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Impact of NGAL (Neutrophil Gelatinase Associated lipocalin) on Outcome of Hepato-renal Syndrome

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ABSTRACT

Background: Renal dysfunction is a severe complication of advanced cirrhosis as well as of acute-on-chronic liver failure (ACLF). Hepato-renal syndrome (HRS) has been defined as a syndrome that occurs in patients with advanced liver disease, characterised by impaired renal function and marked abnormalities in the arterial circulation and over-activity of the endogenous vasoactive systems.

Objective: The aim of this work was to study the role of plasma NGAL level in patients with hepatorenal syndrome in order to identify patients with high risk of renal dysfunction, correlate clinical outcome with therapeutic management and provide a clue on better management to prevent renal deterioration.

Patients and methods: This study was carried out on 50 patients. They were divided into 3 groups in addition to control group; group I of 25 patients with decompensated liver cirrhosis , group II of 25 patients with hepato-renal syndrome , group III of 25 hepato-renal patients who followed up after treatment in addition to 25 healthy individuals as a control group (group IV) .

Results: of NGAL in different study groups were as follows: There was a significant increase of NGAL in Group 2 & 3 compared to Group 1& 2. There was a statistical significance between the four groups ($p< 0.001$).

Conclusions: NGAL could be used in conjuction with serum creatinine to assess the hepato-renal affection and may aid in stratifying patients in need for liver transplant.

Keywords: NGFAL, HRS, MELD, ESLD.

INTRODUCTION

Moreau *et al.* [1] stated that ascites, hepatic encephalopathy and bacterial infection are frequently presented with acute decompensated liver cirrhosis.

About 50% of patients with acute liver decompensation develop several renal dysfunction. Patients with end-stage liver disease (ESLD) who develop hepatorenal syndrome (HRS) have very high mortality rate [2]. Hepatorenal syndrome (HRS) is a multiorgan condition affecting the kidneys and the liver as a natural course in 40 % of patients with cirrhosis and ascites [3].

Liver Transplantation is the best treatment for both type-1 and type-2 HRS [4]. Also medical treatment in the form of administration of vasoconstrictors plus albumin can be effective [5].

Serum creatinine as a marker of renal function in patients with liver cirrhosis has several limitations [6]. Plasma NGAL is a very early & sensitive biomarker in kidney injury because it is less affected by high bilirubin levels, reduced protein diet and muscle wasting as creatinine [7]. Many studies showed that NGAL as valuable marker of GFR in cirrohosis and may predict renal dysfunction [8].

AIM OF THE WORK

The aim of this work was to study the role of plasma NGAL level in patients with hepato-renal syndrome in order to identify patients with high risk of renal dysfunction, correlate clinical outcome with therapeutic management and provide a clue on better management to prevent renal deterioration.

SUBJECTS AND METHODS

This clinical study was carried out on 50 patients admitted to Mahalla Hepatology Teaching Hospital and Tanta Internal Medicine Department in the period of November 2017- November 2018 and divided into three groups:

- ***Group 1:*** it consisted of 25 Patients suffered from decompensated liver cirrhosis.
- ***Group 2:*** it consists of 25 Patients suffered from hepato-renal syndrome.
- **Group 3:** it consists of 25 patients who were diagnosed as hepato-renal syndrome and were followed up after treatment.
- **Group 4**: In addition to 25 apparently healthy individuals served as a control group.

Inclusion Criteria: Cirrhotic patients whose age ranged from 40–70 years were included in this study.

Exclusion criteria: Patients with cancer, cadiomyopathy and pregnant women were excluded.

Written informed consent was taken from all participants in this research**.** They were subjected to the following parameters:

1. Full history taking included age, sex, complaints.
2. Clinical examination with special interest on ascites, vascular spiders, bacterial peritonitis, hepatic encephalopathy & variceal bleeding from portal hypertension.
3. Abdominal U/S and triphasic CT was done when it was indicated.

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The study was approved by the Ethics Board of Tanta University and an informed written consent was taken from each participant in the study.

Laboratory Investigations include:

a) Total bilirubin.
b) International normalized prothrombin time ratio (INR).
c) Creatinine.
d) NGAL using ELISA technique, plasma NGAL was meeasured in all four groups of patients and compared for statistical significance.
e) **MELD Score:** calculated MELD score is derived from a numerical scale used for adult liver transplant.

