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# CONTENTS

# Nutritional Status and Informational Needs for Patients with Liver Cirrhosis

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*liver cirrhosis, Malnutrition, informational needs*

**Background and study aim:** Malnutrition is as an important complication of liver cirrhosis with prognostic implications; a marked knowledge gap exists concerning the information needs of liver cirrhosis patients. The aim of this study is to assess nutritional status and informational needs for patients with liver cirrhosis.

**Subjects and Methods:** A descriptive exploratory design was used in this study. The present study conducted in tropical medicine and gastroenterology units at Zagazig University Hospitals, A purposive sample of 115 patients, The study lasted from the beginning of October 2016 to the end of May 2017 .Three tools were used for collection of data, first tool was a structured interview questionnaire consisted of personal characteristics of patients, second tool was nutritional assessment form, third tool was informational needs questionnaire.

**Results:** The study findings revealed that more two third of studied patients were in the age group of less than 60 years with mean age 53.4±9.3 years, the majority of studied patients were in malnutrition (73.9%). By Subjective Global Assessment (SGA), there are strong correlation between SGA and Child Pugh score, the most important informational needs among the studied patients were medical domains 84.4%. In contrast, psychological domains achieved the least important 27.0%.

**Conclusion:** It can be concluded that patients with liver cirrhosis are suffering from malnutrition and nutritional deficiencies, and also patient with liver cirrhosis had different levels of informational needs. The most prioritized informational needs for patients with liver cirrhosis was Medical domain whereas the least priority was given to the Psychological domain. It also shows that information needs differ based on some socio-demographic and clinical characteristics and physical condition.

## INTRODUCTION

Hepatitis C virus is one of the chief causes of chronic liver disease. Hepatitis C related liver disease encompasses a wide spectrum ranging from chronic hepatitis C to compensated cirrhosis and eventually to decompensated cirrhosis and hepatocellular carcinoma **[1]**. Cirrhosis is the final common pathway for the majority of liver diseases, and is a complex chronic condition that causes population mortality rates of approximately 5–10 per 100,000 person-years worldwide **[2]**. Nutrition is an integral part of health maintenance. Progressive deterioration of nutritional status has been associated with poor outcome in cirrhotic patients **[3]**. Protein-energy malnutrition (PEM) is highly prevalent in patients with liver disease and leads to serious repercussions on the general state, having a direct impact on cirrhotic patient prognosis, deteriorating liver function, adversely affecting the clinical evolution **[4]**. The prevalence of malnutrition in decompensated cirrhosis ranges from 60%-100%, while 20%-30% of patients with compensated cirrhosis are also malnourished **[5]**. The pathogenesis of malnutrition in chronic liver diseases is multifactorial and includes a reduction

in nutrient and calorie intake because of anorexia and dietary restrictions, impaired intestinal absorption, abnormalities of carbohydrate, lipid and protein metabolism and increased pro-inflammatory cytokine levels resulting in a hypermetabolic state that may occur in advanced liver disease stages **[6]**. Therefore for nutritional management of liver cirrhosis patients it is important to precisely assess the patient's nutritional intake and to establish effective nutritional education programs **[7]**. The term supportive care needs encompasses the physical, informational, emotional, practical, social and spiritual needs of an individual with chronic disease. **[8]**. Information need' is defined as a deficiency of information or skill related to a domain of life that is relevant to the patient. Usually patients require information concerning their disease and related care, in addition to side effects, complications, and health-related problems. It is also important to obtain information concerning additional care, daily activities, practical solutions, and financial issues. **[9]**. Inadequate self-management skills and knowledge can lead to serious and detrimental changes in quality of life, as well as increased anxiety, distress, and difficulty coping **[10]**. Patient and family teaching is an important nursing role that may make the difference in the ability of the patient and family to adapt to chronic conditions .Well-informed, educated patients are more likely than uninformed patients to be concerned about their health and to do what is necessary to maintain it **[11]**.

The aim of the study was to assess nutritional status and informational needs for patients with liver cirrhosis.

## SUBJECTS AND METHODS

A descriptive exploratory design was utilized in the study. Study was conducted in tropical medicine and gastroenterology units at Zagazig University Hospitals. Field work of this study was executed in 6 months, starting from October 2016 to May 2017.

**Subjects:**
A purposive sample of 115 adult patients with liver cirrhosis, they were selected randomly.

**Tools for data collection:** three tools were used for data collection**:**
**Tool I:** A Structured interview questionnaire tool for patients was designed by the researcher after revising of related literature and opinions of expertise for content of validity and included the following three parts:

- **Part 1:** Demographic characteristics of patients e.g. (age, sex, marital status, occupation, level of education ….etc).
- **Part 2:** Nutritional profile which contain questions about follow special diet for liver cirrhosis, follow food restriction …etc) **[3]**.
- **Part 3:** Child Pugh score which consists of five items: total bilirubin, serum albumin, international normalized ratio (INR), ascites and hepatic encephalopathy **[12]**.

**Tool II:** Nutritional assessment form for patients was designed by the researcher after revising of related literature and opinions of expertise for content of validity and included the following five parts.
**Part 1:** Subjective Global Assessment **[13]**.
**Part 2:** Risk factor affecting nutritional status.
**Part 3:** anthropometric measurement.
**Part4:** Physical assessment suggestive of malnutrition **[14]**.
**Part 5:** Biochemical measurements.
**Tool III:** Informational Needs Questionnaire **[15]**.

**Content validity and Reliability:**
Content validity was used for the modified tools and the designed booklet to determine whether the tools covered the aim or not. It developed by a jury of 5 experts ,four professors from faculty of Nursing, Zagazig University and one lecturer of medical department from the Faculty of Medicine, Zagazig University, And one lecturer of Biochemistry department from the faculty of medicine. Reliability was done by using Cronbach test **[16].** It was used to examine whether the subjective Global Assessment, Informational Needs Questionnaire had internal consistency or not. The test was done and the agreement percentage was 89%.

**Administrative and Ethical considerations:**
The study was ethically approved from the dean of the faculty of Nursing, the manager of Zagazig University Hospitals, the head of tropical medicine and Gastroenterology department, ethics committee at the faculty of nursing and from Ethical committee of faculty of medicine.

**Statistical Design:**
All collected data were organized, categorized, tabulated, entered, and analyzed by using SPSS (Statistical Package for Social Sciences); a software program version 14, which was applied

to frequency tables and statistical significance. The statistical significance and associations were assessed using, descriptive statistics in the form of frequencies and percentages for qualitative variables, and means and standard deviations and medians and interquartile ranges for quantitative variables, a chi-square test(X2). Spearman rank correlation.

## RESULTS

The first part of our results was the Demographic characteristics and disease characteristic for patients with liver cirrhosis in the study including; gender, age, residence, marital Status, education, occupation and income (Table 1).

The second part of our results was concerned with disease characteristic; it demonstrated that The main cause of liver cirrhosis was viral hepatitis with 102 cases, studied patients were admitted to the hospital related to ascites, hematemesis and black stools (melena) (40.0 %, 37.4%, 20.9%) respectively). According to Child-Pugh, the highest percentage (40.0%) of the studied patients had Child class B while (20.9%) of them were Child class A (Table 2).

The third part of our results was concerned with nutritional status of studied patient by using different method. According to SGA, malnutrition was present in 73.9% of the patients, and of these 49 patients (42.6%) were moderately and 36 (31.3%) were severely malnourished, the table showed 47.8% of studied patient were under weight, according to TSF and MAC it was found 69.6% and 65.2% of studied patient suffering from malnutrition respectively (Fig. 1, Table 3).

The fourth part of our results was concerned with risk factor affecting on nutritional status, it revealed that 84.3% of the enrolled patients confirmed they had dryness of mouth, 69.5% had decreased of appetite, 60.0% inability to prepare meals (Table 4).

The fifth part of our results was concerned with laboratory assessment of the studied patients and revealed that the percentage below and above cut

off more frequent in Hb, PT, Albumin, Na and Ca presenting in 87.0%, 99.1%, 84.3%, 53.0%, 80.9% ( Table 5).

The sixth part of our results was concerned with informational needs for patients; the result explains that the most important informational needs among the studied patients were medical domains 84.4%. In contrast, psychological domains achieved the least important 27.0%. Finally, the total informational needs arranged from moderated to high needs 33.0%, 67.0 % respectively (Table 6).

The seventh part of our results demonstrated that there was statistical significant relation between subjective Global assessment score and patients demographic characteristics, only among their age and income in study (p-value =0.01), p-value =0.003) .There was statistical significant relation between duration of the disease of the patients and their SGA score P-value = 0.0001, there was highly statistically significant relation between SGA score and Child Pugh score p-value = 0.00, there was statistically significant relations between informational needs and patient's age (P- value <0.001), marital status ( P- value = 0.009), and their job (P- value = 0.045). It is noticed that the informational needs was higher among those in younger age group (81.3%), married (71.6%) and employees (93.8). It shows statistically significant negative correlations between total information needs score and number of abnormal signs. On the other hand, number of abnormal signs had statistically significant positive correlation with number of abnormal lab results, statistically significant negative correlation between total informational needs score and patient's age r= 0.520, and Child Pugh r= 0.189. There are also statistically significant positive correlation between SGA and age r= 0.542, duration of illness r= 0.478, Child Pugh r= 0.589, conversely there were significant negative correlation between BMI, weight deficit and SGA score (Tables 7, 8, 9, 10, 11, 12).

In multivariate analysis, indicated that the level of education, female genders were the statistically significant independent positive predictors of higher informational needs score with Standardized Coefficients (0.24, 0.25) respectively. Conversely, patient's age and number of abnormal signs were negative predictors with Standardized Coefficients (-0.41. -0.25) respectively, the model explains 41% of the variation in the information needs score. Age, Child Pugh and duration of illness were the statistically significant independent positive predictors of higher SGA score with Standardized Coefficients (0.22, 0.34, 0.21) respectively. Conversely BMI was statistically significant independent negative predictors of higher SGA score with Standardized Coefficients (-0.25), the model explains 57% of the variation in the SGA scores (Tables 13, 14).

**Table (1):** Personal characteristics of patients in the study sample (n=115)

| Personal characteristic | Frequency | Percent |
|---|---|---|
| **Age:** | | |
| <60 | 80 | 69.6 |
| 60+ | 35 | 30.4 |
| Range | 26.0-65.0 | |
| Mean±SD | 53.4±9.3 | |
| Median | 55.0 | |
| **Gender:** | | |
| Male | 59 | 51.3 |
| Female | 56 | 48.7 |
| **Marital status:** | | |
| Unmarried (single/divorced/widow) | 13 | 11.3 |
| Married | 102 | 88.7 |
| **Job:** | | |
| Employee | 38 | 33.0 |
| Unemployed/housewife | 77 | 67.0 |
| **Education:** | | |
| Uneducated | 71 | 61.7 |
| Educated | 44 | 38.3 |
| **Residence:** | | |
| Rural | 93 | 80.9 |
| Urban | 22 | 19.1 |
| **Income:** | | |
| Sufficient | 40 | 34.8 |
| Insufficient | 75 | 65.2 |

**Table (2):** Disease characteristics of patients in the study sample (n=115)

| | Frequency | Percent |
|---|---|---|
| **Duration of illness (years):** | | |
| <5 | 68 | 59.1 |
| 5+ | 47 | 40.9 |
| Range | 0.0-20.0 | |
| Mean±SD | 4.8±4.0 | |
| Median | 4.0 | |
| **Cause of liver cirrhosis:** | | |
| Viral | 102 | 88.7 |
| Viral + schitosomiasis | 11 | 9.6 |
| Autoimmune | 2 | 1.7 |
| **Child-Pugh:** | | |
| A | 26 | 20.9 |
| B | 46 | 40.0 |
| C | 43 | 39.1 |
| **Hospital admission for:** | | |
| Hematemesis | 43 | 37.4 |
| Ascites | 46 | 40.0 |
| Black stools | 24 | 20.9 |
| Hepatic coma | 16 | 13.9 |
| General symptoms | 12 | 10.4 |

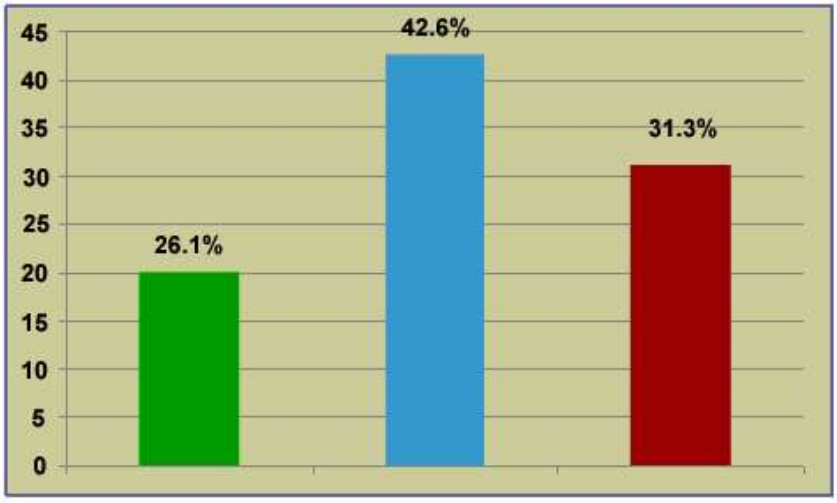


**Figure (1):** Nutritional status among the studied patients according to Subjective Global Assessment (SGA) score, (n=115)

**Table (3):** Anthropometric measurements of patients in the study sample (n=115)

| Anthropometric measurement | Frequency | Percent |
|---|---|---|
| **BMI:** | | |
| <25 | 55 | 47.8 |
| 25- | 37 | 32.2 |
| 30+ | 23 | 20.0 |
| Range | 18.0-76.0 | |
| Mean±SD | 26.4±6.5 | |
| Median | 25.0 | |
| **Malnourished** | | |
| Triceps skinfold | 80 | 69.6 |
| Mid-arm circumference | 75 | 65.2 |
| Calf circumference | 65 | 56.5 |
| Wrist circumference | 45 | 39.1 |

**Table (4):** Risk factors affecting the nutritional status among the studied patients (n=115)

| Risk factors | Frequency | Percent |
|---|---|---|
| **Risk factors**: | | |
| Dryness of mouth | 97 | 84.3 |
| Loss of appetite | 80 | 69.5 |
| Inability to prepare meals | 69 | 60.0 |
| Taste change | 66 | 57.4 |
| Mastication problems | 58 | 50.4 |
| Dyspepsia | 49 | 42.6 |
| Loneliness/ depression | 28 | 24.3 |
| Allergic | 6 | 5.2 |
| **No. of risk factor:** | | |
| Range | 1-5 | |
| Mean±SD | 1.6±1.2 | |
| median | 1.0 | |

**Table (5):** Laboratory investigation among the studied patients (n=115)

| laboratory test | Mean(SD) | below or above cut off % | Percent |
|---|---|---|---|
| SGOT | 116.90±( 99.76) | A | 50.4 |
| SGPT | 68.40±(93.98) | A | 35.7 |
| Albumin | 2.79±( 0.72) | B | 84.3 |
| alkaline phosphate | 105.6± (46.1) | A | 33.9 |
| bilirubin(total) | 3.52± (2.97) | A | 60.0 |
| bilirubin(direct) | 1.82± (1.34) | A | 73.0 |
| prothrombin time | 18.98±( 3.49) | A | 99.1 |
| Hemoglobin | 10.21±( 2.50) | B | 87,0 |
| Hematocrit | 31.75± (7.95) | B | 89.6 |
| Sodium | 132.65±( 7.36) | B | 53.0 |
| Potassium | 3.73± (0.94) | N | 60.0 |
| Calcium | 7.62± (1.22) | B | 80.9 |
| Cholesterol | 106.0 ± (36.7) | B | 67.8 |
| Phosphate | 4.1± (1.2) | N | 70.4 |
| Wbcs | 7.31± (3.19) | N | 72.2 |
| **No. of lab anomalies:**<br>range<br>mean±sd<br>median | 1-13<br>8.8±2.4<br>9.0 | | |

**Table (6):** information needs among the studied patients (n=115)

| Information needs | Frequency | Percent |
|---|---|---|
| **Medical domain :** | | |
| Low | 2 | 1.7 |
| Moderate | 16 | 13.9 |
| High | 97 | 84.4 |
| **Treatment:** | | |
| Low | 8 | 7.0 |
| Moderate | 21 | 18.3 |
| High | 86 | 74.7 |
| **Investigations:** | | |
| Low | 12 | 10.4 |
| Moderate | 46 | 40.0 |
| High | 57 | 49.6 |
| **Physical domain:** | | |
| Low | 4 | 3.5 |
| Moderate | 44 | 38.3 |
| High | 67 | 58.3 |
| **Psychological domain :** | | |
| Low | 49 | 42.6 |
| Moderate | 35 | 30.4 |
| High | 31 | 27.0 |

**Table (7):** Relation between SGA score among the studied patients and their personal characteristics

| Personal characteristics | Subjective Global assessment | | | | | | $X^2$ | p-value |
|---|---|---|---|---|---|---|---|---|
| | Good nutrition | | Moderated malnutrition | | Severe malnutrition | | | |
| | No | % | No | % | No | % | | |
| **Age:** | | | | | | | | |
| <60 | 26 | 32.5 | 35 | 43.7 | 19 | 23.8 | | |
| 60+ | 4 | 11.4 | 14 | 40.0 | 17 | 48.6 | 9.01 | 0.01* |
| **Gender:** | | | | | | | | |
| Male | 15 | 25.4 | 26 | 44.1 | 18 | 30.5 | 0.1 | 0.94 |
| Female | 15 | 26.8 | 23 | 41.1 | 18 | 32.1 | | |
| **Marital status:** | | | | | | | | |
| Unmarried | 3 | 23.0 | 6 | 46.2 | 4 | 30.8 | 0.09 | 0.35 |
| Married | 27 | 26.5 | 43 | 42.2 | 32 | 31.3 | | |
| **Job:** | | | | | | | | |
| Worker | 15 | 39.5 | 15 | 39.5 | 8 | 21 | 5.9 | 0.061 |
| Unemployed/housewife | 15 | 19.5 | 34 | 44.1 | 28 | 36.4 | | |
| **Education:** | | | | | | | | |
| Illiterate | 21 | 29.6 | 29 | 40.8 | 21 | 29.6 | | |
| Basic | 5 | 21.8 | 11 | 47.8 | 7 | 30.4 | 1.47 | 0.83 |
| Secondary | 4 | 19 | 9 | 42.9 | 8 | 38.1 | | |
| **Residence:** | | | | | | | | |
| Rural | 22 | 23.7 | 39 | 41.9 | 32 | 34.4 | 2.65 | 0.26 |
| Urban | 8 | 36.3 | 10 | 45.5 | 4 | 18.2 | | |
| **Income:** | | | | | | | | |
| Sufficient | 10 | 25 | 18 | 45 | 12 | 30 | | |
| Insufficient | 20 | 26.7 | 31 | 41.3 | 24 | 32 | 8.60 | 0.003* |

**(*) statistically significant at p<0.05** **(**) statistically significant at p<0.01**

**Table (8):** Relation between SGA score among the studied patients and their disease characteristics

| Disease characteristics | Subjective Global assessment | | | | | | $X^2$ test | p-value |
|---|---|---|---|---|---|---|---|---|
| | Good nutrition | | Moderated malnutrition | | Severe malnutrition | | | |
| | No | % | No | % | No | % | | |
| **Duration of illness (years):** | | | | | | | | |
| <5 | 29 | 42.6 | 25 | 36.8 | 14 | 20.6 | | |
| 5+ | 1 | 2.1 | 24 | 51.1 | 22 | 46.8 | 17.7 | 0.0001** |
| **Child-Pugh:** | | | | | | | | |
| A | 19 | 73.1 | 3 | 11.5 | 4 | 15.4 | | |
| B | 5 | 10.9 | 29 | 63.0 | 12 | 26.1 | Fisher | 0.00** |
| C | 6 | 14 | 17 | 39.5 | 20 | 46.5 | | |

**(*) statistically significant at p<0.05** **(**) statistically significant at p<0.01**

**Table (9):** Relations between patient's information needs and their personal characteristics

| Personal characteristic | Needs | | | | $X^2$ test | p-value |
|---|---|---|---|---|---|---|
| | Moderate | | High | | | |
| | No. | % | No. | % | | |
| **Age:** | | | | | | |
| <60 | 15 | 18.8 | 65 | 81.3 | | |
| 60+ | 23 | 65.7 | 12 | 34.3 | 24.27 | <0.001* |
| **Gender:** | | | | | | |
| Male | 23 | 39.0 | 36 | 61.0 | | |
| Female | 15 | 26.8 | 41 | 73.2 | 1.93 | 0.16 |
| **Marital status:** | | | | | | |
| Unmarried (single/divorced/widow) | 9 | 69.2 | 4 | 30.8 | | |
| Married | 29 | 28.4 | 73 | 71.6 | Fisher | 0.009* |
| **Job:** | | | | | | |
| Employee | 1 | 6.3 | 15 | 93.8 | | |
| Worker | 9 | 40.9 | 13 | 59.1 | 6.19 | 0.045* |
| Unemployed/housewife | 28 | 36.4 | 49 | 63.6 | | |
| **Education:** | | | | | | |
| Illiterate | 28 | 39.4 | 43 | 60.6 | | |
| Basic | 7 | 30.4 | 16 | 69.6 | 4.72 | 0.09 |
| Secondary | 3 | 14.3 | 18 | 85.7 | | |
| **Residence:** | | | | | | |
| Rural | 32 | 34.4 | 61 | 65.6 | | |
| Urban | 6 | 27.3 | 16 | 72.7 | 0.41 | 0.52 |
| **Income:** | | | | | | |
| Sufficient | 11 | 27.5 | 29 | 72.5 | | |
| Insufficient | 27 | 36.0 | 48 | 64.0 | 0.85 | 0.36 |

**(*) statistically significant at p<0.05** **(**) statistically significant at p<0.01**

**Table (10):** Relations between patient's information needs and their disease characteristics and BMI

| Patient's characteristics | Needs | | | | $X^2$ test | p-value |
|---|---|---|---|---|---|---|
| | Moderate | | High | | | |
| | No. | % | No. | % | | |
| **Duration of illness (years):** | | | | | | |
| <5 | 21 | 30.9 | 47 | 69.1 | | |
| 5+ | 17 | 36.2 | 30 | 63.8 | 0.35 | 0.55 |
| **Child-Pugh:** | | | | | | |
| A | 3 | 11.5 | 23 | 88.5 | | |
| B | 24 | 51.2 | 22 | 47.8 | 1.22 | 0.54 |
| C | 11 | 25.6 | 32 | 74.4 | | |
| **BMI:** | | | | | | |
| Under weight | 22 | 40.0 | 33 | 60.0 | | |
| Over weight | 11 | 29.7 | 26 | 70.3 | 2.72 | 0.26 |
| Obese | 5 | 21. | 18 | 78.3 | | |

