statistical package for social science) version 22.0 on IBM compatible computer (IBM corp., New York, USA, 2012).

Two types of statistics were done

**Descriptive statistics [12]:**

e.g. percentage (%), mean and standard deviation (SD).

**Analytic statistics [13]:**

- *Chi-square test ($\chi^2$):*
  Was used to study association between two qualitative variables

- *Fischer exact test:*
  For 2 x 2 tables when expected cell count of more than 25% of cases was less than 5 and p-value < 0.05 was considered significant.

- *Student t-test:*
  Is a test of significance used for comparison between two groups having quantitative variables.

- *Mann-Whitney test (nonparametric test):*
  Is a test of significance used for comparison between two groups not normally distributed having quantitative variables.

- *Level of significance:*
  Was set as P-value <0.05.

## RESULTS

The patients were 25 male (56.8%) and 19 females (43.2%) their age range was 13-62. The controls were 10 male (50%) and 10 females (50%), their age range was 13-60 (Table 1).

The symptoms were fever in 44 patients (100%), headache in 18 patients (40.9%), abdominal pain in 17 patients (38.6%), rose spots in one patient (2.3%), nausea and vomiting in 10 patients (22.7%), diarrhea in 13 patients (29.5%), constipation in 2 patients (4.5%) and splenomegally in 28 patients (63.6%) (Table 2).

Statistical analysis of results in cases when compared with controls revealed mild anaemia with mean Hb : 11.6 ± 2.4 gm%, mild leucopenia with W.B.C.s: 4.7 ± 3, neutropenia: 39.5 ± 5.7%, lymphocytosis : 58.2 ± 5.6% and diminished platelet count : 222.7 ± 118.3 (Table 3).

Statistical analysis of the results revealed significant elevation of SGOT, SGPT and ALP with non significant bilirubin level. (Table 4)

Statistical analysis of the results revealed sensitivity, specificity, positive and negative predictive values respectively for Widal; 75%, 60%, 80.5%, 52.2% and for culture; 65.9%, 100%, 100%, 57.1% (Table 5).

Statistical analysis of TUBEX validity at cutoff point 5 showing sensitivity, specificity, positive (PPV) and negative predictive values (NPV) respectively; 84.1%, 95%, 97.4% and 73.1% (Table 6).

Statistical analysis of TUBEX test versus blood culture at cutoff point 5 revealed sensitivity; 100% specificity; 65.7%, positive predictive value; 70.7% and negative predictive value; 100% (Table 7).

**Table (1) :** Demographic characteristics of studied groups

| | Cases group (no=44) | | Control group (no=20) | | Mann-Whitney Test | P value |
|---|---|---|---|---|---|---|
| **Age (years)**<br>Mean ± SD<br>Range | 36.6±14.3<br>13-62 | | 36.9±11.8<br>13-60 | | 0.18 | 0.86 |
| | **No** | **%** | **No** | **%** | **$X^2$ test** | **P value** |
| **Gender** | | | | | | |
| Male | 25 | 56.8 | 10 | 50 | 0.26 | 0.61 |
| Female | 19 | 43.2 | 10 | 50 | | |

**Table (2) :** Comparison between studied groups regarding clinical manifestations

| | Cases group (no=44) | | Control group (no=20) | | Fisher's Exact test | P value |
|---|---|---|---|---|---|---|
| | No | % | No | % | | |
| **Fever** | | | | | | |
| Yes | 44 | 100 | 10 | 50 | 27.75 | <0.001** |
| No | 0 | 0.0 | 10 | 50 | | |
| **Headache** | | | | | | |
| Yes | 18 | 40.9 | 0 | 0.0 | 11.38# | 0.001* |
| No | 26 | 59.1 | 20 | 100 | | |
| **Abdominal pain** | | | | | | |
| Yes | 17 | 38.6 | 0 | 0.0 | 10.52# | 0.001* |
| No | 27 | 61.4 | 20 | 100 | | |
| **Rose spots** | | | | | | |
| Yes | 1 | 2.3 | 0 | 0.0 | 0.76 | 0.38 |
| No | 43 | 97.7 | 20 | 100 | | |
| **Nausea & vomiting** | | | | | | |
| Yes | 10 | 22.7 | 0 | 0.0 | 8.31 | 0.004* |
| No | 34 | 77.3 | 20 | 100 | | |
| **Diarrhea** | | | | | | |
| Yes | 13 | 29.5 | 0 | 0.0 | 11.19 | 0.001* |
| No | 31 | 70.5 | 20 | 100 | | |
| **Constipation** | | | | | | |
| Yes | 2 | 4.5 | 0 | 0.0 | 1.53 | 0.22 |
| No | 42 | 95.5 | 20 | 100 | | |
| **Splenomegaly** | | | | | | |
| Yes | 28 | 63.6 | 0 | 0.0 | 22.63# | <0.001** |
| No | 16 | 36.4 | 20 | 100 | | |

# $X^2$ test *Significant difference **Highly significant difference

**Table (3) :** Comparison between studied groups regarding CBC profiles

| | Cases group (no=44) | Control group (no=20) | t-Test | P value |
|---|---|---|---|---|
| **Hb (g/dl)** | | | | |
| Mean ± SD | 11.6±2.4 | 11.9±1.7 | 0.37 | 0.71 |
| Range | 8-16 | 9-15 | | |
| **WBC (cell/mcl)$\times10^3$** | | | | |
| Mean ± SD | 4.7±3.0 | 6.1±1.4 | 3.94# | <0.001** |
| Range | 2.2-14 | 4-10 | | |
| **Platelets (cell/mcl)$\times10^3$** | | | | |
| Mean ± SD | 222.7±118.3 | 303.3±81.4 | 2.79# | 0.005* |
| Range | 100-470 | 145-425 | | |
| **Neutrophils (%)** | | | | |
| Mean ± SD | 39.5±5.7 | 62.1±9.8 | 11.68 | <0.001** |
| Range | 31-49 | 45-80 | | |
| **Lymphocytes (%)** | | | | |
| Mean ± SD | 58.2±5.6 | 35.3±9.4 | 12.12 | <0.001** |
| Range | 49-67 | 19-53 | | |

# Mann-Whitney test *Significant difference **Highly significant difference

**Normal ranges**

| | | |
|---|---|---|
| Hb | Male | 14-17.5 g/dl |
| | Female | 12.3-15.3 g/dl |
| WBCs | | 4-11 cell/mcl (microliter) |
| Platelet | | 150-450 cell/mcl |
| Neutrophil | | 40-80 % |
| Lymphocyte | | 20-40 % |

**Table (4) :** Comparison between studied groups regarding liver functions

| | Cases group (no=44) | Control group (no=20) | t-Test | P value |
|---|---|---|---|---|
| **SGOT (IU/L)**<br>Mean ± SD<br>Range | <br>48.8±7.4<br>35-55 | <br>18.9±6.5<br>10-33 | 15.42 | <0.001** |
| **SGPT (IU/L)**<br>Mean ± SD<br>Range | <br>55.1±7.8<br>40-60 | <br>22.3±9.7<br>10-50 | 13.36 | <0.001** |
| **ALP (IU/L)**<br>Mean ± SD<br>Range | <br>188.4±148.01<br>30-650 | <br>57.5±21.4<br>25-90 | 4.41# | <0.001** |
| **Bilirubin (mg/dl)**<br>Mean ± SD<br>Range | <br>0.97±0.19<br>0.5-1.3 | <br>1.01±0.16<br>0.7-1.3 | 0.96 | 0.34 |

# Mann-Whitney test **Highly significant difference

**Normal ranges :**

SGOT 5-40 IU/L SGPT 7-60 IU/L

ALP 25-100 IU/L Bilirubin 0.2-1.5 mg/dl

**Table (5) :** Validity of Widal test and blood culture in diagnosis of typhoid fever

| | Sensitivity | Specificity | PPV | NPV | Accuracy |
|---|---|---|---|---|---|
| **Widal test** | 75% | 60% | 80.5% | 52.2% | 70.3% |
| **Blood culture** | 65.9% | 100% | 100% | 57.1% | 76.6% |

**Table (6) :** Validity of TUBEX test in diagnosis of typhoid fever

| | AUC | Cutoff point | Sensitivity | Specificity | PPV | NPV | Accuracy |
|---|---|---|---|---|---|---|---|
| TUBEX | 0.95 | 5 | 84.1% | 95% | 97.4% | 73.1% | 87.5% |

AUC-- Area Under the Curve

**Table (7) :** Evaluation of TUBEX test versus blood culture in diagnosis of typhoid fever

| | AUC | Cutoff point | Sensitivity | Specificity | PPV | NPV | Accuracy |
|---|---|---|---|---|---|---|---|
| TUBEX | 0.95 | 5 | 100% | 65.7% | 70.7% | 100% | 81.3% |

AUC-- Area Under the Curve

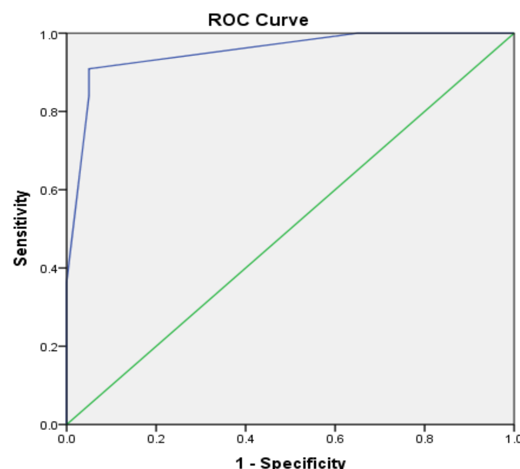


**Fig (1) :** Receiver operator characteristic curve showing the relation between sensitivity and specificity at different cut-off points for TUBEX test

## DISCUSSION

The present study was stressed on the newly emerged rapid antibody test Tubex TF who show simplicity in the procedure, difficulty in interpretation of the results as it is a colorimetric test and some difference in the time of reading of the results which significantly prolonged (30-60 minutes) more than that provided in the pamphlet and closely similar results of sensitivity; 84.1%, specificity, 95%, PPV; 97.4% and NPV; 73.1%. These results agreed with the following studies:

Ley et al. **[14]** found that Tubex has a sensitivity of 79% and a specificity of 89-97% irrespective of control group. The Multi-Test dipstick was the most costly assay, presumably because the dipstick measures antibodies to five different pathogens. Although the TUBEX was the simplest. A limitation of the TUBEX test, which uses a colorimetric reaction, is the potential for difficulty in interpreting the results of hemolyzed samples. Another concern is that the TUBEX may produce a false positive result in persons with recent *S. enterica* serotype *enteritidis* infection and result in inappropriate antibiotic treatment **[15]**.

Dutta et al**.** **[16]** stated that past studies have shown that the use of TUBEX-TF yield highly variable sensitivity and specificity profiles, depending on the country and/or geographical region, study population, and nature of the study. Bangladesh 60% & 85%, Vietnam 79% & 89%, Poland 93% & 95% and Philippines 95% & 80%. This has created difficulties in comparing results between studies and setting worldwide standards for typhoid fever diagnosis. The specificity of TUBEX was extremely good (100%). This finding is not surprising, since previous investigators found *S. typhi* LPS to be very specific **[17]**. Narrowing this antigen to the immunodominant O9 determinant would, in theory, increase the specificity of the assay. Indeed, using an inhibition ELISA to measure anti-O9 antibodies in patients, we observed very good specificity with the test previously. The a-D-Tyvelose is the immunodominant sugar of the O9 determinant. An extremely rare sugar in nature, a-D tyvelose is antigenically different from the b-D-tyvelose found in *T. Spiralis* or the L-tyvelose in *Ascaris lumbricoides* **[18]**. However, the O9 determinant is present not only in *S. typhi* but also in several other serotypes of *Salmonella* (serogroup D) such as *S. enteritidis* and *S. sendai*. However, many of these bacteria are not invasive and may not stimulate a systemic antibody response. The extent to which TUBEX detects infection caused by these salmonellae or the paratyphoid serotypes remains to be investigated **[18]**. Previously, using the ELISA equivalent of TUBEX we found that serum samples from septicemic patients infected with *Salmonella* organisms not belonging to serogroup D (one with *S. choleraesuis*, one with *S. johannesburg*, and one with *S. senftenberg*) were negative in the test, whereas that from a patient infected systemically with *S. sendai* (serogroup D) was weakly positive. Interestingly, serum samples from two patients infected with *S. paratyphi A*, a non-serogroup D organism, were strongly positive in the test. The reactivity was due to the presence in the patients of anti-O12 antibodies, which bound to the O12 determinant in the detecting antigen (LPS) and consequently blocked, by steric hindrance, the binding of the reagent mAb to the adjoining O9 determinant in the LPS **[19]**. Consequently, TUBEX will potentially give positive results for infections caused by any invasive *Salmonella* bacteria which bear the O9 or O12 antigen. Thus, to make TUBEX more specific for typhoid fever, supplementary tests such as those detecting anti- Vi, anti-dH, or anti-OM antibodies, can be included **[19]**.

## CONCLUSION

As typhoid fever remains an important cause of disease in developing countries, Widal test still have debating results while the gold standard blood culture for *Salmonella typhi* in most of the developing world, where widespread antibiotic availability and prescribing are reasons for low sensitivity of blood cultures. On the other hand we found TUBEX results superior to Widal test results in specificity and slightly in sensitivity with closely related specificity to blood culture which is promising as a rapid test.

**Funding:** None.
**Conflicts of interest:** None.
**Ethical approval:** Approved

## REFERENCES

1. Kawano, RL, Leano, SA. , Agdamag, DM. Comparison of serological test kits for diagnosis of typhoid fever in the Philippines. *J Clin Microbiol* 2013 ; 45: 246–247.
2. Baker S, Favorov M, Dougan G. Searching for the elusive typhoid diagnostic. *BMC Infect Dis.* 2010 Mar 5;10:45.

3. Srikantiah P, Girgis FY, Luby SP, Jennings G, Wasfy MO, Crump JA, Hoekstra RM, Anwer M, Mahoney FJ. Population-based surveillance of typhoid fever in Egypt. *Am J Trop Med Hyg.* 2006 Jan;74(1):114-9
4. Merrell DS, Falkow S. Frontal and stealth attack strategies in microbial pathogenesis. *Nature* 2004 Jul 8;430(6996):250-6.
5. Heymann DL. Control of Communicable Disease Manual. An offical report of the American Public Health Association. *American Public Health Association* 2008: 978-0.
6. Song J, Gao X, Galán JE. Structure and function of the Salmonella Typhi chimaeric A(2)B(5) typhoid toxin. *Nature* 2013 Jul 18;499(7458):350-4.
7. Bhutta ZA. Current concepts in the diagnosis and treatment of typhoid fever. *BMJ.* 2006 Jul 8;333(7558):78-82.
8. Rahman M, Siddique AK, Tam FC, Sharmin S, Rashid H, Iqbal A et al. Rapid detection of early typhoid fever in endemic community children by the TUBEX O9-antibody test. *Diagn Microbiol Infect Dis.* 2007 Jul;58(3):275-81.
9. Gilman RH, Terminel M, Levine MM, Hernandez-Mendoza P, Hornick RB. Relativeefficacy of blood, urine, rectal swab, bone-marrow, and rose-spot ultures forrecovery of Salmonella typhi in typhoid fever.*Lancet.* 1975 May31;1(7918):1211-3.
10. Gella FJ, Olivella T, Cruz Pastor M, Arenas J, Moreno R, Durban R, Gomez JA. A simple procedure for the routine determination of aspartate aminotransferase and alanine aminotransferase with pyridoxal phosphate. *Clin Chim Acta.* 1985 Dec31;153(3):241-7.
11. Freter R. Prospects for preventing the association of harmful bacteria with host mucosal surfaces. In Bacterial adherence, pp. 439-458. Springer Netherlands, 1980.
12. Plackett RL. Karl Pearson and the Chi-Squared Test. International Statistical Review. International Statistical Institute (ISI) 1983 ;51 (1):59–72.
13. Corder GW, Foreman DI. Nonparametric statistics for non-statisticians: a step-by-step approach. John Wiley & Sons, 2009.
14. Ley B, Thriemer K, Ame SM, Mtove GM, von Seidlein L, Amos B et al. Assessment and comparative analysis of a rapid diagnostic test (Tubex®)for the diagnosis of typhoid fever among hospitalized children in rural Tanzania.*BMC Infect Dis.* 2011 May 24;11:147.
15. Oracz G, Feleszko W, Golicka D, Maksymiuk J, Klonowska A, Szajewska H. Rapiddiagnosis of acute Salmonella gastrointestinal infection. *Clin Infect Dis*. 2003Jan 1;36(1):112-5.
16. Dutta S, Sur D, Manna B, Sen B, Deb AK, Deen JL et al.Evaluation of new-generatio serologic tests for the diagnosis of typhoid fever: data from a community-based surveillance in Calcutta, India. *Diagn Microbiol Infect Dis*. 2006 Dec;56(4):359-65.
17. Shaheen HI, Girgis NI, Rodier GR, Kamal KA. Evaluation of the response of human humoral antibodies to Salmonella typhi lipopolysaccharide in an area of endemic typhoid fever. *Clin Infect Dis.* 1995 Oct;21(4):1012-3.
18. Lim PL, Tam FC, Cheong YM, Jegathesan M. One-step 2-minute test to detect typhoid-specific antibodies based on particle separation in tubes. *J Clin Microbiol.* 1998 Aug;36(8):2271-8.
19. Lim PL, Ho MY. Diagnosis of enteric fever by inhibition assay using peroxidase-labelled monoclonal antibody and Salmonella typhi lipopolysaccharide. *Aust J Exp Biol Med Sci.* 1983 Dec;61 ( Pt 6):687-704.


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

# Antinuclear Antibody Positivity in Chronic Hepatitis C Patients: Effect on Histopathology and Impact on Early Response to Combined Antiviral Therapy

**Yehia S. Younes[1], Yasser A. Shaheen[1], Hala M. El Feky[1], Maha Z. Omar[1], Rasha SH. Eldesouky[2], Rania Z. Amer[3], Rania I. Mabrouk[4]**

[1]*Hepatology, Gastroenterology and Infectious Diseases Department, Faculty of Medicine, Benha University,Egypt.*
[2]*Community Medicine Department, Faculty of Medicine, Benha University,Egypt.*
[3]*Pathology Department, Faculty of Medicine, Benha University,Egypt.*
[4]*Shebin El-Kom Teaching Hospital , Shebin El-Kom,Egypt*

*Corresponding Author:*
***Yehia S Younes***

*Mobile:*
*+201223571599*

*E mail:*
*dr.yehia56@gmail.com*

*Key words:*
*Antinuclear Antibody (ANA), Interferon (IFN), Early Virological Response (EVR).*

**Background and study aim:** The prevalence of antinuclear antibody (ANA) has been documented in patients with hepatitis C virus (HCV) infection. Hepatitis C virus infection plays an important role in the pathogenesis of immunological derangement, but the mechanism remains unclear. The aim of this study is to detect the significance of ANA positivity and its impact on histopathology and early virological response (EVR) to combined antiviral therapy in chronic HCV patients.

**Patients and methods:** Two hundred Egyptian chronic HCV naïve patients were enrolled in this study. Antinuclear antibody (ANA) was detected by ELISA and it was considered positive with a titer > 1 : 14 by indirect immunofluorescence. Complete laboratory investigations and histological examination were done as a pretreatment work up for all patients. Patients were followed up during treatment and EVR was assessed in ANA positive and negative patients.

**Results:** There was a statistically significant difference between ANA positive and negative patients regarding viral load and histopathological criteria and no significant difference was detected regarding other demographic and laboratory criteria. EVR was close in ANA positive and ANA negative patients (77 for the former Vs. 80 for the later with P = 0.33). No autoimmune manifestations were detected during treatment among positive cases. Except for ALT & AST levels no statistically significant differences were detected between ANA positive and negative cases regarding haematological data, thyroid dysfunction. BMI, ALT levels, viral load and fibrosis stages were independent predictors of EVR.

**Conclusion:** ANA postivity in chronic HCV patients was associated with advanced fibrosis but didn't affect treatment response.

## INTRODUCTION

Hepatitis C virus (HCV) infection is the most common cause of chronic liver disease in the world **[1].** It is estimated that 130-170 million people were chronically infected with HCV at the end of $20^{th}$ century, and 2.4-4.7 million new infections per year **[2].**

Several immunologic abnormalities, such as production of auto antibodies, rheumatoid factor, and cryglobulin, has been associated with HCV infection. Antinuclear antibody (ANA) is one of the most frequently detected auto-antibody **[3].** Its prevalence in HCV infected individuals ranges from 21% to 34%. The mechanism of production of these antibodies in HCV infection remains obscure. It may relate to disturbances in self-tolerance as a result of the molecular mimicry between viral proteins and auto antigens **[4].** Although ANA is the diagnostic hall-mark of systemic lupus erythreматosus (SLE) and type 1 autoimmune hepatitis, its role in chronic HCV infection is unclear **[5].**

The presence of serum ANA is associated with various factors including advancing age, genetic predisposition,

environmental agents, oestrogen-androgen balance, chronic infection and neoplasm **[6].** In some studies ANA positivity had no observed effect on HCV clinical outcome **[7].** The primary goal of HCV therapy is to cure the infection which results in eliminating detectable circulating HCV after cessation of treatment.

IFN-based treatment is frequently associated with significant side effects, some of them related to its immunomodulatory properties which can induce autoimmune phenomena **[8].**

It was recommended that the autoimmune profile namely ANA should be assessed in chronic HCV patients before treatment decision with interferon and ribavirin and consider the presence of active autoimmune disorders as a contraindication for treatment **[9].**

The objective of this study was to detect the significance of antinuclear antibodies (ANA) positivity in chronic HCV patients regarding the effect of its presence on histopathology and early virological response (EVR) to pegylated interferon and ribavirin.

## PATIENTS AND METHODS

**Selection of Patients:**
This prospective study was carried out on 200 adult treatment naive patients with biopsy-proven chronic hepatitis C who were candidate for treatment with pegylated interferon and ribavirin, during the period from April 2012 to December 2012.

Patients were recruited from Hepatology Unit at Shebin El-Kom teaching hospital, a referal center of treatment of chronic HCV in Egypt under the supervision of Ministry of health as a part of the Egyptian national project for combating chronic HCV.

Patients with any other cause of liver disease (as HBV infection, AIH) ,decompensated liver disease, patients with hepatocellular carcinoma (HCC), ischemic cardiovascular diseases, pregnancy or breast feeding, poorly controlled diabetes, psychiatric, ophthalmological or cardiological disorders, substance abuse, patients with organ transplantation, previous treatment with interferon alpha, acute hepatitis, known history of hemolytic anemia were excluded from the study.

***All patients included in the study were subjected to:*** Thorough history taking, complete clinical examination and laboratory investigations including: Complete blood count (CBC), Serum creatinine, Random blood glucose, Complete liver profile (pretreatment then weekly in the first month and monthly unless complications occurred) including :total and direct serum bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum albumin, serum prothrombin, serum alkaline phosphatase).

**Patients Classification:**
The studied populations were divided into 2 groups:

- **Group I**: included 100 patients with positive anti nuclear antibody titer.
- **Group II:** included 100 patients with negative anti nuclear antibody titer.

**Detection of Anti nuclear Antibodies (ANA):**
ANA was detected by using the enzyme-linked immunosorbent assay (ELISA) done at baseline and after 12 weeks of antiviral therapy.

If the ELISA method resulted in a positive or equivocal finding, the sample was titered using indirect immunofluorescence (IFA) assays on Hep-2 cells, and any value less than or equal to 1:14 dilution is negative, and any value more than 1:14 is considerd positive **[10].**

**Histological assessment:**
Liver biopsy was done for all patients before treatment using 16 gauge biopsy needle under complete aseptic conditions. Haematoxyline and eosin stains were used for sections staining for histological assessment and masson trichrome stains for fibrosis detections according to METAVIR scoring system on a scale of F0-4 **[11].** Special concern was directed towards the presence of autoimmune finding in liver biopsy to exclude autoimmune hepatitis. A single experienced pathologist who was unaware of the clinical data evaluated all liver biopsies.

**Treatment protocol and definition of response:**

All patients received 180 µg/week pegylated interferon alfa-2a (Pegasys) plus 1000-1200 mg/day weight-adjusted ribavirin (1000mg /d for patients < 75KG, and1200 mg /d for patients ≥ 75kg) **[12].** Treatment was discontinued if EVR was not achieved; these patients were defined as non responders. Early responders continued treatment for a total of 48 weeks. Complete early virological response (cEVR) was defined by undetectable HCV-RNA at 3 months after

initiation of therapy, while non-responders (NR)were defined by detectable HCV-RNA at 3 months after initiation of therapy.

**Statistical Methods:**
The collected data were tabulated and statistically analyzed using the suitable statistical methods"

For the quantitative data, range, mean and standard deviation were calculated. The difference between two means was statistically analyzed using student (t) test. P value of less than 0.05 was considered statistically significant. Continuous variables were compared using the student's *t*-test or the Mann-Whitney *U* test when appropriate. Categorical variables were compared using the Pearson's $X^2$ test or Fisher's exact test.

## RESULTS

**Patients characteristics and factors associated with ANA postivity:**
This study was conducted on 200 patients with HCV infection (69.5% of them were males) who classiffied into 2 groups: 100 ANA positive and 100 ANA negative patients. Their mean age was 38.5 ± 6.3 years, their mean BMI was 22.3 ± 5.3 and their mean PCR was 1571636.5 ± 52391.21 IU/ml. There was no statistical significant difference between studied groups regarding demographic and laboratory criteria except for smoking and viral load which were significantly higher in ANA positive than ANA negative cases (p= 0.047 & p= 0.041 respectively) (Table 1).

Regarding liver biopsy, there was statistically significant difference between the two studied groups regarding the histological activity and fibrosis stages: 65% of ANA positive patients had high histological activity (≥ A2) versus 60% in ANA negative (p value 0.019). While 62% of group I had advanced fibrosis (F2 + F3) vs 54% of group II with statistically significant difference (p value = 0.021) (Table 2).

**Follow up data and EVR of the studied cases:**
No autoimmune manifestations were reported during treatment among ANA positive cases. TSH level was higher and Hb, WBCs and ANC were lower in ANA positive than ANA negative group but without statistically significant difference (P value 0.076, 0.085, 0.33, 0.096 respectively), EVR rate was close in ANA positive to that of ANA negative patients (77 vs. 80%) without significant difference (P = 0.3) Table (3).

As regard ANA level changes in ANA positive group at 12 weeks, there was increase in ANA level than the baseline in 93 patients (93%) vs 7 patients (70%) who had no changes or decreased ANA level during treatment. Seventy six patients (84%) out of those 93 patients achieved EVR vs 1 patient of the second group with highly significant difference (p value = 0.001) (Table 4).

ANA positivity had no significant impact on EVR by univariate analysis (Table 5). Only BMI, fibrosis stage (F2, F3), ALT and viral load were the predictors of EVR by multi variate analysis (Table 6).

**Table (1):** Demographic and Baseline Characteristics of the Studied Groups in Relation to ANA.

| | | ANA positive cases (>1/14) n=100 | ANA negative cases (≤1/14) n=100 | t. test | P value |
|---|---|---|---|---|---|
| Age | | 41.5±5.69 | 37.41±8.62 | 0.635 | 0.325 |
| Sex | M | 75(75%) | 64(64%) | 0.582 | 0.428 |
| | F | 25(25%) | 36(36%) | | |
| Smoking | | (60)60% | (22)22% | 2.635 | **0.047** |
| BMI | | 28.10±3.24 | 25.60±2.10 | 0.568 | 0.442 |
| DM | | 43(43%) | 35(35%) | 1.452 | 0.642 |
| Hb(gm/dl) | | 12.5±0.19 | 12.7±0.13 | 1.625 | 0.635 |
| WBC(c/mm³) | | 3963.5±5.63 | 4152.6±357.4 | 1.412 | 0.421 |
| ANC(c/mm³) | | 1741.2±356.2 | 1836.4±742.2 | 1.520 | 0.152 |
| Plt(c/mm³) | | 247.5±65.1 | 191.5±32.2 | 0.369 | 0.324 |
| Blood glucose (gm/dl) | | 108.3±12.3 | 103.6±10.5 | 0.472 | 0.159 |
| S.Creat(mg/dl) | | 0.89±0.16 | 0.81±0.16 | 0.530 | 0.752 |
| TSH(mu/ml) | | 2.42±.0.96 | 1.73±0.25 | 1.635 | 0.085 |
| AFP(ng/ml) | | 3.12±1.10 | 4.85±1.42 | 1.753 | 0.247 |
| ALT(IU/L) | | 50.36±12.4 | 43.61±10.25 | 1.996 | 0.324 |
| AST(IU/L) | | 36.24±10.56 | 32.15±11.52 | 0.369 | 0.427 |
| S. albumin(g/dl) | | 4.5 ± 0.2 | 5.3 ± 0.4 | 1.542 | 0.231 |
| Total BIL(mg/dl) | | 0.9±0.16 | 1.0±0.21 | 0.621 | 0.842 |
| Viral load(IU/ml) | | 1341856.5±735220.4 | 1217538.5±25367.1 | 4.624 | **0.041** |

**Table (2):** Hisopathological Criteria of the Studied cases in Relation to ANA.

| | | | | | |
|---|---|---|---|---|---|
| Activity grade N (%) | A1 | 35 (35%) | 40 (40%) | 3.336 | **0.019** |
| | A2 | 47 (47%) | 48 (48%) | | |
| | A3 | 18 (18%) | 12 (12%) | | |
| Fibrosis stage N (%) | F1 | 38 (38%) | 46 (46%) | 2.996 | **0.021** |
| | F2+3 | 62 (62%) | 54 (54%) | | |

**Table (3):** Laboratory Data of Studied Groups at 12 Week

| | ANA- positive Cases(>1/14) | ANA-negative cases(≤1/14) | t .test | P value |
|---|---|---|---|---|
| Hb(gm/dl) | 10.35±2.82 | 11.74±2.27 | 1.332 | 0.085 |
| WBC(cells/cm³) | 2581.5±6.36 | 3159.2±274.1 | 1.259 | 0.332 |
| ANC(c/mm³) | 1654.3±241.6 | 1817.4±716.6 | 1.452 | 0.096 |
| PLT (c/mm³) | 236.5±60.1 | 170.5±32.2 | 0.635 | 0.325 |
| Random blood Sugar(gm/dl) | 107.3±9.31 | 101.6±8.63 | 0.447 | 0.442 |
| S.Creat(mg/dl) | 0.83±0.13 | 0.80±0.14 | 0.625 | 0.258 |
| ALT(IU/I) | 46.23±8.52 | 41.29±10.19 | 2.326 | **0.049** |
| AST(IU/I) | 35.36±11.52 | 30.14±5.22 | 2.639 | **0.047** |
| TSH(IU/ml) | 2.14±.0.12 | 1.15±0.22 | 1.241 | 0.076 |
| AFP(ng/ml) | 3.68±1.74 | 4.32±1.30 | 1.366 | 0.220 |
| EVR | 77 | 80 | 0.825 | 0.33 |

**Table (4):** ANA level changes in ANA-Positive Group (100 patients) at 12 Week

| | No of patients with increase ANA level During treatment | No of patients with Decrease or no change in ANA level during treatment | $X^2$ | p. value |
|---|---|---|---|---|
| **At 12 weeks** | 93 | 7 | 10.336 | 0.001 |
| **EVR** | 84%(76) | 16%(1) | 8.336 | 0.001 |
| **Mean value of Baseline ANA level** | 14.23±2.43 | 15.42±1.32 | 2.532 | 0.053 |
| **Mean value of ANA level after12 weeks** | 16.75±5.36 | 14.52±4.85 | 2.336 | 0.076 |

**Table (5):** Univariate analysis of factors which predict achievement of early virological response (EVR)

| Variables | No EVR(N =43) (21.5%) | EVR(N=157) (78.5%) | P value |
|---|---|---|---|
| **Sex(females)** | 19 | ٤٢ | 0.526 |
| **Age** | 39.63±8.63 | 37.55±7.62 | 0.224 |
| **BMI** | 28.3±0.5 | 21.3±1.3 | **0.046** |
| **ANA positivity** | 23 | 77 | 0.213 |
| **Hb(gm/dl)** | 10.25+2.84 | 11.99+3.41 | 0.526 |
| **WBC(cells/$cm^3$)** | 3869.2+258.6 | 4115.6+366.5 | 0.336 |
| **ANC** | 1785.7+174.6 | 1813.95+259.3 | 0.447 |
| **PLT (c/$mm^3$)** | 251.6+33.6 | 196.3+44.5 | 0.529 |
| **Random blood Sugar (gm/dl)** | 110.4+20.4 | 99.4+15.60 | 0.357 |
| **S.Creat (mg/dl)** | 0.90+0.12 | 0.84+0.36 | 0.859 |
| **ALT (IU/I)** | 52.41+7.63 | 41.29+6.95 | **0.027** |
| **AST (IU/I)** | 37.63+7.19 | 34.19+6.37 | 0.147 |
| **Viral load (IU/ml)** | 1391722.5+63241.4 | 1296438.5+38367.9 | **0.019** |
| **TSH (mU/ml)** | 37.61+7.52 | 33.96+8.63 | 0.352 |
| **AFP (ng/ml)** | 3.68+1.34 | 4.63+0.96 | 0.639 |
| **Fibrosis stage(2,3)** | 40(93%) | 76(48%) | **0.048** |
| **Activity grade(2,3)** | 35 (81%) | 90(57%) | 0.053 |

**Table (6):** Multivariate Logistic Regression Analysis for predictors of EVR

| | r. | P. value | Odd's ratio | 95% confedence interval |
|---|---|---|---|---|
| **BMI** | 0.491 | 0.045 | 0.591 | 0.34-1.57 |
| **Fibrosis stage(2,3)** | 0.457 | 0.048 | 0.718 | 0.36-1.42 |
| **ALT(IU/I)** | 0.436 | 0.036 | 0.638 | 0.35-1.25 |
| **Viral load(IU/I)** | 0.385 | 0.023 | 0.731 | 0.62-2.98 |

## DISCUSSION

Hepatitis C virus infection plays an important role in the pathogenesis of the immunologic derangement, but the mechanism remains unclear. In patients with HCV infection, evidence of altered immune system homeostasis is indicated by a high prevalence of non organ specific autoantibodies (NOSAs) (50%).