We calculate MELD score using a mathematical formula:

MELD Score = 10 * [(0.957 * LN (creatinine in mg/dl)) + (0.378 * LN (total bilirubin in mg /dl)) + (1.12 * LN (INR)) + 6.43)]

LN indicates the natural logarithm.

The range is from 6 (less ill) to 40 (gravely ill). The individual score determines how urgently a patient needs a liver transplant within the next three months.

Blood Sampling:

Five ml of venous blood were withdrawn from all patients and collected into sterile tubes (2 ml on citrated tubes and 3 ml on plain tubes). Citrated plasma and serum were separated after centrifugation at 25°C for 10 minutes and blood chemistry tests were done .Plasma was stored at -20°C until used for determination of plasma NGAL level. Blood samples and needles were disposed in safety boxes which are taken every other day to the incinerator.

Principle:

Human Lipocalin-2/NGAL ELISA Kit is based on standard sandwich enzyme-linked immune-sorbent assay technology.

The purified anti-NGAL antibody was pre-coated onto 96-well plates and the HRP conjugated anti-NGAL antibody was used as detection antibodies. The standards, test samples and HRP conjugated detection antibody were added to the wells subsequently, mixed and incubated, then, unbound conjugates were washed away with wash buffer. TMB substrates (A & B) were used to visualize HRP enzymatic reaction.

TMB was catalyzed by HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the NGAL amount of sample captured in plate. Then reading the O.D. absorbance at 450 nm in a micro plate reader, and then the concentration of NGAL can be calculated.

Preparation of sample and reagents:

1. Sample: Plasma was collected using Na citrate as an anticoagulant , and mixed for 10-20 min, centrifuged at the speed of 2000-3000r.p.m for 20 min of collection . An aliquot is taken from plasma and is stored at -20°C.

2.Wash buffer: Concentrated wash buffer was diluted 30-fold (1-30) with distilled water by adding 20 ml of concentrated wash buffer into 580 ml of distilled water.

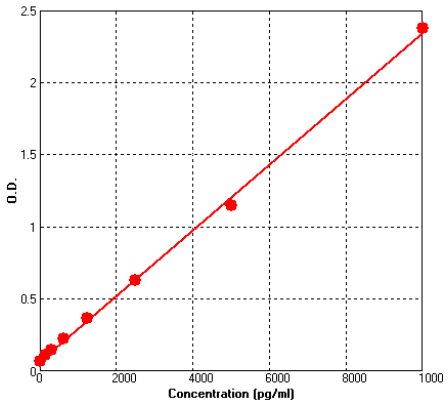
3.Standard: The standard solution should be prepared less than 2hrs prior to the experiment.

a) 10,000pg/ml of standard solution: 200 ml of the 13,500 pg/ml standard was added into 70 ml standard diluent buffer and mixed thoroughly .
b) Six eppendorf tubes were labeled as follows, 5000 pg/ml, 2500 pg/ml, 1250 pg/ml, 625 pg/ml, 312 pg/ml and 156 pg/ml respectively.

Calculation

Relative O.D. was first calculated as follows:

- Relative O.D.= (O.D. of each well) – (O.D. of zero well)
- The standard curve was then plotted "Y" being the relative O.D. of each standard solution and "X" being the respective concentration of the standard solution.
- The NGAL concentration of the samples was then interpolated from standard curve.



Statistics:

Statistical presentation and analysis of the present study was conducted, using the mean, standard deviation and chi-square test by SPSS V.20.

1- Mean value $\left(\bar{X}\right)$ **:**

The sum of all observations divided by the number of observation:

$$\left(\bar{X}\right) = \frac{\Sigma x}{n}$$

Where Σ = sum & n = number of observations.

2- Standard Deviation [SD]: It measures the degree of scatter of individual varieties around their mean:

$$SD = \sqrt{\frac{\Sigma \left| x - \bar{x} \right|^{-2}}{n-1}}$$

3- Analysis of variance [ANOVA] tests (f): according to the computer program SPSS for Windows. ANOVA test was used for comparison among different times in the same group in quantitative data.

4- Chi-square: The hypothesis that the row and column variables are independent, without indicating strength or direction of the relationship. Pearson chi-square and likelihood-ratio chi-square. Fisher's exact test and Yates' corrected chi-square are computed for 2x2 tables.

