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Assessment of Ischemia modified albumen in chronic liver diseases.

ABSTRACT

Aims: Ischemia modified albumin (IMA) level is increased in ischemic conditions and in diseases such as myocardial infarction, systemic sclerosis, advanced cancer, end-stage renal disease and intrauterine disorders**.** The role of IMA in chronic liver diseases and its correlation with disease severity needs further investigations. So we aimed to assess IMA and its ratio to albumin as a marker of advanced liver cirrhosis and their correlation with the disease severity.

Study design: A cross sectional study including125 patients with chronic liver disease (69 males and 31 females) with mean age 54.63 years; and 30 healthy controls .
Place and Duration of Study: Hepatology Unit ,Specialized Medical Hospital and Tropical Department , Mansoura University between June 2014 and March 2015.
Methodology: The patients with chronic liver disease(80 males, 45 females) with age range from 46 to 62 years, 70 cases were chronic HCV, 15 chronic HBV,20 combined HCV,HBV 10 autoimmune liver diseases, 5 NASH, and 5 of unknown cause. They were further subdivided according to Child-Pugh scoring into 50 patients with Child A, 45 patients with Child B and 30 patients with Child C. 35 healthy subjects of matched age and sex were included as control group. Laboratory analysis including CBC, liver profile, prothrombin time ,IMA and IMAR were done to all patients and control.

Results: There was significant increase in IMA and IMAR in studied patient groups versus controls, in Child B versus Child A and in Child C versus both Child A & B. A significant positive correlation was found between both IMA & IMAR with total bilirubin and INR while a significant negative correlation was reported between both IMA & IMAR with albumin, ALT, AST, Hb, WBCs and PLT in liver cirrhosis. At cut off > 0.767 IMA had a sensitivity of 86.99 % and a specificity of 77.1% (AUC; 0.85) and at cut off > 0.213 IMAR had a sensitivity of 92.5 % and a specificity of 88.5% (AUC; 0.95) for detecting liver cirrhosis . For detecting severity of liver cirrhosis, by comparing Child B and Child C, at cut off > 0.968 IMA had a sensitivity of 93.3 % and a specificity of 94.2% (AUC; 0.96) and at cut off > 0.453 IMAR had a sensitivity of 92.8 % and a specificity of 72.7% (AUC; 0.86) for detecting severity of liver cirrhosis.

Conclusion: : IMA and IMAR are sensitive markers in chronic liver disease and better correlated with the degree of decompansation. A further study to assess their role in follow up of treatment response is recommended

Keywords: IMA, IMAR, cirrhosis and chronic liver disease

1. INTRODUCTION

Chronic liver disease is a major health problem in Egypt and is commonly associated with HCV and HBV infections[1] **.** Cirrhosis is often an indolent disease; most of patients remain asymptomatic until the occurrence of decompensation, characterized by ascites, spontaneous bacterial peritonitis, hepatic encephalopathy, or variceal bleeding from portal hypertension. No serologic or radiographic test can accurately diagnose the severity of cirrhosis[2]

Human serum albumin is the most abundant plasma protein, it constitutes about 50% of the total protein content. It is mainly synthesized in liver and it is responsible for about 70% of the plasma oncotic pressure.[3] also it has further biological properties: It binds and transports various water-insoluble molecules, metals, and drugs, also shares in detoxification of endogenous and exogenous substances

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[4]. Furthermore, it is the main circulating antioxidant in the body, being the major extracellular source of reduced sulfhydryl groups [5].

The amino terminal end of albumin molecule is the primary binding site for cobalt and nickel[6] . This site is susceptible to biochemical degradation under various conditions such as ischemia, hypoxia, superoxide-radical injury and acidosis , this decreases binding capacity of albumin for metals and generates variant of albumin with reduced metal binding, known as ischemia modified albumin (IMA)[7].

High serum IMA level was first used as an early marker for the diagnosis of myocardial ischemia and acute coronary syndrome in patients presenting with typical acute chest pain[8]**.** Also, IMA level is known to increase in other ischemic conditions and in diseases as systemic sclerosis, advanced cancer, end-stage renal disease and intrauterine disorders[7].