Among NOSAs, anti-liver/kidney microsomal antibody type 1 has a direct influence over the enhanced severity of liver damage. However, for more frequently observed NOSAs, such as anti smooth-muscle antibody (ASMA) and anti-nuclear antibodies (ANA), there are insufficient data to provide a conclusive answer regarding their pathogenicity **[6].**

This study was conducted on 200 HCV infected patients (139 males &61females) to detect the significance of ANA positivity and its effect on histopathology and EVR to pegylated interferon and ribavirin in chronic HCV infected patients for proper selection of patients for combined antiviral therapy.

In the present study age was non significantly higher in ANA positive group than in ANA negative one [(41.5±5.69) vs. (37.41±8.62) respectively]. As regard sex, males were more likely to have ANA positivity than females (75% vs 25%) but without statistically significant difference (p= 0.4) (Table 1). These results were in consistance with Peng et al. **[5]** and Khairy et al. [**13]** who found that there was no statistical significant difference concerning the demographic data in ANA positive and negative patients. In contrary, Hsieh et al. **[6]** found that women had a significantly higher prevalence than men (41.2 vs 31.0%; p = 0.012). This difference may be due to large number of patients included in the previous study as it was carried out on 614 patients.

Regarding pretreatment evaluation of patients, BMI was higher in ANA positive group than ANA negative one (28.10±3.24 vs. 25.60± 2.10) but without statistical significant difference (p value =0.442) (Tabl 1). This was in agreement with Narciso–Schiavon et al. **[14]** who found no association between ANA positivity and BMI.

On the same hand viral load was higher in ANA positive than ANA negative group with significant difference (p= 0.041). This was in agreement with study done by Chen et al. **[15]** who found that PCR levels in ANA positive group were higher than ANA negative one but without statistical significant difference (p value 0.3). In contrast to the result of the present work, Hsieh et al. **[6]** concluded that antinuclear antibody positivity was associated with lower RNA levels in patients with chronic hepatitis C. This difference might be due to different HCV genotypes that were included in the previous study.

In the present study there was statistically significant difference between ANA positive and ANA negative groups as regards activity grades (p value =0.019)and fibrosis stages (p value= 0.021) (Table 2). This was in agreement with Chretien et al. **[16]** who reported that fibrosis, inflammation and hepatocellular necrosis were significantly more pronounced when NOSA positive patients were compared with NOSA negative patients.

Similarly, Hsieh et al. **[6],** Yee et al. **[7]**, Squadrito et al. **[17],** Takashi et al. **[18]** found significant association between autoantibody reactivity and severe hepatic fibrosis, inflammation, and cirrhosis. ANA positive patients had almost two fold higher chance of having quicker fibrosis. On the other hand Khairy et al. **[13]** concluded that fibrosis stage and necro inflammatory grading were not influenced by ANA positivity in their study. This discripasncy may be attributed to different number of ANA positive cases included in the previous study (59 patients).

In the present study, ALT and AST were significantly elevated in ANA positive than ANA negative group during IFN therapy (p= 0.049 & 0.047 respectively) (Table 3). This came in accordance with Sezaki et al. **[19]** who reported that ALT level may increase in patients with autoimmune features when received IFN- based therapies, and this may be due to IFN- induced immune mediated hepatocyte injury. On the other hand, Khairy et al. **[13]** concluded that ANA negative patients and not those ANA positive patients reported significant elevations of serum transaminases during treatment.

Also in this work, EVR was higher in ANA negative (80%) than ANA positive group (77%) but without significant difference (p value 0.85) (Table 3). This was in consistence with Wasmuth et al. **[20]** who found that absence of NOSA prior to &during combination therapy was associated with favorable treatment response. Similarly, Peng et al. **[5]** reported that patients with higher ANA titers before interferon therapy tend to be interferon-resistant.

Similarly, Gatselis et al. **[21]** found that ANA negativity was the predictor for achieving better treatment response to interferon therapy. On contradiction to the results of the present work, Wu et al. **[22]** demonstrated that EVR was significantly higher in ANA positive patients (77.8%) vs ANA negative (53%) (p value <0.05). This difference might be due to different sample size, patients characters and viral genotypes as the previous study was performed on 69 HCV patients, twenty of them were positive in auto-antibodies, not only ANA but also other auto-antibodies (anti-SMA, anti-AMA, and anti-LKM) and the studied cases had genotype 1 and 2.

In the present study there was statistically significant difference (Table 4) between ANA positive patients who had increased level of ANA during treatment and those who had decreased level of ANA during treatment in achieving EVR (84% vs 16% respectively), p value (0.001) and this was in contrast to what reported by Muratori et al. **[23]** who found that patients whom the NOSA titer developed and increased during treatment were non responders. The difference may be due to small sample size in the previous work (20 patients).

In the present work univariate and multi variate analysis of predictors of EVR revealed that BMI, ALT, viral load and fibrosis stage were an independent predictors of EVR (Table 5 & 6).

This was in consistence with Kim et al. **[24]** who found that rapid normalization of ALT by 4 weeks after treatment might be a useful response factor that is readily available in clinical practice.

This was in contrary to Rodrigues-Torres et al. **[25]** who stated that higher ALT , absence of cirrhosis, younger age and white non-Latino race/ethnicity were associated with successful achievement of RVR and EVR in patients infected with HCV. This difference may be explained by different genotype as the previous study was carried out on genotype 1only.

Regarding BMI, the result of the present study came in agreement with Bressler et al. **[26]** who stated that BMI greater than 30 kg/m², was an independent negative predictor of response to hepatitis C treatment. Also, Rodrigues-Torres et al. **[25]** concluded that lower BMI was associated with achieving EVR. On the other hand, Pattullo et al. **[27]** stated that neither body weight nor BMI influenced virological response.

Regarding viral load, the result of the present work was in consistant with Ridruejo et al. **[28]** who stated that lower virological responce associated with higher viral load. Also, Lee et al. **[29]** found that viral load < 2 million copies/mL was associated with responses 1.5- to two-folds better than cases with high viral load.

Regarding fibrosis stages, the result of the present work agreed with Rodrigues-Torres et al. **[25]**, Paqliaro et al. **[30]** Who stated that short-term and sustained response were independently predicted by lobular structure on pretreatment liver biopsy and by short disease duration.This was contradictory to Lee et al. **[29]** who stated that presence of significant fibrosis/cirrhosis was not important predictive response factor. This difference due to, may be, different sample size (360 patients) of the previous work.

This study concluded that ANA positivity in patients with chronic HCV was associated with advanced fibrosis but it was not a predictor for EVR.

**Funding:**Non.

**Ethical approval:** Written informed consent was taken from all patients before participation in this study.The study protocol was approved by the ethical committee of Benha Faculty of Medicine and its University Hospitals.

**Conflicts of interest:**Non.

## REFERENCES


1. Narciso-Schiavon JL, Freire FCF, Suarez MM, Ferrari MVO, Scanhola GQ, Luccaschiaron Lde. Antinuclear antibody positivity in patients with chronic hepatitis C: clinically relevant or an epiphenomenon?. *J.Clin Viral* 2006; (36): S153.
2. Esmat G, El Kassas M, Hassany M, Gamil ME, Elraziky M. How to optimize HCV therapy in genotype 4 patients. *Liver International* 2013; 33 suppl 1: 41-5.
3. Manns MP, Vogel A. Autoimmune hepatitis, from mechanisms to therapy. *Hepatology* 2006; 43: S132-S144.
4. Bianchi FB, Muratori P, Granito A, Pappas G, Ferri S, Muratori L. Hepatitis C and autoreactivity. *Dig.Liver Dis* 2007; 1 : S22-4.
5. Peng YC, Hsieh SC, Yang DY, Tung CF, Hu WH, Hung WN, Chen GH. Expression and Clinical Significance of Antinuclear Antibody in Hepatitis C Virus Infection. *J Clin Gastroentrol* 2001; 33(5): 402-406.

6. Hsieh MY, Dai CY, Lee LP, Huang JF, Tsai WC, Hou NJ et al. Antinuclear antibody is associated with a more advanced fibrosis and lower RNA levels of hepatitis C virus in patients with chronic hepatitis C. *J Clin Path* 2008; 61: 333-337.
7. Yee LJ, Kelleher P, Goldin RD, Marshall S, Thomas HC, Alberti A et al. Antinuclear antibodies (ANA) in chronic hepatitis C virus infection: correlates of positivity and clinical relevance. *J Viral Hepat* 2004; 11(5):459-464.
8. Manns MP, Wedemeyer H, Cornberg M. Treating viral hepatitis C: efficacy, side effects, and complications. *Gut* 2006; 55:1350-1359.
9. Craxi A, Pawlotsky J, Wedemeyer H, Bjoro K, Flisiak R, Forns X et al. EASL Clinical Practice guidelines: management of hepatitis C virus infection. *J Hepatol* 2011; 55: 245-64.
10. Kavanaugh A, Tomar R, Reveille J, Solomon DH, Homburger HA. Guidelines for clinical use of the antinuclear antibody test and tests for specific autoantobodies to nuclear antigens. *Arch pathol lab Med* 2000; 124:71-81.
11. Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR cooperative study group. *Hepatology* 1996; 24 (2):289-293.
12. Mchutchinson JG, Poynard T. combination therapy with interferon plus ribavirin for the initial treatment of chronic hepatitis C.*Semin liv. Dis* 1999;19 suppl :57-65.
13. Khairy M , El-Raziky M , El-Akel W, Abdelbary MS, Khatab H, El-Kholy B et al. Serum Autoantibodies Positivity Prevelance In Patients With Chronic HCV And Impact On Pegylated Interferon And Ribavirin Treatment Response. *J Gastroentrology & Hepatology* 2013; 65: 1478-3231.
14. Narciso – Schiavon JL, Freire FC, Suarez MM, Ferrari MV, Scanhola GQ, Schiavon Ldel et al. Antinuclear antibody positivity in patients with chronic hepatitis C: clinically relevant or an epiphenomenon? *Eur J. Gastroenterol Hepatol* 2009; 21:350-6.
15. Chen CH, Lee CM, Chen CH, HU TH, Wang JH, Hung CH et al. Prevalence and Clinical Relevance of Serum Autoantibodies in Patients with Chronic Hepatitis C. *Chang Gung Med J* 2010; 33:258-265.
16. Chrétien P, Chousterman M,Abd Alsamad I, Ozenne V, Rosa I, Barrault C et al. Non–organ-specific autoantibodies in chronic hepatitis C patients: association with histological activity and fibrosis.*J Autoimmun* 2009; 32:201-205.
17. Squadrito G, Previti M, Lenzi M, LeRose EP, Caccamo G, Restuccia T et al. High prevalence of non organ specific auto antibodies in hepatitis C virus-infected cirrhotic patients from southern Italy. *Dig Dis Sci* 2003; (48):349-353.
18. Takashi H, Mikio N. Autoantibodies in liver disease: important clues for the diagnosis, disease activity and prognosis. *Hepatitis Monthly J* 2008; (4): 295-303
19. Sezaki H, Arase Y, Tsubota A, Suzuki Y, Kobayashi M, Saitoh S et al. Type C- chronic hepatitis patients who had autoimmune phenomenon and developed jaundice during interferon therapy. *J Gastroenterol* 2003; 38 : 493-500.
20. Wasmuth HE, Stolte C, Geier A, Dietrich CG, Gartung C, Lorenzen J et al. The presence of non-organ-specific autoantibodies is associated with a negative response to combination therapy with interferon and ribavirin for chronic hepatitis. *BMC Infect Dis* 2004; 4:4 doi:10(5):1471-1530.
21. Gatselis NK, Georgiadou SP, Koukoulis GK, Tassopoulos N, Zachou K, Liaskos C et al.Clinical significance of organ and non-organ specific autoanibodies on the response to antiviral treatment of patients with chronic hepatitis C. *Aliment pharmacol ther* 2006; 24 (11-12): 1563.
22. Wu CH, Xu XY, Tian GS, Yu YY. Serum auto-antibodies of patients with chronic hepatitis C and its significance there of infection of hepatitis C virus. *Zhonghua Yi Xue Za Zhi* 2006; 86(6): 390–393.
23. Muratori P, Muratori L, Stroffolini T, Pappas T, Terlizzi P, Ferrari R et al. Prevalence of non-organ specific autoantibodies in HCV-infected subjects in the general population. *Clin Exp Immunol* 2003; 131:118-121.
24. Kim YJ, Jang BK, Kim ES, Park KS, Cho KB, Chung WJ et al. Rapid normalization of alanine aminotransferase predicts viral response during combined peginterferon and ribavirin treatment in chronic hepatitis C patients. *Korean J Hepatol* 2012; 18(1):41-71.
25. Rodriguez-Torres M, Sulkowski MS, Chung RT, Hamzeh FM, Jensen DM. Factors associated with rapid and early virologic response to peg-interferon alfa-2a/ribavirin treatment in HCV genotype 1 patients representative of the general chronic hepatitis C population. *J Viral Hepat* 2010; 17(2): 139–147.
26. Bressler BL, Guindi M, Tomlinson G, Healthcote J. High body mass index is an independent risk factor for nonresponse to antiviral treatment in chronic hepatitis C. *Hepatology* 2003; 38(3):639-644.
27. Pattullo V, Ravindran C ,Mazzulli T, Wong DK, Heathcote EJ.Pegylated interferon plus optimized weight-based ribavirin dosing negate the influence of weight and body mass index on early viral kinetics and sustained virological response in chronic hepatitis C.*J viral Hepat* 2010; 17 (12): 834–838.
28. Ridruejo E. Predictors of Response to Chronic Hepatitis C . *Future Virology* 2012; 7(11):1089-1101.

29. Lee SS, Abdo AA. Predicting antiviral treatment response in chronic hepatitis C: how accurate and how soon? *J. Antimicrob. Chemother* 2003; 51(3): 487-491.
30. Paqliaro L, Craxi A, Cammaa C, Tiné F, Dimarco V, Locono O et al. Interferon-α for chronic hepatitis C: An analysis of pretreatment clinical predictors of response. *Hepatology* 1994; 19 (4): 820-8.

**Peer reviewer: Rashed M 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

# Study of the Relationship between Blood Ammonia Level and Esophageal Varices in Patients with Liver Cirrhosis

**Atef Abo-Alsoud Ali [1], Amira Maher Badawy [1], Ahmed Abdel-Rahman Sonbol [2], Mohammed Elsayed Ayad [3]**

[1]*Tropical Medicine department, Faculty of Medicine, Menoufiya University ,Egypt*
[2]*Clinical Pathology department, Faculty of Medicine, Menoufiya University,Egypt*
[3]*Shebin Elkom Teaching Hospital ,Shebin Elkom ,Egypt*

***Corresponding Author***
***Mohamed Elsayed Ayad***

***Mobile:***
*01063382104*

***E mail:***
*Drayad92@gmail.com*

***Key words:***
*Oesophageal varices, Portosystemic collaterals, Blood ammonia, Upper gastrointestinal endoscopy*

**Background and study aim:** Portal hypertension leads to formation of portosystemic collateral veins as oesophageal varices in patients with liver cirrhosis. Several studies have shown that blood ammonia is a vauable non invasive marker of oesophageal varices. This study is designed to evaluate the possible relation between blood ammonia level and oesophageal varices in patients with liver cirrhosis.

**Patients and Methods:** This study was conducted on three groups of patients and control subjects. Group I included 20 cirrhotic patients without evidence of oesophageal varices. Group II included 40 cirrhotic patients with evidence of varices. Group III included 20 healthy control subjects. Serum level of ammonia were done for all patients and control subjects.

**Results:** There was a highly significant increase in the mean values of blood ammonia in cirrhotic patients with varices in comparison to other patients without varices, with highly significant positive correlation between serum ammonia and size of varices. There was a significant increase in the mean values of blood ammonia in cirrhotic patients with grade III and IV varices [large varices) in comparison to cirrhotic patients with grade I and II varices.

**Conclusions:** Blood ammonia level is a valuable, simple non invasive marker for prediction of oesophageal varices.

## INTRODUCTION

Cirrhosis is defined histologically as a diffuse hepatic process characterized by fibrosis and the conversion of normal liver architecture into structurally abnormal nodules. The progression of liver injury to cirrhosis may occur over weeks to years. Indeed, patients with hepatitis C may have chronic hepatitis for as long as 40 years before progressing to cirrhosis **[1].**

Portal hypertension is a progressive, inevitable consequence of liver cirrhosis, which leads to formation of porto-systemic collateral veins. Among them, oesophageal varices have the greatest clinical impact because their rupture results in variceal hemorrhage that can be fatal. Upper gastrointestinal [GI) endoscopy is the gold standard in the diagnosis of oesophageal varices **[2]**.

Gastroesophageal varices are present in approximately 50% of patients with liver cirrhosis. Their presence correlates with the severity of liver disease. Patients without varices develop them at a rate of 8% per year and the progression from small to large varices occurs in 10 to 20% of cases yearly **[3]**. The most important predictor of hemorrhage is the size of varices, with the highest risk of first hemorrhage occurring in patients with large varices, about 15% per year **[4]**.

Practice guidelines have recommended that all patients with cirrhosis undergo screening upper GI endoscopy to detect oesophageal varices at the time of diagnosis and after that, surveillance endoscopies should be performed every 2 to 3 years in cirrhotic patients without varices and the patients with small varices be endoscoped every 1-2 years, and annually in the setting of decompensation **[5]**. However, these guidelines have not been evaluated prospectively to date ,particularly regarding its cost effectiveness. Since, the point prevalence of medium/large

varices is approximately 15 to 20% **[6]**, the majority of subjects undergoing screening endoscopy either do not have varices or have varices and do not require prophylactic therapy.

Therefore, there is need for identification of non endoscopic, non invasive methods that can accurately predict oesophageal varices, particularly large ones in cirrhotic patients and help to identify patients at greatest risk and thereby reduce the necessity of endoscopic screening **[7]**.

In cirrhosis, the major portion of ammonia carried by portal blood is shunted by portosystemic collaterals into systemic circulation. The raised blood ammonia level may be an indicator of the presence of oesophageal varices as a main part of portosystemic shunts with the most dangerous complications **[8]**).

On the other hand, a recent study found that there was a moderate but significant correlation between blood ammonia level and size of oesophageal varices **[9]**.

## PATIENTS AND METHODS

This study was carried out on patients attending the outpatient clinic and the inpatients of Hepatology and Gastroenterology unit of Shebin Elkom Teaching Hospital in the period between June to December 2014, from which 60 cirrhotic patients were selected, in addition to 20 healthy persons of matched age and sex as controls. The cirrhotic patients were further subdivided into the two groups. Group I included 20 patients without endoscopic evidence of oesophageal varices, Group II included 40 patients with endoscopic evidence of oesophageal varices, this group was subdivided into 4 subgroups according to grade of varices, Group IIa included 10 patients with grade I oesophageal varices, group IIb included 10 patients with grade II oesophageal varices, group IIc included 10 patients with grade III oesophageal varices and group IId included 10 patients with grade IV oesophageal varices. After having an informed consent, all patients and controls were subjected to full history taking, full clinical examination, abdominal ultrasonography, upper gastrointestinal endoscopy and laboratory investigations including: CBC, ESR, RBS, liver function tests, AFP, creatinine & BUN, serum electrolytes, HCV Ab & HBs Ag and serum ammonia were done for all patients.

**Exclusion criteria**

Patients who received endoscopic variceal ligation (EVL) or sclerotherapy, presence of hepatic encephalopathy, active or recent GI bleeding within 4 weeks, portal vein thrombosis on ultrasonography [USG), hepatocellular carcinoma, renal insufficiency evidenced by serum creatinine of >1.3 mg/dl and patients in whom endoscopy is contraindicated.

**Sample collection and measurement of ammonia**

Fasting blood ammonia level was measured in all patients and controls within 1 to 3 days of performing endoscopy. Patients were asked to fast overnight. In the morning, at complete rest, 5 ml of peripheral venous blood was taken from each subject without using tourniquet. Blood was collected into an EDTA evacuated tube. The samples were immediately carried to laboratory gently in an icebox and analyzed within 30 minutes of arrival. In cases of ambulant patient, samples were collected in the laboratory. During analysis, sample was first centrifuged and the plasma was separated from cellular material. Ammonia level was measured by kinetic enzymatic method with glutamate dehydrogenase by using ammonia–liquizyme single reagent provided by Bioassay system, Germany.

**Statistical Analysis :**

All the patient details and study variables were entered in predesigned data collection sheet. Data were analyzed by using statistical software SPSS 13.0. All the quantitative data were expressed as mean ± SD; qualitative data were analyzed by Chi-square test and quantitative data by Student's t-test or Mann-Whitney's U test. Correlation study was done by using Spearman's correlation coefficient test. Performance of the test was assessed by sensitivity and specificity. Receiver operating characteristic [ROC) curve was used to assess the usefulness of the test and performance at different cutoff values. A 'p' value of <0.05 was taken statistically significant and a 'p' value of <0.005 was taken highly statistically significant.

## RESULTS

There was a high statistically significant difference between the mean values of blood ammonia level of cirrhotic patients and control group as shown in table (1).

There was a high statistically significant increase in the mean values of blood ammonia in cirrhotic patients with varices in comparison to other patients without varices as shown in table (1).

The best cutoff value for detection of O.V. was 74 umol/L with sensitivity 97.5%, specificity 80%, positive predictive value (PPV) 90.7% and negative predictive value (NPV) 94.1% as shown in table (3) and figure (1).

On the other hand, there was a high statistically significant decrease in the mean values of platelet count/ spleen diameter ratio in cirrhotic patients with varices in comparison to other patients without varices as shown in table (2). The best cutoff value for detection of O.V was 638.9 with sensitivity 100%, specificity 97.5%, PPV 95.2% and NPV 100% as shown in table (3).

There was a high statistically significant positive correlation between blood ammonia and size of varices as shown in table (4) and figure (2), while there was a high statistically significant negative correlation between platelet count/ spleen diameter ratio and size of varices as shown in table (4).

There was a statistically significant decrease in the mean values of platelet count/spleen diameter ratio in cirrhotic patients with evidence of grade III and IV varices (large varices) in comparison to cirrhotic patients with grade I and II varices as shown in table (5). The best cutoff value for detection of large varices was 436.5 with sensitivity 82.5%, specificity 70%, PPV 84.6% and NPV 66.7% as shown in table (6).

There was a statistically significant increase in the mean values of blood ammonia in cirrhotic patients with evidence of grade III and IV varices (large varices) in comparison to cirrhotic patients with grade I and II varices as shown in table (5). The best cutoff value was 102 umol/L with sensitivity of 95% and specificity of 52.5% in detecting large esophageal varices (grade III and IV varices) in patients with liver cirrhosis. Its PPV was 50% and NPV was 95.5% with accuracy of 66.7% as shown in table (6) and figure (3).

**Table (1):** Blood ammonia level in the studied groups

| | Cirrhotic group with varices (no=40) | Cirrhotic group without varices (no=20) | Total cirrhotic patients (no=60) | Control group (no=20) | t-Test | P value |
|---|---|---|---|---|---|---|
| | Mean ± SD | | Mean ± SD | Mean ± SD | | |
| **Ammonia (umol / L)** | 135.1±24.8 | 65.8±11.3 | 111.9±39.1 | 45.5±4.4 | t1:9.14<br>t2: 11.87 | p1<0.001**<br>p2<0.001** |

**Highly significant difference
t1 and p1: between total cirrhotic patients and control group.
t2 and p2 : between cirrhotic patients with and without varices.

**Table (2):** Platelet count /spleen diameter ratio in cirrhotic patients

| | Cirrhotic group with varices (group II) [no=40) | Cirrhotic group without varices (group I) (no=20) | t-Test | P value |
|---|---|---|---|---|
| | Mean ± SD | Mean ± SD | | |
| **platelet count/spleen diameter ratio** | 431.6±116.1 | 1099.7±356.5 | 10.84 | <0.001** |

**Highly significant difference

**Table (3):** Diagnostic validity of blood ammonia level and platelet count/ spleen diameter ratio for detection of varices

| | AUC | Cutoff point | Sensitivity | Specificity | PPV | NPV | Accuracy |
|---|---|---|---|---|---|---|---|
| **Ammonia (umol/ L)** | 0.991 | 74.0 | 97.5% | 80% | 90.7% | 94.1% | 91.7% |
| **Platelet count/spleen diameter ratio** | 0.999 | 638.9 | 100% | 97.5% | 95.2% | 100% | 98.3% |

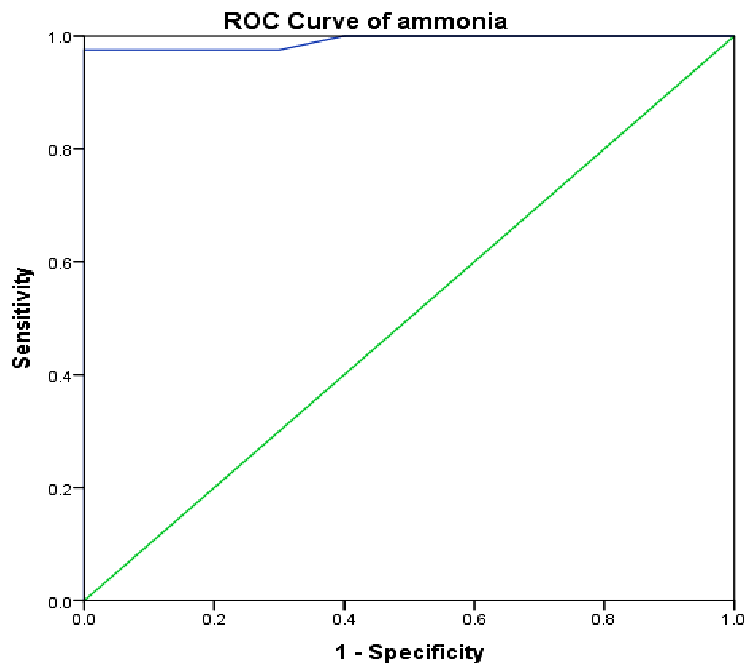


**Fig. (1): ROC curve** for the diagnostic validity of blood ammonia level in detection of oesophageal varices.

**Table (4):** Spearman correlation between size of varices and ammonia and platelet count spleen diameter ratio

| | Size of varices | |
|---|---|---|
| | **r** | **P value** |
| **Ammonia (umol/ L)** | 0.692 | <0.001** |
| **platelet count/ spleen diameter ratio** | -0.461 | 0.003* |

**Highly significant difference
* Significant difference

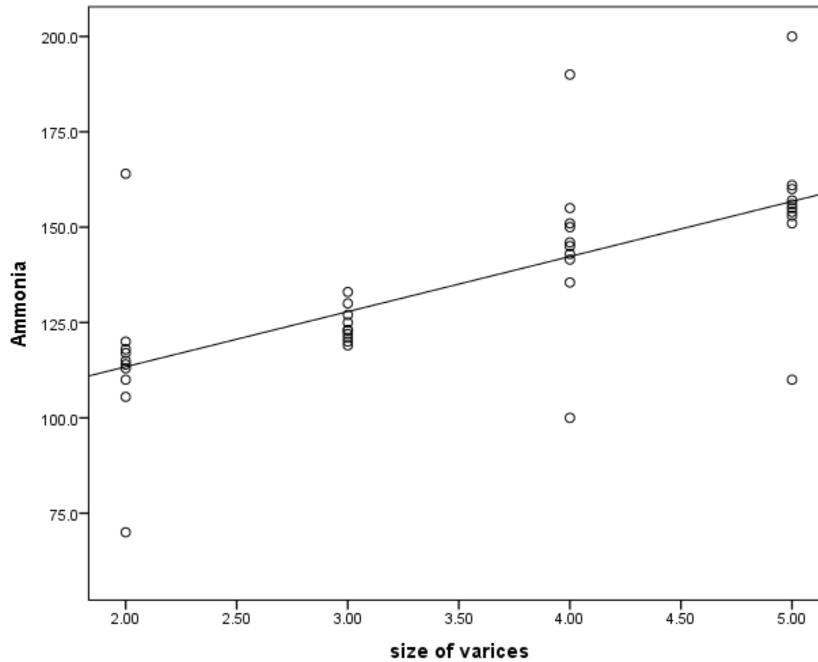


**Fig. (2): Spearman correlation** between blood ammonia concentrations and size of varices

**Table (5):** Comparison between different degrees of varices among cirrhotic group regarding platelet count /spleen diameter ratio and blood ammonia level

| | Cirrhotic group with grade I varices (group IIa) (no=10) | Cirrhotic group with grade II varices (group IIb) (no=10) | Cirrhotic group with grade III varices (group IIc) (no=10) | Cirrhotic group with grade IV varices (group II d) (no=10) | Kruskal Wallis Test | P value | Post Hoc Test |
|---|---|---|---|---|---|---|---|
| | Mean±SD | Mean±SD | Mean±SD | Mean±SD | | | |
| **platelet count /spleen diameter ratio** | 529.7±100.9 | 433.3±85.5 | 400.3±50.3 | 362.9±145.9 | 9.92 | 0.02* | P1=0.19<br>P2=0.02*<br>P3=0.05*<br>P4=0.89<br>P5=0.75<br>P6=0.98 |
| **Ammonia (umol/ L)** | 114.7±22.6 | 124.3±4.5 | 145.7±21.9 | 155.7±21.4 | 9.73 | <0.001** | P1=0.27<br>P2=0.001*<br>P3=<0.001**<br>P4=0.02*<br>P5=0.001*<br>P6=0.25 |

*Significant difference **Highly Significant difference

1------ Comparison between groupIIa and groupIIb .
2------ Comparison between groupIIa and groupIIc.
3------ Comparison between groupIIa and groupIId .
4------ Comparison between groupIIb and groupIIc .
5------ Comparison between groupIIband groupIId .
6------ Comparison between groupIIc and groupIId

**Table (6):** Diagnostic validity of blood ammonia level and platelet count/spleen diameter ratio for detection of large varices [grade III and IV)

| | AUC | Cutoff point | Sensitivity | Specificity | PPV | NPV | Accuracy |
|---|---|---|---|---|---|---|---|
| Ammonia | 0.934 | 102 | 95% | 52.5% | 50% | 95.5% | 66.7% |
| Platelet count/ spleen diameter ratio | 0.864 | 436.5 | 82.5% | 70% | 84.6% | 66.7% | 78.3% |

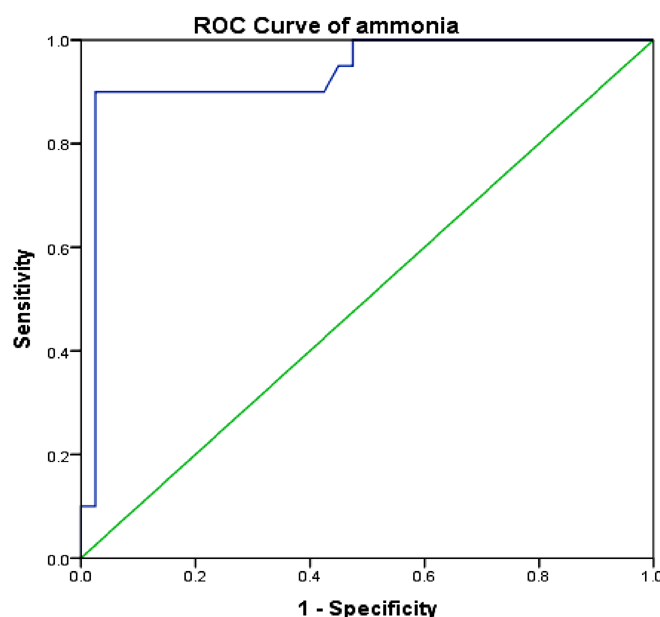


**Fig. (3): ROC curve for** the diagnostic validity of blood ammonia level in detection of large oesophageal varices

## DISCUSSION

The portal venous system has numerous collaterals that interconnect with the systemic circulation. When portal pressure rises above 10 mmHg, potential portosystemic collaterals may develop. Formation of collaterals is a complex process involving the opening, dilatation and hypertrophy of pre-existing vascular channels. It is possible that active neoangiogenesis is involved in the formation of collateral vessels **[10]**.