Chi-square test: For comparison between two groups as regards qualitative data.

$$X^2 = \sum \frac{(O-E)^2}{E}$$

Where:

Σ = Summation.

O = Observed value.

E = Expected value = $\frac{\text{vertical total X horizontal total}}{\text{grand total}}$

Linear Correlation Coefficient [r]:

$$r = \frac{\Sigma \left(X - \bar{X}\right)\left(y - \bar{y}\right)}{\sqrt{\left\{\Sigma \left(X - \bar{x}\right)2 \right\} \left\{\Sigma \left(y - \bar{y}\right)2\right\}}}$$

Where:

X= Independent variable.

Y= Dependent variable

5- ROC-curve:- Receiver Operating Characteristic curve analysis

- **Sensitivity:** Probability that the test results will be positive when the disease is present (true positive rate, expressed as a percentage).
- **Specificity**: Probability that the test results will be negative when the disease is present (true negative rate, expressed as a percentage).
- **PPV:** Positive Predictive value (probability that the disease is present when the test is positive).
- **NPV:** Negative Predictive value (probability that the disease is present when the test is negative).
- **Accuracy:** The ratio of the true positive and true negative on all patients.

RESULTS

There were 16 males (64%) of subjects in Groups 1, 2 & 3 and 14 males (56%) in group 4. Females were 9 (36%) of subjects in Groups 1, 2 & 3 and 11 females (44%) in group 4. No significant difference was found between the four groups p= 0.917 (table 1).

Group 1 had age range of 42.0 – 67.0 years with a mean value of 54.96 ± 6.78 and a median of 56.0 years, while Group 2 & 3 ranged from 45.0 – 70.0 years a with mean value of 58.40 ± 7.11 and a median of 59.0 years. Group 4 ranged from 40.0 – 65.0 years with a mean value of 50.92 ± 7.43 and a median of 50.0 years. There was a statistical significance between the four groups (p =0.001), between group 4 and groups 2 & 3 (p_1 = 0.002) as shown in table (1).

Table (1): Comparison between the different studied groups according to demographic data

	Group1 (n=25)		**Group2 (n=25)**		**Group 3 (n = 25)**		**Group 4 (n = 25)**		**Test of sig.**	**p**
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**		
Sex										
Male	16	64.0	16	64.0	16	64.0	14	56.0	χ^2=0.509	0.917
Female	9	36.0	9	36.0	9	36.0	11	44.0		
Age (years)										
Min. – Max.	42.0 – 67.0		45.0 – 70.0		45.0 – 70.0		40.0 – 65.0			
Mean ± SD.	54.96 ± 6.78		58.40 ± 7.11		58.40 ± 7.11		50.92 ± 7.43		F=6.260	0.001*
Median	56.0		59.0		59.0		50.0			
p_1	0.192		0.002*		0.002*					
Sig. bet. Grps	p_2=0.324,p_3=0.324,p_4=0.192									

χ^2: **Chi square test**

F: **F for ANOVA test**, Pairwise comparison bet. each 2 groups was done using **Post Hoc Test(Tukey)**

p: p value for comparing between the studied groups

p_1: p value for comparing between group4 and other each group

p_2: p value for comparing between group1 and group2

p_3: p value for comparing between group1 and group3

p_4: p value for comparing between group2 and group3

*: Statistically significant at $p \leq 0.05$

Group 1: Decompensated liver cirrhosis
Group2: Hepato - renal syndrome
Group 3: Hepato - renal syndrome post-treatment
Group 4: Control

Table (2) showed that creatinine in group 1 ranged from 1.09 to 1.50 with a mean of 1.31 ± 0.13 and a median of 1.30 mg/dL, while that of group 2 ranged from 1.60 to 6.30 with a mean of 2.80 ± 1.13 and a median of 2.60mg/dL. In Group 3, creatinine ranged from 1.20 to 4.20 with a mean of 2.16 ± 0.83 and a median of 2.0 mg/dL. Finally, that of group 4 ranged from 0.60 to 0.90 with a mean of 0.79 ± 0.11 and a median of 0.80 mg/dL. There was a statistical significance between the four groups ($p < 0.001$), between group 4 & group 1 ($p_1 = 0.001$) and between group 4and groups 2 & 3 ($p_1 < 0.001$). In addition, there was a statistical difference between group 1 & group 2 ($p_2 < 0.001$) and between group 1 & group 3 ($p_3 = 0.001$).