**(*) statistically significant at p<0.05** **(**) statistically significant at p<0.01**

**Table (11):** Correlation matrix of needs score, number of abnormal signs and abnormal lab findings

| Item | Spearman's rank correlation coefficient | | |
|---|---|---|---|
| | Needs score | No. of abnormal signs | No. of abnormal lab |
| Needs score | | | |
| No. of abnormal signs | -.366** | | |
| No. of abnormal lab | -0.11 | .198* | |

**(*) statistically significant at p<0.05** **(**) statistically significant at p<0.01**

**Table (12):** Correlation matrix of needs score, SGA score, number of abnormal signs and abnormal lab findings and patient's characteristics

| Item | Spearman's rank correlation coefficient | | | |
|---|---|---|---|---|
| | Needs score | SGA score | No. of abnormal signs | No. of abnormal lab |
| Age | -.520** | 0.542** | .284** | 0.08 |
| Education | .304** | 0.45 | -.339** | 0.00 |
| Duration of illness | -0.18 | 0.478** | .263** | 0.03 |
| Child-Pugh | -.189* | 0.589** | .313** | .437** |
| Weight deficit | 0.15 | -0.421** | 0.02 | 0.03 |
| BMI | 0.14 | -0.342** | 0.02 | 0.03 |

**(*) statistically significant at p<0.05** **(**) statistically significant at p<0.01**

**Table (13):** Best fitting multiple linear regression models for the Information Needs Score

| Item | Unstandardized Coefficients | | Standardized Coefficients | t-test | p-value | 95% Confidence Interval for B | |
|---|---|---|---|---|---|---|---|
| | B | Std. Error | | | | Lower | Upper |
| Constant | 90.83 | 6.66 | | 13.636 | <0.001 | 77.63 | 104.03 |
| Age | -0.44 | 0.08 | -0.41 | -5.219 | <0.001 | -0.60 | -0.27 |
| Female gender | 5.06 | 1.64 | 0.25 | 3.087 | 0.003 | 1.81 | 8.31 |
| Education | 3.00 | 1.10 | 0.24 | 2.730 | 0.007 | 0.82 | 5.18 |
| No. of abnormal signs | -1.56 | 0.50 | -0.25 | -3.113 | 0.002 | -2.56 | -0.57 |

**Table 14:** Best fitting multiple linear regression models for the Subjective Global Assessment score

| Item | Unstandardized Coefficients | | Standardized Coefficients | t-test | p-value | 95% Confidence Interval for B | |
|---|---|---|---|---|---|---|---|
| | B | Std. Error | | | | Lower | Upper |
| Constant | 76.43 | 7.56 | | 8.355 | <0.001 | 66.98 | 99.89 |
| Age | 6.25 | 3.35 | 0.22 | 2.727 | 0.007 | 2.55 | 15.96 |
| Child pugh | 1.25 | 0.27 | 0.34 | 4.334 | <0.001 | 0.69 | 1.83 |
| BMI | -9.81 | 3.09 | -0.25 | -3.198 | 0.006 | -16.80 | -3.74 |
| Duration of illness | 4.18 | 1.51 | 0.21 | 3.756 | 0.001 | 0.65 | 1.87 |

## DISCUSSION

Liver cirrhosis is the terminal stage in the natural history of chronic liver diseases **[17].** Malnutrition is very common in liver disease and gets worse with the severity of the underlying liver problems **[18].** Malnutrition is defined as clinical and biochemical alteration due to a primary or secondary deficit of nutrients in the daily food intake of a person and it has a high impact on quality of life and on morbidity and mortality **[19].** Complete and reliable information is important to them both during and after treatment. It assists patients in making treatment decisions, managing immediate effects of treatment, and reducing feelings of vulnerability. It can also increase health competence and give patients a sense of control over the illness **[20].**

Discussion of the results will cover these areas in the following sequence; demographic characteristics and diseases characteristic of adult patients with liver cirrhosis under the study, nutritional status among studied patients by using different methods, informational needs of patients with liver cirrhosis and relation and correlation between different variables.

Demographic characteristics and disease characteristic for patients with liver cirrhosis in the study including; gender, age, residence, marital status, education, occupation and income were matched and this help to control other variables that may affect the nutritional status and informational needs for patients with liver cirrhosis in the study**,** The present study classified Child Pugh into three groups Child A, B, C: in the present study indicated that Child P values were more than Child C values (40%, 39.1%) respectively. In the present study, we evaluated the nutritional status of the cirrhotic by different methods and it was observed that subjective global assessment (SGA) diagnosed 73.9 % of patients as malnutrition. The current study result was congruent with the finding of an Egyptian study conducted in Assiut by Khalil **[21]** ,Romeiro and Augusti **[22],** Bakshi and Singh, **[3],** Vieira **[23]**, Salah **[24]**. Campillo **[25]** proposed different BMI cut-off values for patients with liver cirrhosis depending on the presence and severity of ascites. The present study revealed that near of half of studied patients had BMI under 25 kg/m2, this study in line with Bakshi and Singh**[3]** in India that found more than one third of studied sample (37.2) suffering from malnutrition by BMI.

Among the anthropometric methods used, %TSF was the one that most frequently diagnosed malnutrition (69.9%). Although it is theoretically possible that the presence of swelling could hide a depletion in adipose tissue and that the frequency of diagnosis of malnutrition could be reduced by the % TSF method, it has been reported that in patients with chronic liver disease, the upper limbs are not the preferential place of swelling , similar finding was reported by Vieira **[23]** and Monsef **[26].** The present study revealed that; the most common risk factors of malnutrition are dry mouth, loss of appetite, taste alteration, inability to prepare meals, This results partially agree with finding of, Khalil **[21]** and Monsef **[26]** who found that the majority of studied patients had loss of appetite, dryness of mouth , and reported that; multiple factors which are common to the underlying disease directly contribute to malnutrition, among them.

Regarding laboratory investigation: Serum albumin concentration is the most frequently used laboratory measure of nutritional status, the present study revealed that more than three quarter of studied patient have reduction of serum albumin with mean 2.79± 0.72 on the same line Figueiredo **[27]** in Brazil who found decreased in serum albumin with mean 2.7 ± 0.4 Also Vieira **[23]** who found that Serum albumin was reduced in more than half of studied patient and report that The reduction of serum albumin levels in HC patients, principally among those with moderate or severe hepatic insufficiency, could be associated with either malnutrition, due to reduction in food intake and to the worsening metabolism of nutrients, or with the hepatic dysfunction itself which compromises albumin .

Regarding Hemoglobin the present study revealed that the mean value of hemoglobin was decreased 10.21±2.5 on the same line Monsef **[26]** and Dataller and Gines **[28]** who pointed out that anemia is a common manifestation of liver cirrhosis; it may be due to gastrointestinal bleeding, likewise Merli **[29]** who found that the mean value of hemoglobin was (12.9 ± 1.8). This result may be due to difference in severity of diseases in studied patient, regarding informational needs .The present study findings revealed that patients placed the greatest importance on needing information in the medical domain. This result is similar to other studies that have examined informational needs of patients with liver cirrhosis as Ko **[30]**, Volk **[3]**, Ng **[31]**, Burnham **[32]** and Gillespie **[10]** who found that the highest need

for information reported by patients in their study was related to medical domain .The patients in this study expressed a need for information, understanding, and education about liver cirrhosis and possible complications to self-manage their disease and maintain their health. Likewise Papadakos **[33]** who found that patients considered medical information less important because of the knowledge and experience they have gained over the course of their illness.

Although the need for medical information was the greatest, the informational needs regard treatment domain had the second highest importance scores associated with it. This study in line with Alizadeh **[34]** and Fabris **[35]** who found that concerns about the need for specific treatment was reported by majority of studied patients as side effects of treatment and proper medications, this due to the majority of studied patients not received any informational needs regarding treatment from nurse specialist . And disagree with Grogan and Timmins **[36]** and Minuk **[37]** who that found only (11%, 23 % respectively) of studied patients needed information regarding treatment, this due to patient were eager to receive information on treatment.

The next most highly rated domain of need is the physical domain with high score as controlling/ reducing abdominal distention, ways of controlling symptoms (fatigue, pruritus) this was similar to other studies of hepatic patients as Zandi **[38]** who found that managing side effects, including abdominal distension, fatigue and tiredness, were among the highest rated information needs.

The psychological domain received the lowest importance score ,only 27.0% and this is consistent with Chen **[39]** and Janke **[40]** that found studied patients had low score of informational needs regard psychological domain likewise with Conrad **[41]** and jessop **(42)** found that psychological domain had highest important score in informational needs among studied patients .This difference perhaps due to the patient perception that this domain is out of the

As regard to the relation between patients' age & SGA, the present study results indicates that, there was a statistically significant relation between age and SGA score of malnutrition (P= 0.01), this is supported by the finding that, there was a statistically positive correlation between SGA score and age. This finding should be taken cautiously as may confound the relation between liver affection and nutritional status of those patients, because increasing age of the patient is usually associated with high prevalence of geriatric problem that usually affect their nutritional status and may predispose to malnutrition as anorexia, dental problem, this result on same line with Salah **[24]** .Regarding Child- Pugh and its relation with SGA score, the present study revealed that there was a statistical significant relationship between Child- Pugh and SGA, this is further supported by the finding that, there was a statistically significant positive correlation between Child Pugh and SGA. From these results we can observe a trend towards a higher proportion of bad nutritional status in patients with Child-Pugh B & C compared to Child-Pugh A, this indicates that SGA tool has the ability to reflect the degree of severity of liver affection in the study sample. The current study revealed that there was a statistically significant relationship between informational needs and studied patient age p =0.001, this is further supported by the finding that, there was a statistically significant negative correlation between total informational needs and patient with younger age r=.520, these findings might be due to the younger age at the beginning of his life need more information to self- manage their diseases. These findings in accordance with previous studies, Papadakos **[33]**, and with Hassan and Shams **[43]** which demonstrated that younger patients placed more importance on the informational needs when compared to older patients . Likewise other study by Dehghani **[44]** who found that, there was statistically significance positive relation between informational needs and older age ,these finding might be due to that old age predicted higher unmet needs.

Apart from total abnormal signs, the current study revealed that, there was a statistically significant negative correlation between number of abnormal signs, and informational needs. The negative relation between number of abnormal signs, and needs was confirmed in multivariate analysis.

On summary, Malnutrition is highly prevalent among the patients with liver cirrhosis, it varied according to the method used. The most prioritized informational needs for patients with liver cirrhosis was medical domain whereas the least priority was given to the psychological domain. It also shows that information needs differ based on some socio-demographic and clinical characteristics and physical condition.

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# Mean Platelet Volume and Mean Platelet Volume/Platelet Count Ratio as Diagnostic Markers for Hepatocellular Carcinoma in Chronic Hepatitis C Patients

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**Background and study aim:** Hepatocellular carcinoma (HCC) is the most common primary malignant tumor of the liver. AFP is the gold standard tumor marker for HCC, mean platelet volume (MPV) is a parameter obtained from complete blood count (CBC) by automated analyzers, shown to be increased in multiple malignancies and inflammatory conditions.This prospective study was designed to evaluate the diagnostic usefulness of MPV and MPV/ platelet count (PC) ratio in HCC patients due to chronic hepatitis C (CHC) infection.

**Materials and Methods:** One hundred and twenty subjects enrolled in this study, they were divided into 4 equal groups: group I included 30 healthy subjects (control group), group II included 30 patients with chronic viral hepatitis without cirrhosis, group III included 30 cirrhotic patients without HCC and group IV included 30 cirrhotic patients with HCC.MPV, MPV/PC ratio & AFP were evaluated in all groups. Triphasic CT was done for patients of group IV to confirm the diagnosis of HCC. Liver biopsy was done for patients of group II to confirm the diagnosis of chronic hepatitis C.

**Results:** MPV showed higher levels in HCC group compared to control, CHC, and cirrhotic groups with p value(<0.001) but there was no statistically significant difference between HCC group and cirrhotic group (p value=0.49), while MPV/platelet count ratio was higher in cirrhotic group (0.19±0.09 fL/ $10^9$/L) than HCC group (0.14±0.08 fL/$10^9$/L) but also with no significant differences between both groups (p=0.06). There was insignificant positive correlation between MPV and AFP in HCC group (ρ= 0.11 and p value =0.57). Also, there was insignificant negative correlation between MPV/PC and AFP in HCC group (ρ= -0.17 and p value =0.37). In receiver operating characteristic (ROC) curve analysis, MPV had high sensitivity (73.33%), specificity (70 %), and area under curve (AUC) was 0.7, So it more better than MPV/platelet count ratio in diagnosis of HCC which had sensitivity (76.67%), low specificity (56.67%), and AUC was 0.63, while AFP had much higher sensitivity (90%), specificity (98.33%) than both studied parameters (MPV, MPV/PC ratio) with highly statistically difference when compared to MPV (p<0.001) and area under curve (AUC) was 0.9.

**Conclusion:** MPV and MPV/PC ratio are less sensitive and specific than AFP in diagnosis of HCC. So AFP is still the gold standard marker in diagnosis of HCC and MPV and MPV/PC ratio may be used only in association with other markers like AFP to improve sensitivity of tumor detection.

## INTRODUCTION

Hepatocellular carcinoma (HCC) is the second leading cause of cancer deaths in the world with more than 745,000 new deaths annually **[1]**. The prognosis of HCC remains poor, and most patients have a 5-years survival rate of less than 5% mainly because of the late diagnosis **[2]**. Although it is obvious that development of new diagnostic modalities will significantly increase the detection rate of HCC,

there is still a need for detection methods. AFP is the most established tumor marker in HCC and the gold standard by which other markers for the disease are judged **[3]**. Mean platelet volume (MPV) is a parameter of routine blood count which was actively investigated in many liver diseases. MPV was found to be related to metabolic syndrome **[4]**, advanced liver fibrosis **[5]**. And ascitic fluid infection **[6].** There is an inverse relationship between platelet size and number. When platelets decrease in number, bone marrow megakaryocytes are stimulated by thrombopoietin and their nucleus becomes hyperlobulated, with much higher DNA content **[7,8]** producing larger platelets. Thus, platelets with a higher MPV are expected to be seen in destructive thrombocytopenia when megakaryocytic stimulation is present **[9,10]**.

This study was aimed to evaluate the diagnostic usefulness of MPV and MPV/PC ratio in HCC patients due to chronic hepatitis C infection.

## MATERIALS AND METHODS

This prospective study was conducted on 120 subjects with mean age 45.29± 16.44 years in period from April 2014 to March 2016.

**The subjects were divided into 4 groups :**
**Group I:** included 30 healthy subjects served as control group.
**Group II**: included 30 patients with chronic hepatitis C.
**Group III:** included 30 cirrhotic patients without HCC.
**Group IV:** included 30 cirrhotic patients with HCC.

All studied patients were given an informed written concent for participation in this study, the protocol of this study was approved from ethical committe of Benha faculty of medicine, Benha university.

Patients with hepatic malignancy rather than HCC, any signs of inflammation, acute myocardial ischemia, atherosclerosis and cerebrovascular events, ulcerative colitis, Crohn's disease, rheumatoid disease, portal vein thrombosis and patients receiving drugs affecting platelet count or function as Aspirin were excluded from study.

- Diagnosis of CHC infection was done by following criteria:
  1- Detectable HCV Abfor more than 6 months.
  2- Positivity of HCV RNA and confirmed by liver biopsy.
- Diagnosis of liver cirrhosis was based on laboratory investigations and radiological findings.
- Diagnosis of HCC was based on serum level of AFP and imaging modalities( pelvi-abdominal U/S and triphasic CT abdomen)
- All subjects were subjected to full history taking and thorough clinical examination and laboratory investigations including MPV, MPV/PC ratio and AFP, triphasic CT was done to confirm HCC cases.

**Statistical analysis**
Statistical analysis was performed using PASW statistics 18 (SPSS, Chicago, IL USA). Statistically significant differences were analyzed by the X2 test for categorical variables. Continuous variables were tested for normality by the Kolmogorov-Smirnov test. Normally distributed data are presented as mean and standard deviations (SDs). The sensitivity and specificity of MPV level. MPV/PC ratio and AFP for a diagnosis of hepatocellular carcinoma was done under various cut off ranges and receiver operating characteristics (ROC) curves were drawn. Pearson and Spearman correlation between AFP and MPV, MPV/PC ratio in HCC group were calculated. A two tailed P value below 0.05 was considered statistically significant.

## RESULTS

There was highly statistically significant difference between studied groups as regard age and sex. ($p<0.001$).There was male predominance in HCC group with male to female ratio 4 : 1. The mean age was higher in cirrhotic group without HCC than HCC group with no statistically significant difference between HCC and cirrhotic groups. ($p=0.52$), while there was no statistically significant difference between studied groups as regard diabetes mellitus and systemic hypertension as showed in table (1). Highly statistically significant difference was found between studied groups (I, II, III and IV) as regard all studied laboratory parameters, AFP was highly elevated in HCC group than in cirrhotic, chronic hepatitis and control groups with highly statistically significant difference ($P<0.001$) (1239.93± 2881.97 ng /dl Vs 18.35±17.0 ng /dl and 2.2±1.14 ng /dl Vs 0.34±0.31 ng /dl) respectively (Table 2).

MPV and MPV/platelet count ratio show highly statistically significant difference between studied

groups (I,II,III and IV) (P<0.001). HCC group shows higher levels of MPV compared to other groups (10.94±1.88 fL in HCC group Vs 10.74± 1.21 fL in cirrhotic group , 9.13±1.74 fL in HCV group and 8.07±0.86 fL in control group) with p value<0.001, but there was no statistically significant difference between HCC group and cirrhotic group (p value=0.49). While MPV/ platelet count ratio was higher in cirrhotic group (0.19±0.09 fL/$10^9$/L) than HCC group (0.14± 0.08 fL/$10^9$/L), chronic hepatitis group (0.05±0.02 fL/$10^9$/L) and control group (0.03±0.01 fL/$10^9$/L). There was no significant differences between cirrhotic group and HCC group (p=0.06). Table (3) and figure (1,2).There was insignificant positive correlation between MPV and AFP (ρ= 0.11 and p value =0.57),and insignificant negative correlation between MPV/ PC and AFP in HCC group (ρ= -0.17 and p value =0.37) (Table 4). At cut off level >10.7 fl, MPV had high sensitivity (73.33%), specificity (70 %), NPV= (83%), low PPV= (51.2%), accuracy (71.11%) and area under curve (AUC) was 0.7, So it more better than MPV/platelet count ratio in diagnosis of HCC which had at cut off level >0.11 fl/$10^9$/L, low specificity (56.67%) and area under curve (AUC) was 0.63 with no statistically significant difference when compared to MPV (p= 0.14 ).While AFP at cut off >62.3 ng/dl level had much higher sensitivity (90%), specificity (98.33%), PPV= (96.43%), NPV= (95.16%), accuracy (95.56%) and area under curve (AUC) was 0.9, with highly statistically significant difference when compared to MPV (p=<0.001). Table (5) and Figure (3). So, AFP is still better than other studied parameters in diagnosis of HCC.

**Table (1):** Comparison between studied groups as regard baseline data.

| Variables | | Group I (control group) (No.=30) | | Group II (HCV group) (No.=30) | | Group III (cirrhosis without HCC) (No.=30) | | Group IV (HCC group) (No.=30) | | Test | P |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | No | % | No. | % | No. | % | No. | % | | |
| **Sex** | Females | 24 | 80.0 | 15 | 50.0 | 18 | 60.0 | 6 | 20.0 | $\chi^2$= 22.56 | **<0.001**** |
| | Males | 6 | 20.0 | 15 | 50.0 | 12 | 40.0 | 24 | 80.0 | | |
| **Age (years)** | Mean ±SD; (range) | 27.37±9.78; (16-48) | | †37.27±10.32; (20-58) | | †‡59±9.54; (34-79) | | †‡57.53±8.18; (40-74) | | F= 80.32 | **<0.001**** |
| **DM** | No | 27 | 90.0 | 24 | 80.0 | 23 | 76.67 | 20 | 66.67 | $\chi^2$= 4.91 | 0.18 |
| | Yes | 3 | 10.0 | 6 | 20.0 | 7 | 23.33 | 10 | 33.33 | | |
| **HTN** | No | 27 | 90.0 | 26 | 86.67 | 28 | 93.33 | 25 | 83.33 | FET | 0.78 |
| | Yes | 3 | 10.0 | 4 | 13.33 | 2 | 6.67 | 5 | 16.67 | | |

* Significant (P<0.05)
** Highly Significant (P<0.001)
† Significant differences compared to group I
‡ Significant differences compared to Group II

- (HCC group against cirrhosis group as regard age showed p=0.52)

**Table (2)** Comparison between studied groups (I,II, III &IV) regarding laboratory findings.

| Variables | Group I (control group) (No.=30) | Group II (HCV group) (No.=30) | Group III (cirrhosis without HCC) (No.=30 | Group IV (HCC group) (No.=30) | Test | P |
|---|---|---|---|---|---|---|
| | Mean ±SD | | | | | |
| **FBS (mg/dl)** | 94.1 ± 25.85 | 99.17 ± 25.69 | !†131.97 ± 60.14 | !120.73 ± 60.01 | $\chi^2$= 16.48 | **<0.001**** |
| **HB (gm/dl)** | 12.4 ± 1.27 | 13.47 ± 1.41 | !†10.15 ± 1.61 | †‡11.28 ± 2.25 | F= 21.85 | **<0.001**** |
| **WBCs ($10^3$/cmm)** | 6.14 ± 1.68 | 5.81 ± 1.69 | !4.85 ± 2.78 | 5.85 ± 3.04 | $\chi^2$= 10.24 | **0.02*** |
| **Platelets ($10^9$/L)** | 247.77 ± 61.03 | 210.1 ± 74.98 | !†67.07 ± 26.53 | !†103.1 ± 65.24 | F= 61.65 | **<0.001**** |
| **S. creatinine (mg/dl)** | 0.79 ± 0.14 | 0.86 ± 0.20 | !1.16 ± 0.64 | !†1.14 ± 0.59 | $\chi^2$= 24.35 | **<0.001**** |
| **ALT (IU)** | 23.77 ± 6.5 | !50.2 ± 29.47 | !49.03 ± 44.15 | !50.31 ± 28.14 | $\chi^2$= 37.41 | **<0.001**** |
| **AST (IU)** | 24.07 ± 6.9 | !43.18 ± 24.45 | !57.2 ± 34.11 | !60.03 ± 30.99 | $\chi^2$= 48.17 | **<0.001**** |
| **T. bilirubin (mg/dl)** | 0.52 ± 0.45 | 0.72 ± 0.27 | !†3.2 ± 1.93 | !†3.14 ± 3.37 | $\chi^2$= 71.36 | **<0.001**** |
| **S. albumin (gm/dl)** | 4.6 ± 0.37 | !4.24 ± 0.71 | !†2.52 ± 0.39 | †2.78 ± 0.61 | F= 111.24 | **<0.001**** |
| **INR** | 1.01 ± 0.05 | !1.28 ± 0.18 | !1.46 ± 0.34 | !†`1.55 ± 0.44 | F= 19.07 | **<0.001**** |
| **PT(sec)** | 12.15 ± 0.49 | !13.79 ± 1.23 | !†16.25 ± 2.57 | !†16.74 ± 3.33 | F= 28.56 | **<0.001**** |
| **PCR HCV RNA (IU/ml)** | - | 425981.3 ± 386821.5 | - | - | - | - |
| **AFP(ng/dl)** | 0.34 ± 0.31 | !2.2 ± 1.14 | !18.35 ± 17.0 | !†‡1239.93 ± 2881.97 | $\chi^2$= 103.36 | **<0.001**** |