Only few studies were done to assess the role of IMA in chronic liver diseases. ***Jalan et al,*** studied albumin functions in patients with decompensated cirrhosis and reported that albumin functions are impaired in advanced cirrhosis. Additionally, they found that serum IMA level (expressed as an albumin ratio) correlate with the disease severity and may have prognostic use in acute-on-chronic liver failure[9].

So, the aim of this work was to assess IMA and its ratio to albumin as a marker of advanced liver cirrhosis and their correlation with the disease severity

2. material and methods

This study was conducted on 125 patients with chronic liver disease, 80 males and 45 females with age range from 46 to 62 years. The diagnosis of chronic liver disease was based on history taking, complete physical examinations, abdominal ultrasound and laboratory investigations including liver profile and hepatitis markers. Patients were excluded if they received albumin infusion one month prior to the involvement in this study, had infection or hepatorenal syndrome, also patients who had malignancy , vascular disease or thrombosis were excluded. 70 cases were chronic HCV, 15 chronic HBV, 20 combined HCV,HBV ,10 autoimmune liver diseases, 5 NASH, and 5 of unknown cause. They were further subdivided according to Child-Pugh scoring into 50 patients with Child A, 45 patients with Child B and 30 patients with Child C. thirty five healthy subjects, 20 males and 15 females of matched age (46 to 62) were included as control group.

2.1Sampling:

Seven ml venous blood samples were withdrawn and divided as follow; 1ml into EDTA tube for CBC, 1.8 ml into prothrombin tube for INR and the rest into plain tube, left to clot and serum were separated into two aliquots, the first, used for routine analysis and the second was stored at -20℃ I MA assay.

2.1Methods of assay:

CBC was analyzed using automated counter, Sysmex KX-21, USA. Routine laboratory investigations were analyzed using Dimension Xpand plus chemistry auoanalyzer, Siemens.

IMA level was analyzed using the colorimetric method developed by *Bar-Or et al, [10]*. Two hundred microliter (µL) of patient serum was placed into glass tubes then 50 µL of 0.1% $CoCl_2+6H_2O$ (Sigma) was added. After gentle shaking, the mixture was left for 10 minutes to ensure sufficient cobalt albumin binding. Then, 50 µL of 1.5 mg/mL dithiothreitol (DTT) was added as a coloring agent. After 2 minutes, 1 mL of 0.9% NaCl was added quench the reaction. A sample blank was prepared for every sample using the same steps as the sample but 50 µL of distilled water to be used instead of 50 µL of 1.5 mg/mL DTT. Sample absorbencies were measured at 470 against its sample blank.

2.3Statistical analysis:

Data are expressed as mean ± SD. Receiver operator characteristic (ROC) curves with respective points of maximal accuracy for sensitivity and specificity were generated to determine the performance of both IMA and IMAR.. Tukey's Multiple Comparison Test was used to assess the

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significance of group differences. Spearman's rank correlation coefficient was used to examine the correlation between the level of sCD25 and other laboratory parameters. P<0.05 was considered to indicate a statistically significant result. Statistical data were analyzed using Graph Pad Prism 5 version 5.01

3. RESULTS AND DISCUSSION

3.1Results:

Table (1) illustrates some demographic and clinical data of the studied patient groups. Child A included 50 patient (30 males and 20 females) with a mean age of 54.7 ±4.5, Child B included 45 patients (30 males and 15 females) with a mean age of 54.9 ±6.2 and Child C were 30 patients (20 males and 10 females) with a mean age of 56 ±4.7, while the control group included 35 subjects (30 males and 15 females) with a mean age of 54.8 ±5.6. There was no significant difference in age or sex between studied groups and controls (p> 0.05). The causes of chronic liver diseases were chronic HCV in 70 cases, chronic HBV in 15 cases, combined chronic HCV and HBV in 20 cases, autoimmune liver diseases in 10 cases, NASH in 5 cases, and 5 cases of unknown cause (figure 1)

There was significant increase in total bilirubin, INR, ALT, AST, IMA and IMAR in studied patient groups versus controls while a significant decrease in albumin, Hb, PLT and WBCs were reported in them in comparison to controls (table, 2). By comparing Child B versus Child A, there was significant increase in total bilirubin, INR, AST and ALT and significant decrease in albumin and Hb, while there was no difference in WBCs and PLT.