Portosystemic shunts have been shown to be responsible for recurrent or persistent Porto-systemic encephalopathy **[11]**. Ammonia plays a major role in the pathogenesis of hepatic encephalopathy in cirrhotic patients **[12]**.

The generated ammonia, which reaches the liver through the portal vein, is converted to urea by means of urea cycle and excreted from the kidneys. In patients with decreased hepatic functional reserve or those with portosystemic shunt, ammonia level in the blood rises **[8]**.

This study was conducted to evaluate the role of blood ammonia as a non invasive marker for detection of oesophageal varices (O.V) and also for presence of large varices and compared it with platelet count/ spleen diameter ratio that is one of the most important non invasive predictors of O.V.

Blood ammonia values were estimated in cirrhotic groups and control group. The study showed that there was a highly significant difference between the mean ammonia level of cirrhotic and control groups ($P<0.001$). The mean values of serum ammonia in cirrhotic groups was 111.9±39.1 umol/L while it was 45.5±4.4 umol/L in control group. This was in agreement with the study done by Khondaker et al. **[9]** that found that there was a significant increase in blood ammonia level in patients with liver cirrhosis in comparison to healthy individuals but with a low

level of serum ammonia in healthy individuals, that was 28.47 umol/L.

On the other hand, there was a highly significant increase in the mean values of serum ammonia in cirrhotic patients with varices in comparison to other patients without varices. The best cutoff value for detection of O.V was 74 umol/L with sensitivity 97.5% and specificity 80%, that comes in agreement with the study done by El-Hefny et al. **[16]** with a cutoff value of 77.5 umol/L with sensitivity 100% and specificity 95% for detection of O.V, also with the study done by Terantino et al. **[8]** with different cutoff value that was 42 umol/L with sensitivity 97% and specificity 43% for detection of O.V.

This study found that, there was a highly significant decrease in the mean values of platelet count/ spleen diameter ratio in cirrhotic patients with varices in comparison to other patients without varices. The best cutoff value for detection of O.V was 638.9 with sensitivity 100%, specificity 97.5%, PPV 95.2% and NPV 100%, that comes in agreement with the study done by Baig et al. **[14]** with a cutoff value of 1014 with sensitivity 98.1% and specificity 88.6% for detection of O.V.

In the present study, there was a highly significant positive correlation between serum ammonia and size of varices, r (0.692) and P value (<0.001). This comes in agreement with study done by Khondaker et al. **[9]** that found that there was a significant correlation between blood ammonia level and size of varices, r (0.451), P value (<0.05).

On the other hand, there was a significant negative correlation between platelet count/spleen diameter ratio and size of varices, r (-0.461) and P value (<0.05). This comes in agreement with study done by Thomopoulos et al. **[18]** that reported that there was negative correlation between platelet count/ spleen diameter ratio and size of varices, r (-0.431) and P value (<0.05).

In the present study, there was a significant decrease in the mean values of platelet count/spleen diameter ratio in cirrhotic patients with evidence of grade III and IV varices (large varices) in comparison to cirrhotic patients with grade I and II varices. The best cutoff value for detection of large varices was 436.5 with sensitivity 82.5%, specificity 70%, PPV 84.6% and NPV 66.7%. This comes in agreement with study done by Sarangapani et al. **[15]** that found that a cutoff point of 909 had a sensitivity of 88.5% and specificity of 83.5% for detection of large varices.

Blood ammonia, the newly suggested non-invasive marker of esophageal varices showed significant increase in cirrhotic patients with endoscopic evidence of grade III and IV oesophageal varices (large varices) in comparison to cirrhotic patients with grade I and II varices (P<0.05) in the present study.

To test the blood ammonia level as a predictor of large varices, sensitivity and specificity of blood ammonia level at different cutoff values were assessed. Blood ammonia at 102 umol/L had sensitivity of 95% and specificity of 52.5% in detecting large oesophageal varices (grade III and IV varices) in patients with liver cirrhosis. Its PPV was 50% and NPV was 95.5% with accuracy of 66.7%. This comes in agreement with study done by Montasser et al **[17]** with a cutoff value of 133 umol/L with sensitivity 100% and specificity 96% in detecting large varices, also with study done by Khondaker et al. **[9]** that found a significant correlation between blood ammonia level and size of varices, but with a different cutoff point of about 63 umol/l with sensitivity of 95% and specificity of 50% in detecting large oesophageal varices in patients with cirrhosis.

This study proved that blood ammonia level may be a non invasive marker for presence of oesophageal varices and also for detection of large varices. A cutoff value of 74 umol/L for presence of varices and 102 umol/L for detection of large varices. The study compared between blood ammonia level and platelet count/ spleen diameter ratio, that was one of the most important non invasive marker for prediction of O.V, that found that, detection of oesophageal varices was more accurate with platelet count/spleen diameter ratio, but for detection of large esophageal varices, blood ammonia level was more accurate.

Strict precautions should be done for collection, handling, storage and analysis for blood samples to avoid errors for determination of ammonia, also diet and body mass index may affect blood ammonia level **[13]**, this explained different cutoff values of this study and other Egyptian studies from studies done in Italy by Terantino et al. **[8]** and in Bangladesh by Khondaker et al. **[9].**

## CONCLUSION

So finally, it can be concluded that blood ammonia level can detect oesophageal varices at a cutoff value of 74 umol/L and can detect large varices at a cutoff value of 102 umol/L, so blood

ammonia level may be a good tool for non invasive prediction of oesophageal varices.

**Funding:** None.
**Conflicts of interest:** None.
**Ethical approval:** Approved.

## REFERENCES


1. Poynard T, Bedossa T , Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR and DOSVIRC groups . *Lancet* 1987; 349 : 825 – 32 .
2. De franchis R . Revising consensus in portal hypertension : Report of the Baveno V consensus work shop on definitions, methodology and therapy in portal hypertension. *J Hepatol* 2010; 53:762-68.
3. Merli M, Nicolini G, Angeloni S, Rinaldi V, De Santis A, et al. Incidence and natural history of small esophageal varices in cirrhotic patients. *J Hepatol* 2003;38:266-72.
4. Augustin S, Millan L , Gonzalez A. Detection of early portal hypertension with routine data and liver stiffness with asymptomatic liver disease. A prospective study . *J.Hepatol*.2013 ;22:250-60.
5. Lubel JS , Angus PW. Modern management of portal hypertension .*Internal med.J*. 2005; 35[1): 45-49.
6. Garcia-Pegan , Garcia-sancho. Functional aspects on pathophysiology in portal hypertension in liver cirrhosis . *J Hepatol* 2012 ; 57[2),458-61.
7. Berzigotti A, Gilabert R , Abraldes JG. Non invasive prediction of clinically significant portal hypertension and esophageal varices in patients with compensated liver cirrhosis. *Am.J. Gastroenterol*. 2008 ; 103[5),1159-67.
8. Tarantino G, Citro V, Esposito P, Giaquinto S, de Leone A, et al . Blood ammonia levels in liver cirrhosis: A clue for the presence of portosystemic collateral veins. *BMC Gastroenterol* 2009;9:21.
9. Khondaker M, Ahmad N, Al-Mahtab M , Sumi S. Correlation between blood Ammonia Level and oesophageal Varices . *Euroasian J Hepato-Gastroenterol*. 2013; 3: 10-14.
10. Bosch J, Navasa M, Garcia-Pegan JC, Delacy AM , Rodes J. Portal Hypertension. *Medical Clinics of North America* 1989; 73:931-953.
11. Riggo O, Erafti C, Catalano C, Pedeconi F, Mecarelli O et al. High prevalence of Spontaneous Portal-Systemic Shunts in persistent hepatic encephalopathy: A case control study. *J Hepatol*.2005; 42: 1158-1165.
12. Butterworth RF, Giguere JF, Michaud J, Lavoie J Layrargues GP. Ammonia is a key factor in the pathogenesis of Hepatic Encephalopathy. *Molecular and chemical Neuropathology*, 1987; 6: 1-12.
13. Howanitz JH, Howanitz PJ , Skrodzi CA. Influences of specimen processing and storage condition on results for plasma ammonia. *Clin Chem.* 1984; 30:906-908.
14. Baig.WW, Nagaraja MV , Varma M. Spleen diameter for diagnosis of O.V.: Is it feasible? *J Gastroentrol*. 2008 ; 22: 852-8.
15. Sarangapani A, Shanmugam C, Kalyanasundaram M, Rangachari B, Thangavelu P , Subbarayan JK. Non invasive prediction of large O.V. in CLD. *Saudi Journal of Gastroentrology*. 2010; 16[1):38-42.
16. El-Hefny N, Hareedy M and Abdel-Aal R: Blood ammonia level and other non invasive parameters as indicators of portosystemic collaterals in cirrhotic patients: Thesis submitted for partial fulfillment of MSC degree in internal medicine . Assuit university 2013.
17. Montasser M, Abdella H , Samy A. Evaluation of venous ammonia level, splenic longitudinal diameter and portal vein diameter as non invasive indicator for portosystemic collaterals in Egyptian cirrhotic patients . *Open journal of Gastroenterology* 2014; (4) 265-74.
18. Thomopoulos KC, Labropoulou-Karatza C, Mimidis KP, Katsakoulis EC, Iconomou G , Nikolopoulou V.N. Non invasive predictors of the presence of large oesophageal varices in patients with liver cirrhosis. *Digestive and Liver disease* 2003;35 :473-478.


**Peer reviewer: Tarik Zaher** ,Professor of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt.
**Editor: Mohamed Emara**, Lecturer of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt

# Study of the Pattern of Brucellosis in Menoufyia Governorate

**Atef Abo EL-Soud Ali[1], Ayman Mohammed El-Lehleh[1], Somaia Abd El-Mohsen Shehab El-Deen[1], Amira Abd El-Kader El-Hendy[2], EL Sayed Mahmoud Mohammed Mousa[1]**

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

***Corresponding Author:***
***EL Sayed Mahmoud Mohammed Mousa***

***Mobile:***
*+0201144938029*

***E mail:*** *Sayed_m364 @yahoo.com*

***Key words:***
*Human brucellosis, ELISA IgG and IgM test for brucella, Serum samples*

**Background and study aim:** ELISA can determine specific antibody classes against brucella, It is a sensitive, simple and rapid test, thus help to study the pattern of the brucellosis. The aim of this article is to study the pattern of the brucellosis in Menoufyia governorate.

**Patients and methods:** Sera from 150 individuals from confirmed brucellosis cases and 25 healthy individuals were tested for presence of IgG and IgM antibodies by ELISA assay. Culture positivity for brucellosis was used as the reference standard for diagnosis.

**Results:** Serum IgG and IgM for brucellosis by ELISA test had increased values in confirmed brucella cases, ELISA IgM was highly specific (100%) in all groups and sensitive (96%) in acute brucellosis, (100%) in subacute brucellosis and (64%) in chronic brucellosis. While, ELISA IgG specificity in all groups was (80%) and the sensitivity in acute brucellosis was (88%), in subacute and chronic brucellosis was (100%).

**Conclusion:** ELISA IgG and IgM test for brucella is a simple and reliable test in the diagnosis of pattern of the brucellosis.

## INTRODUCTION

Brucellosis is a worldwide zoonosis caused by the facultative intracellular members of the bacterial genus *Brucella* of which at least six species are now recognized; these are highly genetically homogenous and the disease is of major socio-economic importance **[1]**.

Brucellosis is a significant health problem in Egypt and a confirmed cause of 3% of acute febrile illnesses **[2]**.

The estimated annual incidence of brucellosis in Egypt per 100000 population was 64 and 70 in 2002 and 2003, respectively **[3]**.

Diagnosis of the disease is challenging and is frequently delayed or missed because the clinical picture may mimic other infectious and non-infectious conditions, It is often missed because of its non-specific symptoms unless the clinician is aware of the organism and maintains a high degree of suspicion so, diagnosis can be established by laboratory methods such as, serology and blood cultures **[4]**.

In absence of bacteriologic confirmation, a presumptive diagnosis can be made on the basis of high or rising titers of specific antibodies **[5]**.

Therefore, accurate diagnosis of brucellosis is vital for early institution of proper therapy as untreated cases may progress to chronic stage and focal complications **[6]**.

## PATIENTS AND METHODS

This study was carried out in Menoufiya Fever Hospitals and Tropical Medicine Department, Faculty of Medicine, Menoufiya University hospitals.

The study was conducted on two groups: The patient group (group I) is one hundred and fifty patients diagnosed as having brucellosis (based on: Detection of specific antibodies at significant titers by STAT and positive

isolation of Br. species in blood samples). Group I was divided into the following 3 subgroups according to duration of disease**:** group Ia comprised eighty nine patients with acute brucellosis. Group Ib comprised forty two patients with subacute brucellosis. Group Ic comprised nineteen patients with chronic brucellosis .Group II was twenty five normal healthy individuals of matched age and sex as a control group. After having an informed consent; each patient underwent: detailed history taking: personal history, patient's complaints with stress on afternoon fever, profuse sweating and headache, present history with stress on history of contact with animals, drinking unpasteurized milk, ingestion of home made dairy products and duration of symptoms that were reported, past history of chest diseases or other diseases and family history.

Clinical examination with stress on fever, lymphadenopathy, hepatomegaly, slenomegaly, enlarged testes and tenderness of the back.

Investigations: Full routine laboratory investigations: complete urine analysis and stool analysis, complete blood count, Erythrocyte sedimentation rate (ESR) &C-reactive protein (CRP), Liver function tests including serum total and direct bilirubin, ALT, AST and serum albumin, hepatitis viral markers were done for each case with elevated transaminases and bilirubin, Serological diagnostic techniques for brucellosis as agglutination tests was done (Rose bengal slide agglutination test, rapid slide titration test, tube agglutination test for brucellosis and mercaptoethanol agglutination test for brucellosis).

Radiological investigations (Chest radiography, radiographic study of the spine, both sacroilliac joints and peripheral joints, scrotal ultrasonography and abdominopelvic ultrasonography).

IgG and IgM for brucellosis was measured in serum samples of the patients and control groups using ELISA kit.

### Statistical analysis

Data were collected, tabulated and statistically analyzed by computer using SPSS version 20. The following tests; sensitivity, specificity, Significance of results (P value) and Chi square test were calculated to compare the efficacy of ELISA IgG & ELISA IgG assay with blood culture and STAT to assess the pattern of the brucellosis in Menoufyia governorate.

## RESULTS

No statistical significant differences among the studied groups as regarding age and sex as shown in table (I). Patients with chronic and subacute brucellosis were significantly more frequent among residents of rural areas than patients with acute brucellosis and control subjects, while patients with acute brucellosis and control subjects were significantly more frequent among residents of urban areas than patients with chronic and subacute brucellosis as shown in table (2). Constitutional symptoms and symptoms of localization were present in various proportions in the different patients groups. Osteoarticular symptoms were significantly more frequent in the acute stage, weight loss and anxiety were significantly more frequent in the chronic stage while palpitations was significantly more frequent in the subacute stage as shown in table (3). Splenomegaly and hepatomegaly were more frequent in the acute stage than in the subacute or chronic stages as shown in table (4). Fever, pallor, jaundice and signs of localization were present in various proportions in the studied patients groups. splenomegaly, lymphadenopathy and hepatomegaly were significantly more frequent in the acute stage than in the subacute or chronic stages as shown in table (4). Positive titer ≥1/1280 was highly significant more frequent in acute and subacute stages of brucellosis than in chronic stage and control group. Positive titer 1/640 was significantly more frequent in patients groups at different stages of disease than in control group. Positive titer 1/320 was significantly more frequent in subacute and chronic stages than in acute stage and control group. Positive titer 1/160 was significantly more frequent in chronic stage than in acute, subacute stages and control group. Negative serology and positive titer <1/160 were significantly more frequent in control group than patients groups at different stages of disease as shown in table (5). Positive blood culture was highly significantly more frequent in acute and subacute stages than in chronic stage and control group as shown in tables (6). ELISA IgM test was positive in all patients with subacute brucellosis (100%), most patients with acute brucellosis (95.5%), most patients with chronic brucellosis (64%), as shown in table (7) while, ELISA IgG was specific in all groups is (80%) and the sensitivity in acute brucellosis was (87.6%), in subacute and chronic brucellosis was (100%) as shown in table (8). ELISA IgM is highly specific and sensitive

in all groups and ELISA IgG is specific in subacute and chronic groups. blood culture is specific in all groups but with low sensitivity. STAT is sensitive in all patients groups but with low specificity (table 9).

**Table (1):** Shows the age and sex distribution of the studied groups

| | | Patients groups | | | | | | | | Control group | | $\chi^2$ | P value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Group Ia (N=89) | | Group Ib (N=42) | | Group Ic (N=19) | | Total (N=150) | | Group II (N=25) | | | |
| | | No | % | No | % | No | % | No | % | No | % | | |
| Age | <15 y | 17 | 19.1 | 5 | 11.9 | 1 | 5.2 | 23 | 15.3 | 5 | 20 | 0.35 | >0.05 |
| | 15-45 y | 57 | 64 | 28 | 66.6 | 13 | 68.4 | 98 | 65.3 | 16 | 64 | 0.02 | >0.05 |
| | > 45 y | 15 | 16.9 | 9 | 21.5 | 5 | 26.4 | 29 | 19.4 | 5 | 20 | 0.01 | >0.05 |
| Sex | Male | 64 | 71.9 | 26 | 61.9 | 11 | 57.8 | 101 | 67.3 | 21 | 84 | 1.88 | >0.05 |
| | Female | 25 | 28.1 | 16 | 36.1 | 8 | 42.2 | 49 | 32.7 | 5 | 20 | | |

X2:Chi square test.

P value : Significance of results: Non significant difference if P>0.05, Significant difference if P<0.05 and Highly significant difference if P<0.01

**Table (2):** Shows the residence distribution of the studied groups

| Place | Patients groups | | | | | | | | Control group | | $\chi^2$ | P value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Group Ia (N=89) | | Group Ib (N=42) | | Group Ic (N=19) | | Total (N=150) | | Group II (N=25) | | | |
| | No | % | No | % | No | % | No | % | No | % | | |
| Rural | 67 | 75.3 | 40 | 95.2 | 19 | 100 | 126 | 84 | 20 | 80 | 46.6 | <0.01 |
| Urban | 22 | 24.7 | 2 | 4.8 | 0 | 0 | 24 | 12 | 5 | 20 | | |

**Table (3):** Clinical Symptoms of the studied patients groups

| Symptoms | Patients groups | | | | | | | | $\chi^2$ | P value |
|---|---|---|---|---|---|---|---|---|---|---|
| | Group Ia (N=89) | | Group Ib (N=42) | | Group Ic (N=19) | | Total (N=150) | | | |
| | No | % | No | % | No | % | No | % | | |
| Fatigue | 89 | 100 | 33 | 78.5 | 12 | 63.1 | 134 | 89.3 | 29.3 | <0.001 |
| Fever | 81 | 91 | 31 | 73.8 | 11 | 57.9 | 123 | 82 | 12.9 | <0.001 |
| Chills | 85 | 95.5 | 31 | 73.8 | 13 | 68.4 | 129 | 86 | 16.7 | <0.001 |
| Bodyaches | 78 | 87.6 | 23 | 54.7 | 8 | 42.1 | 109 | 72.7 | 25.7 | <0.001 |
| Arthralgia | 74 | 83.1 | 20 | 47.6 | 9 | 47.4 | 103 | 68.7 | 21.3 | <0.001 |
| Back pain | 71 | 79.8 | 20 | 47.6 | 7 | 36.8 | 98 | 65.3 | 20.8 | <0.001 |
| Headache | 71 | 79.8 | 30 | 71.4 | 11 | 57.9 | 112 | 74.7 | 4.3 | >0.05 |
| Appetite loss | 64 | 71.9 | 16 | 38 | 8 | 42.1 | 88 | 58.7 | 15.9 | <0.001 |
| Palpitations | 21 | 23.5 | 21 | 50 | 3 | 15.8 | 45 | 30 | 11.5 | <0.001 |
| Sweating | 67 | 75.2 | 20 | 47.6 | 7 | 36.8 | 94 | 62.7 | 15.5 | <0.001 |
| Nausea/vomiting | 49 | 55 | 7 | 16.7 | 4 | 21 | 60 | 40 | 20.8 | <0.001 |
| Abdominal pain | 46 | 51.6 | 18 | 42.9 | 5 | 26.3 | 69 | 46 | 4.3 | >0.05 |
| Anxiety | 3 | 3.4 | 2 | 4.8 | 10 | 52.6 | 15 | 10 | 43.9 | <0.001 |
| Weight loss | 28 | 31.5 | 23 | 54.8 | 16 | 84.2 | 67 | 44.7 | 20.0 | <0.001 |
| Cough | 10 | 11.2 | 2 | 4.8 | 1 | 5.3 | 13 | 8.7 | 1.8 | >0.05 |
| Scrotal pain | 3 | 3.4 | 3 | 7.1 | 1 | 5.3 | 7 | 4.7 | 0.93 | >0.05 |

**Table (4):** Result of clinical examination of the studied groups

| Findings | Patients groups | | | | | | | | Control group | | $\chi^2$ | P value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Group Ia (N=89) | | Group Ib (N=42) | | Group Ic (N=19) | | Total (N=150) | | Group II (N=25) | | | |
| | No | % | No | % | No | % | No | % | No | % | | |
| Fever | 50 | 56.1 | 7 | 16.7 | 1 | 5.2 | 58 | 38.7 | 0 | 0 | 14.4 | <0.01 |
| Pallor | 35 | 39.3 | 10 | 23.8 | 4 | 21 | 49 | 32.7 | 0 | 0 | 11.3 | <0.01 |
| Hepatomegaly | 7 | 7.9 | 2 | 4.8 | 0 | 0 | 9 | 6 | 1 | 4 | 0.16 | >0.05 |
| Jaundice | 50 | 56.1 | 5 | 11.9 | 3 | 15.8 | 58 | 38.7 | 0 | 0 | 14.4 | <0.01 |
| Lymphadenopathy* | 50 | 56.1 | 7 | 16.7 | 1 | 5.2 | 58 | 38.7 | 0 | 0 | 14.4 | <0.01 |
| Splenomegaly | 53 | 59.5 | 10 | 23.8 | 2 | 10.5 | 65 | 43.3 | 0 | 0 | 17.2 | <0.01 |
| Ascites | 0 | 0 | 0 | 0 | 1 | 5.2 | 1 | 0.6 | 0 | 0 | 0.17 | >0.05 |
| Sacroiliitis | 4 | 4.5 | 2 | 4.8 | 2 | 10.5 | 8 | 5.3 | 0 | 0 | 1.4 | >0.05 |
| Peripheral arthritis | 4 | 4.5 | 0 | 0 | 0 | 0 | 4 | 2.7 | 0 | 0 | 0.68 | >0.05 |
| Spondylodiskitis | 0 | 0 | 2 | 4.8 | 1 | 5.2 | 3 | 2 | 0 | 0 | 0.51 | >0.05 |
| Signs of chest infection | 4 | 4.5 | 0 | 0 | 0 | 0 | 4 | 2.7 | 0 | 0 | 0.68 | >0.05 |
| Signs of epididymoorchitis | 4 | 4.5 | 3 | 7.1 | 1 | 4 | 8 | 5.3 | 0 | 0 | 1.4 | >0.05 |

**Table (5) :** The titers of standard tube agglutination test of the studied groups

| Titers | Patients groups | | | | | | | | Control group | | $\chi^2$ | P value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Group Ia (N=89) | | Group Ib (N=42) | | Group Ic (N=19) | | Total (N=150) | | Group II (N=25) | | | |
| | No | % | No | % | No | % | No | % | No | % | | |
| ≥1/1280 | 53 | 59.5 | 13 | 31 | 1 | 5.2 | 67 | 44.7 | 0 | 0 | 18.1 | <0.001 |
| 1/640 | 18 | 20.2 | 13 | 31 | 5 | 26.3 | 36 | 24 | 0 | 0 | 7.55 | <0.001 |
| 1/320 | 7 | 7.9 | 11 | 26.1 | 9 | 47.3 | 27 | 18 | 0 | 0 | 5.3 | <0.05 |
| 1/160 | 0 | 0 | 0 | 0 | 2 | 10.6 | 2 | 1.3 | 2 | 8 | 4.3 | <0.05 |
| <1/160 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 12 | 18.3 | <0.001 |
| Negative | 11 | 12.4 | 5 | 11.9 | 2 | 10.6 | 18 | 12 | 20 | 80 | 58.3 | <0.001 |

**Table (6):** The results of blood culture of the studied groups

| Blood culture | Patients groups | | | | | | | | Control group | | $\chi^2$ | P value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Group Ia (N=89) | | Group Ib (N=42) | | Group Ic (N=19) | | Total (N=150) | | Group II (N=25) | | | |
| | No | % | No | % | No | % | No | % | No | % | | |
| Positive | 32 | 36 | 15 | 36 | 1 | 5.3 | 48 | 32 | 0 | 0 | 11.0 | <0.001 |
| Negative | 57 | 64 | 27 | 64 | 18 | 94.7 | 102 | 68 | 25 | 100 | | |

**Table (7):** The results of ELISA IgM test of the studied groups

| ELISA IgM test | Patients groups | | | | | | | | Control group | | χ2 | P value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Group Ia (N=89) | | Group Ib (N=42) | | Group Ic (N=19) | | Total (N=150) | | Group II (N=25) | | | |
| | No | % | No | % | No | % | No | % | No | % | | |
| Positive | 85 | 95.5 | 42 | 100 | 12 | 64 | 139 | 92.7 | 0 | 0 | 112.6 | <0.001 |
| Negative | 4 | 4.5 | 0 | 0 | 7 | 36 | 11 | 7.3 | 25 | 100 | | |

**Table (8):** The results of ELISA IgG test of the studied groups

| ELISA IgM test | Patients groups | | | | | | | | Control group | | χ2 | P value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Group Ia (N=89) | | Group Ib (N=42) | | Group Ic (N=19) | | Total (N=150) | | Group II (N=25) | | | |
| | No | % | No | % | No | % | No | % | No | % | | |
| Positive | 78 | 87.6 | 42 | 100 | 19 | 100 | 139 | 92.7 | 5 | 20 | 77.6 | <0.001 |
| Negative | 11 | 12.4 | 0 | 0 | 0 | 0 | 11 | 7.3 | 20 | 80 | | |

**Table (9):** The comparison of the efficacy of ELISA IgM assay with blood culture and STAT

| | ELISA IgM | ELISA IgG | Blood culuture | STAT |
|---|---|---|---|---|
| **Group Ia(89)** | | | | |
| Sensitivity (%) | 95.5 | 87.6 | 36 | 88 |
| Specificity (%) | 100 | 80 | 100 | 80 |
| **GroupIb (42)** | | | | |
| Sensitivity (%) | 100 | 100 | 36 | 92 |
| Specificity (%) | 100 | 80 | 100 | 80 |
| **Group Ic (19)** | | | | |
| Sensitivity (%) | 64 | 100 | 10.5 | 92 |
| Specificity (%) | 100 | 80 | 100 | 80 |

## DISCUSSION

Brucellosis is a worldwide zoonosis caused by the facultative intracellular members of the bacterial genus *Brucella* of which at least six species are now recognized these are highly genetically homogenous and the disease is of major socio-economic importance **[1]**. The present study was planned to study the pattern of the brucellosis in Menoufyia governorate. In the present study we found that, human brucellosis affects all age groups as shown in table (1) this agree with Cetinkaya1 et al. **[7]** who found that human brucellosis affects all age groups. In this study we found that, the highest number of cases was found between 15-45 years old (98 cases, 65.3%), this reflects the magnitude of the socio-economic impact of brucellosis in this area, as it affects mainly the most productive group in the community. This agree with Mantur et al. **[8]** who found that, 29 cases (19.4%) in the age group of >45 years old but the least number of cases, 23 cases (15.3%) was recorded in the age group of <15 years including 1 case (5.3%) of chronic brucellosis. Statistical analysis revealed no significant differences among the three groups with respect to frequencies of disease among different age groups as shown in table (1). We found also that, the highest number of cases was found among males (101 cases, 67.3%), followed by females (49 cases, 32.7%) as shown in table (1). This result is in agreement

with Jama'ayah et al. **[9]** who found that, males are commonly affected by brucellosis and Minas et al. **[10]** who reported that, males were affected more often by brucellosis due to their profession than females. This result disagrees with Hussein and his colleagues **[11]** who reported higher incidence among females. Probably this discrepancy is related to cultural and epidemiological factors as in developing countries females are in contact with domestic animals. In our study we found that, the highest number of cases was found among residents of rural areas (126 cases, 84%) while the least number of cases was found among residents of urban areas (24 cases, 12%) as shown in table (2). This result is in agreement with Kassiri et al. **[12]** who concluded that, the higher prevalence in rural areas may be due to close contact of individuals with livestock. The number of cases was low in urban areas because all commercialized dairy products are produced from pasteurized milk **[10]**. We also found that, all of chronic cases came from rural areas. This is probably due to the delay in visiting physician by shepherds and farmers **[10]**. The main presenting symptoms were as follows : fever (89.3%), fatigue (86%), chills (82%), profuse sweating (74.7%), bodyaches (72.7%), arthralgia (68.7%), back pain (65.3%), appetite loss (62.7%), headache (58.7%), abdominal pain (46%), weight loss (44.7%), nausea and vomiting (40%) and palpitations (30%) as shown in table (3). These findings are in agreement with the results obtained by John et al. **[13]** and Shen **[14]**. The commonest signs of brucellosis were splenomegaly (43.3%), lymphadenopathy (38.7%), hepatomegaly (38.7%) and pallor (32.7%) as shown in table (4). This result was explained by Alişkan **[15]** who reported that, these findings are due to the relatively high concentration of brucella in reticuloendothelial system. These findings are in agreement with the results obtained by Hadda et al. **[16]**. Positive titer ≥1/1280 was highly significant more frequent in acute and subacute stages of brucellosis than in chronic stage and control group. Positive titer 1/640 was significantly more frequent in patients groups at different stages of disease than in control group. Positive titer 1/320 was significantly more frequent in subacute and chronic stages than in acute stage and control group. Positive titer 1/160 was significantly more frequent in chronic stage than in acute, subacute stages and control group. Negative serology and positive titer <1/160 were significantly more frequent in control group than patients groups at different stages of disease as shown in table (5). Mantur et al. **[8]** concluded that, STAT titer ≥1/160 do not signify active infection especially in brucella endemic areas because in areas of endemicity as a high proportion of the population has antibodies against brucellosis. Sisirak and Hukić **[24]** reported that, in countries where the disease is highly endemic, a large proportion of the population may have persistent Br. specific IgG antibodies, hence under such conditions, the detection of specific IgM antibodies is important to diagnose brucellosis in early phase. The present data revealed that isolation of brucella in blood culture was found in 32 cases (36%) in acute brucellosis, 15 cases (36%) in subacute brucellosis and 1 case (5.3%) with chronic brucellosis as shown in table (6). The explanation for the low yield of conventional culture in the present study, appears to be related more to the low number of pathogens in the blood sample and use of different antibiotic treatments for various diagnostic suspicion in other clinical sectors before referring the patients to infectious disease unites than to the technical difficulty of isolation of brucella species from clinical samples. Also bacteraemia in brucellosis may be periodically present and this agrees with Pappas and Papadimitriou **[17]**.