! Significant differences compared to Group I
† Significant differences compared to group II
‡ Significant differences compared to Group III

**Table (3):** Comparison between studied groups (I, II, III &IV) regarding MPV& MPV/Platelet count ratio.

| Variables | Group I (control group) (No.=30) | Group II (HCV group) (No.=30) | Group III (cirrhosis without HCC) (No.=30) | Group IV (HCC group) (No.=30) | Test | P |
|---|---|---|---|---|---|---|
| | Mean ±SD | Mean ±SD | Mean ±SD | Mean ±SD | | |
| **MPV(fL) Normal range** (8.9 ± 1.4 Fl) | 8.07± 0.86 | !9.13± 1.74 | !†10.74± 1.21 | !†.10.94± 1.08 | F= 34.95 | **<0.001**** |
| **MPV/Platelet count (fL/$10^9$/L)** | 0.03± 0.01 | 0.05± 0.02 | †0.19± 0.09 | †‡0.14± 0.08 | F= 43.69 | **<0.001**** |

! Significant differences compared to Group I
† Significant differences compared to group II

- (HCC group against cirrhosis group as regard MPV showed p=0.49)
- (HCC group against cirrhosis group as regard MPV/PC ratio showed p=0.06)

**Table (4):** Correlation between AFP and MPV, MPV/PC ratio in HCC group.

| Variables | HCC group (N0=30) | | | |
|---|---|---|---|---|
| | MPV | | MPV/ PC ratio | |
| | Correlation coefficient | P | Correlation coefficient | P |
| AFP **(ng/dl)** | ρ= 0.11 | 0.57 | ρ= - 0.17 | 0.37 |

r: Pearson correlation coefficient
ρ: Spearman correlation coefficient

**Table (5):** Diagnostic performance of MPV, MPV/platelet count ratio and AFP for diagnosis of HCC

| Variable s | Cutoff point | Sensitivity | Specificity | PPV | NPV | Accuracy | AUC | 95% CI | P* |
|---|---|---|---|---|---|---|---|---|---|
| **MPV (fL)** | 10.7 | 73.33% | 70.0% | 51.2% | 83.0% | 71.11% | 0.7008 | 0.59 - 0.79 | |
| **MPV/PC ($Fl/_{10}{}^{9}/L$)** | 0.11 | 76.67% | 56.67% | 46.9% | 82.9% | 63.33% | 0.6217 | 0.51 - 0.72 | 0.14 |
| **AFP (ng/dl)** | 62.3 | 90.00% | 98.33% | 96.43% | 95.16% | 95.56% | 0.9744 | 0.92- 1.00 | **<0.001**** |

*Compared to MPV

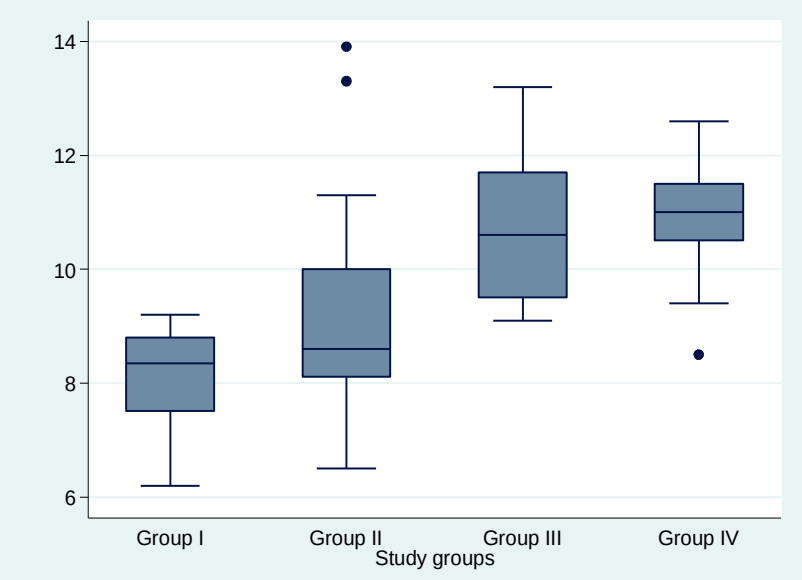


**Figure (1):** Box plot showing MPV level among studied groups

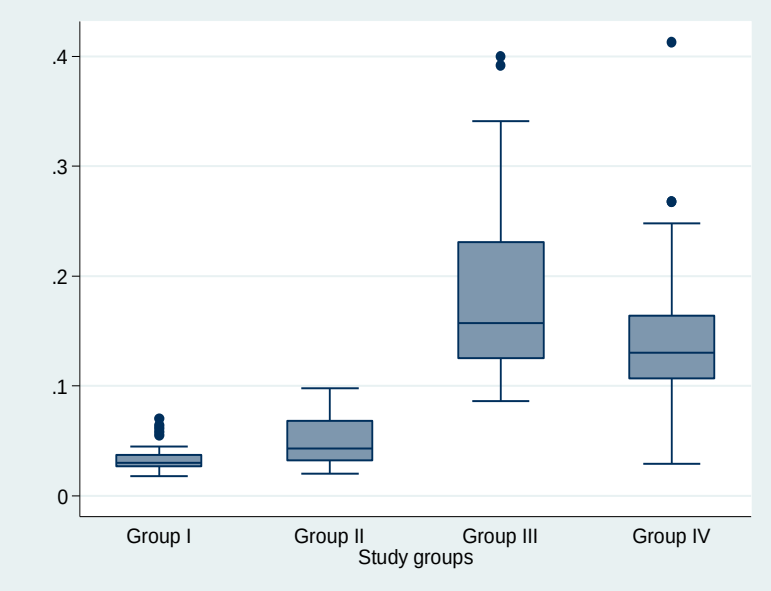


**Figure (2):** Box plot showing MPV / PC ratio among studied groups

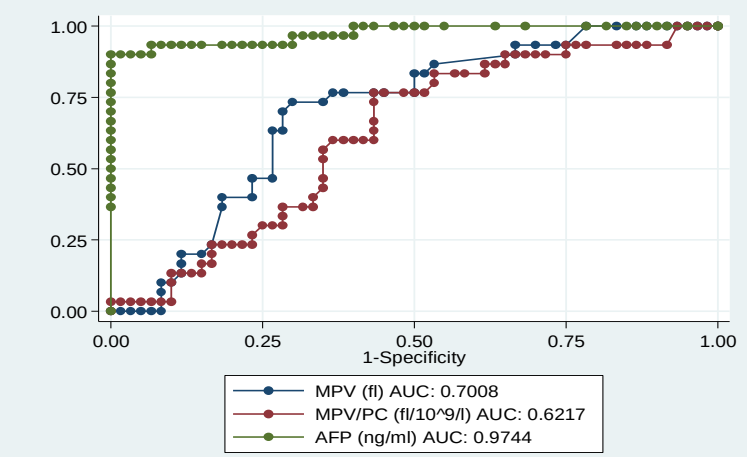


**Figure (3):** Roc curve for diagnostic performance of MPV, MPV/PC ratio and AFP in diagnosis of HCC

## DISCUSSION

Liver cancer is the second cause of cancer related death worldwide (745,000 cases per year) **[1]**, and accounts for 7% of all cancers, representing more than 90% of primary liver cancers **[11]**. Most hepatocellular carcinomas are diagnosed at intermediate or advanced stages and only 30% of patients benefit from curative therapies such as resection, liver transplantation or percutaneous ablation **[12]**. This study was aimed to evaluate the diagnostic usefulness of MPV and MPV/PC ratio in HCC patients due to chronic hepatitis C infection. There was highly statistically significant difference between studied groups as regard age and sex. ($p<0.001$) with male predominance in HCC group (male to female ratio was 4:1),this result was in agreement with previous studies which showed that there was a male predominance among HCC patients with male to female ratio of 3.6:1 **[13,14]**. In contrast to our results, several studies reported that a non significant difference in sex distribution between HCC patients **[15]**. The mean age was higher in cirrhotic group without HCC than HCC group with no statistically significant difference between HCC and cirrhotic groups. ($p=0.52$),this result was in agreement with previous study which showed that the mean

age of HCC patients was 57.95 ± 8.41 years **[14]** and in agreement with another study which documented that the mean age was 56.28 years for the HCC patients **[16]**. Also, another study reported that the most predominant age group for HCC was (40-59) years **[17]**. In contrast to our results, other studies reported that reported that HCC occur in older age (61 years) **[18]** and (66 years) **[19]**. AFP was markedly elevated in HCC group than in cirrhotic, chronic hepatitis and control groups with highly statistically significant difference (P<0.001) (1239.93±2881.97 ng/dl, 18.35±17.0 ng/dl, 2.2±1.14 ng/dl and 0.34±0.31 ng/dl) respectively, these results were in agreement with a previous study who mentioned that marked elevation of AFP level was observed in patients with HCC in comparison with healthy control subjects, patients with CHC and patients LC (239.5±770.7ng /dl in HCC group, 7.1±5.4ng /dl in control group, 10.1±10.7 ng/dl in CHC group and 31.1±83.2 ng /dl in cirrhotic group). (P<0.001)[20].On the same hand, Atta et al. **[14]**, reported a higher mean values for HCC cases than cirrhotic cases. Also Hsia et al. **[21]**, reported that mean value of AFP was higher in HCC group than hepatitis C and control group, and Baig et al. **[22]**, found that, mean AFP levels in HCC patients were 421ng/ml, and concluded that, AFP is a significant marker and an indicator for Hepatocellular carcinoma.

Mean platelet volume (MPV) is a parameter of routine blood count which was actively investigated in many liver diseases.

In this work, HCC group and cirrhotic group show higher levels of MPV compared to chronic HCV group and control group (10.94±1.88 fL in HCC group vs 10.74±1.21 fL in cirrhotic group vs 9.13±1.74 fL in HCV group vs 8.07±0.86fL in control group) with highly statistically significant difference (P<0.001) but there was no statistically significant difference between HCC group and cirrhotic group (p value=0.49). These results matched with those reported by Metwaly et al. **[20]**, who found that MPV were higher in patients with HCC and in patients with liver cirrhosis when compared with controls and patients with CHC (P<0.001). However, no significant differences were found between patients with LC and those with HCC (P=0.94). Also, Kurt et al. **[23]**, reported that mean level of MPV was higher in HCC group than cirrhotic and CHC groups (9.7 fL in HCC group, 9.1 fL in cirrhotic group and 8.6 fL in CHC group).

In our study, MPV/PC ratio was higher in cirrhotic group (0.19±0.09 fL/$10^9$/L) than HCC group (0.14±0.08 fL/$10^9$/L), chronic hepatitis group (0.05±0.02 fL/$10^9$ /L) and control group (0.03±0.01 fL/$10^9$/L) with statistically significant difference (P<0.001) between all the studied groups but no significant differences were found between cirrhotic group and HCC group (p=0.06). Similarly, Metwaly et al. **[20]**, found that MPV/PC ratio was higher in patients with HCC and in patients with liver cirrhosis (P<0.001) when compared with controls and patients with CHC. Although the MPV /PC ratio was higher in cirrhotic group (1.48±0.79fl $10^{-4}$ $\mu l^{-1}$) than HCC group (1.33±0.7fl $10^{-4}$ $\mu l^{-1}$) but did not reach significant level (P= 0.69).Also, these results were consistent with those of Cho et al. **[24]**, who reported that MPV/PC ratio was higher in HCC group than control group with statistically significant difference (P<0.001) (0.058 fL/$10^9$/L in HCC group vs 0.033±0.01 fL/$10^9$/L in control group).

In present study, there was insignificant positive correlation between MPV and AFP in the HCC group (r=0.11, p=0.57) that is in line with Kurt et al. **[23]**, who mentioned that there was no correlation between MPV and AFP (r=0.242). Also, the present study revealed that there was insignificant negative correlation between MPV/PC ratio and AFP in HCC group which was in agreement with Cho et al. **[24]**, who concluded also that there was no correlation between MPV/PC ratio and AFP.

Analysis of MPV by ROC curve in the present work, showed that at cut off level =10.7 fl, MPV had high sensitivity (73.33%), specificity (70 %), and AUC was 0.7 in diagnosis of HCC. These results are concided with the results of Metwaly et al. [20], who demonstrated that at cut off level =10.1 fl, MPV showed a sensitivity (70 %), and specificity (57.3 %) and AUC was 0.67, while Kurt et al. [23], reported that at cut off level = 9.2 fl, MPV showed more low sensitivity (68.3%), and specificity (62.1 %) and AUC was 0.67. This difference may be partially attributed to the different sample size and different studied population as Kurt et al. [23], conducted his study on 230 Turkish patients while our study was conducted only on 120 Egyptian patients.

In the present study, analysis of MPV/PC ratio by ROC curve showed that at cut off level = 0.11 fl/$10^9$/L, MPV/PC ratio had a sensitivity (76.67%), specificity (56.67%) in diagnosis of HCC with

AUC was 0.63. These results are close to the results of Metwaly et al. [20], who demonstrated that at cut off level = 0.82, MPV/ PC ratio showed a sensitivity (79.6 %), and specificity (72.7 %) and AUC was 0.777. While Cho et al. **[24]**, who reported that The AUC for MPV/PC ratio was 0.884 with high sensitivity (74.5%), and specificity (96.5%) at the criterion =0.0491(a vertical arrow). This difference between may be attributed to the different sample size and different studied population as Cho et al. **[24]**, conducted his study on 411 Korean patients while our study was conducted on 120 Egyptian patients.

Regarding AFP, by ROC curve analysis in the present study, at cut off level = 62.3 ng/dl, it showed a higher sensitivity (90.0%), and higher specificity (98.3%) than MPV and MPV/PC ratio with AUC was 0.97 in diagnosis of HCC. This result was agreed with Metwaly et al. **[20]**, who conducted his study on 200 Egyptian patients and found that at cut of level =16.9 ng/dl had high sensitivity (81%) and specificity (82%) in detection of HCC than MPV and MPV/PC ratio with AUC was 0.88, so he concluded that that MPV and MPV/PC ratio are less sensitive and specific than AFP as markers for HCC. Therefore, they may be used only in association with other markers like AFP to improve sensitivity of tumor detection. But this result disagreed with Kurt et al. **[23]**, and Cho et al. **[24]**, who reported higher sensitivity and specificity for MPV and MPV/PC ratio than AFP in their studies. This difference may be attributed to the different sample size, different studied population or difference in HCV genotype.

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# An Overview Study of Malaria Infection in Almaza Military Fever Hospital; An Egyptian Pilot Study

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**Background and study aim:** Malaria is a life-threatening disease caused by parasites that are transmitted to people through the bites of infected female *Anopheles* mosquitoes.The aim of this study is to study the clinical presentations and outcomes of malarial infected cases attending Almaza Military Fever Hospital in Cairo.

**Patients and Methods:** Fifty patients with malarial infection were selected from those admitted to Almaza Military Fever Hospital. The following investigations were done for all cases; (CBC), liver and renal function tests, serological tests (rapid diagnostic test for serum malarial antigens & microscopic examination of peripheral blood film) and abdominal US.

**Results:** The majority of cases (76%) was already diagnosed and was coming from Peace Keeping Mission Forces in Africa. Congo was the most malaria-infected place (36%), then Ivory Coast (26%). Most of cases (80%) had intermittent fever. Six patients (12%) were admitted at ICU. The thick film method was the most sensitive diagnostic test (98%). *P. falciparum* was the commonest species among cases (80%) then *P. ovale* (20%). The best response in studied cases was poly-therapy (84%) while mono-therapy was effective in only 5 patients (10%), (82%) of cases were cured, one patient died and one patient had a relapse while 2 patient (4%) had recurred.

**Conclusion:** Thick film is the most sensitive and informative test among diagnostic test modalities. Combined therapy (polytherapy) is preferable than monotherapy.

## INTRODUCTION

Malaria is a life-threatening disease caused by parasites that are transmitted to people through the bites of infected female *Anopheles* mosquitoes. In 2015, 91 countries and areas had ongoing malaria transmission **[1]**.

Between 2000 and 2015, malaria incidence among populations at risk (the rate of new cases) fell by 37% globally. In that same period, malaria death rates among populations at risk fell by 60% globally among all age groups, and by 65% among children under 5 years **[2]**.

In 2015, approximately 3.2 billion people nearly half of the world's population were at risk of malaria. It was estimated that the number of malaria cases had decreased to 214 million (range: 149–303 million), and the number of deaths to 438 000 (range: 236 000–635 000). Most malaria cases and deaths occur in sub-Saharan Africa. However, Asia, Latin America, and, to a lesser extent, the Middle East, are also at risk **[2]**. In 2015, 97 countries and territories had ongoing malaria transmission. Young children, pregnant women and non-immune travelers from malaria-free areas are particularly vulnerable to the disease when they become infected **[2]**.

Malaria is preventable and curable, and increased efforts are dramatically reducing the malaria burden in many places. Sub-Saharan Africa carries a disproportionately high share of the global malaria burden. In 2015, the region was home to 88% of malaria cases and 90% of malaria deaths **[1]**.

Malaria occurs mostly in poor tropical and subtropical areas of the world. In

many of the countries affected by malaria, it is a leading cause of illness and death. In areas with high transmission, the most vulnerable groups are young children, who have not developed immunity to malaria yet, and pregnant women, whose immunity has been decreased by pregnancy **[3]**. The costs of malaria to individuals, families, communities, nations are enormous **[3]**.

## PATIENTS AND METHODS

This cross-sectional prospective study was held in co-operation between Tropical Medicine Department and Almaza Military Fever Hospital in the period from June 2015 to June 2016. Sample size was calculated to include 50 patients with malarial infection at 95% confidence interval and a power of 0.80 and an expected effect size of 50%.

**Inclusion Criteria:**
Clinical manifestations (mainly fever with or without chills, sweating, headaches, nausea, vomiting, body aches or general malaise; signs; elevated temperatures, increased respiratory rate, weakness, mild jaundice, enlargement of the liver and\or spleen) [3]. As well, positive rapid malaria test.

**The** objectives of the study were explained to all patients who met the eligibility criteria and they were asked to sign a written consent form. Approval of the local ethical committee of the hospital was also obtained.

All the studied cases were subjected to the following:
**Complete history taking and thorough clinical evaluation**
**Laboratory investigations:** Including complete blood count (CBC), liver function tests (ALT, AST), renal function tests (serum creatinine, urea).

**Serological tests:** Rapid diagnostic test for serum malarial antigens**,** and microscopic examination of peripheral blood film stained with Giemsa stain.

**Abdominal ultrasonography :** This was done for all cases.

**Therapy and Follow up:** All patients after diagnosis were treated either by single drug regimen (Alexaquine or Mefloquine) the recommended dose (25 mg /kg) is dividing into two parts given at an arrival of 6-24 h then Primaquine for 14 days **[4]** or by combined drug therapy in case of *P. falciparum* (Artesunate 2.4 mg/kg given intravenously at 0, 12, and 24 hours, and then single daily dose for 2 days + Pyrimethamine & Sulphadoxine three tablets once only) **[5,6]**. Treatment for other manifestation of malaria was done. The patients were followed up by another blood film after 2-3 days to show the parasitaemia and assess the response of the treatment regimen. If parasitaemia was persistent, the patient was shifted to other regimen.

**Clinical outcome of the studied cases:** Patients were discharged from hospital when they had been showed clinical improvement, mainly in the fever, and other manifestation. If any of the previous manifestations was present, we followed-up them after 15 and 30 days from discharge by clinical picture or laboratory investigations.

**Study methods:**
Serum samples: Ten milliliters venous blood was collected from each patient by clean vein puncture using disposable plastic syringes. Two ml of blood were delivered into plastic tube containing EDTA for performing Rapid diagnostic test.

**Malaria P.F/Pan Rapid Test Device (Whole Blood):**
It is a rapid, reliable and simple chromatographic immunoassay for the qualitative detection of circulating *P. falciparum* HRP-II antigen (histidine-rich protein II) and/or Pan-malarial Aldolase antigens found in *Plasmodium falciparum*, *Plasmodium vivax, Plasmodium ovale and Plasmodium malariae* in whole blood (Figure 1).

**1. Procedure:**
The device was labeled with patient name or identification number, then the specimen was transferred by a pipette 10ul of whole blood to well-1 (W1) of the test device, then 3 full drops of buffer was added to well-2 (W2), and the timer was started. We avoided trapping air bubbles in W1. At the end of 5 minutes, we added 1 full drop of buffer to W1, waiting for the colored line(s) to appear. The result was read at 15 minutes (not after 20 minutes).

**2. Interpretation of results:**
- **Positive:** Two or Three distinct colored lines appeared. *P. falciparum or mixed malaria*

*infection:* one line appeared in the control region, one line appeared in P.f line region. *P. falciparum infection:* one line appeared in the control region, and one line appeared in P.f line region. *Non-P. falciparum species infection:* one line appeared in the control region, and one line appeared in Pan line region.

- **Negative:** Only one colored line appeared in the control region.
- **Invalid:** Control line failed to appear. Insufficient specimen volume or incorrect procedural techniques were the most likely reasons for control line failure. The procedure was revised and the test was repeated with a new test device.

**Thin & Thick blood films:** Thin blood films were prepared by placing the edge of the spreader slide in a drop of blood and smearing the blood along the surface. Films were allowed to air-dry and were fixed with absolute methanol. For thick blood films, a blood spot was stirred in a circular motion with the corner of the slide and slides were left to dry without fixation. After drying of blood films, they were stained with diluted Giemsa (1 : 20, vol/vol) for 20 minutes and washed in buffered water. To ensure good quality of staining and standardization of blood film examination and reporting, the amount of blood used to make blood films, especially thick films, was kept as constant as possible and the blood was spread evenly over a specified area of the slide (15 × 15 mm for thick films). Each slide was subjected to preliminary screening using low power objectives (×10 and ×40) followed by examination of at least 100 microscopic fields using high power objectives (×100). Slides were examined by 2 microscopic experts and suspicious slides were examined by a third expert (Figure 2) **[7]**.

**Statistical Analysis:**

The collected data were coded, tabulated, and statistically analyzed using SPSS program (10.0) (Statistical Package for Social Sciences) for Windows XP. Descriptive statistics were done for numerical parametric data as mean, standard deviation (mean ± SD) and minimum & maximum of the range, while they were done for categorical data as number and percentage. Description of qualitative variables was done by frequency and percentage. The diagnostic cut off and the related sensitivity and specificity were determined.