Also when comparing Child C to both Child B and Child A there was significant increase in total bilirubin, INR, AST and ALT and significant decrease in albumin, WBCs, PLT and Hb

A significant positive correlation was found between both IMA & IMAR with total bilirubin and INR while a significant negative correlation was reported between both IMA & IMAR with albumin, ALT, AST, Hb, WBCs and PLT in liver cirrhosis (table 3 & figure 3).

At cut off > 0.767 IMA had a sensitivity of 86.99 % and a specificity of 77.1% (AUC; 0.85) and at cut off > 0.213 IMAR had a sensitivity of 92.5 % and a specificity of 88.5% (AUC; 0.95) for detecting liver cirrhosis (Figure 4). For detecting severity of liver cirrhosis, by comparing Child B and Child C, at cut off > 0.968 IMA had a sensitivity of 93.3 % and a specificity of 94.2% (AUC; 0.96) and at cut off > 0.453 IMAR had a sensitivity of 92.8 % and a specificity of 72.7% (AUC; 0.86) for detecting severity of liver cirrhosis (figure 5).

3.2Discussion:

Albumin is more than a volume expander, it has been proved to undertake various functions including metal chelation, fatty acid transport , drug binding and anti-oxidant activity.[4]
Increased IMA suggest an impaired metal chelation capacity and loss of albumin function. Also, increased IMA level was associated with poor survival [11,12,9]
IMA was shown to be a myocardial infarction marker [13]. However, IMA may increase in non-cardiac ischemic conditions, as liver cirrhosis and metabolic syndrome [14,15]. Also, in diabetic patients , in chronic hypoxia conditions [16]and in chronic hepatic and renal diseases[17].

Patients with advanced cirrhosis almost always have hypoalbuminemia[18]. A reduced albumin level will result in a lower capacity to bind cobalt, leading to high false positive IMA score; so it was suggested that IMA should be expressed relative to the serum albumin concentration (IMAR) to minimize the impact of reduced albumin level.[9]
Thus, in this study we aimed to assess IMA and its ratio to albumin IMAR as a marker of advanced liver cirrhosis and their correlation with the disease severity.

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The present study that assess the serum IMA and IMAR levels in patients with CLD, revealed that IMA and IMAR levels were significantly higher in these patients compared to healthy subjects. Elevation of IMAR which considered a marker of albumin function, and impairment of liver functions in patients with CLD not only decreases the effective circulation volume in association with hypoalbuminemia, but also decreases the get rid of toxic metabolites. [19]
This may be explained by oxidative stress which results from CLD and affects the biological properties of albumin, so becomes more susceptible to proteinase digestion and faster degradation trhan the non-oxidized part, and decreased binding capacity to various substances [4,20]. In healthy adults, a small fraction is highly oxidized to sulfonic acid (human nonmercaptalbumin 2). In contrast, in chronic diseases, the oxidized form of albumin greatly increases, leading to an impairment of its biological activities [20].

In the current study, it was noticed that IMA and IMAR levels were correlated with Child-Pugh score and similar results were reported by Jalan etal. , Chen etal , Cakir et al and Klammt et al[9,15,19, 21]. This increase in IMAR levels with disease severity provides evidence of protein modification, which associated with severity of oxidative stress.[22]
Higher level of IMAR in more advanced decompensated cirrhotic patients(Child B,C)may lead to accumulation of toxic metabolites (tryptophan, endogenous benzodiazepines, bile acids, and fatty acids) in these patients, and may aggravate the development of hepatic encephalopathy.

A significant positive correlation was found between both IMA & IMAR with total bilirubin and INR while a significant negative correlation was reported between both IMA & IMAR with albumin, ALT, AST, Hb, WBCs and PLT in liver cirrhosis. This is consistent with its correlation to liver disease severity. Similar results was found by Chen etal which found that IMA and IMAR were significantly correlated with bilirubin and INR in patients with chronic hepatitis and cirrhosis[15].
In contrast ,Cakir etal found no correlation between serum alanine aminotransferase (ALT), INR levels and serum IMAR level. Only bilirubin level was significantly
correlated with serum IMAR level[19] .This difference between that study and the current study can be explained by firstly; more advanced chronic liver disease(larger number of Child B, C) in our study and Child A in the other study . secondly; different aetiologies of liver disease (most of our cases are chronic HCV, HBV and in the other study, 64%metabolic and 18% cholestatic liver diseases).