ELISA IgM test was positive in all patients with subacute brucellosis (100%), most patients with acute brucellosis (96%), most patients with chronic brucellosis (64%) and negative in all persons in the control group as shown in table (7) and ELISA IgG test was positive in most patients with brucellosis whatever the duration of disease and negative in most persons in the control group as shown in table (8) This result agree with Maha et al. **[18]** who found that, the examined cases using ELISA of clinically suspected brucellosis yielded positive result with ELISA, (80%) were positive for brucella IgM while, (64.6%) were positive for brucella IgG. ELISA IgM was highly specific (100%) in all groups and sensitive (96%) in acute brucellosis, (100%) in subacute brucellosis and (64%) in chronic brucellosis. While, ELISA IgG specificity in all groups (80%) and the sensitivity in acute brucellosis was (88%), in subacute and chronic brucellosis was (100%). A comparative study conducted by Araj et al. **[19]** it was argued that, the ELISA method should be preferred because in chronic and complicated cases, STAT and Rose Bengal tests might miss a serious portion of positive cases,

they reported high sensitivities for ELISA tests of 91% and 100% for IgG ELISA and IgM ELISA, respectively. This result was not similar to that of Sanaei Dashti et al. **[20]** who found that, there are some contradictory reports regarding the diagnostic ability of ELISA in acute brucellosis. Therefore, it is reasonable to further evaluate and standardize the test according to the various geographical regions and populations. ELISA IgM is highly specific (100%) in all patient groups and sensitivity is (95.5%) in acute brucellosis, (100%) in subacute brucellosis and (64%) in chronic brucellosis. ELISA IgG is highly sensitive (100%) in subacute and chronic brucellosis and (87.6%) sensitive in acute brucellosis. ELISA IgG specificity is (80%) in all patient groups. Blood culture is specific in all groups (100%) but with very low sensitivity (36%) in acute brucellosis, (36%) in subacute brucellosis and (10.5%) in chronic brucellosis. STAT is sensitive in all patient groups (88%) in acute brucellosis, (92%) in subacute brucellosis and (92%) in chronic brucellosis. While its specificity is (80%) in all patient groups as shown in table (9).

## CONCLUSION

From this study we can conclude that diagnosis of human brucellosis regardless of the duration or focalization of the disease based not only on positive history of exposure to infection and compatible clinical picture but also on laboratory diagnosis. The conventional blood culture for the diagnosis of human brucellosis is time consuming and poses low sensitivity especially with prolonged durations of the disease and in the presence of focal forms but important in species identification. The ELISA method has higher positivity, higher titers and the advantage of identifying different classes of antibodies in comparison to other agglutination methods. ELISA method should be preferred because in chronic and complicated cases, STAT and Rose bengal tests might miss a serious portion of positive cases. Detection of IgG and IgM is suggestive of subacute brucellosis while detection of IgG or IgM is suggestive for diagnosis of chronic or acute brucellosis respectively. The prevalence of brucellosis is more frequent in spring and summer than autumn and winter.

**Funding:** None.
**Conflicts of interest:** None.
**Ethical approval:** Approved.

## REFERENCES

1. Tharwat M, Amir H , Abd-Elrahman. Evaluation of a rapid immunochromatographic test for detection of brucella abortus ntibodies in egyptian cattle sera and milk. *Alexandria Journal of Veterinary Sciences*. 2014, 40:24-28.
2. Afifi S., Earhart K., Azab M., Youssef F., El Sakka H., Wasfy M. Hospital-based surveillance for acute febrile illness in Egypt. A focus on community-acquired blood stream infections. *AmJ Trop Med Hyg*. 2005, 73(2):392-9.
3. Samaha H., Mohamed T., Khoudair R., Ashour H. Serodiagnosis of brucellosis in cattle and humans in Egypt. *Immunobiology.* 2009, 214(3):223-6.
4. Mutnal M., Purwar S., Metgud S., Nagmoti M., Patil C. PCR confirmation of cutaneous manifestations due to brucella melitensis. *J Med Microbiol.* 2007, 56 (2):283-5.
5. Ertek M., Yazgi H., Zkurt Z., Ayyildiz A, parlak M. Comparison of the diagnostic value of the standard tube agglutination test and the ELISA IgG and IgM in patients with brucellosis .*Turk J Med Sci.* 2006, 36 (3): 159-163.
6. Jain R , Tilak V. Evaluation of different serological techniques in laboratory diagnosis of brucellosis. *J Indian Med Assoc.* 2008, 106(8):520, 522, 524.
7. Cetinkaya1 Z., Aktepe1 OC. , Ciftci1 IH. , Demirel R. Seroprevalence of human brucellosis in a rural area of Western Anatolia, Turkey. *J Health Popul Nutr.*2005, 23(2):137-141.
8. Mantur B., Biradar M., Bidri R., Mulimani M., Kariholu P , Mangalgi S. Protean clinical manifestations and diagnostic challenges of human brucellosis in adults: 16 years' experience in an endemic area. *J Med Microbiol*.2006, 55 : 897-903.
9. Jama'ayah M., Heu J., Norazah A. Seroprevalance of brucellosis among suspected cases in Malaysia. *Malaysian Journal of Pathology***.** 2011, 33(1) : 31 – 34.
10. Minas M., Minas A., Gourgulianis K., Stournara A. Epidemiological and clinical aspects of human brucellosis in Central Greece. *Jpn J Infect Dis.* 2007, 60(6):362-6.
11. Hussein A., Sayed A., El Feki M. Seroepidemiological study on human brucellosis in Assiut governorate. *The Egyptian Journal of Immunology*. 2005, Vol. 12 (1), 49-56.
12. Kassiri H., Amani H., Lotfi M. Epidemiological, laboratory, diagnostic and public health aspects of human brucellosis in western Iran. *Asian Pac J Trop Biomed.* 2013, 3(8):589-594.
13. John K., Fitzpatrick J., French N., Kazwala R., Kambarage D., Mfinanga GS., et al. Quantifying risk factors for human brucellosis in rural Northern Tanzania. *PLoS ONE* 5 2010, (4): 9968.

14. Shen M W. Diagnostic and therapeutic challenges of childhood brucellosis in a nonendemic country. *Pediatrics*. 2008, 121 (5): 1178-1183.
15. Alişkan H. The value of culture and serological methods in the diagnosis of human brucellosis. *Microbiology*. 2008, 42(1):185-95.
16. Hadda V., Khilnani G., Kedia S. Brucellosis presenting as pyrexia of unknown origin in an international traveller: a case report.*Cases J.* 2009, 1;2:7969.
17. Pappas G., Papadimitriou P. Challenges in brucella bacteraemia. *Int J Antimicrob Agents*. 2007, 30 Suppl 1:S29-31.
18. Maha A., Abo-Shadi1 A., Alhanouf H., Elmahi M. Serum Levels of Interferon Gamma in Patients with Brucellosis in a Saudi Hospital .*British Microbiology Research Journal*. 2014, 4(3): 293-305.
19. Araj G., Kattar M., Fattouh L., Bajakian K., Kobeissi S. Evaluation of The brucella immunglobulin G and IgM enzyme-linked immunosorbent assays for diagnosis of human brucellosis. *Clin Diagn Lab Immunol*. 2005, 12:1334-1335.
20. Sanaei Dashti A, Karimi A, Javad V, Shiva F, Fallah F, Alaei MR, et al. ELISA Cut-off Point for the Diagnosis of Human Brucellosis; a Comparison with Serum Agglutination Test. *Iran J Med Sci*. 2012 ;37(1):9-14.



**Peer reviewer: Tarik Zaher** ,Professor of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt. **Mohamed I Radwan** , Assistant Professor of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt.

**Editor: Mohamed Emara**, Lecturer of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt

# Changes in CD4 and CD8 after Interventional Management of Hepatocellular Carcinoma

**Walid El-Sherbiny[1], Nashwa Khairat Abousamra[2], Muhammad Diasty[1], Shaker Wagih Shaltout[1]**

[1]Tropical Medicine Department, Faculty of Medicine, Mansoura University, Egypt.
[2]Clinical Pathology Department, Faculty of Medicine , Mansoura University, Egypt.

***Corresponding Author***
*Walid El-Sherbiny*

***Mobile:***
+201009092903

***E mail:***
*walidelsherbiny1@yahoo.com*

***Key words:***

*CD4, CD8, CD4/CD8 ratio, Hepatocellular carcinoma ablation, RFA, PEI, TACE, MWA*

**Background and study aim :** Hepatocellular carcinoma (HCC) has many curative choices which in some circumstances are equal to or even better than surgery. These strategies for treatment of HCC may induce certain local effects which trigger distinct immunological responses that may have a systemic impact on the natural history of the tumour itself. These responses are validated through the measurement of specific immune cells in the systemic circulation. In this study, we tried to observe and analyze changes in the peripheral immune cells that accompany and follow HCC ablation by different procedures of radiological intervention and compare our results with literature. So, this study may be useful with other criteria in the guidelines for the selection of the optimal therapeutic strategy for each patient.

**Patients and Methods:** This study was conducted on about 50patients diagnosed with HCC who were referred to Tropical Medicine Department at Mansoura University Hospital, Egypt and 20 healthy volunteers as a control. The therapeutic strategy was selected according to the tumor stage and general condition. RFA was performed for12 cases, PEI for 13, MWA for12 and TACE for 13 cases. All Patients were subjected to full history taking, clinical examination, liver function tests, anti HCV antibodies and HBS antigen by 3rd generation ELISA, serum alpha fetoprotein, abdominal ultrasonography, triphasic abdominal computerized tomography and lymphocyte subset assay by flow cytometry 1 day before, and 3 weeks after the treatment.

**Results:** Regarding the immunological status between control and HCC patients, there was a demonstrable difference in the number of cells in both groups, as the control group had higher levels of CD4+ and CD8+ values. In the RFA group, CD4+ cells and CD4/CD8 ratio remarkably increased after treatment (P <0.001), and the CD8+ cells significantly decreased (P <0.002) with concomitant increase in the CD4+/CD8+ ratio (P<0.001). In the PEI group, CD4+ cells markedly increased after treatment (P<0.001), but there were no significant differences in CD8+ cells and CD4/CD8 ratio. In the MWA group, CD4+ cells markedly increased after treatment (P<0.001), with increase in CD4/CD8 ratio (P<0.007) but there were no significant differences in CD8+ cells. In the TACE group, the CD4+ cells and CD4/CD8 ratio dramatically decreased after treatment (P<0.001), and the CD8+ cells increased significantly (P<0.001).

**Conclusion:** Our study has proved to find a relationship between immunity and different models of therapy in HCC patients and demonstrated a positive attitude towards increasing immune cells following ablation technique.

## INTRODUCTION

Hepatocellular Carcinoma (HCC) is currently the sixth most prevalent cancer worldwide and the third leading cause of cancer-related death **[1]**. It is one of the leading causes of morbidity and mortality in patients with liver cirrhosis. Furthermore, it has a rapidly rising incidence, largely driven by the burden of advanced Hepatitis C Virus (HCV) and non-alcoholic steatohepatitis (NASH) cases **[2]**.

Prognosis for patients with HCC depends on tumor stage at diagnosis, with curative options available only for patients diagnosed at an early stage **[3,4]**. Unfortunately, two thirds of patients with HCC are diagnosed at an advanced stage, when curative options no longer exist and median survival is less than 1 year **[5]**.

Local ablative therapies such as radiofrequency ablation (RFA), percutaneous ethanol injection (PEI) and Microwave coagulation therapy (MWA) offer potential cure for tumors detected at an early stage in well selected patients. For intermediate-stage HCC, transarterial chemoembolization (TACE) is the mainstay of treatment **[6].** Also; RFA combined with TACE is an efficient and safe treatment that provides overall survival rates similar to those achieved with surgical resection **[7].**

Previous studies have shown that immune responses are inevitable in HCC and the lymphocytes phenotype and proportion are being valuable in predicting the response and prognosis in HCC **[8-9]**. Studies have shown a significant increase in frequency of regulatory T cells in peripheral blood, tumor and ascites of HCC patients **[8]**. Although, naturally occurring anti-tumour immune responses could be detected in patients with HCC, this response fails to control tumour growth. This failure could be because of the suppressive effects exerted by the tumour cells on anti-tumour immune responses **[11]**.

Ablative techniques share, irrespective of their mechanism of induction of cell death, the ability to stimulate the immune system **[12]**. Interventional therapeutic procedures produce local and systemic effect capable of inducing cellular infiltration which in turn has the ability to mediate immunological response capable of combating tumour growth and proliferation **[13]**. These valuable data could lead us in the future to create the so-called tumor vaccination.

This study aimed to investigate changes in the peripheral immune cells of HCC patients following treatment with different interventional strategies including RFA, MWA, TACE and PEI. So, this study may be useful with other criteria in guidelines for the selection of optimal therapeutic strategy for each patient.

## PATIENTS AND METHODS

This study was a prospective interventional (Randomized Control Trial) study. It was conducted on 50 patients selected from 65 patients diagnosed with HCC who were referred to Tropical Medicine Department at Mansoura University Hospital, Egypt. The study included20 subjects as control. Patients with HCC were 31 males and 19 females and their age ranged from 42 years to 66 years with mean age of (58.42±5.42). According to Child–Turcotte–Pugh classification, 34 patients were classified as class A and 16 as class B.

HCC diagnosis was confirmed by triphasic abdominal computerized tomography scan or dynamic contrast-enhanced MRI. Diagnosis was based on the identification of the typical hallmark of HCC (hypervascular in the arterial phase with washout in the portal venous or delayed phases).

The inclusion criteria were as follows: (i) Patients exhibiting good compliance and providing informed consent, (ii) Patients with primary HCC and naïve to treatment. (iii)Patients with liver cirrhosis of Child-Pugh class A or B .About 15 patients were excluded from the study because they had one or more of the following exclusion criteria: Patients with metastatic tumor, patients with liver cirrhosis of Child- Pugh class C and patients who refused to participate in the study.

This study included 2 main groups: Group A: included 20 healthy control individuals, Group B: included 50 HCC patients. Group B was subdivided into 4 subgroups, subgroup 1(RFA group): Patients in this group were treated with radiofrequency ablation, subgroup2 (MWA group): Patients in this group were treated with microwave ablation, subgroup 3(PEI group): Patients in this group were treated with percutaneous ethanol injection and subgroup4 (TACE group): Patients in this group were treated with transarterial chemoembolization therapy. An informed consent was obtained before patients were enrolled in the study.

All participants in both groups were subjected to full history taking, clinical examination (general and abdominal examination), liver function tests (serum albumin level, serum bilirubin level and international normalized ratio),anti HCV antibodies and HBS antigen by 3rd generation ELISA, serum alpha fetoprotein level(AFP) ,abdominal ultra-sonography, triphasic abdominal computerized tomography and lymphocyte subset assay.

**Lymphocyte subset assay:**

Peripheral blood samples from patients (after obtaining informed consent and Institutional Review Board approval) were collected 1 day before treatment and three weeks after treatment in EDTA containing tubes. After incubation of blood sample with a mixture of fluorescence labeled anti-CD3, and anti-CD8 monoclonoal antibodies for 15 minutes, lysis of red blood cells was done using 10Test 3 lysis solution (Immunotech, Beckman Coulter, Marseille, France). Analysis

was done using the EPICS XL flow cytometer (Coulter Electronic, Fl, USA). For all flow cytometric analysis each sample was run with an appropriate isotype control (Mouse IgG, Dako-cytoformation, Denmark) to define the negatively stained cells. The following antibodies were used: fluorescein isothiocyanate (FITC)–labeled anti-CD3,R-phycoerythrin-cyanine 5 (RPE-CY5)-labeled anti-CD4, phycoerythrin (PE)–labeled anti-CD8. The antibodies were purchased from immunotech, Beckman Coulter, Marseille, France.

**Statistical analysis**

Data were analyzed with SPSS version 21. The normality of data was first tested with one-sample Kolmogorov-Smirnov test. Qualitative data were described using number and percent. Association between categorical variables was tested using Chi-square test. Continuous variables were presented as mean ± SD (standard deviation). The two groups were compared with Student t test while paired t- test were used to compare paired data. Analysis Of Variance (ANOVA test) used for comparison of means of more than two groups.

## RESULTS

This study was conducted on 50 patients selected from 65 patients diagnosed with HCC. They were 31 males (62%) and 19 females (38%) and their age ranged from 42 years to 66 years with mean age of (58.42±5.42). According to Child–Turcotte–Pugh classification, 34 patients were classified as class A and 16 as class B.

In our study, the correlation between the child score and immunological condition of patients was statistically non-significant.

Most of patients had HCV infection (49), and only one patient had HBV as a cause of liver cirrhosis and HCC subsequently (Table1).

Our patients performed a variety of clinical interventional procedurs including RFA (12), PEI (13), MWA (12) and finally TACE (13). All patients survived to the date of follow up with no major complications or morbidity (Fig. 1).

Regarding the immunological status between control and HCC patients, their was a demonstratable difference in the number of cells in both groups, as the control group had higher levels of CD4+ and CD8+ values (Table 2).

Changes in the lymphocyte subset after treatment in the subgroups of HCC patients are shown in Table 3which demonstrate the tendency of partial variables changes. In the RFA group, CD4+ cells and CD4/CD8 ratio remarkably increased after treatment (P<0.001), and the CD8+ cells significantly decreased (P<0.002) with concomitant increase in the CD4+/CD8+ ratio (P<0.001). In the PEI group, CD4+ cells markedly increased after treatment (P<0.001), but there were no significant differences in CD8+ cells and CD4/CD8 ratio. In the MWA group, CD4+ cells markedly increased after treatment (P <0.001), with increase in CD4/CD8 ratio (P<0.007) but there were no significant differences in CD8+ cells. In the TACE group, the CD4+ cells and CD4/CD8 ratio dramatically decreased after treatment (P<0.001), and the CD8+ cells increased significantly (P<0.001).

Statistical comparison between all subgroups shows significant decrease in the levels of AFP after treatment in The PEI group, MWA group and TACE group. (P=0.05, 0.033 and 0.043 respectively). The levels of AFP after treatment also decreased in RFA group but this decrease was statistically non-significant (P=0.136) (Table 4).

**Table (1) :** Demographic data of HCC patients.

| | RFA (n=12) | | PEI(n=13) | | MWA(n=12) | | TACE(n=13) | | Total | P value |
|---|---|---|---|---|---|---|---|---|---|---|
| | No | % | No | % | No | % | No | % | No | |
| **Sex** | | | | | | | | | | |
| Male | 7 | 58.3 | 5 | 38.5 | 9 | 75.0 | 10 | 76.9 | 31 | $X^2$= 5.215 P= 0.157 |
| Female | 5 | 41.7 | 8 | 61.5 | 3 | 25.0 | 3 | 23.1 | 19 | |
| **Age** | | | | | | | | | | |
| Mean ± SD | 57.50±6.01 | | 59.84±5.91 | | 58.33±4.94 | | 57.92±5.13 | | | F=0.436 P=0.728 |
| Min-Max | 42-63 | | 45-66 | | 50-66 | | 50-66 | | | |
| **Etiology** | | | | | | | | | | |
| HCV | 12 | | 13 | | 12 | | 12 | | 49 | |
| HBV | 0 | | 0 | | 0 | | 1 | | 1 | |
| **Child classification** | | | | | | | | | | |
| A | 10 | 83.3 | 6 | 46.2 | 6 | 50.0 | 12 | 92.3 | 34 | $X^2$= .465 P= .024* |
| B | 2 | 16.7 | 7 | 53.8 | 6 | 50.0 | 1 | 7.7 | 16 | |

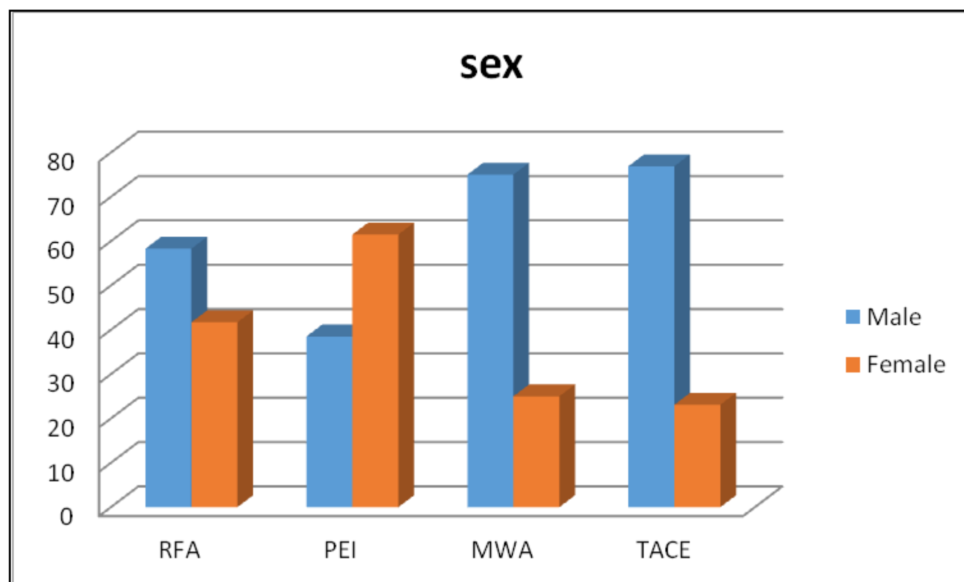


**Fig. (1) :** Different used methods of ablation

**Table (2) :** Difference between CD4+ and CD8+ in control and HCC patients

| GROUP | CD4+ | CD8+ | CD4/CD8 ratio |
|---|---|---|---|
| Control | 44.61±4.2 | 31.1±5.51 | 1.4775±0.07 |
| HCC | 35.26±7.15 | 29.72±6.77 | 1.2480±0.05 |
| Test of sig. p-value | 0.001 | 0.419 | 0.02 |

**Table (3) :** Immune cells before and after ablation procedures.

| Items | Before Mean ± SD | After Mean ± SD | Test of sig. p-value |
|---|---|---|---|
| **RFA group** | | | |
| CD4 | 34.41±5.68 | 43.93±4.42 | Paired t-test= 4.902 P=≤.001* |
| CD8 | 32.94±6.22 | 28.27±5.18 | Paired t-test= 4.027 P= .002* |
| CD4/CD8 | 1.08±0.24 | 1.61±0.39 | Paired t-test= 4.658 P=.001* |
| **PEI group** | | | |
| CD4 | 32.5±7.24 | 40.09±5.66 | Paired t-test= 5.316 P=≤.001* |
| CD8 | 26.87±7.64 | 28.67±6.22 | Paired t-test= 1.156 P= .270 |
| CD4/CD8 | 1.27±0.36 | 1.50±0.59 | Paired t-test= 1.715 P= .112 |
| **MWA group** | | | |
| CD4 | 31.7±3.67 | 39.89±3.98 | Paired t-test= 6.989 P=≤.001 |
| CD8 | 30.23±6.23 | 31.81±6.31 | Paired t-test= .981 P= .348 |
| CD4/CD8 | 1.09±0.26 | 1.30±0.28 | Paired t-test= 3.301 P= .007* |
| **TACE group** | | | |
| CD4 | 42.11±6.47 | 35.5±7.70 | Paired t-test= 4.699 P= .001* |
| CD8 | 29.11±6.15 | 34.86±6.94 | Paired t-test= 4.459 P= .001* |
| CD4/CD8 | 1.52±0.45 | 1.07±0.38 | Paired t-test= 4.348 P= .001* |

**Table (4) :** AFP before and after ablation procedures.

| | **RFA** | **PEI** | **MWA** | **TACE** |
|---|---|---|---|---|
| | **Mean ± SD** | **Mean ± SD** | **Mean ± SD** | **Mean ± SD** |
| AFP before | 43.25±69.73 | 48.30±53.75 | 63.22±65.88 | 75.13±134.95 |
| AFP after | 14.859±19.98 | 25.71±23.20 | 36.23±32.65 | 39.08±87.61 |
| Test of sig. p-value | 0.136 | 0.053 | 0.033 | 0.043 |

## DISCUSSION

For many years, HCC was regarded as a fatal tumour that has no curative treatment except for surgery, which unfortunately was a rare possibility since we are dealing with a cirrhotic liver with all its surgical limitations and morbid systemic effects, however, in recent years the view has dramatically changed and nowadays we have many curative choices which in some circumstances are equal to and even better than surgery. Hepatic interventional procedures are now considered as a cornerstone in management of HCC due its feasibility, relative safety and affordable cost.

The effect of these procedures is not limited to tumour ablation only, but extends to another immunological local and systemic effect **[14]**. Ablation of tumour cells leads to hepatocyte death, formation of necrotic tissue and establishment of chemical debris, this enhancing

environment confers the release of certain chemicals and cytokines which in turn stimulates immunity and causes sensitization of cellular antigen presentation leading to abortion of further tumour growth and even disappearance of small malignant collections **[15]**.

In this study, we tried to observe and analyze changes in the peripheral immune cells that accompany and follow HCC ablation by different procedures of radiological intervention and compare our results with literature.

Most of HCC lesions were caused by liver cirrhosis due to HCV infection; this is explained by the fact that our community has one of the highest rates of HCV infection worldwide **[16,17]**.

When patients with untreated HCC compared with healthy subjects, they expressed a state of immunological impairment manifested by decreased level of CD4+ and CD4/CD8 ratio with no significant change on CD8+ level. Guan et al, in their study has demonstrated alteration of the immune function following any change of lymphocyte subsets **[13]**. In our study, lymphocytes have significantly changed after ablation according to the method used.

Among the RFA group, 12 patients underwent ablation most of them were Child A (10 patients). All parameters were changed following ablation, there was significant increase in CD4+ and CD4/CD8 ratio ($P<0.001$) which means a strong immunological response due to the presence of necrotic cell death which is much more immunogenic than apoptotic cell death, it leads to inflammatory response that triggers signals that lead to dendritic cell (DC) stimulation and maturation. Our results are equal to Zerbini et al who demonstrated efficient antigen loading following DC maturation which can be elicited locally by heat shock proteins (HSPs), release of cytokines, complements and other inflammatory mediators **[18]**.

Gravante et al, has explained this extensive immunological reaction following RFA by release of "danger signals" which triggers adaptive T-cell responses when an adequate antigen – presenting cell (APC) activation is present. These danger signals consist mainly of HSPs which activates DC [12]. The thermal effect of RFA can skip its local effect to extend to non-specific inflammatory stimulation induced by necrotic cells that might help to overcome immune tolerance or anergy towards the transplanted tumour configuring it as "in vivo tumour vaccination" as mentioned by Hansler et al. **[19]**.

Our work is considered as a unique study for immunological aspect of PEI, since few papers are published in the literature to compare the effect of alcohol ablation therapy on the immunological milieu. 13 patients have received multiple sessions of PEI and have proved to be of significance in elevating CD4+ after ablation ($P<0.001$). Absolute alcohol kills tumor cells by direct cytotoxic effects, causing necrosis of the treated region. It diffuses rapidly into cells, causing cellular dehydration and protein denaturation with resultant coagulation necrosis. This is followed by a fibrotic reaction, thrombosis, and occlusion of small vessels **[20]**. This local effect could be a potent stimulus for the immune cells to attract them to the site of inflammations.

Our results are confirmed by Nakayama et al who has injected ethanol in combination with microwave therapy, interleukins and interferon to treat melanoma and HCC, he has found an increase in the infiltration of T lymphocytes and natural killer (NK) cells into the ablated tissues, confirming that an immune response was elicited **[21]**.

Regarding MWA group, both CD4+ and CD4/CD8 ratio has significantly increases ($p<0.001$) which are parallel to studies of Dong and his colleagues who had a maximal response on the third day and he also noticed a lower rate of recurrence with high degree of infiltration **[22]**.

Recent studies have proved the effectiveness of TACE in induction of apoptosis, which provides theoretical evidence at the molecular level for the therapeutic effect of TACE [23]**.**In the present study, all the immunologic indicators were significantly changed. When compared with before treatment, in the TACE group, CD4+ cells and the CD4/CD8 ratio markedly decreased and CD8+ cells increased, suggesting that immunologic function was compromised shortly after treatment. These results are extremely similar to other studies especially to the work of Guan and his colleagues who displayed the same results **[13]**.

The discrepancy between the good clinical ablative power of TACE and the impairment of the immunity following the procedure could be explained by the fact that TACE affects blood vessels and lead to its occlusion which essentially could lead to decrease in the flow of the immune

cells, also the toxic materials used in TACE could have a role in this significant change.