Table (2): Comparison between the different studied groups according to creatinine

Creatinine	Group1 (n=25)	Group2 (n=25)	Group 3 (n = 25)	Group 4 (n = 25)	H	p
Mean ± SD.	1.31 ± 0.13	2.80 ± 0.13	2.16 ± 0.83	0.79 ± 0.11		
Median	1.30	2.60	2.0	0.80		
p_1	0.001*	<0.001*	<0.001*			
Sig. bet. Grps	p_2<0.001*, p_3=0.001*, p_4=0.122					

H: H for **Kruskal Wallis test,** Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (Dunn's for multiple comparisons test)**

p: p value for comparing between the studied groups
p_1: p value for comparing between group4 and other each group
p_2: p value for comparing between group1 and group2
p_3: p value for comparing between group1 and group3
p_4: p value for comparing between group2 and group3
*: Statistically significant at $p \leq 0.05$

Group 1: Decompensated liver cirrhosis
Group2: Hepato - renal syndrome
Group 3: Hepato - renal syndrome post-treatment
Group 4: Control

Table (3) showed that total bilirubin range in group 1 was 1.10 – 11.80 with a mean of 3.83 ± 2.67 and a median of 2.90 mg/dL, while that of group 2 ranged from 1.50 to 38.0 with a mean of 6.84 ± 9.20 and a median of 2.90 mg/dL. In group 3, bilirubin ranged from 1.10 to 31.0 with a mean of 5.48 ± 7.67 and a median of 2.20 mg/dL and in group 4, it ranged from 0.50 to 0.90 with a mean of 0.72 ± 0.14 and a median of 0.70 mg/dL. There was a statistical significance between the four groups ($p < 0.001$) and also a significance difference between group 4 and groups 1,2 & 3 ($p_1 < 0.001$).

Table (3): Comparison between the different studied groups according to total Bilirubin

Total Bilirubin	Group1 (n=25)	Group2 (n=25)	Group 3 (n = 25)	Group 4 (n = 25)	H	p
Mean ± SD.	2.67± 0.83	6.84± 1.20	5.48 ± 1.67	0.14± 0.072		
Median	2.90	2.90	2.20	0.70		
p_1	<0.001*	<0.001*	<0.001*			
Sig. bet. Grps	p_2=0.553, p_3=0.667, p_4=0.313					

H: H for **Kruskal Wallis test,** Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (Dunn's for multiple comparisons test)**

p: p value for comparing between the studied groups
p_1: p value for comparing between group4 and other each group
p_2: p value for comparing between group1 and group2
p_3: p value for comparing between group1 and group3
p_4: p value for comparing between group2 and group3
*: Statistically significant at $p \leq 0.05$

Group 1: Decompensated liver cirrhosis
Group2: Hepato - renal syndrome
Group 3: Hepato - renal syndrome post-treatment
Group 4: Control

Table (4) showed that INR in group 1 ranged from 0.87 to 1.19 with a mean of 1.01 ± 0.10 and a median of 1.0, while that of group 2 ranged from 1.26 to 3.07 with a mean of 1.84 ± 0.47 and a median of 1.63. In group 3, INR ranged from 1.20 to 2.70 with a mean of 1.58 ± 0.34 and a median of 1.50 and in group 4, it ranged from 0.80 to 1.0 with a mean of 0.92 ± 0.06 and a median of 0.9. There was a statistical significance between the four groups ($p < 0.001$) , between group 4 and groups 2 & 3 ($p_1 < 0.001$) , between group 1 and group 2 ($p_2 < 0.001$) , between group 1 and group 3 ($p_3 < 0.001$) and between group 2 and group 3 ($p_4 = 0.013$).