## RESULTS

The current study showed that the majority of patients (No=42 (84%)) were (20-40) years old and 46 of them (92%) were military staff. According to the bases of selection of the studied patient, most of them (No=38 (76%)) were diagnosed as imported malaria coming from Peace Keeping Mission Forces in Africa, while (24%) were presenting with prolonged fever (7-10 days) for investigation. The previous 24% of the studied patients was further classified into; eight patients (16%) were diagnosed as relapsing malaria and four patients (8%) were diagnosed as endemic acquired malaria.

**N.B:** Imported Malaria: Malaria acquired outside a specific geographic area (Egypt). Relapsing malaria: occur after primary attack due to reactivation of hypozoites of parasite in liver up to 3-5 years after primary infection. Endemic Acquired malaria: Malaria acquired at malaria-endemic countries.

The geographical distribution of malaria parasite showed that Congo was the most malaria-infected place (36%), and Ivory Coast was coming next in frequency (26%) (Figure 3).

**Concerning the clinical presentations,** all patients (100%) had a fever as presenting symptoms, associated with rigors & sweating. Three patients (6%) were comatosed, two patients (4%) had convulsion with neck stiffness, five patients (10%) with dark urine had high grade fever and six patients (12%) was admitted at ICU (Table 1). It also, revealed the outcome of the six patients (12%) that was admitted at ICU, two patients were cured, two patients presenting with anemia, one patient presenting with renal impairment and one patient died.

**As regards to the past history of the studied malarial-infect patients;** ten patients (20%) had taken doxycycline, 8 patients (16%) had mefloquine as preventive antimalarial drugs and 32 patients (64%) had no any preventive therapy. Twenty patients among all (40%) had a past history of malaria infection,

**The current study assessed the diagnostic tests**. The thick film method was more sensitive (98%) than thin film test (**Table 2)**. *P.falciparum* was the commonest species among the studied cases (No=40 (80%)) and *P.ovale* coming next in frequency (No=10 (20%)).

The laboratory investigations didn't reveal any significant findings while abdominal ultrasound only revealed that twenty eight patients (56%) had hepatomegaly and twenty four (48%) had splenomegaly.

**Concerning the therapeutic responses of the studied patients,** the best response was detected after poly-therapy (No=42 (84%)) while mono-therapy was effective in only 5 patients only (10%) as had shown in (table 3). Also, it revealed the clinical outcome of the studied cases, forty-one patients (82 %) were cured, one patient (2%) died and one patient (2%) had a relapse while 2 patients (4%) had recurred. Table (4) showed the comparative study between complicated & non-complicated malaria:

**Table (1) :** Severe malarial manifestations & Analysis of the patients that had admitted to ICU

| Severe malarial manifestations | Conscious level | | | Neuro-psychiatric | | | Renal | | Chest | | Other | | | ICU Admission |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Conscious | Drowsy | Comatose | Hallucination | Convulsion | Neck-stiffness | Oliguria | Anuria | Cough | Dysnea | Hypoglycemia | Severe anemia | Black water fever | |
| **Frequency** | 38 | 9 | 3 | 12 | 2 | 2 | 3 | 1 | 9 | 9 | 12 | 7 | 5 | 6 |
| **Percent %** | 76 | 18 | 6 | 24 | 4 | 4 | 6 | 2 | 18 | 18 | 24 | 14 | 10 | 12 |
| **Analysis of 6 cases that had admitted to ICU** | **Pt (1)** | | **Pt (2)** | | **Pt (3)** | | **Pt (4)** | | | | **Pt (5)** | | **Pt (6)** | |
| **Age** | 22 | | 19 | | 32 | | 20 | | | | 23 | | 21 | |
| **Manifestations** | **Cerebral malaria**<br>- Coma.<br>- Hyperpyrexia<br>- Convulsion.<br>- Hallucination.<br>- Neck stiffness.<br>- Sever anemia.<br>- Thrombocytopenia.<br>- Hypoglycemia.<br>- Black water fever. | | | | | | **Sever malaria**<br>-Drowsy.<br>-Hyperpyrexia.<br>-Hallucination.<br>- Sever anemia.<br>- Thrombocytopenia.<br>- Hypoglycemia.<br>- Black water fever.<br>- Anuria. | | | | | | | |
| **Laboratory Features** | - Hypoglycemia < 50 mg/dl.<br>- Metabolic acidosis.<br>- Severe anemia< 7 gm/dl.<br>- Thrombocytopenia< 150/ml<br>- Hemoglobinuria.<br>- Renal impairment.<br>- Hyperparasitamia. | | | | | | | | | | | | | |
| **Malaria species** | ***P. falciparum*** | | | | | | | | | | | | | |
| **Treatment** | **Coma:**<br>- Maintain airway<br>- Ventilator support, cardiac monitoring.<br>- Exclude other treatable causes of coma (e.g. hypoglycemia, bacterial meningitis).<br>- Avoid harmful treatments.<br>**Hyperpyrexia:**<br>- Administer tepid sponging and antipyretic drugs.<br>- Paracetamol is preferred over more nephrotoxic drugs.<br>**Correction of fluids, electrolytes and acid base balance.**<br>**Blood transfusion.** **Specific treatment.**<br>**Artesunate is drug of choice** 2.4mg/kg iv at 0hr, 12hr and 24h then daily for 6 days till pt can take orally then once daily (2 mg/kg per day by mouth) to complete 7 days. Then doxycycline 200 mg daily by mouth for 7 days **[26]**. | | | | | | | | | | | | | |
| **Outcome** | - Anemia | | | Death | | | R. impairment | | | | Cured | | | |
| **Frequency** | - 2 (4%) | | | 1 (2%) | | | 1 (2%) | | | | 2 (4%) | | | |

# Descriptive Data

**Table (2) :** Diagnostic characteristics of Thick and Thin blood film in diagnosis of Malaria

| Character | Value | (95% CI) |
|---|---|---|
| **Thick blood film** | | |
| Sensitivity % | 98 | 93.7-98.0 |
| Specificity % | 100 | 100.0-100.0 |
| Positive Predictive value (PPV) % | 100 | 100.0-100.0 |
| Negative Predictive value (NPV) % | 98.5 | 93.2-98.5 |
| Diagnostic accuracy (DA) % | 100 | 100.0-100.0 |
| **Thin blood film** | | |
| Sensitivity % | 84 | 79.7-84.0 |
| Specificity % | 100 | 100.0-100.0 |
| positive Predictive value (PPV) % | 100 | 100.0-100.0 |
| Negative Predictive value (NPV) % | 93 | 95.8-93.0 |
| Diagnostic accuracy (DA) % | 100 | 100.0-100.0 |

# Descriptive Data N.B: Total=50, CI: Confidence interval

**Table (3) :** Therapeutic responses & Clinical outcome of the studied patients

| **Therapeutic responses** | **Response to single (ttt) of Alexaquine or mefloquine** | **Response to combined(ttt) (Artesunate+ pyrimethamine+ Sulphadoxine)** | **Resistance to single responsive to combined (ttt)** | **Total** |
|---|---|---|---|---|
| **Frequency** | 5 | 42 | 3 | 50 |
| **Percent %** | 10 | 84 | 6 | 100 |

| **Clinical outcome** | **Cured** | **Recurrence** | | **Complications** | | | **Death** | **Total** |
|---|---|---|---|---|---|---|---|---|
| | | **Recurred** | **Relapse** | **Anemia** | **Splenomegaly** | **Renal impairment** | | |
| **Frequency** | 41 | 2 | 1 | 2 | 2 | 1 | 1 | 50 |
| **Percent %** | 82 | 4 | 2 | 4 | 4 | 2 | 2 | 100 |

# Descriptive Data

**N.B:** @ Recurred Malaria; is a new malaria infection after new mosquito bite. @ Relapsed Malaria occurs after primary attack due to reactivation of hypozoites of parasite in liver up to 3-5 years after primary infection.

**Table (4) :** Comparative study between complicated & non-complicated malarial-infected patients

| Variables (No=50) | Non-complicated Malaria (n=44) | Complicated Malaria (n=6) |
|---|---|---|
| **Age** | 19-43 yrs | 19-34 yrs |
| **Symptoms** | Intermittent or Remittent fever.<br>Conscious.<br>Dark urine.<br>Vomiting. | Continuous high grade fever.<br>Comatose or drowsy.<br>Black water fever. |
| **Signs** | Mild to moderate anemia<br>Hypoglycemia.<br>Enlarged liver, spleen or both | Severe anemia.<br>Hypoglycemia<br>Hepatoslenomegaly. |
| **Past history** | Malaria infection | No malaria infection |
| **Chemoprophylaxis** | Yes or incomplete course or no Chemoprophylaxis | Incomplete course or no Chemoprophylaxis |
| **Laboratory Investigations** | **Hb** 7-15 gm/dl<br>**Platelets** 50 -450/ml<br>**Bilirubin** 0.5- 1.0<br>**ALT** 25- 75<br>**AST** 20- 70<br>**Creat** 0.7-1.4 | **Hb** <7 gm/dl<br>**Platelets** <50/ml<br>**Bilirubin** >1.0<br>**ALT** 40- 100<br>**AST** 40 - 100<br>**Creat** >1.4 |
| **U/S** | **Liver size** 13-15 cm<br>**Spleen size** 11-14 cm | **Liver size**>15 cm<br>**Spleen size**>14 cm |
| **ICU** | No- admitted | Admitted |
| **Line of treatment** | Mono therapy **or** Poly therapy | Poly therapy |
| **Outcomes** | - 39 Cured<br>- 2 splenomegally<br>- 3 Recurrence | - 2 Cured<br>- 2 Anemia<br>- 1 Renal impairment<br>- 1 Death |

# Descriptive Data

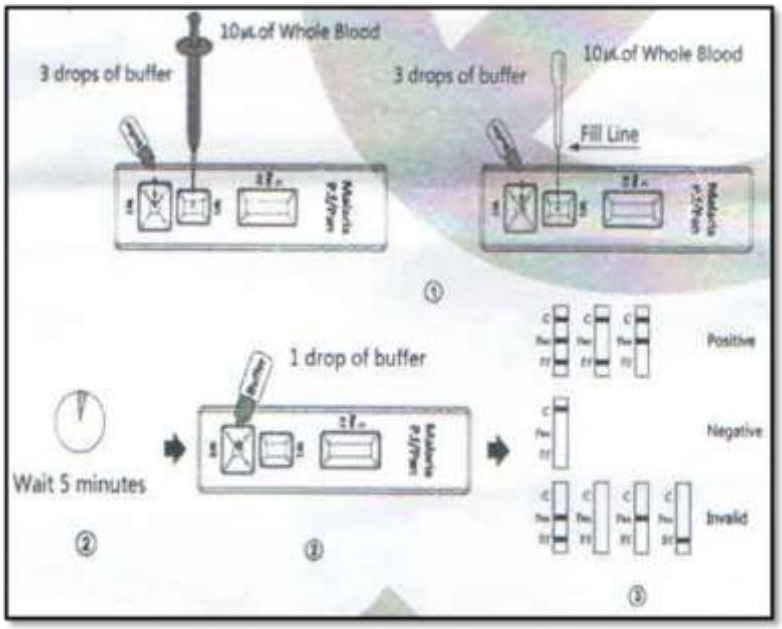


**Figure (1):** Diagram of rapid diagnostic test of whole blood malarial antigen (Bio Tina@Gmbh, www.eu-biotina.com)\

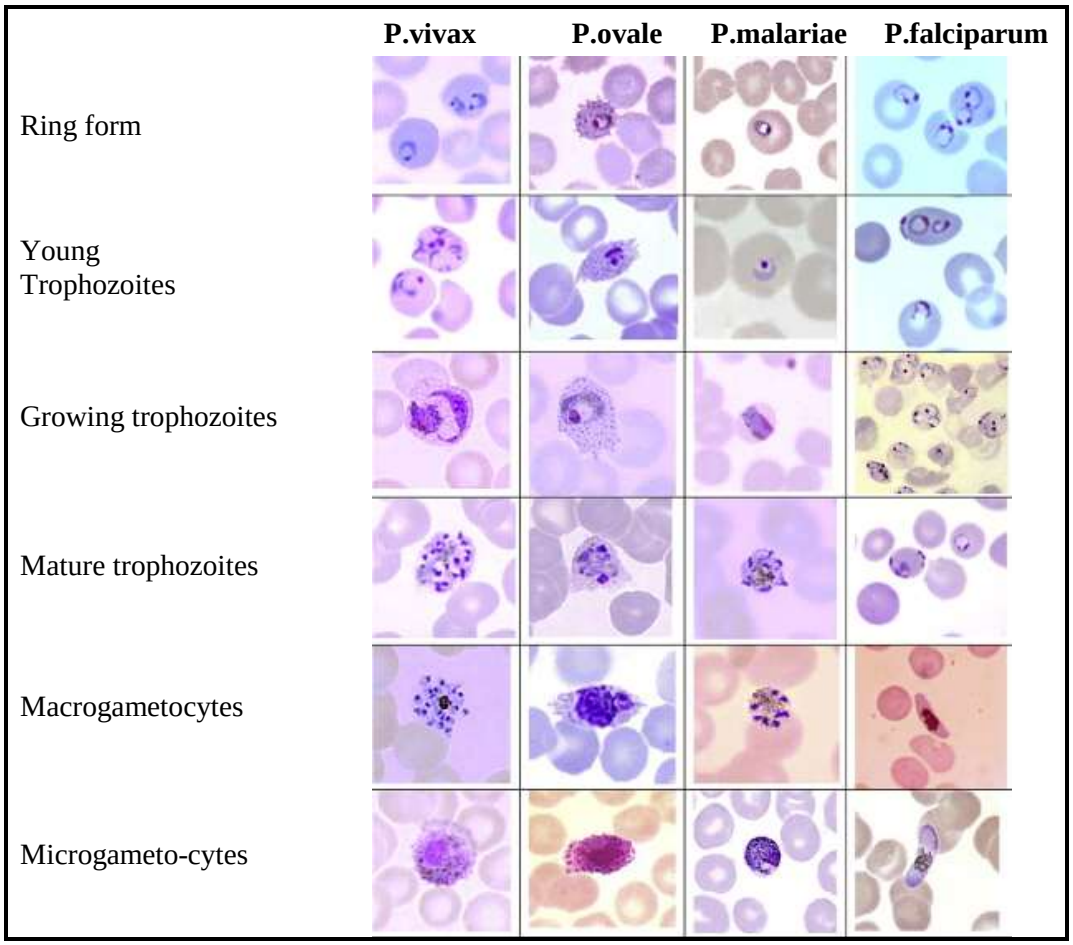


**Figure (2):** Blood stages of *Plasmodium* **[7]**

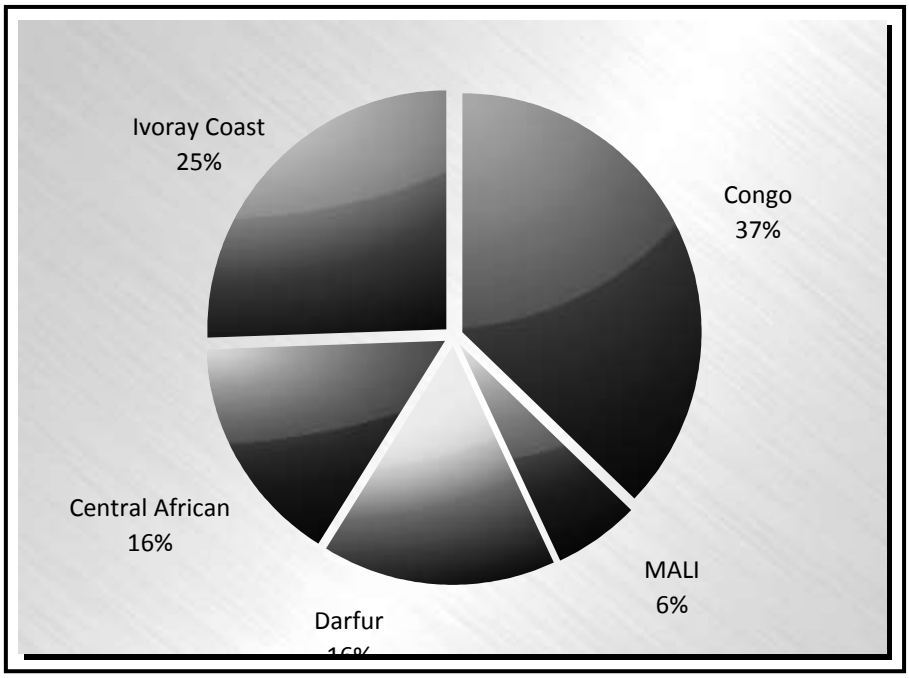


**Figure (3):** Geographical distribution of malaria parasite among studied cases (in Africa).

## DISCUSSION

Malaria remains one of the major killers of humans' population worldwide, threatening the lives of more than one third of the world's population **[1]**. In Egypt, Malaria is eradicated since 1998 until June 14, 2014. Between lates May to mid-June, 19 locally-acquired P. vivax malaria cases were identified in one village of the Aswan Governorate **[8]**. Few Egyptian studies had assessed the status of malaria infection in Egypt.

The study shows that the Egyptian malarial patients were recruited from malarial areas in Africa. Congo was the most malaria-infected place (36 %) and Ivory Coast coming next in frequency (26%). The reasons are not clear, although it is thought renewed fighting by militia groups has made it increasingly difficult for people to access prevention and treatment **[9]**.

Fever was the main presenting symptoms of malaria among all the studied patients (100%). This is in consistent with Ella et al. **[10]** who explained that fever is a characteristic feature of P. falciparum infection. The symptoms of malaria include paroxysms defined by intense chills; fever and sweating caused as new merozoites burst from the erythrocyte and infect more cells **[11].** In the current study the majority of patients experience fever, sweating and rigors (100%), myalgia (86%), headache (76%). This is in consistent with He et al. **[12]** who explained that patients with no or incomplete chemoprophylaxis, like patients in the current study, had fever, sweating, rigors (96%), headache (74%) and myalgia (86%) in those with malaria.

Five patients (10%) had black water fever (black urine, fever, drowsiness, sweating and other symptoms suggestive of severe malaria). This is the clinical manifestation of This is comparable with study carried out by Bodi et al. **[13]** who explained that rainy season, low parasitaemia were the major risk factors significantly associated with haemoglobinuria, black water fever first described in non-immune European expatriates who lived in endemic malaria regions.

Six of the studied malarial patients (12%) in the current study, was admitted at ICU. Three of them (6%) were comatosed have "Glasgow Coma Scale" less than 4 as cerebral malaria and other three patients were drowsy have GCS less than 9 presenting with sever malaria manifestations. These patients had hyperpyrexia, convulsion, hallucination, neck stiffness, severe anemia (<7gm/dl.), thrombocytopenia (<50/ml), hypoglycemia (<50mg/dl) and black water fever, positive rapid diagnostic test and blood smear showed causative species was P. falciparum. This is in agreement with Cserti-Gazdewich et al. **[14]** who explained that 4.5% of his studied malarial patients were comatosed and severe anemia was observed in 65% of patients with severe malaria.

The current study reveals that ten patients (20%) had taken doxycycline, eight patients (16%) had taken mefloquine as chemoprophylaxis and thirty-two patients (56%) had not received any previous preventive antimalarial drugs**.** Twenty patients (40%) among those who received doxycycline and those who did not received any preventive therapy, had past history of previous malarial infection. Though mefloquine was used in (16%) of cases, yet it had a better clinical outcome, that was proved previously by Schlangenhauf **[15]** and also documented in 2006 by federal ministry of health Sudan/WHO/ National malaria central program **[16]**.

A congress of the WHO produced a document entitled new perspectives in Malaria Diagnosis (WHO/MAL/2000.1091) **[17]**. Certain recommendations were presented on non-microscopic rapid diagnostic tests (RDT). The document concluded that results from these test devices were accurate. Also, it was sensitive (98-100%) in detecting of parasitaemia and its ability to distinguish viable parasites from parasite products such as antigens or nucleic acids not associated with viable organisms and also to indicate the prediction of treatment outcomes or resistance to common antimalarial drugs **[18].**

The present study showed that the thick blood film method was the most sensitive test (98%) while the thin film was much easier in identifying Plasmodium species. This is in consistent with Rescigno and Borrow **[19]** who reported that the thick blood film concentrates the layers of red blood cells (RBC) on a small surface by a factor of 20 to 30 and is stained as an unfixed preparation using fields stain or diluted wrights or Giemsa stain. The thick blood film provides enhanced sensitivity of the blood film technique and is much better than the thin film for detecting the low levels of parasitaemia and reappearance of circulating parasites during infection recrudescence or relapse **[19]**.

The thin blood film is often preferred for routine estimation of the parasitaemia because the

organisms are easier to see and count. The ability to count parasites in sequential blood films enables the response to therapy to be monitored, particularly for P.falciparum infections **[20]**.

The Plasmodium falciparum was the commonest species found among the studied cases (80%) and P.ovale is coming next in frequency (20%)**.** These were explained that P. falciparum is found in tropical and subtropical areas, and especially in Africa where this species predominates **[21]**.

Concerning the therapeutic responses of the studied cases, the best response in studied cases was detected with poly-therapy (84%) such as Artesunate plus sulphadoxine plus pyri-methamine (Artecospe) while mono-therapy as Mefloquine was effective in 5 patients (10%). This agrees with Luyendyk et al. **[22]** and documented in 2006 by Federal Ministry of Health Sudan WHO/National malaria central Programme **[16]**. It seems that the efficacy of variable lines of therapy is different according to variations in place and time **[22]**.

Concerning the clinical outcomes of the studied cases, Most of the studied malarial-infected patients (82%) were cured and discharged from hospital when they had been shown clinical improvement, mainly the fever; if present we followed them up after 15 and 30 days from discharge. Some patients (10%) had been discharged from hospital with complications like anemia, renal impairment and splenomegaly for follow up, three patients (6%) admitted again after 2-6 months with recurrence of malarial infection and one patient died. Because he did not received chemoprophylaxis during his trip to Africa, so he was infected with P. falciparum that was complicated by cerebral malaria, this is the same outcome that was described by Suh et al. **[23]** and Geerligs et al. **[24]**. Artesunate was the drug of choice for cerebral malaria 2.4mg/kg iv at 0hr, 12hr and 24h then daily till patient can take it orally, paracetamol is preferred over more nephrotoxic drugs for hyperpyrexia, blood transfusion for severe anemia and correction of fluids, electrolytes and acid base balance **[25,26]**.

Concerning the comparative results between complicated & non-complicated malarial cases in the present study, younger age patients with no past history of malaria infection or chemo-prophylaxis were liable to complicated malaria.

Therefore, Malaria should be considered in the febrile patient even with a normal WBC who coming out of an endemic area, malarial-infected younger age people with no past history of malaria infection or chemoprophylaxis were liable to complicated malaria.