Table (1): Demographic and clinical data of studied patient groups:

Parameter	Control (n=35)	Child A (n=50)	Child B (n=45)	Child C (n=30)
Age (years) **Mean ±SD**	**54.8 ±5.6**	**54.7 ±4.5**	**54.9 ±6.2**	
p-value	**>0.05**			
Sex **Male** **Female**	 **20** **15**	 **30** **20**	 **30** **15**	 **20** **10**
p-value	**>0.05**			
Ascites (N0-%) **No** **Mild** **Moderate** **Severe**	**35 (100%)**	**50 (100%)**	**12 (26.6%)** **33 (73.4%)**	**4 (13.3%)** **6 (20.0%)** **20 (66.7%)**
Hepatic encephalopathy	**No**	**No**	**5 (11%)**	**10 (33.3%)**

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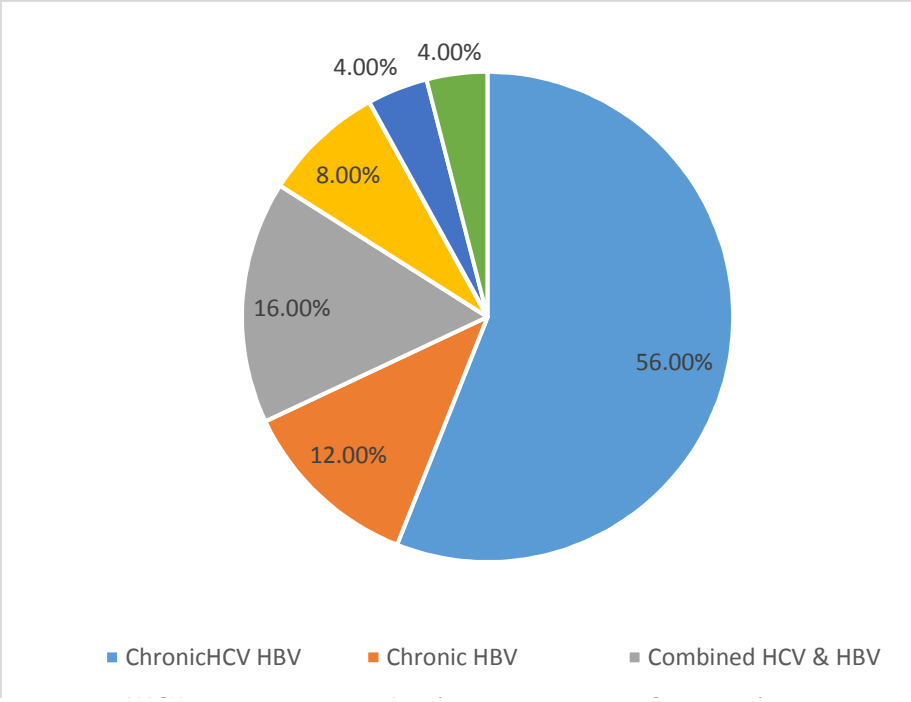

Figure (1): Causes of chronic liver diseases among studied patients

Table (2): Some laboratory parameters of studied patient groups:

Parameter	Control (n=35)	Child A (n=50)	Child B (n=45)	Child C (n=30)	p- value
Hb (gm/dl) Mean ±SD	**13.6 ± 1.2**	**9.8 ± 0.75**	**9.0 ± 0.4**	**7.8 ± 0.41**	**$P_1 < 0.05^*$ $P_2 < 0.05^*$ $P_3 < 0.05^*$**
p-value	**$< 0.05^*$**				
WBCs (10^3/cmm) Mean ±SD	**6.1 ±1.29**	**3.3 ±0.43**	**3.37 ± 0.29**	**3.0 ± 0.54**	$P_1 > 0.05$ $P_2 > 0.05$ $P_3 > 0.05$
p-value	**$< 0.05^*$**				
PLT (10^3/cmm) Mean ±SD	**221.3 ± 21.6**	**88.5 ± 8.2**	**91.2 ± 8.3**	**71.7 ± 31.5**	$P_1 > 0.05$ **$P_2 < 0.05^*$ $P_3 < 0.05^*$**
p-value	**$< 0.05^*$**				
ALT(U/L) Mean ±SD	**22.1 ± 4.6**	**52.5 ± 6.4**	**47.8 ± 6.4**	**35.8 ± 11.0**	**$P_1 < 0.05^*$ $P_2 < 0.05^*$ $P_3 < 0.05^*$**
p-value	**$< 0.05^*$**				
AST (U/L) Mean ±SD	**22.0 ± 2.8**	**51.1 ± 6.0**	**43.0 ± 2.8**	**31.9 ± 7.8**	**$P_1 < 0.05^*$ $P_2 < 0.05^*$ $P_3 < 0.05^*$**
p-value	**$< 0.05^*$**				
T. bilirubin (mg/dl) Mean ±SD	**0.56 ± 0.1**	**1.03 ± 0.15**	**1.6 ± 0.28**	**2.3 ± 0.71**	**$P_1 < 0.05^*$ $P_2 < 0.05^*$ $P_3 < 0.05^*$**
p-value	**$< 0.05^*$**				
Albumin (gm/dl)					**$P_1 < 0.05^*$**