Table (4): Comparison between the different studied groups according to INR

INR	Group1 (n=25)	Group2 (n=25)	Group 3 (n = 25)	Group 4 (n = 25)	F	p
Mean ± SD.	1.01± 0.10	1.84 ±0.17	1.58 ±0.34	0.92± 0.06		
Median	1.0	1.63	1.50	0.90		
p_1	0.756	<0.001*	<0.001*			
Sig. bet. Grps	p_2<0.001*, p_3<0.001*, p_4=0.013*					

F: **F for ANOVA test**, Pairwise comparison bet. each 2 groups was done using **Post Hoc Test(Tukey)**

p: p value for comparing between the studied groups
p_1: p value for comparing between group4 and other each group
p_2: p value for comparing between group1 and group2
p_3: p value for comparing between group1 and group3

p_4: p value for comparing between group2 and group3

*: Statistically significant at $p \leq 0.05$

Group 1: Decompensated liver cirrhosis
Group2: Hepato - renal syndrome
Group 3: Hepato - renal syndrome post-treatment
Group 4: Control

Table (5) showed Meld score in group 1 ranged from 9.0 to 19.0 with a mean of 13.68 ± 2.66 and a median of 13.0, while that of group 2 ranged from 20.0 to 43.0 with a mean of 27.12 ± 5.45, and a median 26.0. In group 3, it ranged from 15.0 to 40.0 with a mean of 22.32 ± 5.86 and a median of 21.0 . There was a statistical significance between the four groups ($p < 0.001$), between group 1 and group 2 ($p_1 < 0.001$) , between group 1 and group 3 ($p_2 < 0.001$) and between group 2 and group 3 ($p_3 = 0.018$) .

Table (5): Comparison between the different studied groups according to Meld score

Meld score	Group1 (n=25)	Group 2 (n= 25)	Group3 (n=25)	H	p
Mean ± SD.	13.68± 2.66	27.12 ±5.45	22.32± 5.86		
Median	13.0	26.0	21.0		
Sig. bet. Grps	$p_1<0.001^*$,$p_2<0.001^*$,$p_3=0.018^*$				

H: H for **Kruskal Wallis test,** Pairwise comparison bet. each 2 groups was done using **Post Hoc Test (Dunn's for multiple comparisons test)**
p: p value for comparing between the studied groups
p_1: p value for comparing between group1 and group2
p_2: p value for comparing between group1 and group3
p_3: p value for comparing between group2 and group3
*: Statistically significant at $p \leq 0.05$

Group 1: Decompensated liver cirrhosis
Group2: Hepato - renal syndrome
Group 3: Hepato - renal syndrome post-treatment

Table (6) showed that NGAL in group 1 ranged from 128.0 to 300.0 with a mean of 208.6 ± 58.19 , and a median of 200.0 ng/dl, while that of group 2 ranged from 230.0 to 410.0 with a mean of 348.24 ± 56.85 and a median of 370.0 ng/dl. In Group 3, NGAL ranged from 180.0 to 360.0 with a mean of 297.8 ± 56.65 and a median of 320.0 ng/dl and in Group 4, it ranged from 41.0 to 54.0 with a mean of 47.04 ± 3.92 and a median of 48.0 ng/dl. There was a statistical significance between the four groups ($p < 0.001$), between group 4 and groups 1, 2 & 3 ($p_1 < 0.001$), between group 1 and group 2 ($p_2 < 0.001$), between group 1 and group 3 ($p_3 < 0.001$) and between group 2 and group 3 ($p_4 = 0.003$).

Table (6): Comparison between the different studied groups according to NGAL (ng/ml)

NGAL (ng/ml)	Group1 (n=25)	Group2 (n=25)	Group 3 (n = 25)	Group 4 (n = 25)	F	p
Mean ± SD.	208.6 ±8.19	348.2 ±6.85	297.8 ±6.65	47.04 ±3.92		
Median	200.0	370.0	320.0	48.0		
p_1	$<0.001^*$	$<0.001^*$	$<0.001^*$			
Sig. bet. Grps	$p_2<0.001^*$,$p_3<0.001^*$,$p_4=0.003^*$					

F: **F for ANOVA test**, Pairwise comparison bet. each 2 groups was done using **Post Hoc Test(Tukey)**
p: p value for comparing between the studied groups
p_1: p value for comparing between group4 and other each group
p_2: p value for comparing between group1 and group2
p_3: p value for comparing between group1 and group3
p_4: p value for comparing between group2 and group3
*: Statistically significant at $p \leq 0.05$

Group 1: Decompensated liver cirrhosis
Group2: Hepato - renal syndrome
Group 3: Hepato - renal syndrome post-treatment
Group 4: Control

Figure (1) and table (7) showed that the AUC for MELD score was 1.000 while for NGAL was 0.943**.** The cutoff for MELD score was >19 while for NGAL was >290ng/ml. MELD score sensitivity, specificity ,PPV , NPV were all 100 %, while NGAL 's sensitivity was 80 %, specificity was 96%, PPV was 95.2% and NPV was 82.8 %.