**Funding:** Non.
**Conflicts of interest:** The authors declare that there is no conflict of interest.
**Ethical approval:**Informed consent was taken from each patient. The research protocol was duly approved by the Ethical Committee of Faculty of Medicine, Mansoura University **.**

## REFERENCES


1- Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2010; 127:2893–917.
2- El-Serag HB. Epidemiology of viral hepatitis and hepatocellular carcinoma. *Gastroenterology* 2012; 142:1264–73.
3- Katherine A, McGlynn, W. Thomas London. The Global Epidemiology of Hepatocellular Carcinoma: Present and Future. *Clin Liver Dis* 2011;15 223–243.
4- Shu G, Zhao W, Yue L, Su H, Xiang M. Antitumor immunostimulatory activity of polysaccharides from Salvia chinensis Benth. *J Ethnopharmacol.* 2015; pii: S0378-8741(15)00224-X.
5- Altekruse SF, McGlynn KA, Dickie LA, Kleiner DE. Hepatocellular carcinoma confirmation, treatment, and survival in surveillance, epidemiology, and end results registries, 1992-2008. *Hepatology* 2012; 55:476–82.
6- Padhya KT, Marrero JA, Singal AG. Recent advances in the treatment of hepatocellular carcinoma. *Curr Opin Gastroenterol* 2013; 29:285–92.
7- Kagawa T, Koizumi J, Kojima S, Nagata N, Numata M, Watanabe N, et al. Transcatheter arterial chemoembolization plus radiofrequency ablation therapy for early stage hepatocellular carcinoma: comparison with surgical resection. *Cancer* 2010; 116: 3638-3644.
8- Korangy F, Höchst B, Manns MP, Greten TF. Immune responses in hepatocellular carcinoma. *Dig Dis* 2010; 28: 150-154.
9- Patel KR, Liu TC, Vaccharajani N, Chapman WC, Brunt EM. Characterization of inflammatory (lymphoepithelioma-like) hepatocellular carcinoma: a study of 8 cases. *Arch Pathol Lab Med.* 2014; 138(9):1193-202.
10- Ormandy LA, Hillemann T, Wedemeyer H, Manns MP, Greten TF, Korangy F. Increased populations of regulatory T cells in peripheral blood of patients with hepatocellular carcinoma. . Cancer Res 2005; 65: 2457–2464.
11- Alisa A, Ives A, Pathan AA, Navarrete CV, Williams R, Bertoletti A, et al. Analysis of CD4+ T-cell responses to a novel alpha-feto-protein-derived epitope in hepatocellular carcinoma patients. *Clin Cancer Res* 2005; 11: 6686–94.
12- Gravante G, Sconocchia G, Ong SL, Dennison AR, Lloyd DM. Immunoregulatory effects of liver ablation therapies for the treatment of primary and metastatic liver malignancies. *Liver Int* 2009;
13- Guan HT, Wang J, Yang M, Song L, Tong XQ, Zou YH. Changes in immunological function after treatment with transarterial chemoembolization plus radiofrequency ablation in hepatocellular carcinoma patients. *Chin Med J* 2013; 126 (19).
14- Schueller G, Kettenbach J, Sedivy R, Stift A, Friedl J, Gnant M, et al. Heat shock protein expression induced by percutaneous radio-frequency ablation of hepatocellular carcinoma in vivo. *Int J Oncol* 2004; 24: 609–13.
15- Schueller G, Kettenbach J, Sedivy R, Bergmeister H, Stift A, Fried J, et al. Expression of heat shock proteins in human hepatocellular carcinoma after radiofrequency ablation in an animal model. *Oncol Rep* 2004; 12: 495–9.
16- El-Zanaty F, Way A. Egypt Demographic and Health Survey 2008. Egyptian: Ministry of Health. Cairo: El-Zanaty and Associates, and Macro International; 2009.
17- Lavanchy D. Evolving epidemiology of hepatitis C virus. *Clin Microbiol Infect.* 2011; 17(2):107–115.
18- Zerbini A, Pilli M, Fagnoni F, Pelosi G, Pizzi MG, Schivazappa S, et al. Increased immune stimulatory activity conferred to antigen-presenting cells by exposure to antigen extract from hepatocellular carcinoma after radio-frequency thermal ablation. *J Immunother* 2008; 31: 271–82.
19- Hansler J, Wissniowski TT, Schuppan D, Witte A, Bernatik T, Hahn EG,et al. Activation and dramatically increased cytolytic activity of tumor specific T lymphocytes after radio-frequency ablation in patients with hepatocellular carcinoma and colorectal liver metastases. *World J Gastro-enterol* 2006; 12: 3716-3721.
20- Lee MJ, Mueller PR, Dawson SL, Gazelle SG, Hahn PF, Goldberg MA, et al. Percutaneous ethanol injection for the treatment of hepatic tumors: indications, mechanism of action, technique, and efficacy. *AJR Am J Roentgenol.* 1995 ; 164(1):215-20.

21- Nakayama J, KokubaH, Kobayashi J, Yoshida Y, Hori Y. Experimental approaches for the treatment of murine B16 melanomas of various sizes. II: injection of ethanol with combinations of beta-interferon and microwaval hyperthermia for B16 melanomas with a size of greater than 10 mm in diameter. *J Dermatol Sci* 1997;15: 82–8.
22- Dong BW, Zhang J, Liang P, Yu XL, Su L, Yu DJ, et al .Sequential pathological and immunologic analysis of percutaneous microwave coagulation therapy of hepatocellular carcinoma. *Int J Hyperthermia* 2003; 19: 119–33.
23- Lu W, Li YH, He XF, Zhao JB, Chen Y, Mei QL. Necrosis and apoptosis in hepatocellular carcinoma following low-dose versus high-dose preoperative chemoembolization. *Cardiovasc Intervent Radiol* 2008; 31: 1133-1140.

**Peer reviewers:Nadia Mohammad Elwan,** Professor of Tropical Medicine , Faculty of Medicine, Tanta University, Egypt, Member of Supreme Council Promotion Committee for Tropical Diseases; **Mohamed I Radwan, A**ssistant Professor of Tropical Medicine and Hepato-Gastroenterology, Faculty of Medicine , Zagazig University , Egypt.
**Editor : Mohamed H Emara**; Lecturer of Tropical Medicine, Faculty of Medicine , Zagazig University , Egypt.

# Role of Insulin Resistance and Cytokeratin 18 on the Recurrence of Hepatocellular Carcinoma after Radiofrequency Ablation

**Soha E. Khorshed**[1] **, Alaa Fayed**[2] **, Lamiaa Mahmoud Kamel**[3] **, Shereen M. Awad**[4]

[1]*Tropical Medicine Department, Faculty of Medicine Zagazig University, Egypt*
[2]*Clinical Oncology and Nuclear Medicine Department, Faculty of Medicine Zagazig University, Egypt*
[3]*Clinical pathology Department, Faculty of Medicine Zagazig University, Egypt*
[4]*Family Medicine Department , Faculty of Medicine Zagazig University, Egypt*

*Corresponding Author*
***Soha E. Khorshed***

***Mobile:****+201224578358*

***Email:****sohaesmat@hotmail.com*

***Key words:***
*Hepatocellular carcinoma, homeostatic model assessment of insulin resistance, Caspase-generated cytokeratine-18, radiofrequency ablation*

**Background and study aim:** Many studies have reported that insulin resistance raises the risk of primary hepatocellular carcinoma (HCC). Caspase-generated cytokeratin-18 (CK-18) fragments are produced during apoptosis of hepatic cells. The present study aims to evaluate the value of serum CK-18 and the impact of insulin resistance on recurrence of HCV related HCC in patients treated with radiofrequency ablation (RFA).

**Patients and Methods:** The present study was conducted on 60 HCV patients. Group I: 30 patients with HCC treated by RFA and group II: 30 non HCC cirrhotic patients. Insulin resistance was estimated by the homeostatic model assessment of insulin resistance (HOMA-IR) and CK-18 level was measured for all patients using ELISA.

**Results:** HOMA-IR and CK-18 levels were significantly elevated in HCC patients when compared to non HCC cirrhotic patients. There was a significant increase in CK-18 and HOMA-IR levels in patients with local recurrence versus those with no recurrence ($p<0.001$). Univariate analysis revealed that HOMA-IR ($p=<0.001$) and CK-18 ($p=<0.001$) were significant predictors of recurrence of HCC.

**Conclusion:** CK-18 and HOMA-IR are potential prognostic markers as they can predict the recurrence of HCC.

## INTRODUCTION

Hepatoceullar carcinoma (HCC) is a common cause of cancer-related death and its incidence is increasing worldwide **[1]**. Egypt has a rising rate of HCC. The currently increasing incidence of HCC in Egyptians may be due to the increased prevalence of hepatitis C virus (HCV) as a primary risk factor **[2]**. Diabetes mellitus (DM) increased the risk of chronic non-alcoholic liver disease and HCC in male patients without concomitant liver disease **[3]**. Insulin resistance (IR) is a condition where body cells fail to respond to the normal actions of insulin leading to hyperglycemia **[4]**.

Steatohepatitis is associated with IR and characterized by inflammation, apoptosis of liver cells, fat and fibrotic tissue accumulation in the liver, which may progress to cirrhosis and HCC. IR seems to play a fundamental role in the pathogenesis of non-alcoholic fatty liver disease (NAFLD) **[5]**.

Current data suggest that an alteration in the regulation of hepatocyte apoptosis could play an important role in hepatic damage, steatohepatisis and HCC progression **[6]**.

The HCV core protein induces insulin resistance by increasing tumor necrosis factor α which disturb tyrosine phosphorylation of insulin receptor substrate-1 thus, diabetes mellitus including insulin resistance seems to be closely associated with various liver diseases that can lead to HCC **[7]**.

Liver cell damage in chronic HCV infection is mediated by the induction of apoptosis. During apoptosis, there is an activation of specific intraceullar protesases (caspases) that able to cleave

different substrates, including cytokeratin-18 (CK-18), a major inter-mediate filament protein in the liver. The action of these caspases shows a neoepitope in CK-18($M_{30}$) which is not detectable in alive or necrotic cells **[8]**.

When surgery is not possible for HCC, there are several minimally invasive options for tumor ablation either chemical or thermal **[9-11]**. The most frequent event observed during the follow up of curatively treated HCC patients is intrahepatic recurrence **[12-14]**. Which may be either local tumor progression or intrahepatic distant recurrence **[15]**.

Radiofrequency ablation (RFA) is among therapies that offer a high rate of complete response and potential cure . RFA induces tumor destruction by heating the tumor tissue to a temperature that exceeds 60$^{o}$C. This heating is generated from a high frequency alternating current that is delivered through an electrode placed in the center of the tumor. The generated heat result in coagulative necrosis of the tumor tissue with denaturation of the intra-cellular proteins and dissolution of the cell membrane lipid bilayers **[16]**.

The objective of this study is to evaluate the impact of insulin resistance and cytokeratin 18 on HCC recurrence after initial treatment with RFA.

## PATIENTS AND METHODS

The present study was carried out in Tropical Medicine, Radiotherapy, Clinical Pathology and Family Medicine Departments, Zagazig University. The study included 60 chronic HCV patients; the diagnosis of HCV was based on positive anti-HCV antibodies. Patients were divided into 2 groups: group I: 30 patients with HCV related HCC, group II: 30 patients with HCV related cirrhosis without HCC.

Exclusion criteria included diabetes mellitus, treatment with any medication known to affect glucose tolerance or insulin secretion, alcoholics, pregnant and breast feeding women.

This study was approved by the local Ethical Committee of Faculty of Medicine, Zagazig University and an informed written consent was obtained from each participant in the study.

**All patients were subjected to the following:**

- Full history taking and clinical examination.
- Venous blood samples were taken from all patients, serum was separated from the cellular fraction via centrifugation at 5,000 RPM for 3 minutes. Serum samples were kept at-80°C till the time of assay. Serum CK-18 assay was performed using CK-18 M30 apoptosis ELISA assay (PEVIVA, Alexis, Günwald, Germany).
- HCVAb in serum was detected by ELISA third generation, using kit from ORTHO. Catalogue number; 631300942.
- Insulin resistance using homoestasis model assessment of insulin resistance (HOMA-IR) which was calculated on the basis of fasting values of plasma glucose and insulin according to the HOMA model formula; HOMA-IR= fasting insulin (mIU/L) x fasting glucose (mmol/L)/ 22.5 **[17]**.
- AFP level was measured on Cobas E411 immunoassay (Roche Diagnostics, USA) **[18]**.
- Routine laboratory tests include liver function tests: Albumin, total bilirubin, ALT, AST; were analyzed using Cobas 501 (Roche diagnostics, Switzerland) and platelet count was done by automated cell counter Cell Dyne (APOTT, USA).

**Radiological investigations including:**

a) Abdominal ultrasonography (US) to assess liver cirrhosis, hepatic focal lesions as regard its number, size and for needle guidance during percutaneous RFA**[19]**.

b) Triphasic CT scan to confirm the diagnosis of HCC, diagnosis of HCC based on the typical hypervascular tumor stain on angiography and typical dynamic-study findings of enhanced staining in the early phase and attenuation in the delayed phase**[20]**.

**Tumor ablation:**

The technique was started by a small puncture into the skin using a scalpel (No 11). We used a common, commercially available RFA technique and system (RITA 1500X RF generator and RITA StarBurst XL, RITA Medical Systems, Mountain View, California). Grounding was achieved by attaching 2 pads to the patient's thighs. After administration of analgesia as well as local anesthesia, the electrode needles were introduced into the tumor under ultrasonographic guidance, then a gradual unfolding of the electrodes was obtained, and the generator was

activated to achieve RF energy and maintain an average temperature of 105°C. At first, the electrodes were moved by 2 cm, then the electrode needles were pushed forward and unfolded gradually to 3 cm, 4 cm and 5 cm until they reached or crossed the borders of the tumor according to the ablation range, delivering RF energy for 5 minutes for every intermediate step and for 7 to 10 minutes in the final step of the procedure. The ablation area was intended to cover the tumor as well as at least 0.5 to 1.0 cm of the surrounding tissue **[21]**. In case of tumor recurrence, RFA was repeated while in cases of multiple new focal lesions or metastases other treatment options were performed **[22]**.

**Follow up of therapy**

Triphasic CT, HOMA-IR, serum AFP and CK-18 levels were performed at 1, 3 and 6 months after the last session of RFA to evaluate the response to treatment and its effect on these parameters.

**Statistical Analysis**

Data were collected, tabulated and statistically analyzed using SPSS 22.0 for windows (SPSS Inc., Chicago, IL, USA) & MedCalc 13 for windows (MedCalc Software bvba, Ostend, Belgium). Quantitative variables were expressed as mean ± SD and median (range). Mann Whitney U test was used to compare between two groups of independent non-normally distributed data. Friedman test was used to compare more than two groups of dependent non-normally distributed data. The Spearman's rank correlation coefficient (r) was calculated to assess the relationship between CK-18 & selected study parameters. Receiver Operating Characteristic (ROC) curves were obtained to calculate the optimized cutoff point for CK-18, AFP, and HOMA-IR to reach the best compromise in the prediction of recurrence. The cutoff point with maximum sensitivity and specificity (validity) is used as the recommended cutoff point and also Area Under Curve (AUC) was calculated. Survival (S) was calculated as the time from diagnosis to death or the most recent follow-up contact (censored). Stratification of S was done according to CK-18 & HOMA-IR subgroups. These time-to-event distributions were estimated using the method of Kaplan-Meier plot, and compared using two-sided exact log-rank test. All tests were two sided with $p<0.05$ was considered statistically significant (S), $p<0.01$ was considered highly statistically significant (HS), and $p>0.05$ was considered non statistically significant (NS).

## RESULTS

This study was conducted on 30 HCV related HCC patients and 30 HCV related cirrhotic patients. Their demographic, clinical and biochemical characteristics are shown in Table 1. There was a statistically significant difference in the studied markers (AFP, CK-18 and HOMA-IR) among subgroups of Child classes as well as tumor size in HCC patients (Table 2). There was positive significant correlation between CK-18 and maximum tumor diameter, AFP and HOMA-IR. Also, there was positive significant correlation between HOMA-IR and maximum tumor diameter and AFP (Table 3 & Figure 1).

After treatment with RFA, the level of HOMA-IR, CK18 and AFP showed significant reduction in the responded patients [1 month 26 patient, 3 months 21 patient and lastly, after 6 months 18 patients] (Table 4).

Most patients with recurrent tumor were in Child class B and all patients with recurrence had maximum tumor diameter (3-5cm) (P<0.001). CK18 and HOMA-IR level were significantly increased in patients with recurrent HCC versus those without recurrence [324.8(317.9-340.9)] versus 308.5 (287.8-317.2) ng/ml, P<0.001 and 3-4(2.9-6.4) versus 2.3(1.29), P<0.001) respectively (Table 5).

According to receiver operating characteristic curve (ROC) the most accurate predictor of recurrence in HCC patients was CK-18 (Table 6 & Figure 2). Six months recurrence-free survival in total was 60% (Table 7). Analysis of the data using Kaplan-Meier estimates of survival probability for patients with serum CK-18 ≤318.9 ng/mL and HOMA-IR ≤2.7 showed significant longer free survival and higher overall survival probability when compared to CK-18> 318.9 and HOMA-IR>2.7 (p value=<0.001) (Tables 7 and 8, Figures 3 and 4).

**Table (1):** Demographic, clinical and biochemical characteristics of studied groups

| Demographic, clinical and biochemical characteristics | HCC patients (N=30) | Cirrhotic patients (N=30) | *t* | p-value |
|---|---|---|---|---|
| **Demographic characteristics** | | | | |
| Age(year) | 53 (25 – 68) | 50 (34 – 57) | -1.672‡ | 0.095 |
| Sex | | | | |
| Male | 27 (86.7%) | 25 (83.3%) | 0.180* | 0.672 |
| Female | 3 (13.3%) | 5 (16.7%) | | |
| **Clinical characteristics** | | | | |
| Splenomegaly | 8 (26.7%) | 0 (0%) | 10.479* | 0.001 |
| Lower limb edema | 5 (16.7%) | 2 (6.7%) | 1.731* | 0.188 |
| Hepatic Encephalopathy | 1 (3.3%) | 0 (0%) | 1.552* | 0.213 |
| Child-Pugh classification | | | | |
| Child A | 21 (70%) | - | - | |
| Child B | 9 (30%) | - | - | |
| Diameter of focal lesion (cm) | 3.5 (1.9 – 5) | | | |
| **Biochemical characteristics** | | | | |
| AST (IU/L) | 83.5 (43 – 203) | 17 (12 – 23) | -7.705‡ | <0.001 |
| ALT (IU/L) | 77.5 (41 – 216) | 16 (11 – 21) | -7.706‡ | <0.001 |
| Total bilirubin (mg/dL) | 1.4 (0.7 – 3.8) | 0.6 (0.2 – 1.5) | -6.531‡ | <0.001 |
| Serum albumin (g/dl) | 2.8 (2.2 – 3.4) | 2.8 (1.1 – 5.1) | -0.360‡ | 0.719 |
| Platelet count ($x10^3/mm^3$) | 160 (96 – 210) | 273.5 (233 – 298) | -7.705‡ | <0.001 |
| AFP | 215.5 (2 – 398) | 2.7 (0.8 – 8.7) | -7.579‡ | <0.001 |
| CK-18 (ng/mL) | 431.4 (287.8 – 557) | 92.6 (81.4 – 100) | -5.797‡ | <0.001 |
| HOMA-IR | 3 (1 – 24) | 1.3 (1 – 2.2) | -5.250‡ | <0.001 |

Continuous variables were expressed as the median (range); Categorical variables were expressed as number (percentage); ‡ Mann Whitney U test;* Chi-square test; p< 0.05 is significant.

**Table (2):** AFP, CK-18 and HOMA-IR difference among different demographic and clinical subgroups of HCC patients (N=30)

| | AFP | CK18 | HOMA-IR |
|---|---|---|---|
| **Sex** | | | |
| Male (n=27) | 62 (2 – 321) | 314.4 ± 13.4 | 2.6 (1 – 6.3) |
| Female (n=3) | 49 (12 – 63) | 308.8 ± 10.7 | 2.5 (2 – 2.7) |
| Test | -0.899‡ | 0.702• | -0.729‡ |
| p-value | 0.369 | 0.489 | 0.466 |
| **Child-Pugh classification** | | | |
| Child A (n=21) | 41.7 ± 25.1 | 310.9 (287.8 – 317.9) | 2.3 ± 0.5 |
| Child B (n=9) | 187.9± 65.1 | 328.2 (318.9 – 340.9) | 4.2 ± 1.2 |
| *t* | -6.532• | -4.279‡ | -4.381• |
| p-value | <0.001 | <0.001 | <0.001 |
| **Diameter of focal lesion** | | | |
| < 3 cm (n=4) | 11.5 (2 – 12) | 292.2 ± 3.76 | 1.6 (1 – 2) |
| 3 – 5 cm (n=26) | 65.5(23 – 321) | 317.2 ± 10.55 | 2.8 (2 – 6.3) |
| *t* | -3.173‡ | -4.639• | -3.065‡ |
| p-value | 0.002 | <0.001 | 0.002 |

Continuous variables were expressed as the mean ± SD for normally distributed data or median (range) for non-normally distributed data; ‡ Mann Whitney U test; • Independent samples Student-t test;* Chi-square test; p< 0.05 is significant.

**Table (3):** Correlation of CK-18 level and HOMA-IR with other studied parameters in HCC patients (N=30)

| Studied parameters | CK-18 level | | HOMA-IR | |
|---|---|---|---|---|
| | R | P | r | P |
| Mean diameter of focal lesion | + 0.986 | <0.001 | + 0.980 | <0.001 |
| AFP | + 0.999 | <0.001 | + 0.995 | <0.001 |
| CK-18 | --- | --- | + 0.994 | <0.001 |
| HOMA-IR | + 0.994 | <0.001 | --- | --- |

r Spearman rank correlation coefficient; p< 0.05 is significant.

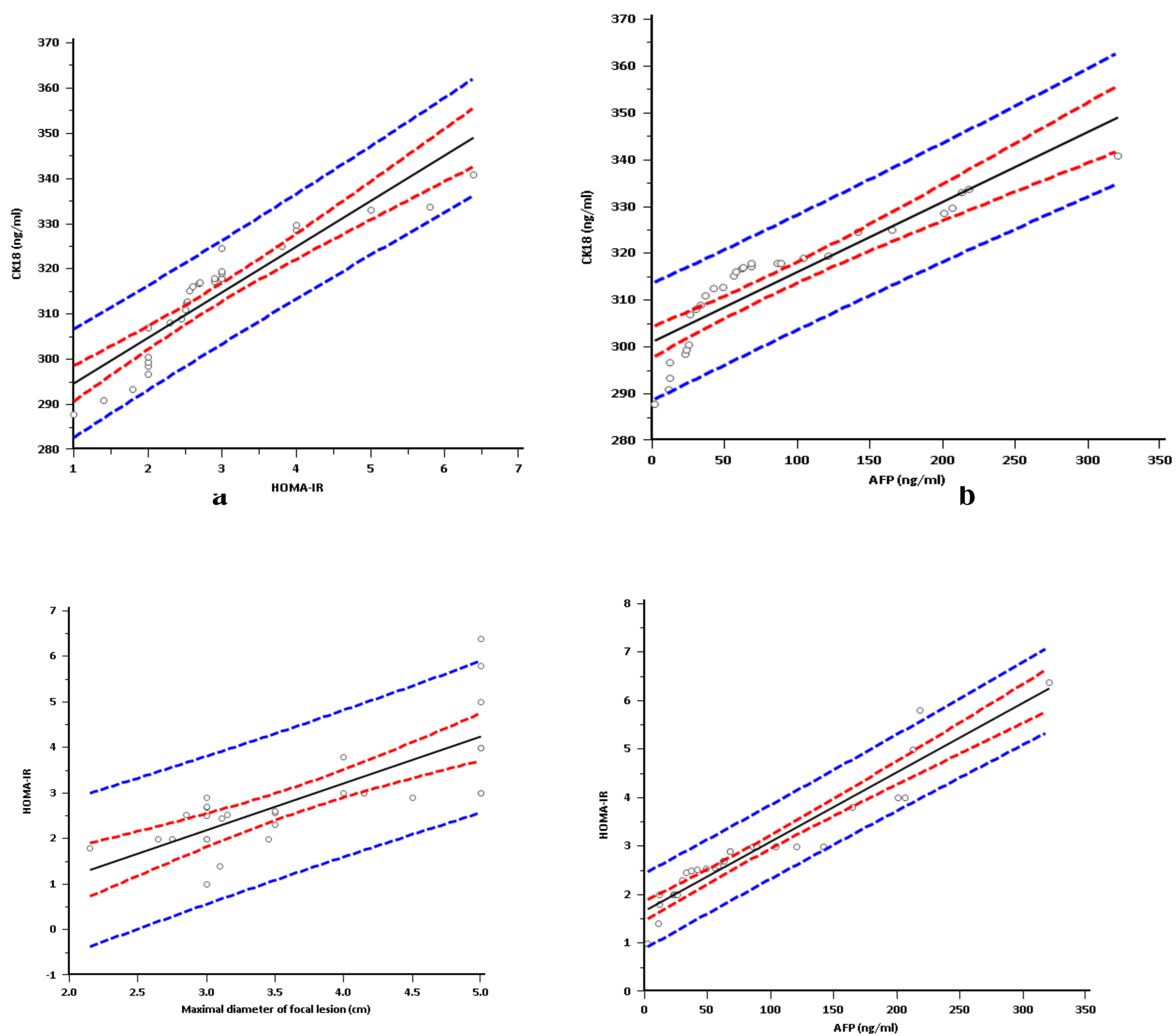


**Figure (1):** Scatter plot with regression line shows correleation between the studied markers in HCC patients (N=30). Blue lines representing the 95% confidence interval (CI) & red lines representing the 95% prediction interval of regression line. (a) CK-18 & HOMA-IR, (Spearman's rank correleation coefficient r= +0.994, p<0.001); (b) CK-18 & alpha-fetoprotein level (Spearman's rank correleation coefficient r= +0.999, p<0.001); (c) HOMA-IR & maximal diameter of focal lesion, (Spearman's rank correleation coefficient r=0.980, p<0.001); (d) HOMA-IR & alpha-fetoprotein level, (Spearman's rank correleation coefficient r=0.995, p<0.001).

**Table (4):** Analysis of AFP, CK-18 and HOMA-IR in HCC patients after treatment

| | AFP | CK18 | HOMA-IR |
|---|---|---|---|
| Before treatment (n=30) | 42 (2 – 121) | 312.6 (287.8 – 319.4) | 2.5 (1 – 3) |
| 1 month after treatment (n=26) | 21.6 (10.5 – 33.5) | 247.8 (220 – 257.5) | 1.4 (0.2 – 2.01) |
| 3 month after treatment (n=21) | 19 (11.9 – 33.5) | 216.4 (203.6 – 228) | 1.1 (1 – 1.8) |
| 6 month after treatment (n=18) | 15 (10.8 – 66.8) | 192 (146.2 – 202) | 1.02 (0.3 – 1.3) |
| $X^2$ | 38.053 | 69.000 | 55.339 |
| p-value | <0.001 | <0.001 | <0.001 |

Continuous variables were expressed as the median (range); $\chi^2$ Friedman test; p< 0.05 is significant.

**Table (5):** Univariate analysis difference in characteristics of patients with recurrent HCC versus those without recurrence

| Parameter | No recurrence (N=18) | Recurrence (N=12) | Test | p-value |
|---|---|---|---|---|
| | | | | |
| **Age** | 40.2 ± 9.4 | 50.7 ± 4.3 | 3.598• | 0.003 |
| **Sex** | | | | |
| Male | 15 (83.3%) | 12 (100%) | 2.222* | 0.136 |
| Female | 3 (16.7%) | 0 (0%) | | |
| **Clinical characteristics** | | | | |
| Child-Pugh classification | | | | |
| Child A | 18 (100%) | 3 (25.0%) | 19.286* | <0.001 |
| Child B | 0 (0%) | 9 (75.0%) | | |
| Diameter of focal lesion | | | | |
| < 3 cm | 4 (22.2%) | 0 (0% | | |
| 3 – 5 cm | 14 (77.8%) | 12 (100%) | -4.633‡ | <0.001 |
| **Biochemical characteristics** | | | | |
| AST (IU/L) | 74.5 (59 – 122) | 70.5 (43 – 99) | -1.673‡ | 0.076 |
| ALT (IU/L) | 64 (45 – 109) | 45 (41 – 90) | -2.375‡ | 0.034 |
| Total bilirubin (mg/dL) | 1.7 ± 0.8 | 1.6 ± 0.6 | 0.275• | 0.785 |
| Serum albumin (g/dl) | 2.8 ± 0.3 | 2.9 ± 0.3 | -0.639• | 0.528 |
| Platelet count ($x10^3/mm^3$) | 149.8 ± 32.1 | 153.2 ± 33.4 | -0.281• | 0.781 |
| AFP | 35.1 ± 20.3 | 161.2 ± 73.8 | -5.772• | <0.001 |
| CK18 | 308.5 (287.8 – 317.2) | 324.8 (317.9 – 340.9) | -4.574‡ | <0.001 |
| HOMA-IR | 2.3 (1 – 2.9) | 3.4 (2.9 – 6.4) | -4.572‡ | <0.001 |

Continuous variables were expressed as the mean ± SD for normally distributed data or median (range) for non-normally distributed data; Categorical variables were expressed as number (percentage); ‡ Mann Whitney U test; • Independent samples Student-t test;* Chi-square test; p< 0.05 is significant.

**Table (6):** Diagnostic performance of AFP, CK-18 and HOMA-IR in prediction of recurrence in HCC patients (N=30).

| Cutoff value | Sensitivity (95%CI) | Specificity (95%CI) | PPV (95%CI) | NPV (95%CI) | LR+ (95%CI) | LR- (95%CI) | Accuracy (95%CI) | AUC (95% CI) |
|---|---|---|---|---|---|---|---|---|
| **AFP > 63** | 100% (73.2-100) | 94.4% (72.7-99.9) | 92.3% (64-99.8%) | 100% (80.5-100) | 18 (16.1-20.1) | 0 | 96.6% (72.9-999) | 0.998‡ (0.880-1.000) |
| **CK-18 > 317.2** | 100% (73.5-100) | 100% (81.5-100) | 100% (73.5-100) | 100% (81.5-100) | 18 (16.1-20.1) | 0 | 100% (78.3-100) | 1.000§ (0.884-1.000) |
| **HOMA-IR > 2.7** | 100% (73.5-100) | 94.4% (72.7-99.9) | 92.3% (64-99.8%) | 100% (80.5-100) | 18 (16.1-20.1) | 0 | 96.6% (72.9-999) | 0.998* (0.880-1.000) |

‡p<0.001 (HS); § p<0.001 (HS);*p<0.001 (HS)

ROC curve: Receiver Operating Characteristic curve; PPV: Positive Predictive Value; NPV: Negative Predictive Value; LR+: positive Likelihood Ratio; LR-: negative Likelihood Ratio; AUC: Area Under Curve; 95%CI: 95% Confidence Interval; p< 0.05 is significant.