## CONCLUSION

In Egypt, no endogenous malaria cases have been reported since1998 until 2014 a malaria outbreak was discovered in Aswan. Malaria cases in Egypt are usually coming from endemic area in Africa. Thick film is the most sensitive and informative test among all diagnostic test modalities. Mortality due to malaria disease is unusual, and may result from improper chemoprophylaxis and missed diagnosis or late treatment. Combined therapy (polytherapy) is preferable than monotherapy, as it is more effective among the studied malarial cases. Tools of prophylaxis are insect repellents, protective clothes, bed nets and chemoprophylaxis.

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# A study of the Role of 25 Hydroxy-Cholecalciferol Level on Non Alcoholic Fatty Liver Disease (NAFLD) in a Cohort of Egyptian Patients

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*Key words: NAFLD, vitamin D supplementation, fatty liver*

**Background and study aim:** Epidemiological and experimental data correlated hypovitaminosis D to the pathogenesis of NAFLD. So, the aim of this study was to evaluate the role of vitamin D in NAFLD patients with hypovitaminosis D.

**Patients and Methods:** We studied 78 consecutive patients with biopsy-proven NAFLD. Patients were divided into 2 groups according to serum level of 25 (OH) D; group I; have deficient 25 (OH) vitamin D (<50nmo/L) and group II; have sufficient 25 (OH) vitamin D (50-70 nmol/L). Liver injury profile (ALT, AST), lipid profile (LDL, HDL and triglycerides), inflammatory marker (CRP) as well as histopathological assessment according to NAS scoring were evaluated at baseline. Vitamin D supplementation for 24 weeks was given for both populations with follow up evaluation of laboratory parameters at the end of the study.

**Results:** Patients with deficient 25 (OH) vitamin D levels had significantly more severe NAFLD than those with sufficient 25 **(**OH) vitamin D levels at baseline. After 24 weeks of high dose vitamin D supplementation there was significant improvement in lipid profile (LDL, HDL, and triglycerides), hepatic transaminases (ALT, AST) and CRP in NAFLD patients with hypovitaminosis D, but no significant changes in NAFLD patients with sufficient vitamin D.

**Conclusion:** Correction of hypovitaminosis D may have beneficial effects on NAFLD in patients with moderate to severe activity but no effects in case of sufficient vitamin D.

## INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) has become the most common chronic liver disease worldwide **[1]**, with an estimated prevalence of 20-30%in general population and 75% in patients with type 2 DM, obesity and metabolic syndrome **[2,3]**. NAFLD is a pathological condition characterized by aberrant triglycerides accumulating in the hepatocytes, in some cases accompanied by necro-inflammatory activity and fibrosis (steatohepatitis) and potentially evolving into liver cirrhosis **[4]**. However, beside lifestyle intervention, no established therapy of NAFLD has been identified yet **[5].** Non-alcoholic fatty liver disease is the hepatic component of metabolic syndrome where the insulin resistance increases non esterified fatty acids release from adipose tissue and favor their deposition into hepatocytes **[6]**.

Low vitamin D levels have been associated with the histological severity of NAFLD/NASH **[7]**. Overall, a 26 % additional risk for vitamin D deficiency has been reported in NAFLD subjects **[8]**. A strong epidemiological overlap also exists between NAFLD and hypovitaminosis D prevalence, as both conditions are widely spread among obese dys-metabolic individuals **[9]**. It has been recently recognized that vitamin D has other functions in addition to its role in bone metabolism **[10]**. It has an emerging role in regulating inflammation and immunomodulation **[11,12]**. Also, it is known to suppress pro-inflammatory cytokines and increases IL-10 level **[13]**.

Furthermore, data from meta-analyses point towards the existence of an association between low circulating vitamin D levels and NAFLD **[8]**. However, Sharifi et al. **[14]** and Barchetta et al. **[15]** reported no beneficial effect of vitamin D supplementation in NAFLD patients.

So, the aim of the present study was to evaluate the effect of vitamin D supplementation on specific sub-populations of patients with fatty liver not studied in the previous trials, namely NAFLD patients with moderate to severe liver disease.

## PATIENTS AND METHODS

Study population was recruited among patients referred to hepatology clinic of Zagazig university hospitals, Egypt. Between December 2016 and June 2017, 78 patients proved to have NAFLD were divided according to 25 (OH) cholecalciferol serum level into 2 groups: Group I: patients have deficient 25 (OH) cholecalciferol level (<50 nmol/L). Group II: patients have sufficient but sub-optimal 25 (OH) cholecalciferol level (50-75 nmol/L).

To be eligible for the study, patients had to satisfy the following criteria: fatty liver detected by abdominal ultrasonography (US) and confirmed by histopathology, ALT is more than AST, negative tests for HBsAg and HCV Abs.

**Exclusion criteria include :**
History of alcohol abuse (as defined by an average daily consumption of alcohol >30 g/day in men and >20 g/day in women), cirrhosis, autoimmune hepatitis and other causes of liver disease (hemochromatosis, Wilson's disease), hyper/ hypoparathyroidism, known hypersensitivity to cholecalciferol, hypercalcemia, hypercalciuria, nephrolithiasis, nephrocalcinosis.

All patients were subjected to:

1- Thorough history taking with full clinical examination and all medications were carefully recorded at baseline visits and drug alterations regarding anti-diabetic agents, anti-hypertensive treatments. Statins were not allowed throughout the study.

2- The body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters.

3- Serum 25 (OH)-cholecalciferol was measured as an indicator of vitamin D status **[16]**.

4- Liver ultrasound scanning was performed to assess the presence of hepatic steatosis.

5- Evaluation of lipid profile (LDL, HDL, triglycerides), glycemic control (HbA1c %) and liver enzymes (ALT, AST) before and after vitamin D supplementation.

6- Liver biopsy for all patients at base line, to confirm the diagnosis and for histological grading of NAFLD, using Kleiner NAFLD activity score (NAS). This score is the sum of three histological components; steatosis (0-3), lobular inflammation (0-3) and ballooning degeneration (0-2). It ranges from 0 to 8 **[17]**.

7- Vitamin D supplementation in the form of oral solution of cholecalciferol (50,000 IU/week) for 24 weeks for both groups.

**Statistical Analysis**
Data were analyzed with SPSS version 16 for data processing and statistics. Quantitative data were expressed as mean±standard deviation (SD) and were analyzed by independent t test for unpaired data. Paired quantitative data were analyzed by paired t test. While Wilcoxon test has been used to analyze qualitative paired data and Man-Whitney test has been used for qualitative unpaired data. P-value was considered significant if <0.05 and highly significant if <0.001.

**Ethical Considerations**
Since the patients have elevated liver enzymes with no obvious cause, some patients had family history of liver diseases and liver malignancy and imaging evident of focal fat and focal sparing, liver biopsies were done to detect the underlying cause and written consent was obtained from all patients before the study.

The study protocol was reviewed and approved by the Institutional Review Board of the faculty of Medicine, Zagazig University, Egypt.

## RESULTS

Out of 78 patients who met the inclusion criteria of the study, 40 patients had deficient 25 (OH) cholecalciferol (<50 nmol/L) and allocated to group I, while 38 patients had sufficient but sub-optimal 25 (OH) cholecalciferol (50-75 nmol/L) and allocated to group II (mean for group I; 21.41±12.3 vs. group II; 61.97±6.84, while the

mean for healthy subjects was 73±7.53) (Figure 1). No major adverse events occurred during the study, highlighting safety of the dosing and no change in the ongoing therapies throughout the study. Regarding the cause of NAFLD, there was no statistically significant difference between both populations (data were not shown).

The mean age of all patients was 41.14±9.88 years with males represented 65%. Both groups were age and sex matched. After oral cholecalciferol supplementation, serum 25 (OH) D levels significantly increased in both groups (mean for group I; 21.41±12.3 to 45.2±12.5 vs. mean for group II; 61.97±6.84 to 77.42±20.1).

As regard baseline biochemical parameters, there were statistically significant increases in LDL; (133.2 ± 43.69 vs. 101.61 ± 22.18, p value <0.001), triglycerides; (185.8±73.6 vs. 124.1±34.1, p value <0.001), transaminases (ALT; 65.26±38.88 vs. 49.66±26.10, P value; 0.042, AST; 55.13±22.08 vs. 40.71±25.26, P value; <0.001), and histological grading in group I when compared to group II, while HDL showed statistically significant decrease (38.88±9.98 vs. 44.32±7.62, p value 0.008).

After 24 weeks of vitamin D supplementation, there was statistically significant improvement in LDL, HDL, triglycerides, ALT and AST in group I (Table 2), while there was non-significant change in group II (Table 2).

Finally, it was noted that at baseline there was non-significant difference between both groups regarding the anti-inflammatory marker (CRP); 3.4±1.3 vs. 3.4±1.3, p value 0.213. After vitamin D supplementation there was statistically significant improvement of CRP in both populations.

When comparing both groups after vitamin D supplementation, there were non-significant differences regarding hepatic indicators, lipid profile and HbA1c%, but there were significant differences in CRP (Table 3).

Moreover, we performed a correlation analysis that showed a significant association between NAFLD scores, serum 25(OH)-cholecalciferol levels, lipid profile and histological grading (Table 4).

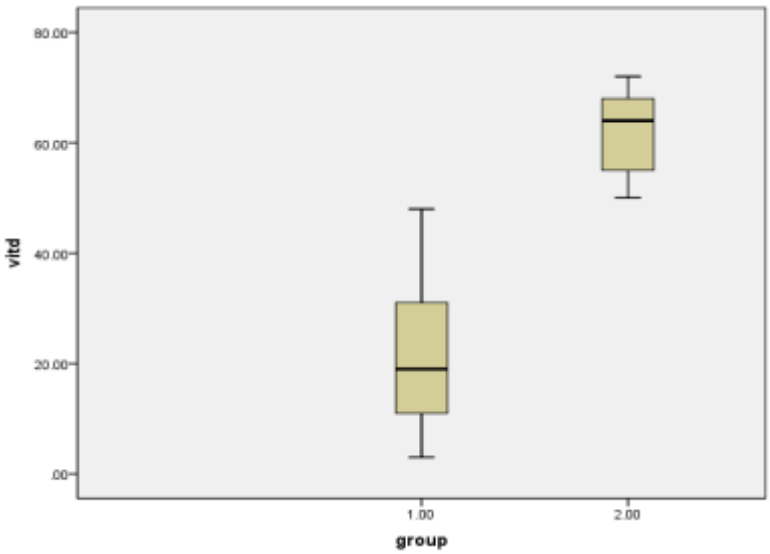


**Figure 1:** vitamin D levels among both populations

**Table (1);** Baseline clinical and biochemical characteristics of both groups:

| | **Group I (NO=40) X±SD** | **Group II (NO=38) X±SD** | **P value** |
|---|---|---|---|
| Age | 41.70±9.66 | 40.58±10.10 | o.619 |
| Gender | Male:28 Female:12 | Male:23 Female :15 | 0.477 |
| BMI | 29.190±2.337 | 28.979±3.143 | 0.739 |
| 25 (OH) D (nmol/L) | 21.41±12.30 | 61.97±6.84 | <0.001 |
| LDL (mg/dl) | 133.2±43.69 | 101.61±22.18 | <0.001 |
| HDL (mg/dl) | 38.88±9.98 | 44.32±7.62 | 0.008 |
| Triglycerides (mg/dl) | 185.8±73.6 | 124.1±34.1 | <0.001 |
| HbA1c (%/mmol/mol) | 8.17±1.67 | 7.31±2.27 | 0.062 |
| AST (IU/l) | 55.13±22.08 | 40.71±25.26 | 0.009 |
| ALT (IU/l) | 65.26±38.88 | 49.66±26.10 | 0.042 |
| CRP (mg/dl) | 3.4±1.3 | 3±1.5 | 0.213 |
| NAS | 5±2.05 | 3±2 | <0.001 |

**Table 2:** Comparison of characteristics before and after study treatment in both groups

| | **Group I (No=40)** | | **P** | **Group II (No=38)** | | **P** |
|---|---|---|---|---|---|---|
| | **Baseline X±SD** | **24 weeks X±SD** | | **Baseline X±SD** | **24 weeks X±SD** | |
| **Vitamin D(nmol/L)** | 21.41±12.30 | 45.20±12.46 | <.001 | 61.97±6.84 | 81.5±9.27 | <.oo1 |
| **ALT (IU/l)** | 65.26±38.88 | 50.05±28.04 | <.001 | 49.66±26.10 | 48.34±22.45 | 0.699 |
| **AST (IU/l)** | 55.13±22.08 | 35.42±19.32 | <.001 | 40.71±25.26 | 40.68±23.52 | .914 |
| **LDL (mg/dl)** | 133.20±43.6 | 115.96±35.24 | <.001 | 97.79±16.22 | 98.05±16.07 | 0.826 |
| **HDL (mg/dl)** | 38.88±9.98 | 41.91±7.66 | <.003 | 44.76±7.02 | 45.08±6.71 | 0.275 |
| **Triglycerides (mg/dl)** | 185.8±73.6 | 139.3±68 | <.001 | 124.13±34.13 | 122.61±31.1 | 0.250 |
| **HbA1c% (%/mmol/mol)** | 8.17±1.67 | 6.70±1.52 | <0.046 | 7.31±2.27 | 6.69±1.52 | 0.157 |
| **CRP (mg/dl)** | 3.4±1.3 | 1±0.6 | <0.001 | 3±1.5 | 1.5±0.8 | <0.001 |

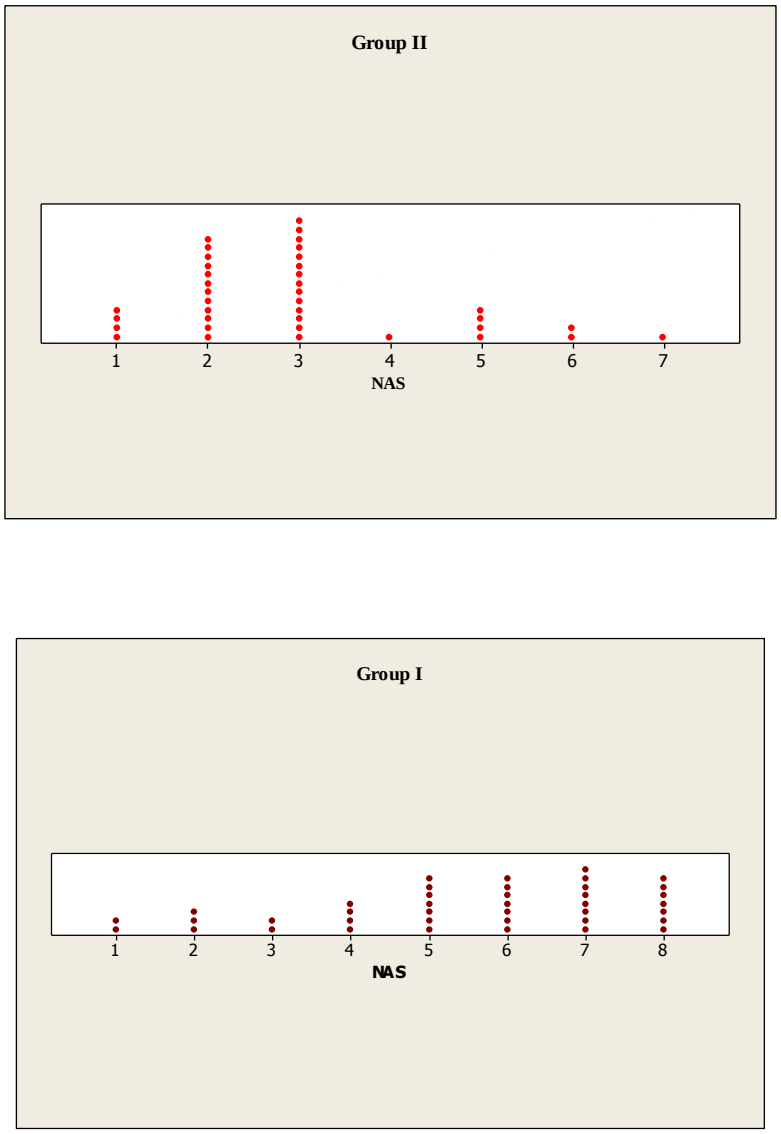


**Figure 2:** Distribution of NASH according to activity grade.

**Table 3 :** Biochemical characteristics after vitamin D supplementation in both groups

| | **Group I NO=40 X±SD** | **Group II NO=38 X±SD** | **T test** | **P value** |
|---|---|---|---|---|
| Vitamin D (nmol/L) | 45.20±12.5 | 77.42±20.1 | -8.61 | <0.01 |
| ALT (IU/l) | 50.05±28.04 | 48.34±22.5 | 0.30 | 0.768 |
| AST (IU/l) | 35.42±19.32 | 40.68±9.67 | -1.51 | 0.136 |
| LDL (mg/dl) | 106.1±24.4 | 98.1±16.1 | 1.73 | 0.088 |
| HDL (mg/dl) | 41.91±7.66 | 45.08±6.71 | -1.95 | 0.055 |
| Triglycerides (mg/dl) | 139.1±55 | 122.6±31.1 | 1.64 | 0.106 |
| HbA1c% (%/mmol/mol) | 6.70±1.51 | 6.69±1.52 | 0.03 | 0.977 |
| CRP (mg/dl) | 1±0.6 | 1.5±0.8 | 22.36 | <0.001 |

**Table 4:** Correlation between vitamin D and different parameters before vitamin D supplementation

| Term | Coefficient | P value |
|---|---|---|
| **BMI** | -0.069 | .548 |
| **LDL** | -0.530 | <0.001 |
| **HDL** | 0.402 | <0.001 |
| **Triglycerides** | -0.545 | <0.001 |
| **NAS** | -0.928 | <0.001 |

## DISCUSSION

Vitamin D has been proposed as a potential therapeutic option for liver damage in NAFLD and non-alcoholic steatohepatitis (NASH) **[18]**. However, most studies aiming to test the efficacy of high dose vitamin D supplementation on NAFLD did not obtain any improvement in either fatty liver content, histological parameters related to NASH, or transaminases **[19]**. The present study was designed to investigate the role of 24-week oral vitamin D on specific group of NAFLD patients.

In this study, all subjects affected by NAFLD have sub-optimal serum 25 (OH)-cholecalciferol levels compared to age and sex matched subjects (data not shown); however, subjects with deficient levels display a significantly more severe liver disease than those with sufficient levels. Previously, an association between low 25 (OH)-cholecalciferol levels and the histological severity of NASH was suggested by Targher et al. **[7]**.

In this study, participants were recruited among patients referred to hepatology clinic. This allowed selection of patients with more severe liver disease as compared to those studied in previous trials **[14,15]**. The baseline biochemical tests as well as the histological activities were significantly different among study populations.

While vitamin D supplementation in the form of oral cholecalciferol (50,000 IU/week) for 24 weeks had beneficial effects on hepatic injury indicators, metabolic profile and CRP among patients with hypovitaminosis D, it could not restore the advanced liver injury among control patients with sufficient vitamin D.

In another study, similar response in patients with normal or reduced 25(OH)-cholecalciferol levels at the baseline was observed after vitamin D supplementation **[15]**. The reasons for this discrepancy between the results may be due to the differences in the disease activity, duration of the disease, doses of vitamin D supplementation and other possible cellular mechanisms between vitamin D and inflammatory cytokines not fully understood.

Also, Sharifi et al. **[14]** had reported that, twice a month 16-weeks cholecalciferol supplementation had no effects compared to placebo on amino-transferases, and insulin resistance in non-diabetic subjects selected on the basis of US-detected fatty liver and upper normal ALT levels. In this study, the intervention was limited to 4 months and the dose of cholecalciferol was 50.000 IU/2weeks. Moreover the disease activity was mild. These differences may explain the discrepancy with the recent study.

On the other hand, although there was no statistically significant difference at baseline regarding CRP, there was significant improvement in CRP among all patients, high lightening the anti-inflammatory effect of vitamin D **[21]**. In agreement with this result, significant improvement of the inflammatory marker (hs.CRP) has been reported after vitamin D supplementation in NAFLD patients **[14]**.

Serum vitamin D is inversely correlated to lipid profile and histological grading (table 4) in our study as well as other published reports **[7]**.

## CONCLUSION

Correction of hypovitaminosis D may has beneficial effects in NAFLD patients with moderate to severe activity, while vitamin D supplementation has no effect on NAFLD patients with sufficient vitamin D level. Larger, randomized, placebo-controlled trials are required to better evaluate the efficacy of vitamin D supplementation on NAFLD.

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# Risk of Ischemic Heart Disease in Patients with Non-alcoholic Fatty Liver Disease

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*Key words:*
*Fatty liver-Ischemic Heart disease - Metabolic syndrome*

**Background and study aim:** Non-alcoholic fatty liver disease (NAFLD) has an increasing prevalence worldwide. It has also been closely associated with obesity and metabolic syndrome - two conditions known to be associated with ischemic heart disease (IHD). The aim of this study was to assess the association between NAFLD and ischemic heart disease.

**Subjects and Methods:** 140 patients with NAFLD and 70 non-NAFLD subjects were selected. Full history taking, clinical examination and laboratory tests including blood sugar, lipid profile and liver profile were done. Ultrasonography was performed to prove NAFLD while ECG and echo-cardiography were used for detection of myocardial ischemia.

**Results:** Of the NAFLD group, the frequency of mild, moderate and severe NAFLD was 42.9%, 30% and 27.1% respectively. Subjects with NAFLD had a significantly higher BMI, waist circumference and weight compared to those of non NAFLD group (p=0.014, 0.0218 and <0.001 respectively). Independent risk factors for NAFLD were obesity, DM, high LDL, low HDL, waist circumference, glycated hemoglobin and IHD with odds ratios 1.09, 2.12, 1.01, 1.15, 1.13, 1.37 and 1.17 respectively. While independent risk factors for IHD included obesity, DM, high LDL, total cholesterol, triglycerides and the presence of NAFLD with odds ratios 1.31, 1.23, 1.19, 1.132, 1.68 respectively.

**Conclusion:** NAFLD was independently associated with increased risk of myocardial ischemia**.**

## INTRODUCTION

NAFLD has become the predominant cause of chronic liver disease in many parts of the world. An overall global prevalence of 25.24% was reported with the highest figures were derived from the Middle East (31.79%) and South America (30.45%) **[1].**

NAFLD is considered a multisystem disease affecting several extrahepatic organs and regulatory pathways **[2].** The major focus of NAFLD-related diseases has involved chronic liver disease, cardiovascular diseases and type 2 DM. There is also emerging evidence that NAFLD is linked to other diseases such as sleep apnea, colorectal cancer, osteoporosis, psoriasis and polycystic ovary syndrome **[3].**

Liver biopsy is considered the gold standard for diagnosing steatosis, but it is an invasive method associated with many adverse effects. Since US is relatively precise for the diagnosis of NAFLD, low-cost, risk-free and widely available, it has been a frequently used method **[4].** Magnetic resonance imaging either by spectroscopy or by proton density fat fraction is an excellent noninvasive quantitative modality for assessment of steatosis and is being widely used in NAFLD clinical trials **[5].**

Patients with NAFLD have been shown to have increased mortality. The main cause of morbidity and mortality in these patients was cardiovascular disease **[6]**. Several studies have linked fatty liver to ischemic heart disease but they present controversial results. Some demonstrated an increased cardiovascular risk in NAFLD while others argued against this relationship. So, the role of NAFLD as an independent cardiovascular risk factor is still debated **[7]**.