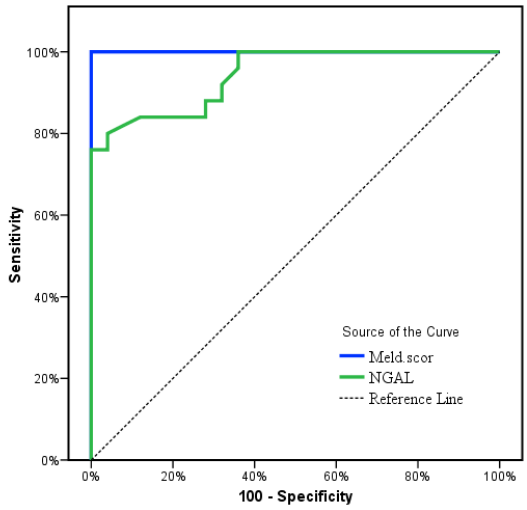


Figure (1): ROC curve for different parameters to diagnose group (Hepato - renal syndrome) from group 1(Decompensated liver cirrhosis)

Table (7): Agreement (sensitivity, specificity) for different parameters to diagnose group (Hepato - renal syndrome) from group 1(Decompensated liver cirrhosis)

	AUC	P	95% C.I	Cut off	ensitivity	pecificity	PPV	NPV
Meld score	1.000	<0.001*	1.0 – 1.0	**>19**	100.0	100.0	100.0	100.0
NGAL (ng/ml)	0.943	<0.001*	0.885 – 1.001	**>290**	80.0	96.0	95.2	82.8

AUC: Area Under a Curve, p value: Probability value, CI: Confidence Intervals, NPV: Negative predictive value PPV: Positive predictive value, *: Statistically significant at $p \leq 0.05$

Figure (2) and table (8) showed that the AUC for MELD score was 0.769 while for NGAL was 0.757**.** The cutoff for MELD score was ≤ 20, while for NGAL was ≤ 320 ng/ml. MELD score sensitivity was 44%, specificity was 96%, PPV was 91.7% and NPV was 63.2 %, while NGAL 's sensitivity was 56% , specificity was 64%, PPV was 60.9% and NPV was 59.3 %.

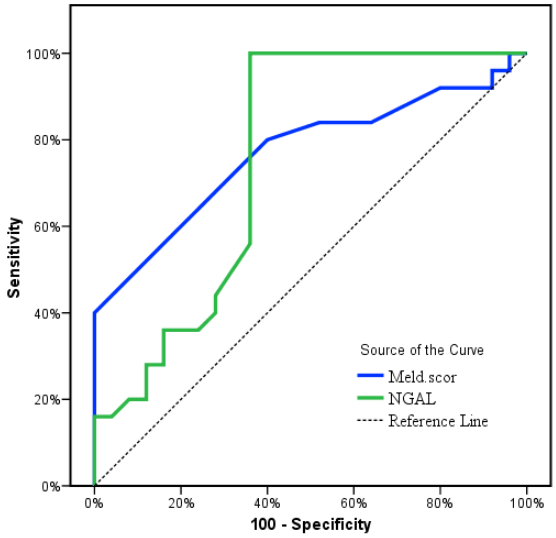


Figure (2): ROC curve for different parameters to diagnose group 3 (Hepato - renal syndrome post-treatment) from group2 (Hepato - renal syndrome)

Table (8): Agreement (sensitivity, specificity) for different parameters to diagnose group 3 (Hepato - renal syndrome post-treatment) from group 2(Hepato - renal syndrome)

	AUC	P	95% C.I	Cut off	ensitivity	pecificity	PPV	NPV
Meld score	0.769	0.001*	0.635 – 0.903	**≤20**	44.0	96.0	91.7	63.2
NGAL (ng/ml)	0.757	0.002*	0.614 – 0.899	**≤320**	56.0	64.0	60.9	59.3

AUC: Area under a Curve, p value: Probability value, CI: Confidence Intervals, NPV: Negative predictive value PPV: Positive predictive value, *: Statistically significant at $p \leq 0.05$