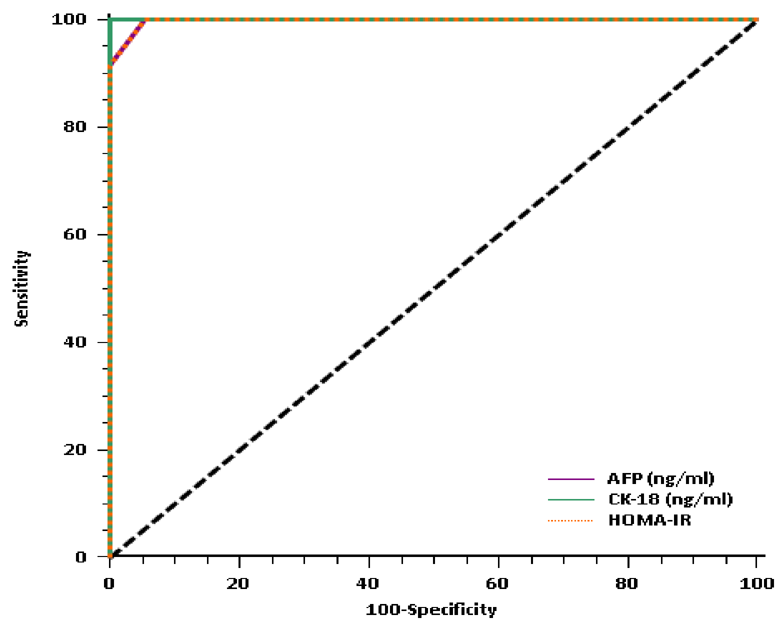


**Figure (2):** Receiver Operating Characteristic (ROC) curves of AFP, CK18 and HOMA-IR in prediction of recurrence in HCC patients (N=30).

**Table (7):** Recurrence free survival (RFS) in HCC patients (N=30).

| Characteristics | All (N=30) | AFP | | | CK-18 | | | HOMA-IR | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | ≤63 (n=17) | >63 (n=13) | p-value | ≤317.2 (n=18) | >317.2 (n=12) | p-value | ≤2.7 (n=17) | >2.7 (n=13) | p-value |
| 1 month RFS (%) | 86.7% | 100% | 69.2% | | 100% | 66.7% | | 100% | 69.2% | |
| 3 month RFS (%) | 70% | 100% | 30.8% | <0.001§ | 100% | 25% | <0.001§ | 100% | 30.8% | <0.001§ |
| 6 month RFS (%) | 60% | 100% | 7.3% | | 100% | 0% | | 100% | 7.3% | |
| Recurrent | 12 (40%) | 0 (0%) | 12 (92.3%) | <0.001* | 0 (0%) | 12 (100%) | <0.001* | 0 (0%) | 12 (92.3%) | <0.001* |
| Non-recurrent | 18 (60%) | 17 (100%) | 1 (7.7%) | | 18 (100%) | 0 (0%) | | 17 (100%) | 1 (7.7%) | |
| Max. tumor diameter < 3 cm | 4 (13.3%) | 4 (23.5%) | 0 (0%) | 0.060* | 4 (22.2%) | 0 (0%) | 0.079* | 4 (23.5%) | 0 (0%) | 0.060* |
| Max. tumor diameter 3 – 5 cm | 26 (86.7%) | 13 (76.5%) | 13 (100%) | | 14 (77.8%) | 12 (100%) | | 13 (76.5%) | 13 (100%) | |

Qualitative data are presented as number(%);*Chi-square test.; § Log rank test; p< 0.05 is significant.

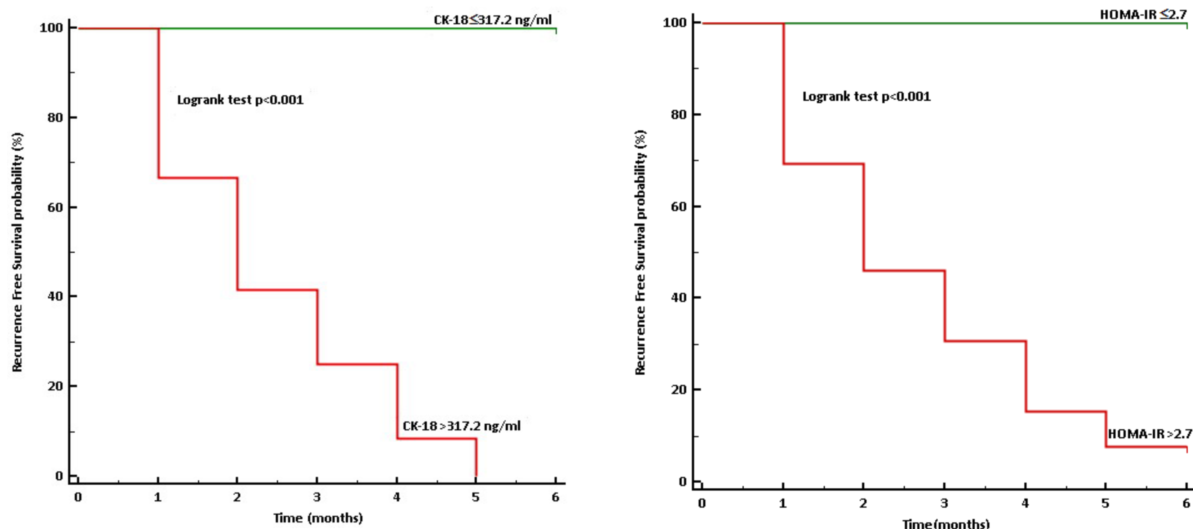


**Figure (3):** Kaplan-Meier estimates recurrence free survival probability in relation to time in HCC patients (N=30). (a) as regard CK-18 subgroups. (b) as regard HOMA-IR subgroups.

**Table (8):** Overall survival (OS) in HCC patients (N=30).

| Characteristics | All (N=30) | AFP ≤104 (n=22) | AFP >104 (n=8) | AFP p-value | CK-18 ≤318.9 (n=22) | CK-18 >318.9 (n=8) | CK-18 p-value | HOMA-IR ≤2.9 (n=19) | HOMA-IR >2.9 (n=11) | HOMA-IR p-value |
|---|---|---|---|---|---|---|---|---|---|---|
| Median OS (months) | NR | NR | 2.5 | --- | NR | 2.5 | --- | NR | 4 | --- |
| 1 month OS (%) | 100% | 100% | 100% | <0.001§ | 100% | 100% | <0.001§ | 100% | 100% | <0.001§ |
| 3 month OS (%) | 83.3% | 100% | 37.5% | | 100% | 37.5% | | 100% | 54.5% | |
| 6 month OS (%) | 73.3% | 100% | 0% | | 100% | 0% | | 100% | 27.3% | |
| Dead | 8 (26.7%) | 0 (0%) | 8 (100%) | <0.001* | 0 (0%) | 8 (100%) | <0.001* | 0 (0%) | 8 (72.7%) | <0.001* |
| Alive | 22 (73.3%) | 22 (100%) | 0 (0%) | | 22 (100%) | 0 (0%) | | 19 (100%) | 3 (27.3% | |
| Max. tumor diameter < 3 cm | 4 (13.3%) | 4 (18.2%) | 0 (0%) | 0.195* | 4 (18.2%) | 0 (0%) | 0.195* | 4 (21.1%) | 0 (0%) | 0.102* |
| Max. tumor diameter 3 – 5 cm | 26 (86.7) | 18 (81.8%) | 8 (100%) | | 18 (81.8%) | 8 (100%) | | 15 (78.9%) | 11 (100%) | |

Qualitative data are presented as number(%);*Chi-square test.; § Log rank test; p< 0.05 is significant.

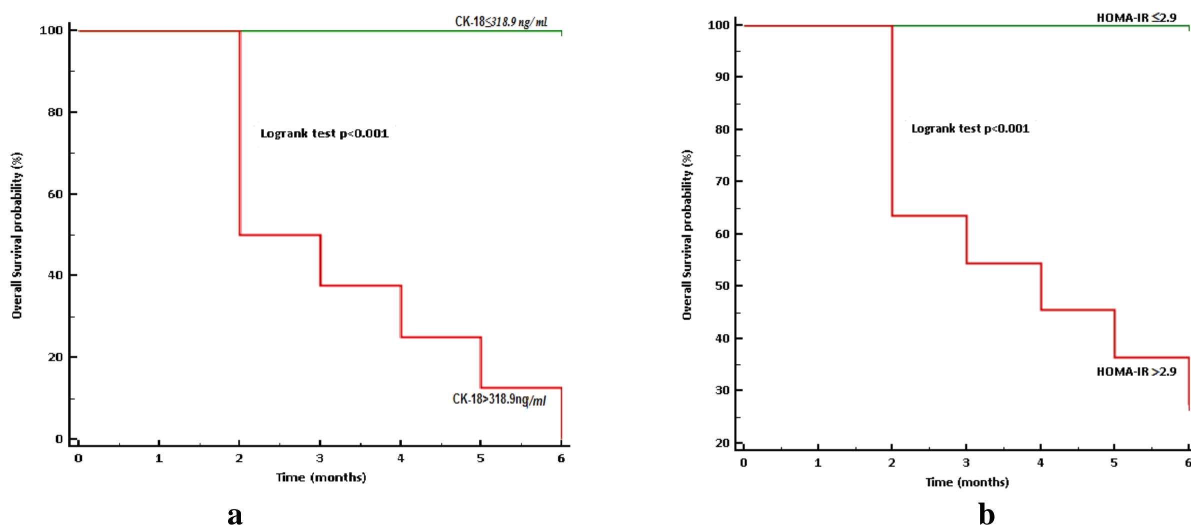


**Figure (4):** Kaplan-Meier estimates survival probability in relation to time in HCC patients (N=30). (a) as regard CK-18 subgroups. (b) as regard HOMA-IR subgroups.

## DISCUSSION

Hepatocellular carcinoma is a worldwide malignancy, and the incidence rate has increased significantly over the past few decades **[23]**. The reason for this increase has not yet been explained clearly, although more than 50 % of this increase has been attributed to hepatitis virus or alcoholic liver disease **[24]**. Insulin resistance is frequently seen in patients with HCV **[25]**. Although in the general population, lack of exercise and overeating are major causes of insulin resistance, in patients with HCV infection, hepatic inflammation, activated inflammatory cytokines, and HCV-induced impairment of insulin and lipid signaling molecules are also important factors for the development of insulin resistance **[26]**.Therefore, the prevalence of insulin resistance is higher in patients with HCV infection compared to that in the general population and patients with other hepatobiliary disorders **[27-28]**.

In the present study HOMA-IR was significantly higher in HCC patients compared to cirrhotic patients. In consistence with our results, Donadon et al. **[29]** found that the mean levels of HOMA-IR increases progressively among chronic hepatitis C, liver cirrhosis and HCC patients. There is association of IR with cancer in various organs **[30]**. The mechanisms by which insulin acts as carcinogenic factor are: First; insulin functions as a growth factor by phosphorylating insulin receptor substrate 1 and second; hyperinsulinemia increases peripheral lipolysis and hepatic accumulation of free fatty acids. The excess B-oxidation in mitochondria and microsome leads to the production of reactive oxygen species **[31]** that play a significant role in carcinogenesis **[32-33]**.

The results of the present study demonstrated highly significant increase in HOMA-IR level in patients with recurrent lesions versus those without recurrence. Also, HOMA-IR had significant correlation with age, tumor diameter, AFP and CK-18.

The strong relation of recurrent HCC to HOMA-IR may be due to the possibility of the presence of microvascular invasion and angiogensis. This finding not only basically agree with previous studies that suggested an association between insulin resistance and carcinogenesis **[34-35]**, but also, suggests that insulin resistance is a significant risk factor for early recurrence of HCC and thus might be a critical target to prevent the recurrence. Muto and his colleagues described that oral supplementation with Branched-chain amino acid (BCAA) granules inhibited liver carcinogenesis in HCV-related liver cirrhosis with DM and obesity **[34]**.

Current data reveled that there was correlation between HOMA-IR and CK-18 (P=<0.001). These finding could be explained by the importance of IR in the induction of hepatocytes apoptosis such that HCV replication and production in infected hepatocytes can induce apoptosis and a release of inflammatory cytokines/chemokines, such as tumor necrosis factor alpha (TNFα), transforming growth factor-Beta (TGFβ), interferon-gamma (IFNγ), interleukin 10 (IL-10), IL-12, IL-22, chemokine receptor (CCR5) ligands, and regulated on activation, normal T cell expressed and secreted (RANTES) **[36-37]**. These inflammatory cytokines/ chemokines can stimulate hepatocyte apoptosis through different pathways and during apoptosis there is an activation of specific intracellular proteases that able to cleave different substrates, including cytokeratin-18(CK-18) **[38]**.

By following up HCC patients who performed RFA, this study revealed that the median HOMA-IR level was significantly decreased at 1, 3 and 6 months progressively in responding patients; Our results were in line with Kenji Imai et al. **[39]** who proved that IR raises the risk of HCC development in patients with chronic HCV.

Our data revealed that the median serum AFP level was significantly higher in HCC group compared to HCV related cirrhotic patients. This finding was in agreement with Arrieta et al. **[40]** who said that AFP level was significantly higher in patients with HCC compared to patients with liver cirrhosis.

After follow up of patients in HCC group who were treated by RFA, our data showed that the median serum AFP level before treatment was significantly decreased at 1, 3 and 6 months progressively in all cured HCC patients. Similarly Berry et al. **[41]** concluded that the serum AFP level and changes in its level strongly predict survival independently of the tumor burden.

Liver cell damage in chronic hepatitis C (CHC) virus infection is mediated by the induction of apoptosis. The key morphological alteration of apoptosis is mediated by family of intracellular

cysteine protease, called caspases **[42]** which cleave a number of different substrates inside the cells including (CK-18) the major intermediate filament protein in the liver **[43]**. Kawai et al. **[44]** revealed that CK-18 plays an important role in tumor genesis of hepatocellular carcinoma

In the present study, there is a significant increase in CK18 level in HCC patients compared to cirrhotic patients. Moreover CK-18 was significantly higher in patients with recurrent tumor versus non recurrent. These could be explained by increase in the spontaneous apoptosis of HCC cells which released into blood vessels.

We have also observed significant increase of CK-18 level in large tumor size. This could be explained by the crosstalk between angiogenesis, cytokeratin-18, and insulin resistance **[45]**. McHugh and co-workers found that microvascular invasion is strongly associated with the tumor size and AFP >100 ng/ml and greatly increase the risk of recurrence after transplantation for HCC **[46]**.

It is vital to assess patients' prognosis to determine the suitable treatment and improve the outcome thus we investigated risk factors of recurrence which could help in its prevention and follow up of patients. The present study revealed that Child class B, large tumor size (3-5cm), increased serum CK-18, AFP and HOMA-IR levels were risk factors that predict HCC recurrence Also, Lai et al. **[47]** found a strong correlation between AFP level, tumor dimensions and microvascular invasion which all are predictors of HCC recurrence. Carcinogenesis may develop when the homeostatic balance between cell survival and apoptosis is disturbed in our study; we found that the CK-18 and HOMA-IR response independently predicted the recurrence free survival (together with Child-Pugh score and tumor size). We can expect more favorable prognosis in patients with CK-18 <317.2 and HOMA-IR had response than that of those with CK-18 >317 and HOMA-IR >2.7. The close observation of the non-response patients might be required to detect disease progression.

The main limitations of our study included small sample size in limited observation period and there was no correlation between tissue and serum CK-18 levels.

Finally, we can conclude that CK-18 correlates with HOMA-IR and is related to HCC size which makes them potential prognostic markers for follow up of patients after therapeutic strategies to predict recurrence of HCC.


## Acknowledgements

The authors would like to express their sincere gratitude to the patients and staff of Tropical Medicine Department and to the laboratory technicians, for their great endeavor. We'd like to appreciate Dr. Inas M. Elfiki, Lecturer of Radiology, for her great help.

**Funding:** None


**Ethical Approval:** This study was approved by the ethical committee of Faculty of Medicine, Zagazig University and all patients provided written informed consent before participation in any protocol specific procedure.

**Conflicts of interest :**There are no conflict of interests

## REFERENCES


1-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.

2- 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; 11:5193–8.

3- El Serage HB, Tran T, Everhart JE. Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. *Gastroentrology* 2004; 126:460-8.

4- Lebovitz HE. Insulin resistance : definition and consequences. *Exp clin Endocrinol Diabetes* 2001; 109:136-48.

5- García-Monzón C, Martín-Pérez E, Iacono OL, Fernández-Bermejo M, Majano PL, Apolinario A, et al. Characterization of pathogenic and prognostic factors of non-alcoholic steatohepatitis associated with obesity. *Hepatology* 2000; 33:715-724.

6- Feld Stein AE, Canbay A, Angulo P, Taniai M, Burgart LJ, Lindor KD, et al. Heaptocyte apoptosis and fas expression are prominent features of human non alcoholic steatohepatitis. *Gastro-entrology* 2003 Aug;125(2):437-43.

7- Shintani Y, Fujie H, Miyoshi H, Tsutsumi T, Tsukamoto K, Kimura S, et al. Hepatitis C Virus infection and diabetes : direct involvement of the virus in the development of insulin resistance. *Gastroentrology* 2004; 126:840-8.

8- Ueno T, Toi M, Linder S. detection of epithelial cell death in the body by cytokeratin 18 measurement. *Biomed Pharmacother* 2005; 59 (suppl.2) s359-s362.

9- Shiina S, Tagawa K, Niwa Y, Unuma T, Komatsu Y, Yoshiura K, et al. Percutaneous ethanol injection therapy for hepatocellular carcinoma: results in 146 patients. *AJR Am J Roentgenol* 1993; 160: 1023-8.
10- Rossi S, Buscarini E, Garbagnati F, Di Stasi M, Quaretti P, Rago M, et al. Percutaneous treatment of small hepatic tumors by an expandable RF needle electrode. *AJR Am J Roentgenol* 1998; 170:1015-22.
11- Seki T, Wakabayashi M, Nakagawa T, Itho T, Shiro T, Kunieda K, et al. Ultrasonically guided percutaneous microwave coagulation therapy for small hepatocellular carcinoma. *Cancer* 1994; 74:817-25.
12- Choi D, Lim HK, Rhim H, Kim YS, Lee WJ, Paik SW, et al. Percutaneous radiofrequency ablation for early-stage hepatocellular carcinoma as a first-line treatment: long-term results and prognostic factors in a large single-institution series. *Eur Radiol* 2007; 17:684-92.
13- Raut CP, Izzo F, Marra P, Ellis LM, Vauthey JN, Cremona F, et al. Significant long-term survival after radiofrequency ablation of unresectable hepatocellular carcinoma in patients with cirrhosis. *Ann Surg Oncol* 2005;12:616-28.
14- Tateishi R, Shiina S, Teratani T, Obi S, Sato S, Koike Y, et al. Percutaneous radiofrequency ablation for hepatocellular carcinoma. An analysis of 1000 cases. *Cancer* 2005;103:1201-9.
15- Kim YS, Rhim H, Cho OK, Koh BH, Kim Y. Intrahepatic recurrence after percutaneous radiofrequency ablation of hepatocellular carcinoma: analysis of the pattern and risk factors. *Eur J Radiol* 2006; 59:432-41.
16- Shiina S, Tatesishi R, Arano T, Uchine K, Enooku K, Nakagawa H, et al. Radiofrequency Ablation of Hepatocellular carcinoma. *Am J Gastroenterol.* 2012; 107(4):569-77.
17- Mathews D, Hosker IP, Rudenski AS, Naylor BA, Treacher DF, Truner RC .Homostasis model Assessment :Insulin resistance and B.cell function from fasting plasma glucose and insulin concentration in man. *Diabetologia* 1985; 28(7):412-9.
18- Farinate F, Marino D, De Giorgio M, Bakian A, Contarini M, Cursaro C, et al. Diagnostic and prognostic role of alphafetoprotein in hepatocellular carcinoma: Both or neither? *Am J Gastrenterol* 2006; 101:524-32.
19- Lencioni R, Crocelti L, Della Pina MC, Cioni D. Guidelines for imaging focal lesions in liver cirrhosis. *Expert. Rev. Gastroenterol Hepatol* 2008; 2:697-703.
20- Lee KH, O' Malley ME, Haider MA, Triphasic CT of Hepatocellular carinoma. Am J Roentyenol 2004; 182:643-9.
21- Tanaka S, Shimada M, Shirabe K, Taketomi A, Maehara S, Tsujita E, et al. Surgical radio-frequency ablation for treatment of hepatocellular carcinoma: an endoscopic or open approach. *Hepatogastroenterology* 2009, 56(93):1169-1173.
22- Bruix J, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; 53(3): 1020-2.
23- Goa C, Yao SK. Diabetes mellitus a "ture" independent risk factor for hepatocellular carcinoma ? *Hepatobiliary Pancreat Dis Int.*2009; 8: 465–473.
24 Yuen MF, Hou JL, Chutaputti A. Hepatocellular carcrinoma in the Asia pacific region. *J Gastroenterol Hepatol* 2009;24: 463–353.
25- Davila JA, Morgan RO, Shaib Y, McGlynn KA, El-Serag HB. Diabetes increases the risk of hepatocellular carcinoma in the United States: a population based case control study. *Gut* 2005;54: 533–539.
26- Tazawa J, Maeda M, Nakagawa M, Ohbayashi H, Kusano F, Yamane M, et al. Diabetes mellitus may be associated with hepatocarcinogenesis in patients with chronic hepatitis C. *Dig Dis Sci.*2002;47:710–715
27- Franz MJ, Bantle JP, Beebe CA, Brunzell JD, Chiasson JL, Garg A, et al. Evidence-based nutrition principles and recommendations for the treatment and prevention of diabetes and related complications. *Diabetes Care*; 2002;25:148–198.
28- Nagao Y, Sata M. High incidence of multiple primary carcinomas in HCV-infected patients with oral squamous cell carcinoma. *Med Sci Monit* 2009;15 :CR453–CR459.
29- Donadon V, Balbi M, Perciaccante A, Casarin P. Insulin resistance and hyperinsuliemia in patients with chronic liver disease and hepatocellular carcinoma.*Clinical Medicine Endocrinology and Diabetes* 2009; 2:25-33.
30- Hotamisligil GS. Inflammation and metabolic disorders. *Nature* 2006;444:860-867
31- Pessayre D, Berson A, Fromenty B, Mansouri A. Mitochon-dria in steatohepatitis. *Semin Liver Dis* 2001; 21:57-69.
32- Cerutti PA, Trump BF. Inflammation and oxidative stress in carcinogenesis. *Cancer Cells* 1991;3:1-7
33- Dreher D, Junod AF. Role of oxygen free radicals in cancer development. *Eur J Cancer* 1996; 32A: 30-832 .
34- Muto Y, Sato S, Watanabe A, Moriwaki H, Suzuki K, Kato A, et al. Overweight and obesity increase the risk for liver cancer in patients with liver cirrhosis and long-term oral supplementation with branched chain amino acid granules inhibits liver carcinogenesis in heavier patients with liver cirrhosis .*Hepatology research*2006;35(3):204-14.

35- Shimizu M, Shirakami Y, Iwasa J, Shiraki M, Yasuda Y, Hata K, et al. Supplementation with branched-chain amino acids inhibits azoxymethane-induced colonic preneoplastic lesions in male C57BL/KsJdb/db mice. *Clin Cancer Res* 2009; 15: 3068–75.
36- Barrett S, Collins M, Kenny C, Ryan E, Keane CO, Crowe J. Polymorphisms in tumor necrosis factor-alpha, transforming growth factor-beta, interleukin-10, interleukin-6,interferon-gamma, and outcome of hepatitis C virus infection. *J Med Virol* 2003; 71: 212–218.
37- Moreno C, Gustot T, Nicaise C, Quertinmont E, Nagy N, Parmentier M et al. CCR5 deficiency exacerbates T-cell-mediated hepatitis in mice. *Hepatology* 2005; 42: 854–862.
38- Valva P, De Matteo E, Galoppo MC, Gismondi MI, Preciado MV. Apoptosis markers related to pathogenesis of pediatric chronic hepatitis C virus infection: M30 mirrors the severity of steatosis. *J Med Virol* 2010; 82: 949–957.
39- Imai K, Takai K, Nishigaki Y, Shimizu S, Naiki T, Hayashi H. Insulin resistance raises the risk for recurrence of stage I hepatocellular carcinoma after curative radiofrequency ablation in hepatitis C virus-positive patients: A prospective, case series study. *Hepatology Research* 2010; 40:376-382
40- Arrieta O, Cacho B, Morales-Espinosa D, Ruelas-Villavicencio A, Flores-Estrada D, Hernández-Pedro N. The progressive elevation of alpha fetoprotein for the diagnosis of hepatocellular carcinoma in patients with liver cirrhosis. *BMC Cancer* 2007; 728-39.
41- Berry K, Ioannou GN. Serum alpha protein level independently predicts post transplant survival in patients with hepatoccellular carcinoma. *Liver Transpl.* 2013; 19(6):643-45.
42- Bantel H, Lügering A, HEidemann J, Volkam X, Poremba C, Strassburg Cp, et al. Detection of apoptotic caspase activation in sera from patients with chronic HCV infection is associated with fibrotic liver injury. *Hepatology.* 2004; 40(5): 1078-87.
43- Danial NN, Korsmeyer SJ. Cell death: Critical control points.*Cell* 2004;116:205-219.
44- Kawai M, Saegusa Y, Kemmochi S, Harada T, Shimamoto K, Shibutani M, et al. Cytokeratin 8/18 is a useful immunohistochemical marker for hepatocellular carcinoma. *J Vit Med Sci* 2010; 72:263-9
45- Kitade M, Yoshiji H, Noguchi R, Ikenaka Y, Kaji K, Shirai Y, et al. Crosstalk between angiogenesis, cytokeratin-18, and insulin resistance in the progression of non-alcoholic steatohepatitis, *World J Gastroenterol*. 2009 7; 15(41): 5193–5199.
46- Mcltugh Pp, Gilbert J, Vera S, Kock A, Ranjan D, Gedoly R. alpha-fetoprotein and tumer size are associated with microvascular invasion in explanted livers of patients undergoing transplantation with hepatocellular carcinoma. HPB (oxford) 2010;1256-61.
47- Lai P, Meli M, Ginanni-Corradini S, Mennini G, Gentili F, Malinaro A, et al. Predictive factors of recurrence of hepatocellular carcinoma after liver transplantation a multivariate analysis. *Transplant Proc* 2009; 41:1306-9.

**Peer reviewers:Maysaa Saed**, Professor of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt. **Amira Suliman**, Professor of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt. **Mohamed El-Sebaee**, Department of Internal Medicine, Faculty of Medicine, Tanata University, Egypt.
**Editor: Mohamed Emara**, Lecturer of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt.

# Nurses Knowledge and Practice Regarding Gastrointestinal Endoscopy and Suggested Nursing Guidelines

**Wafaa Mohamed Amer, Nadia Mohamed Taha , Howida Kameel Zaton**
*Medical Surgical Nursing Department, Faculty of Nursing, Zagazig University,Egypt*

*Corresponding Author:* ***Wafaa Mohamed Amer***

*Mobile: 01002134911*

*E mail: wafaaamer22@yahooc om*

*Key words: Nurses, knowledge, practice, attitude, gastrointestinal endoscopy, training, education*

**Background and study aim:** The increased of demand for GIT endoscopies necessitates the assistance of highly trained endoscopy nurses to perform her role to take care of patients undergoing different GIT endoscopies through the whole phases of endoscopy.The aim of this study is to assess knowledge and practice of nurses regarding to Gastrointestinal Endoscopy procedure and suggested nursing guideline.
**Methods:** The present study conducted in Gastrointestinal Endoscopy Unit at Zagazig University Hospitals. The study subject includes all available endoscopy 35 nurses. Three tools were used for collection of data, first questionnaire sheet to collect knowledge about socio demographic characteristics of study nurses and questions to assess nurses' knowledge regarding endoscopy as definition, structure, complications, nursing role, guidelines and types of endoscopy and her role in the pre-procedure, during procedure and post- procedure. Second tool was nursing attitude. Third tool was an observational checklist to assess nurses' practice in the pre procedure, during procedure and post procedure in endoscopy and Suggested nursing guideline.
**Results:** The study revealed that more than half of the nurses had their age equal 25 years or less and most of them had diploma degree and that more than half of nurses had their working experience range from 8 years to less than 28 years. Most of studied nurses had satisfactory level of nurses' knowledge regarding Gastrointestinal Endoscopy. The majority of nurses had positive attitude. The majority of nurses had unsatisfactory nurses' level of practice before, during and after GI endoscope, discharge instructions and manual disinfection of endoscopy. Also, there were statistical significant relationship between total nurses' knowledge regarding GI endoscopy and their age, training and work duration and not significant with nurse qualification.
**Conclusion:** We can conclude that the nurse age, training and working duration are affecting the level of nurses' knowledge regarding gastrointestinal endoscopy including; general precautions, basic steps to clean and disinfection in endoscopy unit. The majority of nurses had positive attitude. While training and qualification can affect dealing with patients in addition to nurses' level of practice before, during and after GI endoscope, discharge instructions and manual disinfection of endoscopy. So, we recommend adequate education and training of all nurses working with gastrointestinal endoscopy unit, with continuous evaluation of nurses' work practice. Periodic evaluation may help to take decision regarding training programs to increase theoretical and practical experience. Further studies are necessary to identify effects of educational programs on nurses' performance in gastrointestinal endoscopy unit.

## INTRODUCTION

Gastrointestinal (GI) endoscopy is an important tool for the identification and treatment of disorders of the gastrointestinal tract. Gastrointestinal endoscopy aids in there diagnosis and allows minimally-invasive therapeutic techniques to replace more aggressive interventions such as surgery. These advances have reduced mortality and hospital stay of patients with gastro-intestinal disorders undergoing these procedures. All staff in any setting where gastrointestinal endoscopy is performed must adhere to infection control principles that will maintain a safe environment, free from the possibility of spreading disease to patients and

co-workers. This is true regardless of the setting (Hospital Clinic, Ambulatory Care Center and Office), all types of gastrointestinal (GI) procedures performed. Nursing professionals who work in endoscopy units, providing patients with the care required before (pre procedure), during and after the procedure (post procedure), should have specific training to be able to carry out their duties in these units and to be able to manage the materials and equipment required, so that they may contribute to the success of these procedures. The specific knowledge and the development of the functions of these nurses aims to establish a close collaboration with the endoscopist to minimize the complications of the technique, reduce the patient's potential anxiety, and improve the applicability and results of gastrointestinal endoscopy **[1]**. Endoscopy nurses play a critical role in the provision of safe, high quality Preparing endoscopy. Nurses have many tasks. Preparing the endoscopic room, with the right instrument and necessary devices for examination of the upper or lower GI tract, is very important. It is also crucial that the nurse gives the right information about the procedure to the patient, to relieve anxiety and to give explanations about the modality of the endoscopic procedure. During the procedure the nurse must help the endoscopist and when indicated, the anesthesist. After the completion of the procedure, the nurse must carry-on with the reprocessing of the endoscopic instrument and of the devices. Specialized and dedicated nurses who attended courses to keep up-dated are indispensable in this field because of the constant evolution of the endoscopic instruments and techniques. Possible complications must be kept in mind to be recognized and to be treated in an early phase. The endoscopy-trained nurse must know the anatomy of the examined segments but should however integrate this knowledge with the care of the patients. Nurses should also contribute to clinical research regarding endoscopy **[2]**.