**The aim:**
To assess the possible relationship between non-alcoholic fatty liver disease and its associated risk factors on one hand and ischemic heart disease on the other hand.

## SUBJECTS AND METHODS

**Subjects :**
The study was conducted on 210 patients (140 patients with ultrasonography-proven NAFLD and 70 subjects with no evidence of NAFLD serving as controls) attending Benha Teaching Hospital and Benha university hospital during the period from March 2016 to April 2017. All patients informed about the research and asked for their permission and consent before enrollment in the study.

**Inclusion criteria:**
1. Patients of both sexes were included.
2. Age: 18-65.
3. Ultrasonography- proven NAFLD.

**Exclusion criteria:**
1. Advanced liver disease.
2. Hepatocellular carcinoma.
3. Comorbid liver disease and life threatening illness.

All Patients were subjected to thorough history taking, clinical examination including body mass index, weight and waist circumference and laboratory assessment including aspartate amino-transferase (AST), alanine aminotransferase (ALT), total & direct bilirubin, total serum cholesterol, serum triglycerides (TG), serum high-density lipoprotein (HDL) cholesterol, fasting glucose, HbA1C, hepatitis B surface antigen, and an antibody to hepatitis C virus.

**Diagnosis of NAFLD:** abdominal ultrasonography performed by an experienced radiologist was used for diagnosis of fatty liver. The criteria used were: increased echogenicity relative to the kidney, distal attenuation and loss of intra-hepatic architectural details. Fatty liver was classified according to the following items: echogenicity relative to kidneys (0-3), blurring of gall bladder wall (0-3), blurring of hepatic veins (0-3), blurring of portal vein (0-3), far gain attenuation (0-3). Grading was defined as mild (total scores of 2-6), moderate (7-10), and severe (11-15) fatty liver **[8]**. Diagnosis of NALFD was done following exclusion of significant alcohol intake, other causes of chronic liver disease and use of drugs that could cause fatty liver**.**

**Diagnosis of IHD:** Was done using a combination of electrocardiography (ECG) and echocardiography.

**Statistical methods:**
The collected data were analyzed using SPSS software (statistical package for social science) version 22.0 on IBM compatible computer. Continuous variables were expressed as mean ± standard deviation (M ± SD), and analyzed using Student's *t*-test. Categorical variables were expressed as number and percentage and analyzed using Chi-square test or Fisher's exact test. Multivariate logistic regression analysis was used. Variables found to be significant on the univariate analysis to identify independent factors associated with NAFLD and IHD. The level of significance was set at a *P*-value of <0.05.

## RESULTS

A total number of 210 participants were selected and divided into two groups; NAFLD group included 140 patients with ultrasonography-proven NAFLD (77 males & 63 females) with mean age 45.81±12.46 years and non-NAFLD group of 70 subjects with mean age 45.32± 10.56 years. Of the NAFLD group, the frequency of mild, moderate and severe NAFLD was 42.9%, 30% and 27.1% respectively. Subjects with NAFLD had a significantly higher BMI, waist circumference and weight compared to those of non NAFLD group (p=0.014, 0.0218 and <0.001 respectively). On the other hand, no significant difference was found between the two groups regarding the age, sex or height.

Regarding the evidence of myocardial ischemia, a statistically significant difference was found between NAFLD and non NAFLD groups with respect to the presence wall motion abnormalities

and ischemic changes in ECG as shown in (Table 2). There was also a highly statistical significant difference among different grades of NAFLD ($p<0.001$).

On multivariate logistic regression analysis, independent risk factors for NAFLD were obesity, DM, high LDL, low HDL, waist circumference, glycated hemoglobin and IHD with odds ratios 1.09, 2.12, 1.01, 1.15, 1.13, 1.37 and 1.17 respectively (Table 3). While independent risk factors for IHD included obesity, DM, high LDL, total cholesterol, triglycerides and the presence of NAFLD with odds ratios 1.31, 1.23, 1.19, 1.13, 1.68 respectively (Table 4). Both IHD and NAFLD shared common risk factors such as obesity, presence of diabetes and dyslipidemia (increased LDL, low HDL). The risk of IHD was also significantly more in higher grades of NAFLD**.** The odds ratios for the different grades of NAFLD were; 1.56, 2.11 and 2.61, for mild, moderate and severe NAFLD respectively.

**Table (1):** Clinical and laboratory characteristics of NAFLD and non-NAFLD groups

| | **NAFLD** | **Non NAFLD** | **Chi-square/ *t*-test** | |
|---|---|---|---|---|
| | **No (%)** | **No (%)** | **t/$X^2$** | **P-value** |
| **Sex** | | | | |
| Female | 63 (45%) | 31 (44.29% ) | 0.10 | 0.922 |
| Male | 77 (55%) | 39 (55.71%) | | |
| **Smoking** | 51 (36.4%) | 20 **(**28.6%) | 1.287 | 0.257 |
| **BMI** | | | | |
| **Normal** | 24 (17.1%) | 13 (18.6%) | 6.802 | 0.033* |
| **Over weight** | 41 (29.3%) | 32 (45.7%) | | |
| **Obese** | 75 (53.6%) | 25 (35.7%) | | |
| **DM** | 90 (64.3%) | 13 (18.5%) | 58.372 | <0.001** |
| | **Mean ± SD** | **Mean ± SD** | **t/$X^2$** | **P-value** |
| **Age (year)** | 48.81 ± 12.46 | 49.32 ±10.56 | | |
| **Height (cm)** | 159.0 ± 7.4 | 161.4 ± 8.4 | 25.146 | 0.321 |
| **Weight (kg)** | 75.5 ± 10.0 | 65.9 ± 8.2 | 9.422 | <0.001** |
| **Waist circumference (cm)** | 96.5 ± 11.4 | 90.3 ± 12.9 | 11.393 | 0.0218* |
| **BMI** (kg/m2) | 31.81±5.23 | 25.4±3.45 | 4.713 | 0.014* |
| **HDL** (mg/dL) | 38.4 ± 12.1 | 48.2 ± 12.2 | 3.487 | 0.004* |
| **LDL** (mg/dL) | 125.5 ± 32.3 | 104.3 ± 24.1 | 5.461 | 0.013* |
| **Total cholesterol** (mg/dL) | 215.6 ± 39.1 | 177.1 ± 30.8 | 3.730 | 0.009* |
| **Serum triglycerides** (mg/dL) | 179.7 ± 64.7 | 129.9 ± 52.8 | 3.256 | 0.012* |

**Table (2):** ECG and echocardiographic findings in different grades of NAFLD

| | Grades of NAFLD | | | | | |
|---|---|---|---|---|---|---|
| | Non | Mild | Moderate | Severe | Chi-square | |
| | N (%) | N (%) | N (%) | N (%) | $X^2$ | P-value |
| **Wall motion abnormalities** | | | | | | |
| Negative | 63 (90%) | 39 (84.8%) | 32 (76.2%) | 39 (75%) | 50.066 | <0.001** |
| Positive | 7 (10.00%) | 7 (15.2%) | 10 (23.8%) | 13 (25%) | | |
| **ECG changes** | | | | | | |
| Negative | 61( 87.2%) | 38 (82.6%) | 31 (73.8%) | 35 (67.3%) | 57.875 | <0.001** |
| Positive | 9 (12.8 %) | 8 (17.4%) | 11 (26.2%) | 17 (32.7%) | | |

**Table (3):** Multivariate logistic regression analysis of the risk factors for NAFLD

| | 95% Confidence Interval | | Odds ratio | P-value |
|---|---|---|---|---|
| | Lower | Upper | | |
| **Obesity** | 0.527 | 1.237 | 1.09 | 0.004* |
| **DM** | 0.875 | 3.547 | 2.12 | <0.001** |
| **IHD** | 0.943 | 2.1 65 | 1.17 | 0.005* |
| **Age** | 0.087 | 0.271 | 0.16 | 0.252 |
| **Waist circumference** | 1.00 | 1.15 | 1.13 | 0.019 * |
| **LDL** | 1.00 | 1.18 | 1.01 | 0.022* |
| **HDL** | 0.109 | 1.844 | 1.15 | 0.024* |
| **Total Cholesterol** | 0.498 | 0.977 | 0.52 | 0.156 |
| **S. Triglycerides** | 0.261 | 0.843 | 0.40 | 0.092 |
| **HBA1C** | 1.18 | 1.03 | 1.37 | 0.028* |

**Table (4):** Multivariate logistic regression analysis of the risk factors for IHD

| | 95% Confidence Interval | | Odds ratio | P value |
|---|---|---|---|---|
| | Lower | Upper | | |
| **Obesity** | 0.870 | 1.275 | 1.195 | 0.035* |
| **DM** | 0.932 | 2.085 | 1.234 | 0.032* |
| **NAFLD:** | 1.051 | 2.754 | 1.685 | 0.023* |
| **Mild.** | 1.071 | 2.524 | 1.56 | 0.021* |
| **Moderate.** | 1.356 | 3.341 | 2.11 | 0.016* |
| **Severe.** | 1.421 | 3.396 | 2.61 | 0.011* |
| **Age** | 0.068 | 0.573 | 0.105 | 0.373 |
| **Waist circumference** | 0.132 | 0.506 | 0.211 | 0.089 |
| **HDL** | 0.256 | 0.834 | 0.058 | 0.458 |
| **LDL** | 0.99 | 1.537 | 1.132 | 0.004* |
| **Total Cholesterol** | 1.012 | 1.732 | 1.31 | 0.010* |
| **S. Triglycerides** | 1.003 | 1.089 | 1.01 | 0.053 |
| **HBA1C** | 0.112 | 0.937 | 0.514 | 0.654 |

## DISCUSSION

Metabolic syndrome features are not only highly prevalent in NAFLD patients, but components of metabolic syndrome also increase the risk of developing NAFLD **[9]**. There is a growing body of evidence that NAFLD and its risk factors carry an increased risk of cardiovascular disease independent of traditional cardiovascular risk factors and metabolic syndrome **[10]**. The most common cause of mortality in patients with NAFLD was presumed to be myocardial ischemia irrespective of other co-morbidities **[11].** The possible contributing mechanisms are complex and heterogeneous including genetic predisposition, insulin resistance and dyslipidemia, chronic inflammation, oxidative stress, adiponectin deficiency and altered production of pro- and anticoagulant factors [**12]**.

The current study aimed to investigate the association ultrasonography-diagnosed NAFLD and risk of IHD diagnosed by ECG and myocardial motion abnormality by echocardiography. In this study NAFLD group had a significantly higher BMI and waist circumference than the non NAFLD group. This was also observed by Loomis et al. who reported a 5- to 10-fold increased risk of NAFLD in the obese and 10- to 14-fold risk in the morbidly obese subjects. They concluded that both prevention of weight gain and weight reduction are important for prevention and management of NAFLD **[13]**. This finding also agrees with what reported by Sasaki et al. who observed that all grades of obesity are associated with NAFLD **[14].**

In the current study, there was a statistically significant positive association between diabetes mellitus and NAFLD as the diabetic patients had an odds ratio of 2.12 regarding the risk of developing NAFLD as revealed on multivariate analysis. This result agrees with several studies reported a high prevalence of NAFLD in patients with diabetes mellitus and a mutual association between NAFLD and DM **[15,16].** Our study showed also a highly statistical significant difference between NAFLD and non-NAFLD groups as regarding the frequency of dyslipidemia. Patients with NAFLD had significantly higher levels of LDL cholesterol and triglycerides and lower levels HDL cholesterol than those of non-NAFLD group. Similar to our study Alper et al., Sun et al. and Kim et al. illustrated that high levels of triglycerides, LDL-cholesterol were significantly associated with increased risk of IHD **[17-19].**

Regarding the relationship between NAFLD and IHD, our study revealed a significantly positive association between degree of NAFLD diagnosed by ultrasonography and presence of ischemic heart disease. Finding was documented on both the univariate and the multivariate logistic regression analysis. The odds ratios for the various grades of NAFLD versus non- NAFLD group were 1.56, 2.11 and 2.61 for mild, moderate and severe NAFLD respectively. These data coincide with Pais et al. and Francque et al. who reported that there was a significant positive association between NAFLD and atherosclerotic process.They concluded that NAFLD represents an independent risk factor for coronary vascular disease **[20, 21]**. Our results also come in accordance with ***Zib et al., 2016*** in their prospective study *on* 4119 subjects after exclusion of coronary vascular disease or any known liver disease at baseline with a mean period of 7.6 years follow up **[12]**. They concluded that NAFLD was independently associated with the risk of adverse cardiovascular events. Furthermore, in a cross-sectional study on diabetic patients, Targher et al. found ultrasonography-diagnosed NAFLD to be associated with prevalent cardiovascular disease independent of classical risk factors, glycemic control, medications and metabolic syndrome features [**22**]. On the other hand**,** Chan et al. in their cross-sectional study on Asian diabetic patients reported that independent factors associated with IHD were older age, greater waist circumference, lower levels of physical activity and higher levels of HbA1c. They concluded that ultrasonography-diagnosed NAFLD was not associated with IHD. They explained this discrepancy in results by the differences in the study populations [**23**]. Pickhardt et al. also stated that steatosis was not found to be an independent risk factor for cardiovascular events on multivariate logistic regression analysis after controlling for other confounding risk factors **[24**].

Our study also showed that both NAFLD and IHD share common risk factors such as increased BMI, presence of diabetes and dyslipidemia. This result agree with what revealed by Lin et al. and Corey et al. who declared that the higher rates of IHD risk are associated with factors including hypertension, dyslipidemia, hyperglycemia and overweight. They also reported that IHD risk increased with the severity of fatty liver. They explained that patients with NAFLD have pro atherogenic lipid profile due to hepatic lipid concentration and insulin resistance **[25,26]**.

Some limitations of our study warrant to be mentioned. First, NAFLD patients in our study were not histologically diagnosed by biopsy. We diagnosed NAFLD using transabdominal ultra-sonography that does not identify fatty infiltration <30% and subjected to intra-and inter observer differences when making a diagnosis. However, it is a safe, available, of low cost, reasonably reliable and noninvasive method. To overcome this point, ultrasonography was performed by a single experienced operator. Second, assessment of ischemic heart did not depend on coronary angiography which is the gold standard but we used ECG and echocardiography instead. The usage of these non-invasive methods for diagnosing and grading NAFLD and also for detection of IHD risk have acceptable reliability and are practical tools of screening purpose **[8].** In addition, this was not a population-based study covering a wide geographic area or a large number of patients. So our results needs to be furtherly evaluated for validation on larger cohorts.

## CONCLUSION:

The current study revealed that presence of NAFLD is associated with increased risk of IHD. This association was not only due to common risk factors shared between the two entities such as obesity, dyslipidemia and diabetes, but also a direct independent positive relationship was found between them. So NAFLD can be considered as an independent predictor of myocardial ischemia.

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# Role of Hepatitis C Virus Core Antigen Assay in Blood Donors Screening at Zagazig University Hospitals

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*Key words:*
*HCV; HCV core antigen; HCV window phase*

**Background and study aim:** Hepatitis C virus (HCV) infection is a major public health problem worldwide. Blood donations screening achieved mainly by serological identification of HCV-Antibody (Ab), has largely reduced HCV transmission. HCV Core Antigen (CAg) tests have been introduced to supplement anti-HCV tests and HCV PCR analyses. CAg may be a useful screening test for identifying window phase of HCV infected patients whom are candidate for blood donations. The study aimed to evaluate diagnostic performance of HCV core antigen in comparison to HCV-RNA quantification and anti-HCV-Ab analyses in attendances of blood bank of Zagazig University hospitals.

**Patients and Methods:** The study was performed on 92 participants attending the blood banks of Zagazig University Hospitals for blood donation between May 2015 to November 2017. The participants were classified into two groups; group A, which included 46 donors (32 males and 14 females) with negative HCV antibody and group B, which Included 46 patients (30 males and 16 females) with positive HCV antibody. Clinical assessment, HCV AB detection by ELISA, Prototype ELISA for HCV core antigen for presence of HCV core antigen and HCV RNA Quantitative were done for all participants.

**Results:** No significant differences between both studied regarding sex and age. A high significant relation between HCV AB positivity and negativity as regard HCV PCR was found in both groups. A high significant relation between HCV core antigen positivity and negativity as regard HCV PCR. There was high significant relation between HCV core antigen positivity and HCV PCR in group A patients. There was a high significant relation between HCV core antigen positivity and HCV PCR in positive HCV antibody patients and statistically a high significant relation between HCV core antigen negativity and HCV PCR in group B patients.

**Conclusion:** HCV core Ag can be identified by serological ELISA. This assay is cheap, easily performed, and compatible with HCV PCR. Its application may prevent the vast majority of HCV transmissions caused by the transfusion of window phase donations.

## INTRODUCTION

Hepatitis C virus (HCV) is an overall disease. In 2008 about 15% of the Egyptian population, aged 15–59 years had antibodies to HCV and 10% (approximately 5 million persons) had chronic HCV infection **[1].** HCV transmission is continuous in Egypt, and the incidence rates have been estimated to be 2.4 per 1,000 person/ year **[2].**

The incubation period for newly acquired HCV infection ranges from two weeks to six months, however, viral replication can be detected as early as one week after exposure. It has been shown that total HCV core antigen levels correlate with HCV RNA levels. Core antigen kinetics run parallel to HCV RNA kinetics during chronic HCV infection. **[3].**

Diagnostic tests for hepatitis C can be divided into two general categories; serological assays that detect antibody to HCV (anti-HCV) and molecular assays that detect and/or quantify HCV RNA genomes within an infected patient. Serological assays have been subdivided into screening tests for anti-HCV, such as the enzyme immunoassay

(EIA), and supplemental tests such as recombinant immunoblot assay (RIBA). Supplemental anti-HCV tests are designed to resolve false-positive testing by EIA. Detection of HCV RNA in patient specimens by polymerase chain reaction (PCR) provides evidence of active HCV infection and is potentially useful for confirming the diagnosis and monitoring antiviral response to therapy **[4].**

Anti-HCV assays have several disadvantages, such as a high rate of false positivity, lack of sensitivity if used in the early window period of 45 to 68 days after infection, the inability to distinguish between acute (ongoing active, viremia), past (recovered), and persistent (chronic) infections, in addition to the possibility of false negativity with samples from immunocompromised patients, who may not have an adequate antibody response. Also, PCR analysis for measuring viral loads has some drawbacks including; expensive and requirement of technical skills **[5]**.

HCV core antigen (Ag) tests have been introduced to supplement anti-HCV tests or HCV PCR analysis **[6,7]**. It can be identified by routine serological ELISA in specimens from the early antibody–negative phase of HCV infection. It may be a useful test for identifying window phase blood donations from antibody negative donors infected with HCV **[8]**. These quantitative HCV Ag assays could be used for diagnosis of HCV infection as well as for monitoring of antiviral therapy **[9].** Furthermore, HCV Ag assay could also be useful in monitoring immuno-compromised patients and those undergoing regular hemodialysis **[10].**

The work aimed to evaluate the diagnostic performance of HCV core antigen in comparison to HCV-RNA quantification and anti-HCV-Ab analyses in blood donors.

## PATIENTS AND METHODS

**Study design and settings :**
This comparative cross section study included ninety-two participants who attended the blood bank of Zagazig University Hospitals. Between May 2015 to November 2017. Written consents have been taken from all included participants.

**Target population and sampling :**
Individuals attending the blood bank of Zagazig University Hospitals. Participants were classified into two groups; group A, which Included 46 patients (32 males and 14 females) with negative HCV antibody and group B, which Included 46 patients (30 males and 16 females) with positive HCV antibody.

**Exclusion criteria :**
Patients who had HBV infection, history of immune-suppressive drugs, DM and any manifestations of chronic liver disease were excluded from the study.

**Methods and study tools :**
All participants were subjected to the following workup:

- Thorough history taking and full clinical examination with special stress on manifestations of chronic liver disease.
- Laboratory workup including HBsAg, HCV Ab detection by ELISA, HCV core antigen Prototype by ELISA and HCV Quantitative RNA assay.

**Specimen collection and preparation:**
Venous blood sample withdrawn from each participant under complete aseptic conditions using wide bore needle and slowly withdrawn from antecubital vein to avoid RBCs hemolysis. The sample was added to a sterile vacutainer dry tube, allowed to be clotted for 30 minutes, and then separated by centrifugation in PCR unit. The resulted serum was divided into 2 aliquots under strict sterile conditions and stored at -80°C to be used for HCV-Ab levels, HCV core antigen and PCR. HCV antibodies (HCV-Ab) and hepatitis B surface antigen (HBsAg) were detected by ELISA (Dia Sorin Diagnostics, Italy). This kit complements qualitative methods depended on enzyme linked immunosorbent assays (ELISA). The procedures were done according to manufacturer's instructions **[11]**. HCV core Antigen was measured by QuickTiter™ HCV Core Antigen ELISA Kit CELL (BIOLABS, INC, USA). HCV quantitative RNA detection by Real time PCR was detected using a Cobas AmpliPrep/Cobas TaqMan HCV kit (Amplicor, Roche Diagnostics, Branchburg, NJ). The lower limit of detection of this assay was 16 IU/ml **[12].**

**Statistical analysis :**
Data were checked, entered and analyzed using SPSS 22 for Windows. Data were expressed as mean ± SD for quantitative variable, number and percentage for qualitative one. Chi-squared ($X^2$) or t test and paired t test were used when

appropriate. P<0.05 was considered significant. P<0.001 was considered high significant.

## RESULTS

Demographic data showed non significant difference between both groups as regard gender and age (p>0.05) (Table 1). positive HCV core Ag in group (A) was 4 cases (8.7%) and 37 cases (80.4%) in group (B) (Table 2). A high significant relation between HCV AB positivity and negativity as regard HCV PCR in both groups (p<0.001) (Table 3). A high significant relation between HCV core antigen positivity and negativity as regard HCV PCR (p<0.001) (Table 4). A high significant relation between HCV core antigen positivity and HCV PCR in group A patients (p<0.001) (Table 5). A significant relation between HCV core antigen positivity and HCV PCR in positive HCV antibody patients and also, a statistically significant relation between HCV core antigen negativity and HCV PCR in group B patients (p<0.001) (Table 6). positive HCV core Ag in 41 patients; 37 patients with positive HCV Ab and 4 patients with negative HCV ab in patients of both groups(Table7). The ability of HCV core Ag test to detect positive infected cases is 97.4% with positive predictive value of 100%, and the ability to exclude negative cases from truly negatives is 100% with negative predictive value of 70%, test accuracy is 97.8% (Table 8).