DISCUSSION

Renal dysfunction is a severe complication of advanced cirrhosis as well as of acute-on-chronic liver failure (ACLF). Hepato-renal syndrome (HRS) has been defined as a syndrome that occurs in patients with advanced liver disease, characterised by impaired renal function and marked abnormalities in the arterial circulation and over-activity of the endogenous vasoactive systems. Hepato-renal syndrome has been classified into two different clinical types: type-1 HRS, characterised by a rapidly progressive reduction of renal function, defined by a doubling of the serum creatinine to a level 2.5 mg/dl in less than 2 weeks, and type-II HRS, in which the renal failure does not have a rapidly progressive course **(9).**

Plasma NGAL levels have been suggested to be as a valuable plasma biomarker in detecting early renal dysfunction in several clinical situations **(10).** This study was performed to investigate the possible role of NGAL as a marker of renal function, in the setting of acute decompensated liver cirrhosis and HRS **(11).** Many studies in several clinical situations have underlined that the NGAL increased two hours after the induction of acute kidney injury (AKI), before the serum creatinine elevation **(12).**

Regarding sex distribution, they were 16 males (64%) of subjects in groups 1, 2 and 3 and 14 males (56%) in group 4. Females were 9 (36%) of subjects in groups 1, 2 and 3 and 11 females (44%) in group 4. Similar results were reported in a study done by **Daniel *et al.*** **(13)** which reported a mean age of 56.3 ±11.8 years, and 63.4% were males. According to an analysis by the National Center for Health Statistics done in 2005, **Rogers *et al.*** **(14)** reported that men are two-fold more likely to die from chronic liver disease and cirrhosis than women and this related to gender-specific differences in exposure to risk factors for developing cirrhosis.

Men are more likely to be infected with HBV and HCV because of smoking, alcohol intake and they have increased iron stores.

In our study, significant differences between the four groups were shown with respect to the plasma levels of creatinine and NGAL, which were markedly lower in patients without development of renal dysfunction. However, only NGAL, was found to be predictive for renal dysfunction development.

The results showed that serum bilirubin was elevated in hepato-renal syndrome and decompensated groups which is in agreement with reports of **El Bassat *et al.*** **(15).**

Regarding INR, our results showed significantly higher international normalized ratio in HRS patients and decompensated group, which is in agreement with **Zhang *et al.*** **(16).**

Baseline levels of creatinine and NGAL, were significantly higher in patients who were about to develop hepato-renal syndrome during follow-up ($P < 0.0001$ for all) and considered as a predictive for 90-day transplant-free mortality **(13)**. In contrast to our results, **Ariza *et al.*** **(17)** reported that plasma NGAL did not predict acute-on-chronic liver failure development; these results may be attributed to limited number in this study.

This biomarker could be of assistance in the pre- and post-transplant evaluation of patients with liver cirrhosis. Some studies indicated that NGAL is a good prediction tool for early post-transplant acute kidney injury and tacrolimus-induced acute kidney injury in patients with liver transplantation. In the pre-transplant setting, kidney damage biomarker could recognize patients with structural renal impairment in need of simultaneous liver and kidney transplant **(18).**

In our study, MELD score showed a significant difference between the four groups, which was lower in patients without development of renal dysfunction and higher in patients with hepato-renal syndrome with $p < 0.001$. After treatment, MELD score decreased so it can control the liver transplantation waiting list. Similar results were obtained and showed independent predictive factor associated with acute kidney injury that was MELD score which emphasized the importance of the severity of liver disease and circulatory dysfunction in the development of acute kidney injury with $p< 0.001$ **(14).**

Despite significantly raised levels found in patients with renal impairment, plasma NGAL was not useful in distinguishing between the causes of kidney dysfunction in patients with liver cirrhosis. This result reported by **Fagundes *et al.*** **(19)** disagree with our study .

CONCLUSIONS

NGAL could be used in conjunction with serum creatinine to assess the hepato-renal affection and may aid in stratifying patients in need for liver transplant.

RECOMMENDATIONS

- Further studies have to be done on a large number of patients for more comprehensive statistical analysis and better conclusions.
- NGAL may be used in conjunction with other diagnostic tools to early detect development of HRS in patients with liver cirrhosis.

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