The Gastrointestinal Endoscopy Center at Zagazig University Hospital offers a highly specialized medical service for many clients suffering from different GIT problems. The monthly reports of that center indicated that during the first four months of 2013, more than 500 GIT endoscopy are performed each month (Registration Department of Gastrointestinal center, Zagazig University, 2013). This high frequency of GIT endoscopies necessitates the assistance of highly trained endoscopy nurses to perform her role for care of patients undergoing different GIT endoscopies through the whole phases of endoscopy according to the American Society for Gastrointestinal Endoscopy 2013. These phases are: the pre-procedure: (the period of time prior to beginning of the endoscopy) the procedure :(the period from initiation of sedation and analgesia till the completion of the endoscopic intervention) and the post-procedure: (the period from the completion of the endoscopic intervention till patient discharge). So, we aimed to assess knowledge and practice of nurses regarding gastrointestinal endoscopy and suggested nursing guideline.

**Aim of the Work :**

To assess knowledge and practice of nurses regarding gastrointestinal endoscopy and suggested nursing guideline.

## SUBJECTS AND METHODS

This descriptive exploratory study was conducted in Endoscopy Unit at Zagazig University Hospitals. Field work of this study was executed in six months from January 2014 to the end of September, 2014.

**Subjects:**

All available endoscopy nurses (35) were recruited in the study setting . Newly graduated nurses less than one year experience were excluded.

**Tools for data collection:** three tools were used for data collection

**1-Nurses' knowledge interviewing questionnaire sheet :** it consisted of five parts:

**Part I :** Socio demographic characteristics of the nurses

**Part II:** Questions to assess nurses' knowledge regarding gastrointestinal endoscopy and nursing care for haematemsis cases in the form of open-end question and multiple choice question (MCQs).

**Part III:** Questions to assess nurses' knowledge regarding practice before, during and after Gastrointestinal Endoscopy. It included open-end question and (MCQs).

**Part VI:** Questions to assess nurses' knowledge regarding general precaution in endoscopy unit in the form of true⁄ false questions.

**Part V:** Questions to assess nurses' knowledge regarding basic steps to clean and disinfect GI Endoscopy in the form of true⁄ false questions.

Subjective nurses' attitude towards gastrointestinal endoscopy was assessed with questionnaire.

**2- Nurses' practice observational checklist 1:**

**Part I :** It is concerned with gastrointestinal endoscopy insertion. It is composed of 28 questions to cover four parts:-

A- Before insertion of GI endoscope.
B- During insertion of GI endoscope.
C- After insertion of GI endoscope.
D- Discharge instructions of GI endoscope.

**Part II:** It is concerned with a checklist to assess nurses' practice before reprocessing for GI Endoscopes. It is composed of 16 items to cover six parts:

A- Pre-procedure, during and post procedure and pre-cleaning for infection control.
B- Leakage Testing.
C- Manual Cleaning
D- High Level Disinfectant (HLD
E- Manual Disinfecting

The nurses had satisfactory level of practice when the total score equal or above 60 % and unsatisfactory if it is below 60%.

**Content validity and reliability :**

It was established for to assure the content validity by a panel of 5 expertise's, in medicine and medical surgical nursing at Zagazig University, who revised the tools for clarity, relevance, comprehensiveness, understanding, and ease for implementation. According to their opinion minor modification were applied and reliability test was done for Thai stress test. The reliability analyses were conducted for the two scales and the total scale of the TST by using Cronbach's Alpha and Split Half Method (Odd-Even technique). The Alpha coefficient of the TST total test was 0.84. The values of the two scales were ranged from 0.83 to 0.86. The Split Half coefficient of the TST total test was 0.88. The values of the two scales were ranged from 0.85 to 0.91. This showed that the reliability coefficients are in the middle to high values.

The researcher met of them individually and explained the purpose of the study and their role in filling the questionnaire sheet, then giving them the questionnaire sheet to fill it. Distribution of the questionnaire sheet was done every day at the end of morning shift for nurses working who was in the morning shift. The researcher was interviewed with each nurses individually to fulfill the questionnaire sheet, the time required for completion of the questionnaire sheet was ranged from 30- 45 minutes. Observation was done continuously, every day. Each nurse was observed in the morning shift, for pre-procedure, during procedure and post-procedure GIT endoscopy, three times and the mean (SD) of these observation was done to assess her performance. Also the researcher was observing nurses practical skills about studied procedures. The time needed to complete the checklist ranged from 45 minutes-1 h.

**Statistical Design :**

After the collection of data, Data were checked, entered and analyzed using SPSS version 19 for data processing. All the quantitative data were expressed as mean ± SD and were analyzed by independent t-test, while, qualitative data were expressed as number and percentage and analyzed by Chi-square test. Statistic- P value of $<0.05$ indicates significant results and $P<0.001$ indicates highly significant results.

## RESULTS

The first part of our results was the socio demographic characteristics of the nurses in the study including; gender, age, work duration, years of experience, training and HBV vaccination (Table 1).

The second part of our results was concerned with the level nurses knowledge, most of the included nurses had satisfactory level of knowledge regarding gastrointestinal endoscopy and nursing care for heamatemsis cases (80%). Moreover, all of them were aware with purpose of endoscope (100%), more than three quarters of nurses had satisfactory level of total knowledge regarding practice before, during and after GI endoscopy. All nurses were aware with documenting emergency signs after GIT endoscopic insertion and knowledge about the guideline was satisfactory. All of them were aware with hand washing, wearing surgical gloves during endoscopy, continuous hand washing in contact with patients, keep clean and short nail, Needle must be put in container, avoiding smoking in endoscopy unit. Most of them (80%) had satisfactory level of knowledge regarding general precautions in endoscopy unit. All nurses had satisfactory level of knowledge regarding endoscope disinfection to kill bacteria, notification to maintenance unit in case of leakage after

disinfection, use cidex for disinfection and sterilization of endoscope. The majority of nurses (80%) had satisfactory level of knowledge regarding basic steps to clean and disinfect GI Endoscope. Otherwise, unsatisfactory level of knowledge were reported regarding use 70% of alcohol for disinfection and endoscope in disinfectant solutions for 10 min between patients (60%, 40%, respectively) (Tables 2,3,4,5).

The third part of our results was concerned with the attitude of the nurses towards the provided care in endoscopy unit. It revealed that The majority of nurses (80%) had satisfactory attitude, all of them agreed on the importance of providing comfortable place for patients, necessity to take HBV vaccine after injury by endoscope (100%). On the other hand, some of them showed unsatisfactory attitude regarding dealing with heamatemesis and melena of patients during endoscopy, satisfied when dealing with patients during endoscopy and necessity to explain to patients procedure of endoscope (Table 6).

The fourth part of our results showed that nurses' practice pre, during and post GI endoscopic procedure in addition to discharge instructions was satisfactory. Regarding assessing patient's demographic data, explanation of the procedure to patient, fulfilling the requested investigations, assessing the vital signs and time passed after the procedure, giving the patients the follow up plan and discharge instructions. Otherwise, the majority of studied nurses had unsatisfactory nurses' level of practice pre, during and post GI endoscopic procedure, apart from unsatisfactory level of practice as regarding how to prepare for of Sungkstagen intubation , perform enema at in the morning before procedure, insertion nasogastric tube at night before procedure, ensure blood transfusion, Provides emotional support, Connected with IV line, keep patient in a side lying position, documents patient's level of consciousness, checks patient swallowing and record the discharge of the patient from hospital (alone or accompanied), mention the type diet, common symptoms and complications (Tables 7,8,9,10).

Also, this part illustrated the practice of the nurses regarding infection control measures, manual cleaning and disinfection of the endoscope, all the nurses had satisfactory level of practice before reprocessing for GI Endoscopes for wearing the mask and sterile gloves and performing leakage testing, immersing endoscope in enzymatic detergent, manual disinfecting of endoscope, removing all valves and removable parts, storing the endoscope with the angulations locks, hanging the endoscope with the insertion tube, ensure the insertion tube is set to maximum flexibility and disinfected scopes were properly hunged and dried. While almost all nurses had unsatisfactory level of applying infection control measures regarding wearing overshoes, perform pre-cleaning in the procedure room immediately after each procedure, wear appropriate (PPE), connect the leakage tester connector to the output socket on the light source, manipulate the angulations knobs and video switches and dry the leakage tester connector cap, practice for manual cleaning and disinfecting of endoscopy (Tables 11,12,13).

Over all we can say that nearly 70% of our nurses had satisfactory level of knowledge, 80% of nurses had satisfactory level of attitude. With unsatisfactory level of practice in most of them (94.3%) (Table 14).

The fifth part of our results demonstrated that there was significant relationship between total nurses' knowledge about GI endoscopy and their age, training and work duration (P value 0.004, 0.001 and 0.004 respectively) but there was no significant relation with nurse Qualification (p=0.342), while, nurses practice showed statistical significant relationship between total nurses' practice and the attendance to training and nurses qualification, dealing with patients with GI Endoscopy and their personal characteristics (P value 0.367, 0.074 and 0.367) and qualification (P= 0.001). While there was no statistical significance differences between nurses practice and their age years of experience (P value 0.119, 0.009, 0.399 respectively) and qualification (P= 0.001). In addition to the statistical significance relationship between total nurses' knowledge, practice in dealing with patient, endoscopy reprocessing and qualification (Tables 15,16,17). There was no significant association between nurses regard knowledge and Practice in dealing with the patients or with endoscope. Also, there was no statistical significant correlation between total nurses knowledge and practice their total (p value 0.152,0.409).

**Table (1):** Socio demographic characteristics of nurses including age, gender, work duration, years of experience, and their training

| Socio demographic data | No (n=35) | % |
|---|---|---|
| **Age** (years) >25- | 18 | 51.4 |
| 35- | 7 | 20.0 |
| <45 | 10 | 28.6 |
| Mean± (SD)<br>Rang | 39.7± 11.6<br>26-58 | |
| **Sex** Female<br>Male | 35<br>0 | 100.0<br>0.0 |
| **Qualification**<br>Diploma degree<br>Associate degree<br>Bachelor degree | <br>28<br>4<br>3 | <br>80.0<br>11.4<br>8.6 |
| **Work conditions & training** | **No ( n=35)** | **%** |
| **Work duration (years)**<br>8-<br>18-<br>>28 | <br>18<br>7<br>10 | <br>51.4<br>20.0<br>28.6 |
| Mean± SD<br>Range | 19.8 ± 11.1<br>8-38 | |
| **Experience (years) per departments**<br>**Haematemesis**<br><10years<br>≥10years | <br><br>7<br>7 | <br><br>20.0<br>20.0 |
| **GIT Endoscopy**<br><10years<br>≥10years | <br>11<br>7 | <br>31.4<br>20.0 |
| Mean SD<br>Range | 19.8 ± 11.1<br>8-38 | |
| **Training** Yes<br>No | 14<br>21 | 40.0<br>60.0 |
| **Vaccination (HBV)** Yes<br>No | 32<br>3 | 91.4<br>8.6 |

**Table (2):** Distribution of nurses' level of knowledge regarding Gastrointestinal Endoscopy and nursing care for heamatemsis cases (N = 35)

| Items | Unsatisfactory | | Satisfactory | |
|---|---|---|---|---|
| | No | % | No | % |
| Definition of endoscope | 7 | 20.0 | 28 | 80.0 |
| Types of diagnostic GI Endoscopy | 7 | 20.0 | 28 | 80.0 |
| Types of therapeutic GI Endoscopy | 11 | 31.4 | 24 | 68.6 |
| Composition of endoscope | 7 | 20.0 | 28 | 80.0 |
| Uses of endoscope | 0 | 0.0 | 35 | 100.0 |
| Purpose of endoscope | 0 | 0.0 | 35 | 100.0 |
| Complications of endoscope | 14 | 40.0 | 21 | 60.0 |
| Complications of endoscope procedure | 14 | 40.0 | 21 | 60.0 |
| Nursing care for haematemesis cases | 11 | 31.4 | 24 | 68.6 |
| Nurse duties with patient during haematemesis | 31 | 88.6 | 4 | 11.4 |
| keeping open airway during haematemesis | 7 | 20.0 | 28 | 80.0 |
| **Total knowledge** | **7** | **20.0** | **28** | **80.0** |

**Table (3):** Distribution of nurses' level of knowledge regarding Practice before, during and after Gastrointestinal Endoscopy (N=35)

| Items | Unsatisfactory | | Satisfactory | |
|---|---|---|---|---|
| | No | % | No | % |
| **Gastrointestinal Endoscopy** | | | | |
| Before GIT EI | 11 | 31.4 | 24 | 68.6 |
| During GIT EI | 4 | 11.4 | 31 | 88.6 |
| After GIT EI | 7 | 20.0 | 28 | 80.0 |
| **Total knowledge** | | | | |
| Knowledge about guideline | 8 | 22.9 | 27 | 77.1 |
| Nursing guidelines after GIT endoscope insertion | 4 | 11.4 | 31 | 88.6 |
| Documented emergency signs after GIT endoscope insertion | 0 | 0.0 | 35 | 100.0 |
| Written nursing guidelines in GIT endoscopy unit | 18 | 51.4 | 17 | 84.6 |
| **Total knowledge** | **10** | **28.6** | **25** | **71.4** |

**Table (4) :** Distribution of nurses knowledge regarding general precautions in endoscopy unit (N = 35)

| Items | Unsatisfactory | | Satisfactory | |
|---|---|---|---|---|
| | No | % | No | % |
| Hand washing after toilet | 0 | 0.0 | 35 | 100.0 |
| Hand washing before drinking and eating | 0 | 0.0 | 35 | 100.0 |
| Hand washing after contact with blood & discharge | 0 | 0.0 | 35 | 100.0 |
| Hand washing after contact with infected articles | 0 | 0.0 | 35 | 100.0 |
| Hand washing after each patient | 0 | 0.0 | 35 | 100.0 |
| Hand washing after each endoscopy | 0 | 0.0 | 35 | 100.0 |
| Nurse can transmit infection to patients | 0 | 0.0 | 35 | 100.0 |
| Wear mask during endoscopy | 4 | 12.9 | 31 | 87.1 |
| Wear gown during endoscopy | 7 | 20.0 | 28 | 80.0 |
| Wear surgical gloves during endoscopy | 0 | 0.0 | 35 | 100.0 |
| Wear thick gloves in contacts with infected blood | 11 | 31.4 | 24 | 68.6 |
| Continuous hand washing in contact with patients | 0 | 0.0 | 35 | 100.0 |
| Wear gloves in contact with blood and discharges | 0 | 0.0 | 35 | 100.0 |
| Necessary hand washing after glove removal | 0 | 0.0 | 35 | 100.0 |
| Wear clean uniform | 31 | 87.1 | 4 | 12.9 |
| Keep clean and short nail | 0 | 0.0 | 35 | 100.0 |
| Needle must be but in contender | 0 | 0.0 | 35 | 100.0 |
| Follow up every 6 months | 18 | 51.4 | 17 | 48.6 |
| Not necessary avoiding patients with influenza | 7 | 20.0 | 28 | 80.0 |
| Eating and drinking in endoscopy unit | 24 | 68.6 | 11 | 31.4 |
| Avoid smoking in endoscopy unit | 0 | 0.0 | 35 | 100.0 |
| **Total knowledge** | **7** | **20.0** | **28** | **80.0** |

**Table (5):** Distribution of nurses knowledge regarding basic steps to clean and disinfect GI Endoscope (N = 35)

| Items | Unsatisfactory | | Satisfactory | |
|---|---|---|---|---|
| | No | % | No | % |
| Disinfect endoscope to kill bacteria | 0 | 0.0 | 35 | 100.0 |
| Uses of disinfectants prevent infection | 7 | 20.0 | 28 | 80.0 |
| Clean endoscope with soaps before immersing in disinfectants | 11 | 31.4 | 24 | 68.6 |
| Remove valves of endoscope and clean separately | 11 | 31.4 | 24 | 68.6 |
| Use 70% alcohol for disinfection | 21 | 60.0 | 14 | 40.0 |
| Rinse endoscope with water from inside and outside after disinfection | 4 | 11.4 | 31 | 88.6 |
| Put endoscope in disinfectant solutions for 30 min in beginning and end of the day. | 11 | 31.4 | 24 | 68.6 |
| Use k-y Jelly or others for oiling valves | 7 | 20.0 | 28 | 80.0 |
| Notification to maintenance unit in case of leakage after disinfection | 0 | 0.0 | 35 | 100.0 |
| Use cidex for disinfection and sterilization of endoscope | 0 | 0.0 | 35 | 100.0 |
| Put endoscope in disinfectant solutions for 10 min between patients | 14 | 40.0 | 21 | 60.0 |
| **Total knowledge** | **7** | **20.0** | **28** | **80.0** |

**Table (6) :** Distribution of attitude of nurses providing care in endoscopy unit. (N = 35)

| | **Attitude** | | | | | |
|---|---|---|---|---|---|---|
| | **Agree** | | **Sometimes** | | **Not agree** | |
| | **No** | **%** | **No** | **%** | **No** | **%** |
| Importance of providing comfortable place for patients | 35 | 100.0 | 0 | 0.0 | 0 | 0.0 |
| Dealing with patients not affect my psychological state | 18 | 51.4 | 10 | 28.6 | 7 | 20.0 |
| Not necessary to take HBV vaccine after injury by endoscope | 35 | 100.0 | 0 | 0.0 | 0 | 0.0 |
| fairness of endoscopic patient blocks delay providing nursing care | 25 | 71.4 | 10 | 28.6 | 0 | 0.0 |
| Working not turn me harder | 18 | 51.4 | 10 | 28.6 | 7 | 20.0 |
| Hand washing only kill any organism | 35 | 100.0 | 0 | 0.0 | 0 | 0.0 |
| Sympathized with endoscopic patients with advanced cases | 31 | 88.6 | 4 | 11.4 | 0 | 0.0 |
| Necessary to leave endoscope in cidex for 20 minutes | 35 | 100.0 | 0 | 0.0 | 0 | 0.0 |
| Necessary to hand washing before dealing with patients | 35 | 100.0 | 0 | 0.0 | 0 | 0.0 |
| Patient has right to know used articles and endoscope environment | 25 | 71.4 | 4 | 11.4 | 6 | 17.1 |
| Necessary to explain to patients procedure of endoscope | 18 | 51.4 | 7 | 20.0 | 10 | 28.6 |
| Disinfecting tongue depressor not transmit infection | 31 | 88.6 | 4 | 11.4 | 0 | 0.0 |
| Not satisfied when deal with melena of patients during endoscopy | 25 | 71.4 | 10 | 28.6 | 0 | 0.0 |
| Necessary to clean endoscope with water and soap before but in cidex | 28 | 80.0 | 0 | 0.0 | 7 | 20.0 |
| I feel happy in case of stopping of patients bleeding | 28 | 80.0 | 7 | 20.0 | 0 | 0.0 |
| Important to improve nurse , patients and relative relationships | 21 | 60.0 | 7 | 20.0 | 7 | 20.0 |
| Not satisfied when deal with heamatemesis of patients during endoscopy | 25 | 71.4 | 10 | 28.6 | 0 | 0.0 |
| **Total attitude** | +ve | | | | -ve | |
| | **28** | | **80.0** | | **7** | **20.0** |

**Table (7):** Distribution of nurses' level of practice before GI endoscope insertion (N = 35)

| **A) Pre –Procedure** | **Unsatisfactory** | | **Satisfactory** | |
|---|---|---|---|---|
| | **No** | **%** | **No** | **%** |
| **General preparation** | | | | |
| Introduce herself to the patient | 35 | 100.0 | 0 | 0.0 |
| Assess Patient's demographic data | 0 | 0.0 | 35 | 100.0 |
| Assess Allergic to medication | 35 | 100.0 | 0 | 0.0 |
| Assess Patient's past history | 0 | 0.0 | 35 | 100.0 |
| Assess Patient's History about reason(s)for endoscope | 0 | 0.0 | 35 | 100.0 |
| Assess Patient's Vital signs and pulse oximetry | 11 | 31.4 | 24 | 68.6 |
| Explains procedure to patient prior to start it | 0 | 0.0 | 35 | 100.0 |
| Discusses guidelines given to patient before the procedure | 0 | 0.0 | 35 | 100.0 |
| Obtains an informed consent from patient | 18 | 51.4 | 17 | 48.6 |
| Fulfilled every requested lab and radiographic investigations. | 0 | 0.0 | 35 | 100.0 |
| Ensures removal of denture, jewels, nail varnish and make up. | 11 | 31.4 | 24 | 68.6 |
| Ensures that the patients wears gown. | 18 | 51.4 | 17 | 48.6 |
| Administers analgesic, sedation, anti-anxiety agents and medications | 18 | 51.4 | 17 | 48.6 |
| **Specific preparation of upper GIT** | | | | |
| Ensure fasting for 6-8 hours before the procedure. | 11 | 31.4 | 24 | 68.6 |
| Ensure fasting extra hours in case of 'occlusion of oesophagus'. | 0 | 0.0 | 35 | 100.0 |
| **Specific preparation of lower GIT:** | | | | |
| Ensure oral liquid diet for 24 hours before procedure. | 0 | 0.0 | 35 | 100.0 |
| Nasogastric tube at night before procedure. | 18 | 51.4 | 17 | 48.6 |
| Administer Rectal suppositories last night before procedure as described. | 0 | 0.0 | 35 | 100.0 |
| Perform Enema at the morning before procedure. | 35 | 100.0 | 0 | 0.0 |
| Perineal care after enema & chaving. | 35 | 100.0 | 0 | 0.0 |
| **Specific preparation of Endoscopy** | | | | |
| Ensure Prepares for blood transfusion | 18 | 51.4 | 17 | 48.6 |
| Ensure Prepares for Sungkstagen intubation | 35 | 100.0 | 0 | 0.0 |
| **Total practice score** | 31 | 88.6 | 4 | 11.4 |

**Table (8) :** Distribution of nurses' level of practice during GI endoscope (N = 35)

| During procedure : | Unsatisfactory | | Satisfactory | |
|---|---|---|---|---|
| | No | % | No | % |
| Positioning the patient Properly according to each procedure. | 35 | 100.0 | 0 | 0.0 |
| Explain each step during procedure. | 0 | 0.0 | 35 | 100.0 |
| Provides emotional support. | 18 | 51.4 | 17 | 48.6 |
| Regular checks vital sign during procedure. | 11 | 31.4 | 24 | 68.6 |
| Observes patient closely. | 35 | 100.0 | 0 | 0.0 |
| Connected with IV line. | 18 | 51.4 | 17 | 48.6 |
| Perform suctioning from mouth in case of secretions. | 35 | 100.0 | 0 | 0.0 |
| Records medication, vital signs, time of start and end of the procedure,- reports of the procedure | 11 | 31.4 | 24 | 68.6 |
| **Total practice score** | **30** | **85.7** | **5** | **14.3** |

**Table (9) :** Distribution of nurses' level of practice after GI endoscope (N = 35)

| Post-procedure | Unsatisfactory | | Satisfactory | |
|---|---|---|---|---|
| | No | % | No | % |
| Keep patient in a side lying position | 35 | 100.0 | 0 | 0.0 |
| Documents patient's level of consciousness. | 35 | 100.0 | 0 | 0.0 |
| Assess vital signs every 30 minutes for 2 hours. | 0 | 0.0 | 35 | 100.0 |
| Report abnormalities immediately. | 11 | 31.4 | 24 | 68.6 |
| Checks patient is swallowing. | 35 | 100.0 | 0 | 0.0 |
| Record discharge of patient from hospital (alone or accompanied). | 18 | 51.4 | 17 | 50.6 |
| Record patient's discharge as regard to: | | | | |
| Time after the procedure by two to three hours. | 0 | 0.0 | 35 | 100.0 |
| Physical condition. | 11 | 31.4 | 24 | 68.6 |
| Psychological condition. | 35 | 100.0 | 0 | 0.0 |
| Complications detected or not. | 11 | 31.4 | 24 | 68.6 |
| **Total practice score after endoscopy** | **31** | **88.6** | **4** | **11.4** |

**Table (10) :** Distribution of nurses' level of practice regarding Discharge instructions (N= 35)

| Discharge instructions | Unsatisfactory | | Satisfactory | |
|---|---|---|---|---|
| | No | % | No | % |
| **Notified the patient about :** | | | | |
| Notifies the patient about the follow up plan. | 0 | 0.0 | 35 | 100.0 |
| Types of activities | 30 | 85.7 | 5 | 4.3 |
| The types of diet | 35 | 100.0 | 0 | 0.0 |
| Medications schedule. | 18 | 51.4 | 17 | 48.6 |
| Common symptoms and sensations | 35 | 100.0 | 0 | 0.0 |
| The referral place. | 31 | 88.6 | 4 | 11.4 |
| Signs of complications | 35 | 100.0 | 0 | 0.0 |
| Give the patient written discharge instruction | 0 | 0.0 | 35 | 100.0 |
| **Total practice score** | **30** | **95.7** | **5** | **4.3** |

**Table (11) :** Distribution of nurses' level of practice regarding infection control for GI Endoscopes (N = 35)

| A):Apply Infection Control Pre –Procedure | Unsatisfactory | | Satisfactory | |
|---|---|---|---|---|
| | No | % | No | % |
| **Nurses application of ICP before procedure:-** | | | | |
| Wear overshoes. | 35 | 100.0 | 0 | 0.0 |
| Wearing the mask and goggles | 0 | 0.0 | 35 | 100.0 |
| Wash hands | 30 | 85.7 | 5 | 4.3 |
| Wear sterile gown | 11 | 31.4 | 24 | 68.6 |
| Wear sterile gloves | 0 | 0.0 | 35 | 100.0 |
| **During Procedure** | | | | |
| Adherence to infection control principles restrictedly | 35 | 100.0 | 0 | 0.0 |
| **Post procedure Pre-Cleaning:** | | | | |
| Perform pre-cleaning in the procedure room immediately after each procedure. | 35 | 100.0 | 0 | 0.0 |
| Wipe the insertion tube with an enzymatic detergent | 34 | 97.1 | 1 | 2.9 |
| Suction enzymatic detergent through the instrument. | 35 | 100.0 | 0 | 0.0 |
| Suction air through the instrument channel for 10 seconds. | 30 | 85.7 | 5 | 4.3 |
| Attach the air/water channel cleaning adapter | 35 | 100.0 | 0 | 0.0 |
| Use the special cleaning adapters as recommended. | 35 | 100.0 | 0 | 0.0 |
| Discard disposable valves. | 30 | 85.7 | 5 | 4.3 |
| Place valves and removable parts in beaker of detergent solution. | 33 | 94.3 | 2 | 5.7 |
| Inspect and attach the water resistant cap | 35 | 100.0 | 0 | 0.0 |
| Cover the endoscope and transport to the reprocessing area. | 11 | 31.4 | 24 | 68.6 |
| **Leakage Testing:** | | | | |
| Perform leakage testing. | 0 | 0.0 | 35 | 100.0 |
| Wear appropriate protective pariar (PPE). | 35 | 100.0 | 0 | 0.0 |
| Fill basin for leakage testing. | 35 | 100.0 | 0 | 0.0 |
| Connect the leakage tester connector to the output socket on the light source. | 35 | 100.0 | 0 | 0.0 |
| Check that the leakage tester is emitting air . | 11 | 31.4 | 24 | 68.6 |
| Attach the leakage tester's connector to the water resistant cap and endoscope is pressurized. | 34 | 97.1 | 1 | 2.9 |
| Immerse the entire endoscope in the water | 33 | 94.3 | 2 | 5.7 |
| Manipulate the angulations knobs and video switches | 35 | 100.0 | 0 | 0.0 |
| Remove the endoscope from the water. | 34 | 97.1 | 1 | 2.9 |
| Disconnect the leakage tester for the air supply | 33 | 94.3 | 2 | 5.7 |
| Disconnect the leakage tester from the water resistant cap. | 34 | 97.1 | 1 | 2.9 |
| Dry the leakage tester connector cap. | 35 | 100.0 | 0 | 0.0 |

**Table (12):** Distribution of nurses' level of practice for manual Cleaning of endoscope (N= 35)

| Manual Cleaning: | Unsatisfactory | | Satisfactory | |
|---|---|---|---|---|
| | No | % | No | % |
| **Manual cleaning :** | | | | |
| Immerse endoscope in enzymatic detergent | 0 | 0.0 | 35 | 100.0 |
| What type of enzymatic detergent is being used? | 35 | 100.0 | 0 | 0.0 |
| Does the staff have manufacturer's instructions | 35 | 100.0 | 0 | 0.0 |
| Is enzymatic detergent beginning diluted | 31 | 88.6 | 4 | 11.4 |
| Is the sink marked for proper level of water | 35 | 100.0 | 0 | 0.0 |
| Is the enzymatic detergent freshly prepared | 34 | 97.1 | 1 | 2.9 |
| Brush biopsy/suction channel in the insertion tube | 34 | 97.1 | 1 | 2.9 |
| Brush biopsy/suction channel in the universal cord | 34 | 97.1 | 1 | 2.9 |
| Brush suction valve housing and instrument channel | 34 | 97.1 | 1 | 2.9 |
| Use suction channel cleaning adapter | 34 | 97.1 | 1 | 2.9 |
| Attach the channel plug and injection tube | 34 | 97.1 | 1 | 2.9 |
| Use all channel cleaning adapters and brushes. | 35 | 100.0 | 0 | 0.0 |
| Disconnect the channel plug, injection tube | 34 | 97.1 | 1 | 2.9 |
| Soak the endoscope in the detergent solution. | 35 | 100.0 | 0 | 0.0 |
| Brush and flush the valves. | 35 | 100.0 | 0 | 0.0 |
| Thoroughly dry the exterior of the endoscope. | 34 | 97.1 | 1 | 2.9 |
| Inspect the endoscope for residual debris. | 35 | 100.0 | 0 | 0.0 |
| Center use single use scope cleaning brushes? | 35 | 100.0 | 0 | 0.0 |
| Center use re-usable scope cleaning brushes | 34 | 97.1 | 1 | 2.9 |
| Prepare compatible valves and removable parts | 34 | 97.1 | 1 | 2.9 |
| **High Level Disinfectant (HLD) :** | | | | |
| Name and type of high level disinfectant: | 35 | 100.0 | 0 | 0.0 |
| Verify the correct exposure time for HLD | 35 | 100.0 | 0 | 0.0 |
| Verify the temperature required | 35 | 100.0 | 0 | 0.0 |
| Center has a copy of all written mfr instructions | 35 | 100.0 | 0 | 0.0 |
| Confirm the HLD is labeled | 35 | 100.0 | 0 | 0.0 |
| HLD is discarded at the maximum days of re-use | 35 | 100.0 | 0 | 0.0 |