**Table (1):** Comparison between the studied groups as regard demographic data

| Demographic data | Group (A) (N=46) | | Group (B) (N=46) | | Test | p-value (Sig.) |
|---|---|---|---|---|---|---|
| | No. | % | No. | % | | |
| **Sex** | | | | | | |
| Male | 32 | 69.5% | 30 | 65.2% | 0.377‡ | 0.539 (NS) |
| Female | 14 | 30.5% | 16 | 34.8% | | |
| **Age (years)** | | | | | | |
| ≤ 20 years | 13 | 28.2% | 12 | 26% | 0.040‡ | 0.980 (NS) |
| 20-30 years | 15 | 32.6% | 16 | 34.8% | | |
| 30-40 years | 11 | 23.9% | 10 | 21.7% | | |
| ≥ 40 years | 7 | 15.3% | 8 | 17.5% | | |

‡ Chi-square test. * Independent samples Student's t-test.
• Mann Whitney U test. P< 0.05 is significant. Sig.: Significance.

**Table (2):** HCV seromarkers in both groups

| HCV seromarker | Group A (N=46) | | Group B (N=46) | |
|---|---|---|---|---|
| | No. | % | No. | % |
| **HCV Ab** | | | | |
| Negative | 46 | 100% | 0 | 0% |
| Positive | 0 | 0% | 46 | 100% |
| **HCV core Ag** | | | | |
| Negative | 42 | 91.3% | 9 | 19.6% |
| Positive | 4 | 8.7% | 37 | 80.4% |

‡ Chi-square test. P< 0.05 is significant. Sig.: Significance.

**Table (3):** HCV RNA levels among studied groups

| HCV RNA levels by PCR | Group A (N=46) | | Group B (N=46) | | Test‡ | p-value (Sig.) |
|---|---|---|---|---|---|---|
| | No. | % | No. | % | | |
| <16 IU/ml | 41 | 89.1% | 7 | 15.2% | 64.720 | <0.001 (HS) |
| 16 <$10^4$ IU/ml | 2 | 4.3% | 16 | 34.8% | | |
| >$10^4$ – <$10^6$ IU/ml | 1 | 2.3% | 15 | 32.6% | | |
| >$10^6$ IU/ml | 2 | 4.3% | 8 | 17.4% | | |

<16 IU/ml (below level of detection) ‡ Chi-square test.
P< 0.05 is significant. Sig.: Significance.

**Table (4):** Relation of HCV core antigen with HCV RNA levels by PCR in patients

| HCV RNA levels by PCR | HCV core Ag | | | | Test‡ | p-value (Sig.) |
|---|---|---|---|---|---|---|
| | Negative (N=51) | | Positive (N=41) | | | |
| | No. | % | No. | % | | |
| <16 IU/ml | 48 | 94.1% | 0 | 0% | 95.693 | <0.001 (HS) |
| 16 <$10^4$ IU/ml | 2 | 3.94% | 15 | 36.6% | | |
| >$10^4$ – <$10^6$ IU/ml | 1 | 1.96% | 15 | 36.6% | | |
| >$10^6$ IU/ml | 0 | 0% | 11 | 26.8% | | |

<16 IU/ml (below level of detection) ‡ Chi-square test.
P< 0.05 is significant. Sig.: Significance.

**Table (5):** Relation between HCV core Ag and HCV PCR in group (A)

| HCV PCR | Negative HCV Ab (group A) (N=46) | | | | Test | p-value (Sig.) |
|---|---|---|---|---|---|---|
| | Negative HCV core Ag (N=42) | | Positive HCV core Ag (N=4) | | | |
| | No. | % | No. | % | | |
| Negative | 41 | 97.62% | 0 | 0% | 43.137‡ | <0.001 (HS) |
| Positive | 1 | 2.38% | 4 | 100% | | |
| <16 IU/ML | 41 | 97.6% | 0 | 0% | 47.586‡ | <0.001 (HS) |
| 16-$10^4$ IU/ML | 0 | 0% | 2 | 50% | | |
| $10^4$-$10^6$ IU/ML | 1 | 2.4% | 1 | 25% | | |
| >$10^6$ IU/ML | 0 | 0% | 1 | 25% | | |

‡ Chi-square test. P< 0.05 is significant. Sig.: Significance.

**Table (6):** Relation between HCV core Ag and HCV PCR in group (B)

| HCV PCR | Positive HCV Ab (group B) (N=55) | | | | Test | p-value (Sig.) |
|---|---|---|---|---|---|---|
| | Negative HCV core Ag (N=9) | | Positive HCV core Ag (N=37) | | | |
| | No. | % | No. | % | | |
| Negative | 7 | 77.78% | 0 | 0% | 37.447‡ | <0.001 (HS) |
| Positive | 2 | 22.22% | 44 | 100% | | |
| <16 IU/ML | 7 | 77.8% | 0 | 0% | 37.913‡ | <0.001 (HS) |
| 16-$10^4$ IU/ML | 1 | 11.1% | 13 | 35.1% | | |
| $10^4$-$10^6$ IU/ML | 1 | 11.1% | 13 | 35.1% | | |
| >$10^6$ IU/ML | 0 | 0% | 11 | 29.8% | | |

P<0.05 is significant. Sig.: Significance.

**Table (7):** HCV core antigens in all HCV patients with different serological presentations

| | HCV core Ag | | | |
|---|---|---|---|---|
| | Negative (N=51) | | Positive (N=41) | |
| | No. | % | No. | % |
| HCV ab +ve | 9 | 17.6% | 37 | 90.2% |
| HCV ab –ve | 42 | 82.4% | 4 | 9.8% |
| <16 IU/ml | 48 | 94.1% | 0 | 0% |
| 16 <$10^4$ IU/ml | 2 | 3.94% | 15 | 36.6% |
| >$10^4$ – <$10^6$ IU/ml | 1 | 1.96% | 15 | 36.6% |
| >$10^6$ IU/ml | 0 | 0% | 11 | 26.8% |

<16 IU/ml (below level of detection)

**Table (8):** Validity data of HCV-core Ag in relation to PCR in diagnosis of HCV infection

| Cut off value | AUC | P-value | Sensitivity | Specificity | PVP | PVN | accuracy |
|---|---|---|---|---|---|---|---|
| **69.5** | **1.00** | **0.000** | **97.4%** | **100%** | **100%** | **70%** | **97.8%** |

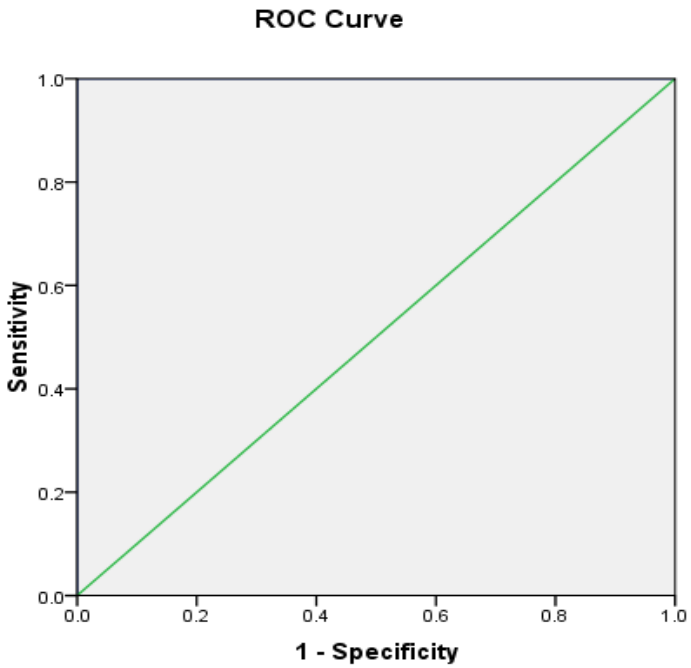


**Figure (1):** Receiver operating curve (ROC) for validity of HCV-core Ag in relation to PCR in diagnosis of HCV infection

## DISCUSSION

WHO declared HCV infection a global health problem, with approximately 3~4% of the world's population (roughly 170-200 million people) infected with hepatitis C. In the US, approximately 3 million people are chronically infected, many of them still undiagnosed while the Egyptian prevalence rate of HCV antibody has been estimated to be 10-13% of the general population. It is well known that in HCV infection, liver fibrosis progresses as the period of infection prolongs, may reach liver cirrhosis and it may reach liver cirrhosis and if it progresses to liver cirrhosis, the risk of HCC also increases **[1]**.

HCV core antigen levels correlate well with HCV RNA levels. Core antigen kinetics runs closely parallel to HCV RNA kinetics during chronic HCV infection. The incubation period for newly acquired (acute) HCV infection ranges from two weeks to six months, however, viral replication can be detected as early as one week after exposure **[3]**.

We try to confirm the importance of HCV core antigen detection as an alternative diagnostic tool for active and chronic HCV infection that can replace HCV RNA in early detection of active HCV infection in blood donners attending blood banks especially in poor country like Egypt.

This study observed that there was no significant difference among both groups as regard age and sex. Regarding patients of group (A), 32 of them were male (69.5%) and 14 were female (30.5%). Regarding patients group (B), 30 of them were male (65.2%) and 16 of them were female (34.8%). This may be attributed to the fact that the prevalence of chronic HCV infection in Egypt is higher among men than women (12% and 8%, respectively) **[1].**

We reported a statistically significant relation between HCV core antigen positivity and HCV PCR in both study groups. This result agrees with the results obtained by Catherine Goudy et al. 2005 [13], who confirmed a 96.7% sensitivity of the HCV core Ag assay with a high significant relation between positivity and negativity of HCV core Ag and HCV PCR in positive HCV antibody patients.

Our results are partially agreed with Reddy et al. **[14]**, who reported a 60% sensitivity of HCV core Ag assay in their study that included 111 chronic renal failure patients undergoing hemodialysis. The cause of discrepancy in the results may be related to compromised immune response.

We reported 4 cases with –ve HCV ab and +ve HCV RNA by PCR. This finding is supported by Morgan et al. **[15]** who confirmed presence of HCV antigen during the early, RNA-positive phase of anti-HCV seroconversion.

This study showed a high significant relation between HCV core antigen positivity and negativity as regard HCV PCR. This goes in harmony with Medici et al. **[16]**, they reported that circulating HCV core antigen first became detectable at approximately the same time as HCV RNA. The residual risk of HCV transmission because of the antibody-negative viremia 'window phase' has been documented. They have indicated that this residual risk might be substantially eliminated by testing blood donations for HCV core antigen or HCV RNA.

Morgan et al. **[15]** evaluated the sensitivity of this prototype test in specimens from individuals undergoing seroconversion following HCV infection. They added that HCV core antigen can be identified by routine serological ELISA in specimens from the early antibody-negative phase of HCV infection. A test for HCV core antigen may be a useful test for identifying window phase of blood donations from antibody negative donors infected with HCV.

## CONCLUSION

HCV core antigen ELISA assay is a simple and reliable direct method for detection of acute and chronic HCV infection. Since this assay is based on ELISA technology, it can be easily performed in most laboratories with low cost and this is a very important in devolving countries with low economic resources. HCV core Ag is compatible with HCV PCR, its wide application may prevent the vast majority of HCV transmissions caused by the transfusion of window phase donations. Moreover, HCV core antigen serve as a good method for direct HCV detection in patients during pre- seroconversion period 'window phase' when the antibody assays are negative. Also, it can be used for monitoring of antiviral therapy.

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# Assessment of Alpha-1-Acid Glycoprotein as a new Biomarker for Hepatocellular Carcinoma

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*Key words: Alpha-1-acid Glycoprotein; Alpha-fetoprotein; Hepatocellular Carcinoma*

**Background and study aim:** The outcome of patients with hepatocellular carcinoma (HCC) remains poor because of late diagnosis. We aimed to evaluate the performances of serum alpha -1-acid glycoprotein (AAG) for the diagnosis of HCC, especially for HCC with low alpha-fetoprotein (AFP).

**Patients and Methods:** Ninety patients included in this study, 60 had HCC, and 30 (50%) of these were AFP low HCC (AFP ≤20 ng/mL). The remaining 30 patients were chronic hepatitis C and cirrhosis without HCC as control group. Plasma AAG was analyzed using quantitative enzyme immunoassay technique.

**Results:** Serum level of AAG was significantly elevated in low AFP HCC group compared with high AFP HCC and cirrhotic without HCC group, 1307.20 ± 9627 vs (850.82 ± 795.14 and 309.77± 220.17 respectively). Receiver operating characteristic (ROC) curve showed that the best cut off for AAG and AFP was 740 µg/ml and 20 ng/mL respectively. The area under the curve of AAG was significantly higher than that for AFP (0.95 vs 0.92) respectively. AAG at a cut-off value of 740 µg/ml provides higher sensitivity (73.3% vs 62%, respectively) and specificity (74.0%, and 71%, respectively) in low AFP HCC than high AFP HCC

**Conclusion:** The role of AFP in the diagnosis of HCC is limited; AAG had better performance in diagnosing HCC patients with low AFP. So Serum level of AAG might be used as a potential diagnostic marker for hepatocellular carcinoma.

## INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignancies in the world **[1]**. Alfa-fetoprotein (AFP) has been the most widely used plasma marker for diagnosis, surveillance and as a prognostic indicator of HCC patients' survival **[2]**.

Several studies indicated that high plasma levels of AFP are related to poor prognosis, as well as histologic grade of malignancy **[3]**. Those with high plasma AFP level at the time of HCC diagnosis have more unfavourable outcomes compared to patients with low AFP level **[4]**, however, AFP has a low sensitivity in detection of HCC even it often increase in the absence of HCC **[5]**.

Alpha-1-acid glycoprotein (AAG) is an acute phase protein, synthesized predominantly in the liver. Cytokines can cause plasma AAG level to increase as a part of an inflammatory response **[6]**. The concentration of AAG significantly increased under the pathologic state of infection, inflammation and tumor and its level may change in various liver diseases as in patients with acute hepatitis and patients with liver cancer **[7]**. The plasma level of AAG has been suggested to be a potential marker for diagnosing cirrhosis and HCC **[8]**. It was shown that combination of AAG and AFP improves the accuracy of HCC diagnosis **[9]**.

In this study we aimed to evaluate the role of AAG in the diagnosis of hepato-cellular carcinoma (HCC), and its clinical significance in HCC patients with low AFP (≤20 ng/ml) and in HCC patients with high AFP (>20 ng/ml).

## PATIENTS AND METHODS

**Patients:**

This observational study was conducted on ninety patients admitted to Hepatology and Gastroenterology Department, Beni-seuf general Hospital during the period from March 2016 to September 2016 and a written informed consent was obtained from all participants prior to recruitment and divided into three groups:

- Group A: included 30 HCC patients with low AFP (≤20ng/ml).
- Group B: included 30 HCC patients with high AFP >20ng/ml).
- Group C: included 30 patients with HCV cirrhotics without HCC served as control group.

Liver cirrhosis was documented by clinical evaluation, laboratory investigation and evidence of cirrhosis by abdominal U/S. The diagnosis of HCC was confirmed by triphasic CT according to American association of study of liver diseases **[10]**. The diagnosis of HCV was dependent on detection of HCV-Ab and confirmed by HCV RNA positivity

Patients bellow 18 years and more 70 years, extremely ill patients, patients with malignancies other than HCC, patients with hepatic metastatic lesions and patients with a previous history of HCC treatment were excluded from the study

**Methodology :**

The enrolled patients were subjected to full history taking, thorough clinical examination and laboratory investigation including complete blood count (CBC), liver and kidney profile tests Hepatitis C Virus (HCV) and Hepatitis B Virus (HBV)) markers and AFP. Severity of liver cirrhosis assessed by using Modified Child-Pugh score.

**Blood sampling and biochemical assays:**

Fasting venous blood samples (5ml) were collected by well-trained laboratory technicians under complete aseptic conditions then distributed as follows:

a- 1 mL of whole blood was collected in an EDTA vacutainer and mixed gently for complete blood count measurement that was performed by automated hematology system (Sysmex XE 5000; Sysmex America, Inc., Mundelein, IL, USA).

b- 4 mL of venous blood samples were collected in plain test tubes containing no anticoagulant, allowed to clot for 30 mins at room temperature, then centrifuged for 15 mins at 1000× g. The serum was removed, aliquoted then stored at ≤−20°C until assayed and thawed immediately before the measurement, the separated serum was used for the following assays :

- Biochemical tests using Beckman CX4 chemistry analyzer (NY, USA, supplied by the Eastern Co. For Eng, Egypt), these tests including :
  - Fasting blood glucose level.
  - Liver function tests: Serum albumin, total and direct bilirubin, Liver enzymes including aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl transpeptidase (GGT).
  - Kidney function tests : including serum creatinine.
- Viral infection status ( HCVAb and HBS Ag) were assayed using an enzyme immunoassay (EIA) Kit (Abbott, Axyam USA ).
- Serum AFP level (ng/ml)was assyed using an enzyme immunoassay (EIA) Kit (Roche Mannheim, Germany) .
- Serum AAG levels were measured using human ELISA (sandwich technique) kits provided by Human AGP1/alpha 1 acid glycoprotein PicoKine™ ELISA Kit (Boster Biological Technology, Pleasanton CA, USA, Catalog # EK1486) for research use only with assay range 1.56-100 µg/ml.

**Assay procedure of AAG :**

100 of Standard or diluted sample was added to the bottom of micro ELISA plate well, covered with a new plate sealer, then incubated for 90 minutes at 37 degree, then 100 µL of biotinylated anti human AGP1 antibody was added, covered with a new adhesive strip and incubated at 37 degree for another 60minutes. Aspiration and wash was performed three times followed by addition of 200 µLof Avidin biotin Peroxidase complex (ABC) and incubated at room temperature for another 30 minutes, wash plate 5 times again.90 µL of substrate was added to each well and incubated in the dark for 15-20 minutes. Finally, 100 µL of Stop Solution was added to each well, where the color turned to yellow immediately and the optical density (OD) was read at 450 nm within 30 minutes.

**Calculation of results:**

The duplicate readings for standard and samples was averaged and subtracted the average zero

standard optical density. A standard curve was created by plotting the mean OD value on the Y-axis against the concentration on the X- axis and a fit curve was drawn by some professional software and a best fitting equation of standard curve was calculated using OD values and concentrations of standard samples.

**Statistical Analysis:**

The statistical analysis was conducted using STATA/SE version 11.2 for Windows (STATA corporation, College Station, Texas). Data are reported as means ± SD, Non-normally distributed data were analyzed by the chi-square test. Spearman pearman correlation test was used to analyse the relation between categorical data. The ROC curve used to determine the most sensitive and specific cut-off value for AAG for diagnosing HCC. Corresponding distribution tables were consulted to get the "P" (probability value). Statistical significance was accepted at P value <0.05.

## RESULTS

This study was conducted on 90 cases. The epidemiologic characteristics of the three patients' groups were summarized in table 1, divided into three groups: Group A included 30 HCC patients with low AFP (73.3% males and 26.7% females), with a mean (SD) of 59.83± 6.16years.

Group B: included 30 HCC patients with high AFP (83.3% males and 16.7% females), with a mean (SD) of 62.17±5.416years. Group C: included 30 cirrhotic patients with chronic hepatitis C (served as control group). 46.6% males and 53.4% females, their mean ages was 62.17±5.416 years. There were highly statistically significant differences in age and gender among the studied groups(p=0.003 and 0.001 respectively) .

**As regard biochemical and molecular parameters :**

There was a highly statistical significant difference between studied groups as regard ALT, AST, serum albumin, serum bilirubin, PC, INR, serum AFP, and serum AAG levels. AAG was high in HCC patients with low AFP than HCC patients with high AFP and cirrhotic patients without HCC (1307.20±962.77 Vs 850.82±795 and 309.77±220 respectively) table 2.

Majority of studied patients in group A were Child Class B (43.3) in group B and group C were Child Class A (40 and 63.3 respectively) table 3.

Tables 5 showed that AAG at a cut-off value 740 µg/ml had high sensitivity, specificity, PPV and NPV in diagnosis of HCC with low level of AFP than HCC with high level of AFP (73.3 vs 68.1, 74 vs 71, 95.5 Vs 91 and 82.3 vs 88) respectively with area under the curve o.95 vs o.81 respectively.

**Table (1):** Demographic characteristics of the patients

| Data | Group (A) (HCC with low AFP) n=30 | Group (B) (HCC with high AFP n=30 | Group (C) (without HCC) n=30 | P value |
|---|---|---|---|---|
| Age (year)<br>• Range<br>• Mean ± SD | <br>46-72<br>59.83±6.16 | <br>44-69<br>62.17 5.41 | <br>35- 56<br>45.57±6.20 | <br>0.003 |
| Sex<br>• Male (n.%)<br>• Female (n.%) | <br>22/30(73.3)<br>8/30(26.7) | <br>25/30(83.3)<br>5/30(16.7) | <br>14/30(46.6)<br>16/30(53.4) | <br><br>0.001 |
| Residence<br>• Urban (n.%)<br>• Rural (n.%) | <br>12(40)<br>18(60) | <br>14(46.6)<br>16(53.3) | <br>13(43.3)<br>17(56.60 | <br><br>0.52 |

**Table (2):** Biochemical parameters of studied groups

| Labs | Group (A) (HCC with low AFP) n=30 | Group (B) (HCC with high AFP) n=30 | Group (C) (without HCC) n=30 | P value |
|---|---|---|---|---|
| Hb mg/dl | 11.26±1.34 | 11.85±1.03 | 11.08±1.08 | 0.397 |
| WBCs $x10^{3/mm3}$ | 7.01±2.55 | 6.10±2.93 | 7.28±2.58 | 0.215 |
| Platelets $x10^{3/mm3}$ | 139.54±35.35 | 153.97±74.80 | 122.03±30.67 | 0.201 |
| ALT u/l | 80.77±49.9 | 94.03±51.3 | 42.73±37.3 | 0.008* |
| AST u/l | 92.13±60.1 | 89.93±58.3 | 43.60±31.3 | 0.006* |
| Albumin g/dl | 3.12±0.5 | 2.93±0.6 | 3.57±0.6 | 0.02* |
| Bilirubin mg/dl | 2.97±3.4 | 2.99±3.5 | 1.84±0.7 | 0.003* |
| PC % | 57.83±8.5 | 59.49±9.3 | 71.67±7.8 | 0.012* |
| INR | 1.55±0.21 | 1.42±0.23 | 1.13±0.11 | 0.04* |
| Creatinine mg/dl | 1.48±0.73 | 1.16±0.56 | 0.8±0.6 | 0.003* |
| HCV RNA by PCR $x10^3$ | 950.8±752.7 | 761.26±670.6 | 829.7±782.8 | 0.14 |
| AFP | 9.89±4.27 | 460±98.84 | 14.78±9.35 | 0.001* |
| AAG N: 50-120 µg/ml | 1307.20±962.77 | 850.82±795.14 | 309.77±220.12 | <0.001* |

**Table (3):** Severity of liver cirrhosis assessed by Child-Pugh classification between studied groups

| Child grade | Group (A) (HCC with low AFP) n=30 | Group (B) (HCC with high AFP) n=30 | Group (C) (Cirrhotic without HHC) n=30 | P-value |
|---|---|---|---|---|
| | n (%) | n (%) | n (%) | |
| Child A | 11(36.6) | 12(40) | 21(63.3) | 0.008* |
| Child B | 13(43.3) | 11(36.6) | 8(33.3) | 0.13 |
| Child C | 6(20) | 7(23.3) | 1(3.33) | 0.02* |

**Table (4):** Correlation between AAG and different parameters among HCC groups

| Variable/AFP | Spearman correlation | P value |
|---|---|---|
| Child-Pugh classification | 0.41 | 0.02 |
| Tumor number | 0.32 | 0.01 |
| Tumor size | 0.16 | 0.09 |
| AFP | 0.54 | 0.03 |

**Table (5):** Diagnostic performance of AAG in diagnosis of HCC

| AAG (µg/ml) | Cut-off Value | Sensitivity % | Specificity % | PPV % | NPV % | Area under curve (AUC) |
|---|---|---|---|---|---|---|
| Group (A) n=30 | 740 | 73.3 | 74 | 95.5 | 82.3 | 0.95 |
| Group (B) n=30 | | 68.1 | 71 | 91 | 88 | 0.81 |

This table shows that AAG at a cut-off value 740 µg/ml had high sensitivity, specificity, PPV and NPV in diagnosis of HCC with low level of AFP than HCC with high level of AFP.