**Table (13) :** Distribution of nurses' level of practice for manual Disinfecting of endoscope (N = 35)

| Manual Disinfecting: | Unsatisfactory | | Satisfactory | |
|---|---|---|---|---|
| | No | % | No | % |
| Test the HLD (MEC) with each use. | 35 | 100.0 | 0 | 0.0 |
| Immerse the entire endoscope in a basin of HLD | 30 | 85.7 | 5 | 14.3 |
| Attach the adapters | 33 | 94.3 | 2 | 5.7 |
| Flush the HLD solution to purge air | 35 | 100.0 | 0 | 0.0 |
| Disconnect the channel plug, injection tube, | 35 | 100.0 | 0 | 0.0 |
| Soak the endoscope in HLD solution | 33 | 94.3 | 2 | 5.7 |
| Flush air thru the endoscope channels | 35 | 100.0 | 0 | 0.0 |
| Immerse the endoscope in fresh sterile water. | 35 | 100.0 | 0 | 0.0 |
| Soak the valves and removable parts in HLD . | 33 | 94.3 | 2 | 5.7 |
| Confirm that endoscopes and channels are rinsed with sterile or filtered water after exposure to HLD, then with alcohol rinse per mfr instructions, and then forced air for drying. | 35 | 100.0 | 0 | 0.0 |
| **Rinsing:** | | | | |
| Test the HLD efficacy | 33 | 94.3 | 2 | 5.7 |
| Properly place the endoscope in the basin | 35 | 100.0 | 0 | 0.0 |
| Attach the scope connectors/adapters to the AER | 35 | 100.0 | 0 | 0.0 |
| Run the AER and ensure the endoscope is soaked | 33 | 94.3 | 2 | 5.7 |
| Remove the endoscope promptly after cycle completed | 35 | 100.0 | 0 | 0.0 |
| Perform the terminal steps does not perform | 33 | 94.3 | 2 | 5.7 |
| The elevator-wire channel require manual disinfecting | 35 | 100.0 | 0 | 0.0 |
| **Endoscope Handling:** | | | | |
| Ensure that the insertion tube is not coiled | 35 | 100.0 | 0 | 0.0 |
| Position the control knobs upright | 33 | 94.3 | 2 | 5.7 |
| Transport the endoscope using both hands. | 33 | 94.3 | 2 | 5.7 |
| **Endoscope Storage:** | | | | |
| Ensure that an alcohol flush was performed | 35 | 100.0 | 0 | 0.0 |
| Remove all valves and removable parts | 0 | 0.0 | 35 | 100.0 |
| Store the endoscope with the angulation locks | 0 | 0.0 | 35 | 100.0 |
| Ensure the insertion tube is set to maximum flexibility. | 0 | 0.0 | 35 | 100.0 |
| Hang the endoscope with the insertion tube | 0 | 0.0 | 35 | 100.0 |
| Disinfected scopes are properly hung and dried | 0 | 0.0 | 35 | 100.0 |
| **Grand reposes practice score** | **33** | **94.3** | **2** | **5.7** |

**Table (14) :** Total knowledge, attitude and practice of nurses. (N = 35)

| | Unsatisfactory | | Satisfactory | |
|---|---|---|---|---|
| | No | % | No | % |
| **Total knowledge** | 8 | 22.9 | 27 | 77.1 |
| **Total attitude** | 7 | 20.0 | 28 | 80.0 |
| **Total practice** | 33 | 94.3 | 2 | 5.7 |

**Table (15) :** Relation between total nurses' level of knowledge regarding GI endoscopy and their socio demographic characteristics, training, qualification and work duration. (N= 35)

| Socio demographic data | Unsatisfactory No=8 | | Satisfactory No.=27 | | X2 | P |
|---|---|---|---|---|---|---|
| | No | % | No | % | | |
| **Age (years)** 25- | 0 | 0.0 | 18 | 66.7 | 11.05 | 0.04 |
| 35- | 4 | 50.0 | 4 | 14.8 | | |
| 45- | 4 | 50.0 | 5 | 18.5 | | |
| **Training** Yes | 0 | 0.0 | 14 | 51.9 | 14.97 | <0.001 |
| No | 8 | 100.0 | 3 | 48.1 | | |
| **Qualification** | | | | | 2.15 | 0.342 |
| Diploma degree | 8 | 100.0 | 21 | 77.8 | | |
| Associate degree | 0 | 0.0 | 4 | 14.8 | | |
| Bachelor degree | 0 | 0.0 | 2 | 7.4 | | |
| **Work duration (years)** | | | | | 11.05 | 0.04 |
| 8- | 0 | 0.0 | 18 | 66.7 | | |
| 18- | 4 | 50.0 | 4 | 14.8 | | |
| 28-38 | 4 | 50.0 | 5 | 18.5 | | |

**Table (16):** Relation between total nurses' level of practice regarding dealing with patients with GI Endoscopy and their some personal data. (N= 35)

| Personal characteristic | Unsatisfactory No.=31 | | Satisfactory No.=4 | | X2 | P |
|---|---|---|---|---|---|---|
| | No | % | No | % | | |
| **Age (years)** 25- | 14 | 45.2 | 4 | 100.0 | 4.27 | 0.119 |
| 35- | 7 | 22.6 | 0 | 0.0 | | |
| 45 | 10 | 32.3 | 0 | 0.0 | | |
| **Training** Yes | 10 | 32.3 | 4 | 100.0 | 6.77 | 0.09 |
| No | 21 | 67.7 | 0 | 0.0 | | |
| **Qualification** | | | | | 27.59 | <0.001 |
| Diploma degree | 28 | 90.3 | 0 | 50.0 | | |
| Associate degree | 3 | 9.7 | 1 | 25.0 | | |
| Bachelor degree | 0 | 0.0 | 3 | 25.0 | | |
| **Work duration (years)** | | | | | 1.83 | 0.399 |
| 8- | 15 | 48.4 | 3 | 75.0 | | |
| 18- | 6 | 19.4 | 1 | 25.0 | | |
| 28-38 | 10 | 32.3 | 0 | 0.0 | | |

**Table (17) :** Relation between total nurses' level of practice regarding reprocessing GI Endoscope and their socio demographic characteristics. (N= 35)

| Personal data | Unsatisfactory No.=33 | | Satisfactory No.=2 | | X2 | P |
|---|---|---|---|---|---|---|
| | No | % | No | % | | |
| **Age (years)** 25- | 16 | 48.5 | 2 | 100.0 | 2.0 | 0.367 |
| 35- | 7 | 21.2 | 0 | 0.0 | | |
| 45- | 10 | 30.3 | 0 | 0.0 | | |
| **Training** Yes | 12 | 36.4 | 2 | 100.0 | 3.18 | 0.074 |
| No | 21 | 63.6 | 0 | 0.0 | | |
| **Qualification** | | | | | 22.63 | <0.001 |
| Diploma degree | 27 | 81.8 | 0 | 0.0 | | |
| Associate degree | 5 | 15.2 | 0 | 0.0 | | |
| Bachelor degree | 1 | 3.0 | 2 | 100.0 | | |
| **Work duration (years)** | | | | | 2.0 | 0.367 |
| 8- | 16 | 48.5 | 2 | 100.0 | | |
| 18- | 7 | 21.2 | 0 | 0.0 | | |
| 28-38 | 10 | 30.3 | 0 | 0.0 | | |

## DISCUSSION

Endoscopy nurses play a critical role in the provision of safe, high quality endoscopy. Nurses have many tasks. Prepare the endoscopic room with the right instrument and necessary devices for examination of the upper or lower GI tract, is very important. It is also crucial that the nurse gives the right information about the procedure to the patient, to relieve anxiety and to give explanations about the modality of the endoscopic procedure. During the procedure the nurse must help the endoscopist and, when indicated, the anesthesist. After the completion of the procedure, the nurse must carry-on with the reprocessing of the endoscopic instrument and of the devices **[2]**.

So, we attempted to assess knowledge and practice of nurses regarding Gastrointestinal Endoscopy and suggested nursing guideline. The study included 35 nurses working in endoscopic Units at Zagazig University Hospitals. Two types of tools were used for collection of data. Nurses' knowledge questionnaire sheet developed by the researcher to assess nurses' knowledge regarding endoscopy as definition, structure, complication, nursing role, guidelines, types of endoscopy and their role in the pre-procedure, during procedure and post-procedure phases. Observational checklist for endoscopy to assess nurses practice in the pre procedure, during procedure and post procedure phases.

All the nurses included in this study were females and their age ranged from 26-58 years. Robinson, Moreau and McCann **[3]** reported that the common pattern representing nurses' characterized with increased number of female nurse as compared with males.

This study revealed that 80% had of them diploma degrees. More than half of nurses had experience of working in gastrointestinal endoscopy unit range from 8-28 years. Two fifths of them attended training courses during their work in GIT Endoscopy and the majority of them had got (HBV) Vaccine. Ramsey and his colleague **[4]** founded in their study that, most of the nurses did not receive any special education or in-service training about endoscope reprocessing practices. Most of the authors reported that education and training, including competency testing, at least annually. This helps professional nurse to keep up to date on the most recent developments in nursing and to be able to manage the demands of nursing practice. Educational program and training courses are two components of staff development. It is recommended that continuous education in nursing is needed to promote development of knowledge, skills and attitudes of nurses and to improve the quality of care given for their patients. Also the formed training courses played an important role in enhancing and updating nurses' knowledge and performance **[5-8]**.

In this study four fifths of nurses had satisfactory level of nurse's knowledge regarding Gastro-intestinal Endoscopy and nursing care for heamatemsis cases. The low level of knowledge in the initial baseline data knowledge assessment for the nurses is reflected on practice of the nurse **[9]**. These results are in agreement with those of Bertleff et al. **[10]** who noted that nurse's

knowledge and practice improved immediately after attending to the training programs, the outcome of these programs was higher among younger ages.

This study revealed that the more than three quarters of nurses had satisfactory level of total knowledge before, during and after gastrointestinal tract endoscope insertion and their knowledge about guideline was satisfactory. This agree with Majeski **[6]** who that state professional endoscopic nurses should performed the nurse observe the level of conscious until the sedation off and observe for the signs and symptoms of risks associated with GI endoscopy including abnormal reaction. These results disagree with Ali **[11]**. In Assuit University in Medical Audit of Upper (GIT) that show that the complications including haematemesis occurs during the procedure due to failure in the management of the upper GIT bleeding. The importance of the provided guidelines for nurses as well as competence of the procedure is required in some situations for the safety of the patient. They consider it as a "necessary evil "as reported by California Department of Health Services (CDHS) **[12]**. Therefore, if they are forced to do it, they need to know how to do it properly without harming themselves by follow the Universal precaution during the procedure. So, healthcare facilities and healthcare providers should establish procedures to ensure that reusable devices are, cleaned, and sterilized according to the manufacturer's instructions **[13]**.

This study revealed that the majority of nurses had satisfactory level of knowledge regarding general precautions in endoscopy unit. Health-care workers in developing countries inconsistently practice universal precautions and are regularly exposed to blood in the course of their work via needle stick injuries, splash incidents, and direct contact Cotton et al. **[14]**, Kennedy et al. **[15]** and Sagoe et al. **[16]** examined occupational exposure to blood and risk of blood-borne virus infection among health-care workers.

The findings of the current study suggest that basic health care workers do not have sufficient knowledge of universal precautions. The majority of respondents (59%) did not answer universal precaution knowledge questions. Only 22% of the workers reported accurate knowledge of universal precautions as an effective barrier between care health workers and patients to prevent the transmission of infections. Hospital based workers are washing their hands before and after attending to each patient. This hand washing practice was similar to that of western countries **[17,18]**. These levels of knowledge were very low compared to other parts of world **[19]**.

This study revealed that the majority of nurses had satisfactory level of knowledge regarding basic steps to clean and disinfect GI Endoscope. Levels of knowledge among the nurses in the present study about (wearing protective clothes, transferring endoscope for cleaning, pre-manual cleaning stage, test leak, manual cleaning stage, rinsing, sterilization and dryness, dangerous of inadequate endoscope disinfection, storage and documentation). California Department of Health Services (CDHS) **[12]** stressed up on that nurses need to know how to do it properly without harming themselves by follow the Universal precaution during the procedure. This also was confirmed by Alfa et al. **[13]**. On the other hand this was not satisfactory for Ramsey and his colleagues **[4]**, who recommended that, continues educational and training guideline program for endoscopies reprocessing will help in effective performance and control infection and they proved this by the significant improvements in the post-guidelines program phase.

This study revealed that the majority of nurses had a positive attitude towards providing care in endoscopy unit. These results are in agreement with those reported in a survey in the UK, which predicted an important albeit restricted role for nursing endoscopy. Clinicians from the UK considered diagnostic gastroscopy and sigmoido-scopy appropriate and diagnostic colonoscopy and therapeutic endoscopies inappropriate for NE. The UK audit however did not specifically investigate the attitude towards screening endoscopies **[20]**.

The majority of nurses in our study had unsatisfactory level of practice before, during and after GI endoscope insertion, infection control, cleaning and manual disinfection of endoscope (wearing protective clothes, transferring endoscope for cleaning, pre-manual cleaning stage, test leak, manual cleaning stage, rinsing, sterilization and dryness, dangerous of inadequate endoscope disinfection, storage and documentation). Therefore, they have to know and follow the Universal precaution during the procedure. It was recommended that, healthcare facilities and healthcare providers should establish procedures and provide training for staff to ensure that reusable

devices are, cleaned, and sterilized according to the manufacturer's instructions **[13]**. Although, El-Shamaa **[21]** in her study, reported that, the majority of nurses have a satisfactory level of knowledge about universal Precautions and infection control policies in the endoscopic unite.

The lack in nurses' knowledge and practice for endoscope's contamination may lead to inadequate reprocessing. Carl, Alvarado and Mark **[22]** reported that the most common factors associated with disease transmission inadequate manual cleaning, inadequate exposure of surfaces to the disinfectant, inadequate rinsing and drying, and use of automated endoscope re-processors. Exogenous infections arise from microorganisms introduced into the patient's body by the flexible endoscope or by the accessories used in the procedure, such infections are preventable with strict adherence to accepted reprocessing guidelines **[23]**. Similar to this result, Weber and Rutala **[24]** reported that, outbreaks associated with flexible endoscopy have most often been associated with breaks in the cleaning and/or disinfection/ sterilization stage of flexible endoscope reprocessing. The currently used reprocessing protocols provide a very narrow margin of safety and any slight deviation from the recommended steps may result in an increased risk of infection transmission by flexible endoscopes **[25]**. In the same consequence, and its relation to test leak, Canadian Standards Association **[26]** reported that, during the manual cleaning process, trained personnel should inspect devices for functionality and damage. It was recommended that, during the endoscopic procedure and while cleaning endoscopes, endoscopy personnel should wear protective attire (including gloves, masks, eye protection, and moisture-resistant gowns or aprons) as needed to protect themselves from exposure to blood and body fluids **[13]**.

As regard, the nursing care for the patient undergoing upper endoscopy(pre, during and after a procedure) our results was in line with Majeski **[6]** who stated that professional endoscopic nurses should observe the level of consciousness until the sedation off and signs and symptoms of risks associated with upper GI endoscopy including abnormal reaction to sedatives, bleeding from biopsy accidental puncture of the upper GI tract swallowing difficulties, throat, chest, and abdominal pain that worsens, vomiting of bloody or passage of dark of stool, fever. However, it was reported that complications occurred in 8% of the studied groups and the type of the complications were (failure of control of upper GIT bleeding 37.5%, syncope in 37.5%, respiratory arrest 12.5% and myocardial infarction in 12.5%). Haematemesis which occurs during the procedure is due to failure in the management of the upper GIT bleeding. Myocardial infarction related to inappropriate selection of the patients and bad preparation before the procedure and respiratory arrest **[11]**.

The present study demonstrated that total nurses' knowledge regarding GI endoscopy is significantly related to their age, training and work duration but it is not related to nurse qualification. There was statistically significant relationship between total nurses' practice regarding dealing with patients with GI Endoscopy, attendant to training and nurses qualification. While only training and qualification showed significant relation with the total nurses' practice regarding dealing with patients with GI Endoscopy, guidelines which improve nurses' knowledge and practice are attributed to the changes in nurses' practice which became adequate and based on satisfactory knowledge. Infection Control and Prevention is a critical part of the orientation continuing education and maintaining consistent excellence in **[27,28]**.

Finally we can conclude that the nurse age, training and working duration could affect the level of nurses' knowledge regarding gastrointestinal endoscopy including; general precautions, basic steps to clean and disinfection in endoscopy unit. The majority of nurses had positive attitude. While training and qualification can affect dealing with the patients in addition to nurses' level of practice before, during and after GI endoscope, discharge instructions and manual disinfecting of endoscopy. So, we recommend adequate education and training of all nurses working with gastrointestinal endoscopy unit, with continuous evaluation of nurses' work practice. Periodic evaluation may indicate interference with training programs should be included both theoretical and practical. Further studies are necessary to identify effects of educational programs on nurses' performance in gastrointestinal endoscopy unit.

**Funding:** None
**Ethical approval**: The study was ethically approved from Zagazig University Hospitals and Faculty of Nursing, Zagazig University
**Conflicts of interest :**There are no conflict of interests

## REFERENCES


1- Gómez M, Llach J. Role of nurses in gastrointestinal endoscopy. *Gastroenterol Hepatol* 2009, 32:44-47.

2- Salvetto M. The nurse's role in endoscopic ultrasonography. *Minerva Med* 2007,98:269-70 (Abstract)

3- Robinson J, Moreau D, McCann J. Hand Book of Medical-Surgical Nursing , 3rd ed.,Pennsylvania: Springhouse 2002, pp. 303-310.

4- Ramsey AH, Oemig TV, Davis JP,Massey JP, Torok TJ. An outbreak of bronchoscopy-related *Mycobacterium tuberculosis* infections due to lack of bronchoscope leak testing.*Chest* 2002, 121:976-81

5- Nelson DB. Recent advances in epidemiology and prevention of Gastrointestinal related infections, *Current Opinions in Infectious Diseases* 2011, 18, 326- 330

6- Majeski J, Lynch W, Durst G. Esophageal perforation during esophagogastroduodenoscopy. *Am J Surg*. 2009 Nov;198(5):e56-7.

7- Petersen BT, Chennat J, Cohen J, Cotton PB, Greenwald DA, Kowalski TE et al. ASGE Quality Assurance in EndoscopyCommittee, Rutala WA; Society for Healthcare Epidemiology of America.Multisociety guideline on reprocessing flexible GI endoscopes: 2011. *Infect Control Hosp Epidemiol.* 2011 Jun;32(6):527-37.

8- Smith, NE , Timby, BK. Introductory medical surgical nursing, Caring for clients with disorders of the upper gastrointestinal tract, 10th ed. Philadelphia, Wolters Kluwer Health, Lippincott Williams & Wilkins 2010, Pp (583- 585).

9-Day T, Wainwright SP, Wilson-Barnett J. An evaluation of a teaching intervention to improve the practice of endotracheal suctioning in intensive care units. *J Clin Nurs*. 2001 Sep;10(5):682-96.

10-Bertleff M, Helm JA, Bemelman WA, Vandeham AC, Vandeharst E , Oei HI. Randomized Clinical Trial of Laparoscopic Versus Open Repair of the Perforated Peptic Ulcer: The LAMA Trial Randomized Clinical Trial of Laparoscopic Versus Open Repair of the Perforated Peptic Ulcer: The LAMA Trial. *World Journal of Surgery* 2009;33 (7): 1368- 1377.

11-Ali R. Hamed Shehata. Thesis; Gastroenterology Department, Assiut university 2013.

12-California Department of Health Services (CDHS). State Health Department Releases Revised Nurse-To-Patient ratios for public comment 2003: Available at:http://www.dhs.ca.gov6/4/2012.10 am.

13-Alfa MJ, Olson N , Degagne P. Automated washing with the Reliance Endoscope Processing System and its equivalence to optimal manual cleaning. *AJIC* 2006, 34:561

14-Cotton PB, Williams CB, Hawes RH, Saunders BP. Practical Gastrointestinal Endoscopy, the Fundamentals, Philadelphia: Lippincott Williams and Wilkins, 2008. Sixth edition, chapter 3, p.2.

15- Kennedy J, Oconnor P and Sadovnick AD. Multiple peptic ulcers influenced By place of residence. *American Journal of Nursing* 2006;93 (9): 162-167.

16- Sagoe-Moses C, Pearson RD, Perry J, Jagger J. Risks to health care workers in developing countries. *N Engl J Med* 2001, 345: 538-541.

17- Stein AD, Makarawo TP, Ahmad MF. A survey of doctors' and nurses knowledge, attitudes and compliance with infection control guidelines in Birmingham teaching hospitals. *J Hosp Infect* 2003, 54: 68-73.

18- Paudyal P, Simkhada P, Bruce J. Infection control knowledge, attitude, and practice among Neplease health care workers. *Am J Infect Control* 2008, 36: 595-597.

19- Reda AA, Vandeweerd JM, Syre TR, Egata G. HIV/AIDS and exposure of healthcare workers to body fluids in Ethiopia: attitudes toward universal precautions. *J Hosp Infect* 2009, 7: 163-169.

20- Pathmakanthan S, Murray I, Smith K, Heeley R, Donnelly M. Nurse Endoscopists in United Kingdom health care: a survey of prevalence, skills and attitudes. *J Adv Nurs* 2001; 36: 705–10.

21- El-Shamaa ET. Developing A Control Action Plan For Infection Prevention At The Endoscopy Unit. *International Journal Of Academic Research*2010, 2(4):

22- Carl J. Alvarado , Mark R. APIC guideline for infection preventionand control Flexible endoscopy. *Am J Infect Control* 2000;28:138-55.

23- Public Health Agency of Canada. Infection Prevention and Control Guideline for Flexible Gastrointestinal Endoscopy and Flexible Bronchoscopy, is available on Internet at the following address: http://www.phac-aspc.gc.ca, 2010.

24- Weber DJ, Rutala WA. Lessons from outbreaks associated with ronchoscopy. *J Hosp Infect* 2001; 22:403-8.

25- Cowen AE. The clinical risks of infection associated with endoscopy. *Can J Gastroenterol* 2001, 15:321-31.

26- Canadian Standards Association. Decontamination of Reusable Medical Devices. Mississauga, Ontario; 2008. Report No.: Z314.8-08

27- Mehta AC, Prakash UBS, Garland R, et al. American College of Chest ysicians and American Association for Bronchology Consensus Statement: Prevention of Flexible Bronchoscopy-Associated Infection. *Chest* 2005; 128:1742-55.

28- Vergis AS, Thomson D , Pieroni P, Dhalla S. Reprocessing Flexible Gastrointestinal Endoscopes After a Period of Disuse: Is It Necessary? .*Endoscopy* 2007; 39:737-9.

**Peer reviewers:Maysaa Saed**, Professor of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt. **Amira Suliman**, 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.

# Spontaneous Cryptococcal Peritonitis Successfully Treated with Fluconazole

**Maysaa Abdallah Saeed[1] and Tarig S.A. Al Khuwaitir[2]**

*[1]Tropical Medicine Department, Faculty of Medicine, Zagazig University, Egypt.*
*[2]Medicine Department, King Saud Medical City, Kingdom of Saudi Arabia*

***Corresponding Author***
*Maysaa Abdallah Saeed*

***Mobile***:*+201275594979*

***E mail:***
*dr.maysaaabdallah@windowslive.com*

***Key words***: *cryptococcal peritonitis; Fluconazole*

## ABSTRACT

Spontaneous cryptococcal peritonitis is an uncommon infection that predominantly encountered in immunocompromised. Its clinical presentation is indistinguishable from spontaneous bacterial peritonitis and often results in fatal outcome. Liver disease is an under recognized predisposition for cryptococcal disease. We report a case of spontaneous cryptococcal peritonitis in HIV-negative, non-diabetic female patient with chronic hepatitis C related cirrhosis. Cryptococcus infection was diagnosed by India ink and culture of ascetic fluid. The patient was successfully treated with fluconazole and she was well at the follow up of 9 months. Early clinical suspicion and initiation of antifungal are of paramount to save the patient's life.

## INTRODUCTION

Spontaneous bacterial peritonitis (SBP) is a common complication in cirrhotic patients with ascites. Clinically it manifests, as fever, abdominal pain and abdominal tenderness. The diagnosis is confirmed by the presence of >250 neutrophils/mm$^3$ in the ascetic fluid and by demonstration of bacteria on Gram stained smear or peritoneal fluid culture **[1]**. Cryptococcal peritonitis is an uncommon infection and it is rarely reported. Delayed diagnosis of cryptococcal peritonitis due to its rarity and the fact that its presentation is indistinguishable from SBP often results in fatal outcome **[2]**. We present a case of spontaneous cryptococcal peritonitis in a patient with decompensated cirrhosis who successfully treated with fluconazole.

## Case Report

A 55-years old HIV-negative, non-diabetic, Egyptian female was admitted to King Saud Medical City with complaints of abdominal pain and increasing ascites for two weeks. She was diagnosed as chronic hepatitis C- related cirrhosis. On admission she was afebrile, conscious, had jaundice and bilateral lower limbs edema. Tense ascites and generalized abdominal tenderness were detected on abdominal examination. Her laboratory parameters revealed: total leukocytic count: 6.7x10$^3$ cells/mm$^3$ with 75% neutrophils, hemoglobin: 9 g/dl, platelets: 90000/mm$^3$, INR: 1.3, creatinine:1 mg/dl, total bilirubin: 5.9 mg/dl (direct 3.6 mg/dl), ALT:65 U/L, ALP: 90 U/L and serum albumin 2 g/ dl. Abdominal ultrasound revealed cirrhotic liver with ascites. Abdominal paracentesis revealed slightly turbid ascetic fluid, cell count of 600/mm$^3$ with predominant neutrophils (60%), albumin level of 0.8 g/L, glucose: 55mg/dl and bacterial culture did not show any growth. Her chest X-ray did not show any infiltrate. Patient was treated empirically with cefotaxime 2 grams every 8 hours. On hospital day three she developed fever so the patient was referred to Infectious Diseases Unit where paracentesis was requested in addition to India ink. Repeated paracentesis revealed similar cytological findings without any decrease in ascetic fluid cell count. India ink preparation showed encapsulated budding yeast cells with morphology suggestive of *Cryptococcus neoformans* (*C. neoformans*). Intravenous fluconazole started immediately in a dose of 200 mg once daily. Ascetic fluid culture taken from the first day of admission grew *C. neoformans* after 9 days incubation. Blood and urine cultures did not reveal growth of any organisms. The patient became afebrile

3 days after starting fluconazole and showed gradual clinical improvement. Ascetic fluid culture was sterile 3 weeks later. The patient was discharged on oral fluconazole 100 mg once daily and it was continued for 6 months, with careful monitoring of liver function tests. The patient was well at the follow up of 9 months.

## DISCUSSION

*Cryptococcus neoformans* causes significant infection in immunocompromised individuals with HIV infection, organ transplantation, malignancy and prolonged glucocorticoid therapy **[3,4,5]**. The respiratory tract is considered to be the usual portal of entry. However, the GIT has been proposed as a potential site either following ingestion or possible direct inoculation of *C. neoformans* into the blood stream following upper GI bleeding or overgrowth of fungus after antibiotic use **[3,6]**. Our patient, had history of previous antibiotic exposure and upper GI bleeding 2 weeks before development of cryptococcal peritonitis. This situation raise the possibility of translocation of the Cryptococci from GI into the blood stream.

Review of previous reports reveals a striking association between chronic liver disease and cryptococcal peritonitis **[7]**. Patient with cirrhosis are immunocompromised for several reasons. First, there is a relative complement deficiency leading to reduced opsonic recognition of fungi, as well as deterioration of humoral immunity **[2]**. In addition, the function of polymorphonuclear leukocyt (PMLs) is reduced **[8,9]**. The clinical presentation of spontaneous fungal peritonitis (SFP) may be similar to that of spontaneous bacterial peritonitis (SBP) but at times it may be occult. The typical peritoneal cell count is polymorphonuclear predominant ( greater than or equal to 250 cell/$mm^3$) but can be mononuclear with lymphocytic predominance **[10]**. Our patient had decompensated cirrhosis, presented with clinical picture similar to that of SBP and her ascetic fluid analysis revealed 360 PMLs/$mm^3$ which did not show any reduction after 2 days of cefotaxime. The development of fever and the persistence of high PMLs in the ascetic fluid after 2 days of starting antibiotic raise the possibility of SFP in our patient so we asked for India ink preparation after which the diagnosis was confirmed.

Clinician should consider a diagnostic work up and empirical treatment for cryptococcal peritonitis if cell counts in ascetic fluid revealed an elevated leukocyte count but bacterial culture remains negative after 48 hours. Two recent reports in the literatures suggest performing cytology and India ink preparation on ascetic fluid and testing cryptococcal antigen, in the serum and ascetic fluid, may assist in the diagnostic evaluation of patients with suspected cryptococcal peritonitis **[11,12,13]**.

There are no studies evaluating treatment of cryptococcal infection involving sites other than lungs and central nervous system (CNS). In general infection at a single site in the absence of CNS disease, fungemia or risk factors for immunosuppression may be managed with fluconazole for 6-12 months **[14]**. Our patient had no fungemia and was successfully treated with fluconazole for 6 months. Her ascetic fluid fungal culture was sterile 3 weeks after the index one.

The mortality rate in cirrhotic patients developing cryptococcal peritonitis is high, about 70% to 80% of the patients died **[3,15]**. The very poor outcome of these patients is likely to be associated with the underlying advanced liver dysfunction, systemic dissemination and delay in antifungal treatment **[10]**. In our patient the diagnosis of cryptococcal peritonitis was suspected early and consequently fluconazole started at appropriate time so she was saved. We concluded that *Cryptococcus neoformans* should be considered as a causative organism for spontaneous peritonitis in cirrhotic patients. Early clinical suspicion and timely initiation of antifungal are of paramount.

## REFERENCES


1- Such J, Runyon BA. Spontaneous bacterial peritonitis. *Clin Infec Dis* 1998; 27: 669-674.

2- Saif MW, Raj M. Cryptococcal peritonitis complicating hepatic failure: Case report and review of literature. *J of Applied Research* 2006; 6 (1): 43-50.

3- Mabee CL, Mabee SW, Kirkpatrick RB, Koletar SL. Cirrhosis: a risk factor for cryptococcal peritonitis. *Am J Gastroenterol* 1995; 90:2042- 2045.

4- Srikanta D, Santiago-Tirado FH, Doering TL. Cryptococcus neoformans: historical curiosity to modern pathogen. *Yeast* 2014; 31: 47-60.

5- Negroni R. Cryptococcosis. *Clin Dermatol*.2012; 30: 599-609.

6- Poblete RB. Cryptococcal peritonitis. *Am J Med* 1987; 82: 665- 667.

7- Yinnon AM, Solages A, Treanor JJ. Cryptococcal peritonitis: report of a case developing during continuous ambulatory peritoneal dialysis and review of the literature. *Clin Infec Dis* 1993; 17: 736- 741.

8- Rimola A, Soto R, Arroyo C, Plera C, Rodes J. Reticuloendothelial system phagocytic activity in cirrhosis and its relation to bacterial infections and prognosis. *Hepatology* 1984; 4: 53-58.

9- Tritto G, Frances R, Shah N, Stadlbauer V, Davis N, Mookeriee RP. Plasma from stable patient with cirrhosis transmit sever neutrophil phagocytic dysfunction which is independent of the presence of bacterial DNA and associated with increased expression of TLR-4. *Hepatology* 2009; 50: 311A.

10- Park WB, Choe YJ, Lee KD, Lee SC, Kim HB, Kim NJ, et al. Spontaneous cryptococcal peritonitis in patient with liver cirrhosis. *Am J Med* 2006; 119: 169-171.

11- Albert-Braun S, Venema F, Bausch J, Hunfeld KP, Schafer V. Cryptococcus neoformans peritonitis in a patient with alcoholic cirrhosis: case report and review of the literature. *Infection* 2005; 33: 282-288.

12- El-Kersh K, Rawasia WF, Chadda U, Guradiola J. Rarity revisited cryptococcal peritonitis. *BMJ* Case Rep published on line. 2014.

13- Jean SS, Fang CT, Shau WY, Chen YC, Shang SC, Hsueh PR, et al. Cryptococcaemia: clinical features and prognostic factors. *QJM* 2002; 95: 511-518.

14- Perfect JR, Dismukes WE, Dromer F. Clinical practice guidline for the management of crypto-coccal diseases: 2010 updated by the infectious diseases society of America.*Clin Infect Dis* 2010; 50: 291.

15- Singh N, Husain S, De Vera M, Gayowski T, Cacciarelli TV. Cryptococcus neoformans infection in patient with cirrhosis,including liver transplant candidates. *Medicine* (Baltimore) 2004; 83:188-192.