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

| AFP (ng/ml): | Cut-off Value | Sensitivity % | Specificity % | PPV % | NPV % | Area under curve (AUC) |
|---|---|---|---|---|---|---|
| HCC patients | 20 | 63.2 | 79 | 84.9 | 82.7 | 0.92 |

This table shows that AFP level of (20 ng/dl) had low sensitivity (63.2%), good specificity (79%) and high PPV, NPV (84.9 and 82.7) respectively.

## DISCUSSION

Prognosis and survival of patients with HCC is affected by the HCC stage at the time of diagnosis. So reliable markers would greatly improve the chances of early detection Of HCC **[7]**.

Surveillance for HCC usualy depends on AFP and abdominal ultrasonography. yet, the low sensitivity and specificity of AFP make it as a poor biomarker with abdominal ultrasonography for early detection of HCC **[11]**. so, a good new biomarker is required for early detection of HCC. Plasma level of AAG has been suggested as a new biomarker for HCC and cirrhosis **[7]**. The aim of this study was to asses the role of AAG in the diagnosis of HCC, especialy with low alfa-fetoprotein (AFP) (≤20 ng/ml) and in HCC with high alfa-fetoprotein (AFP) (>20 ng/ml). In the current study HCC patients were older than cirrhotic HCV patients, (as the mean age in the HCC group A was 59.83 ± 6.16, group B was 62.17±5.41 while in chronic liver disease group C was 45.57±6.20). This finding was in agreement with Shaker who reported that the peak age group for HCC was from 50-70 years with a mean age of 58.7 years **[12]**. Also in agreement with El-Zayadi et al. who found that patients of the age group 40-59 years were at 3.7 times, and of age group 60 years were at 11 times more risk to develop HCC **[13]**. Also, there was a male predominance among the HCC groups, (as the number of males were 22 male to 8 female in group A and the number of males were 25 male to 5 female in group B). This finding was in agreement with Sharaf-Eldin et al. **[14]** and Holah et al. **[15]** who reported that males predominated with male to female ratio of 4.7:1 and 5:1 among HCC patients respectively.

Also, El-Serag and Rudolph **[16]** reported that males have higher liver cancer rates than females, with male: female ratios usually averaging between 2:1 and 4:1 the reasons for higher rates of liver cancer in males may relate to gender specific differences in exposure to risk factors.

The higher incidence of HCC in males might be due to the stimulatory effects of androgen and the protective effect of estrogen. The biological activity of natural progesterone on HCC is controversial and lacks clear investigations **[17]**. Men are more likely to be infected with HBV and HCV, consume alcohol, smoke cigarettes and have increased iron stores. Higher levels of androgenic hormones, body mass index, and increased genetic susceptibility may also adversely affect male risk **[16]**. In the present study, HCC cases were more from rural areas, this result was in agreement with Shaker et al. who stated that the incidence of HCC is more common in rural areas than urban **[12]**. In the present study, AST, AST and serum bilirubin were higher in HCC patients. These results were in agreement with Abu El Makarem et al. who reported that AST and serum bilirubin are higher in HCC patients than cirrhotics **[18]**.

Other liver biochemical profile in this study as serum albumin, prothrombin concentration and INR were lower in HCC patients than in cirrhotic patients. This result was in agreement with Wong et al. and Baghdady et al. who reported the same results **[19,20]**.

In this study, most of the patients with HCC were Child A and B, followed by Child C, These results were in agreement with Alves et al. who reported that (53.1%) of HCC cases were Child B followed by Child A (31.3%) then Child C (12.5%) **[21]**. And El-Sawy who reported that (40%) 0f advanced HCC cases were Child B, followed by Child A (32.7%) then Child C (27.3%) **[22]**.

In the current study there was significant positive correlation between AAG and severity of liver disease (Child-Pugh grade), tumor number and AFP but there was no significant correlation between AAG and the size of the tumor, this goes in agreement with a study by Kanget et al. that found AAG had similar sensitivity value in differentiating HCC regardless of the tumor size **[13]**.

AFP used as a diagnostic test with a cut-off value 20 ng/ml provides sensitivity is only 63.2% and the specificity is 73%, This goes in agreement

with Gonzalez and Keeffe defining an elevated AFP level >20 ng/ml, confers a sensitivity of 60% and specificity of 80% **[24]**, While Jiang et al. reported that sensitivity of AFP was about 79.7% and specificity was about 80.3% in HCC cases **[25]**.

In this study, ROC analysis of AAG used as a diagnostic test suggested that a cut-off value of 740 µg/ml provides sensitivity, specificity, PPV and NPV for group A of (73.3%, 74.0%, 95.5%, 82.3%) respectively, and for group B(68%, 71%, 91%, 88%) respectively. This shows increase in sensitivity and specificity of AAG in low AFP HCC (group A) more than that in high AFP HCC (group B), so these results allows us to purpose that determining AAG concentration could be especially powerful in patients with HCC especially with non-diagnostic AFP concentrations.

The results of our study are supported by Bachtiar et al. who reported that the level of AAG was low in patients with AFP high HCC but was higher in patients with AFP low HCC. the clinical performance of AAG increased in low AFP HCC( <20 ng/ml) as Sensitivity, accuracy, PPV and NPV of AAG in patients with AFP-low HCC were (82%, 90%, 91%, and 89%, respectively) while in patients with AFP-high HCC Sensitivity, accuracy, PPV, and NPV of AAG were (62%, 82%, 89%, and 79%, respectively). At cut-off value of 800 µg/ml for AAG and 20 ng/ml for AFP **[9]**.

## CONCLUSION

Serum Alpha 1 Acid Glycoprotein (AAG) concentration could be used as a potential marker for hepatocellular carcinoma.

### Consent

All authors declared that written informed consent was obtained from the patients for publication of this paper.

### Ethical Approval

Ethical clearance was obtained from Beni-seuf general Hospital's ethics committee.

### Competing Interests

Authors have declared that no competing interests exist.

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# Fecal Calprotectin in Patients with Hepatic Encephalopathy

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**Background and study aim:** Calprotectin is a cytoplasmatic protein of neutrophilic granulocytes and it is an established marker for the assessment of localized intestinal inflammation .Bacterial translocation is known to play an important role in the pathogenesis of certain complications of cirrhosis such as hepatic encephalopathy (HE). This study amid to assess: the value of fecal calprotectin in the diagnosis of hepatic encephalopathy, relationship between level of fecal calprotectin and the degree of hepatic encephalopathy.

**Patients and Methods:** This cross sectional study was conducted on 90 patients attended to the Hepatology, Gastroenterology and Infectious Diseases Department of Benha University Hospital between March and July 2016. All medical diseases which are known to influence the level of fecal calprotectin were excluded (as: gastrointestinal bleeding or inflmmatory bowel disease). The degree of liver insufficiency was assessed according to the Child Pugh classifiation and Model of End Stage Liver Disease (MELD); and degree of hepatic encephalopathy by West-Haven criteria, and the number connection test.

**Results:** The mean value of fecal calprotectin in patients with overt HE was 304.4 ± 41.05 µg/g, and in patients with MHE was 74.4 ± 23.9 µg/g and in the group of liver cirrhosis without encephalopathy was 57.55± 8.92 and in healthy group was 25.22 ± 8.63, respectively with high significant difference ($p<0.001$). There was no significant correlation between fecal calprotectin and (age, psychometric test, Child-Pugh classification, MELD score and West-Haven criteria).

**Conclusion:** This study confirmed significantly higher values of fecal calprotectin in patients with hepatic encephalopathy. Among patints with OHE and patients of MHE, no significant correlation between fecal calprotectin and age, psychometric test, Child classification, MELD score and West-Haven criteria were detected.

## INTRODUCTION

Calprotectin is a calcium and zinc-binding protein, representing more than 60% of the cytosolic proteins in neutrophils. The presence of calprotectin in feces quantitatively relates to neutrophil migration into the gastrointestinal (GI) tract **[1]**. Therefore, it is considered as a valid marker of intestinal inflammation because it is released during cell activation and death **[2]**.

As the GI tract of cirrhotic patients shows various alterations of its mucosal barrier including infiltrates of neutrophils, calprotectin might be a promising diagnostic parameter to diagnose the onset and course of hepatic encephalopathy **[3]**.

There is extensive literature about the diagnostic significance of fecal calprotectin in patients with inflammatory bowel disease, non-steroidal anti-inflammatory enteropathy and patients with irritable bowel disease, studies about the value of calprotectin in patients with cirrhosis are extremely sparse **[4,5]**.

Fecal calprotectin in cirrhosis was firstly investigated by Yagmur and others who found significantly elevated values in patients with advanced cirrhosis **[6]**. Significantly increased levels of fecal calprotectin in patients

with cirrhosis confirmed by Gundling and others in their study **[7]**.

Alempijević and his colleagues reported high significant values of fecal calprotectin in hepatic encephalopathy patients in comparison to liver cirrhosis and healthy subjects with significant correlation between FCC values and grades of hepatic encephalopathy according to West-Haven criteria **[8]**.

## PATIENTS AND METHODS

This study was performed on 90 patients attended to the Hepatology, Gastroenterology and Infectious Diseases Department of Benha University Hospital between March and July 2016 and divided into 4 groups. Group I: included 25 cirrhotic patients with minimal hepatic encephalopathy. Group II: included 25 cirrhotic patients with overt hepatic encephalopathy. Group III: included 20 cirrhotic patients without hepatic encephalopathy. Group IV: included 20 healthy subjects. The study was conducted according to the research plan of Hepatology, Gastroenterology and Infectious Diseases Department of Benha University.

Diagnosis of liver cirrhosis was based on clinical clues from the patient's medical history, physical examination, laboratory tests (CBC, ALT, AST, bilirubin, PT, PC, INR, urea, creatinin, HCVAb, HBSAg) and abdominal ultrasonography.

Quantitative estimation of calprotectin level in stool by an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (EDI$^{TM}$ Quantitative fecal calprotectin ELISA, USA - KT- 849).

The degree of liver in sufficiency was assessed according to Child-Pugh classification of severity of liver disease and Model for End-Stage Liver Disease (MELD).

The degree of hepatic encephalopathy was assessed according to Number connection test and West-Haven criteria.

## RESULTS

The study was conducted on 90 patients (54 male and 36 female), the mean age 49.92±8.86 years (61% of patients were hepatitis C, 45% were hepatitis B).

This study showed statistical significant difference between studied groups regarding age {mean age was highest in group I (52.72±9.03)}. Regarding clinical examination, ascites was more frequent in group I and III (100%) and jaundice was more frequent in group II (76%) .

This study showed statistically significant difference between the studied groups as regard to platelet count ,serum albumin, INR, bilirubin, SGOT and SGPT{platelet was lowest in group II (114.12±59.27)], [Serum albumin was lowest in group I (1.99±0.35), [INR was highest in group II (1.51±0.30)], [T. bilirubin was highest in group II (2.44±1.46)], [SGPT was highest in group II (58.4±34.67)] and [SGOT was highest in group II (78.4±39.25)]. This study showed no statistical significant difference between studied groups as regard Hb and WBCs as shown in table (1)

As regard MELD score, there was no statistical significant difference between studied groups. But, there was statistical significant difference between diseased groups regarding Child classification [Child C was more frequent in group II (72%)] while child B was more frequent in group III (75%) as shown in Table (2)

As regard number connection test. There was statistical significant difference between studied groups: {in group II there were 92 % (grade III: forced termination) in comparison to group I there were 64 % grade (I – II) as shown in table (3)

This study showed statistical significant difference between studied groups regarding fecal calprotectin {fecal calprotectin was highest in group II (304.4±41.05) in comparison to (74.4±23.9) in group I, (57.55±8.92) in group III and (25.22± 8.63) in group IV}as shown in table (4)

Among cases of group I (MHE), no significant correlation between fecal calprotectin and age, psychometric test, Child classification and MELD score. Among patients with OHE (group II), no significant correlation between fecal calprotectin and age, psychometric test, Child classification, MELD score and West-Haven criteria as shown in Table (5).

**Table (1):** Laboratory data of the studied groups

| | Group I MHE (n : 25) | Group II OHE (n : 25) | Group III Without HE (n : 20) | Group IV (n : 20) | Test | P value |
|---|---|---|---|---|---|---|
| **Hb mean ±SD** | 11.17±1.74 | 11.16±1.39 | 10.7±1.06 | 11.11±0.85 | F=0.59 | 0.62 |
| **Plt mean ±SD** | 127.8±62.97 | 114.12±59.27 | 133.4±61.64 | 196.85±71.88 | F= 7.04 | 0.001** |
| **WBCs mean ±SD** | 6.52±2.88 | 6.72±4.59 | 6.41±2.22 | 6.71±2.09 | F=0.05 | 0.99 |
| **RBCs mean ±SD** | 3.75±0.62 | 3.69±0.37 | 3.84±0.92 | 3.89±0.92 | F=0.34 | 0.80 |
| **T Bilirubin mean ± SD** | 2.16±1.51 | 2.44±1.46 | 1.89±1.65 | 0.98±0.14 | F=4.74 | <0.05 |
| **S albumin mean ±SD** | 1.99±0.35 | 2.16±0.58 | 2.5±0.54 | 3.77±0.24 | F= 67.3 | 0.001** |
| **SGPT mean ±SD** | 55.48±35.63 | 58.4±34.67 | 44.55±13.45 | 32.5±3.97 | F=4.14 | <0.05 |
| **SGOT mean ±SD** | 70.52±45.69 | 78.4±39.25 | 63.3±23.27 | 28.8±4.07 | F=8.99 | 0.001** |
| **Proth.concentration mean ±SD** | 57.08±12.33 | 54.76±11.3 | 66.05±13.45 | 89.6±2.56 | F= 45.4 | 0.001** |
| **INR mean ±SD** | 1.47±0.24 | 1.51±0.30 | 1.39±0.24 | 1.06±0.05 | F=16.83 | 0.001** |

P value <0.05 was considered statistically significant

**Table (2):** Assessment of severity of liver disease in cirrhotic groups

| | Group I OHE (n : 25) | Group II MHE (n : 25) | Group III Without HE (n : 20) | $X^2$ test | P value |
|---|---|---|---|---|---|
| **MELD score** | 14.92±5.6 | 15.36±5.63 | 13.2±5.25 | F=0.92 | 0.40 |
| **Child classification** | | | | | |
| **A** | 0 | 0 | 0 | | |
| **B** | 12 (48.0) | 7 (28.0) | 15 (75.0) | FET= 89.94 | 0.001** |
| **C** | 13 (52.0) | 18 (72.0) | 5 (25.0) | | |

**Table (3):** Number connection test in HE patients

| | Group I OHE (n : 25) | Group II MHE (n : 25) | FET | P value |
|---|---|---|---|---|
| **Number connection test** | | | | |
| **0-I** | 8 (32.0) | 0 (0.0) | | |
| **I-II** | 16 (64.0) | 0 (0.0) | 56.22 | 0.001** |
| **II-III** | 1 (4.0) | 2 (8.0) | | |
| **III (FT)** | 0 (0.0) | 23 (92.0) | | |

**Table (4):** Fecal calprotectin level in the studied groups

| | Group I MHE (n : 25) | Group II OHE (n : 25) | Group III Without HE (n : 20) | Group IV (n : 20) | F Test | P value |
|---|---|---|---|---|---|---|
| **Fecal calprotectin mean ±SD** | 74.4±23.9 ug/g | 304.4±41.05 ug/g | 57.55±8.92 ug/g | 25.22±8.63 ug/g | F= 584.7 | 0.001** |

**Table (5):** Correlation between fecal calprotectin and other variables among patients with (OHE) and patients with (MHE)

| Patients with (MHE) | | | Patients with (OHE) | | |
|---|---|---|---|---|---|
| **Fecal calprotectin gp I** | **r test** | **P value** | **Fecal calprotectin gp II** | **r test** | **P value** |
| **Age** | 0.11 | 0.60 | **Age** | 0.15 | 0.48 |
| **Psychometric test** | 0.16 | 0.46 | **Psychometric test** | 0.05 | 0.82 |
| **Child score** | -0.09 | 0.67 | **Child score** | -0.39 | 0.054 |
| **MELD score** | -0.20 | 0.34 | **MELD score** | 0.08 | 0.71 |
| | | | **West-Haven criteria** | -0.25 | 0.23 |

## DISCUSSION

The diagnosis of HE continues to be a major clinical problem. Patients may present with mild cognitive impairment. It is important to recognize HE at their early stages because adequate treatment of the condition reduces morbidity and mortality **[7]**.

Pathological bacterial translocation plays an important role in the pathogenesis of HE. Cal-protectin is representing more than 60% of the cytosolic proteins in neutrophils. As the GI tract of cirrhotic patients shows various alterations of its mucosal barrier including infiltrates of neutrophils, calprotectin might be a promising diagnostic parameter to diagnose the onset and course of HE **[3]**.

In this study, there was statistically significant difference between all groups as regard age (P<0.05). Mean age was highest in MHE group in comparison to OHE group cirrhotic group and control group (52.72±9.03), (51.52±6.63), (48.7± 8.27) (42.75±11.51) respectively. Likewise, Akhtar and his colleagues reported that incidence of hepatic encephalopathy increased in elderly people in the study done on 294 elderly patients (age 65-97) **[9]**. This variation in age may be attributed to the diversity of eitiologies of liver cirrhosis and the impact of the course of disease progression.

As regard to sex, residence and smoking, there were no statistical significant difference between studied groups. This was in accordance with Butterworth **[10]**. But, this was against Manabendra and his colleagues who reported that incidence of hepatic encephalopathy was more in male than in female **[11]**.

Regarding clinical examination, jaundice was more present in OHE group (76%) and ascites was more frequent in [MHE group and cirrhotic group (100%)]. This was in agree with Hartmann and his colleagues and Thornton who reported that the incidence of hepatic encephalopathy is increased following the development of ascites and increased degree of jaundice **[12,13]**.

As regarding to hematological criteria, Platelet count showed highly statistical significant difference between studied groups (0.001). with the lowest level in OHE group in comparison to MHE group, cirrhotic group and control group (114.12 ± 59.27), (127.8±62.97), (133.4±61.64), (196.85± 71.88) respectively. This comes in accordance with Gangireddy and his colleagues who reported that thrombocytopenia is a well-known complication in patients with liver cirrhosis and worsened with hepatic encephalopathy **[14]**. But, there was no difference regarding (Hb concentration and WBCs).

As regarding to liver profile, INR was highest in OHE (1.51±0.30) and serum albumin was lowest in MHE group (1.99±0.35). This was in agreement with Lee who reported that decreased synthetic capacity of liver as albumin and prothrombin observed in liver cirrhosis and aggravated with progression of disease and development of hepatic encephalopathy **[15]**. Bilirubin was highest in OHE group (2.44±1.46) this comes in accordance with Hartmann and his colleagues who found increased degree of jaundice among HE patients compared with cirrhotic patients **[16]**. SGPT was highest in OHE group (58.4±34.67) and SGOT was highest in overt hepatic encephalopathy group (78.4±39.25). This study found no significant difference between the studied groups regarding viral markers.

In this study, there was significantly statistical difference between studied groups regarding Child-Pugh classification. Child C was more predominant in OHE group (72 %) and Child B was more predominant in cirrhotic group (75%).

In this study, comparison between studied groups as regard fecal calprotectin showed statistically high significant difference with the lowest value in control group (25.22±8.63 µg/g) followed by

cirrhotic group (57.55±8.92 µg/g) followed by MHE group (74.4±23.9 µg/g) and the highest value in OHE group (304.4±41.05 µg/g) despite of a careful exclusion of other causes of abnormal calprotectin results e.g. GI bleeding.

These results come in accordance with Alempijević and his colleagues **[8]**, Fatma and his colleagues **[17]**, Gundling and his colleagues **[7]** and Yagmur and his colleagues **[6]** who found that median FCCs in high grade hepatic encephalopathy (380.7 ±107.4 µg/g), (489±23 µg/g), (median 321.6 µg/g) and (median 429.38±74.90 µg/g) respectively.

In this study, there was no significant correlation between fecal calprotectin and (age, psychometric test, Child-Pugh classification, MELD score and West-Haven criteria).

Alempijević and his colleagues reported nearly similar results and found no significant correlation between fecal calprotectin and Child-Pugh classification. Also there was no significant correlation with MELD score and psychometric test **[8]**. But, Alempijević and his colleagues reported significant correlation between FCC values and grades of hepatic encephalopathy according to West-Haven criteria **[8]**.

This was in partial disagreement with Gundling and his colleagues who revealed significant correlation between elevated FCCs and HE grading as measured by West-Haven criteria (p=0.001) and significant correlation with Child-Pugh classification and M ELD score (P<0.001) and (P=0.018), respectively **[7]**.

Also, Yagmur and his colleagues reported significant correlation between FCCs and Child-Pugh classification (P<0.001) and HE grading as measured by West–Haven criteria (p=0.001) but, no significant correlation with MELD score **[6]**.

Finally, fecal calprotectin may serve as a good screening tool for diagnosis of hepatic encephalopathy.

## CONCLUSION

Fecal calprotectin level was shown to be significantly higher in cirrhotic patients in comparison with normal subjects. Fecal calprotectin level was shown to be significantly higher in cirrhotic patients presented with HE in comparison to cirrhotic patients.

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