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Mean ±SD	**4.1 ± 0.15**	**3.2 ± 0.16**	**2.7 ± 0.18**	**2.0 ± 0.43**	**$P_2 < 0.05^*$**
p-value	**< 0.05***				**$P_3 < 0.05^*$**
INR					$P_1 > 0.05$
Mean ±SD	**1.03 ± 0.03**	**1.3 ± 0.15**	**1.3 ± 0.19**	**1.46 ± 0.19**	**$P_2 < 0.05^*$**
p-value	**< 0.05***				**$P_3 < 0.05^*$**
IMA					**$P_1 < 0.05^*$**
Mean ±SD	**0.6 ± 0.27**	**0.86 ± 0.21**	**1.1 ± 0.45**	**1.56 ± 0.62**	**$P_2 < 0.05^*$**
p-value	**< 0.05***				**$P_3 < 0.05^*$**
IMAR					**$P_1 < 0.05^*$**
Mean ±SD	**0.142±0.066**	**0.269±0.07**	**0.411±0.16**	**0.792±0.36**	**$P_2 < 0.05^*$**
p-value	**< 0.05***				**$P_3 < 0.05^*$**

P: each patient group versus control, p_1 : Child A versus Child B, p_2: Child A versus Child C, p_3: Child B versus Child C

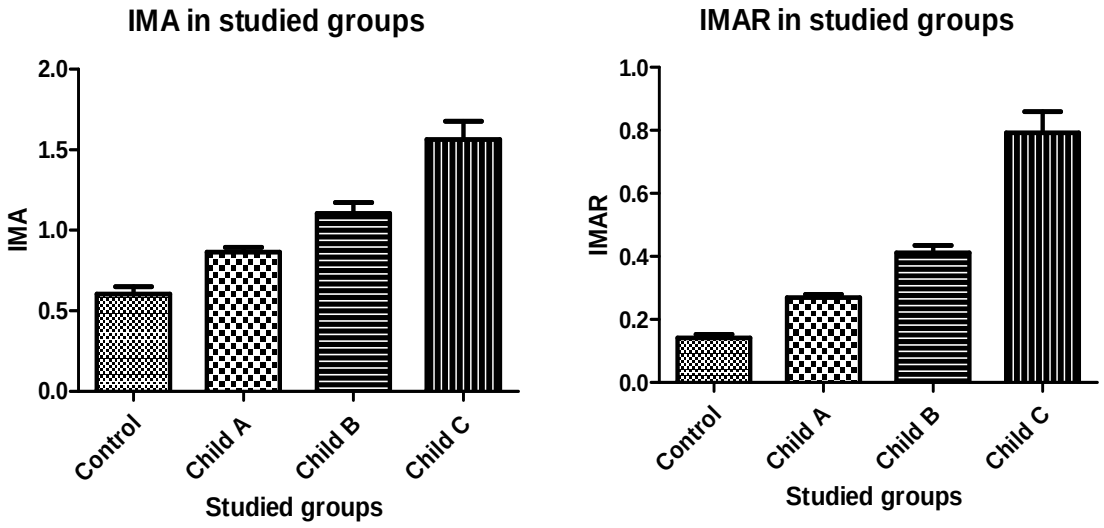

Figure (2): IMA in studied patient groups

Table (3): Correlation co efficient between IMA & IMAR and laboratory parameters:

	IMA		IMAR	
	R	**P-value**	**R**	**P-value**

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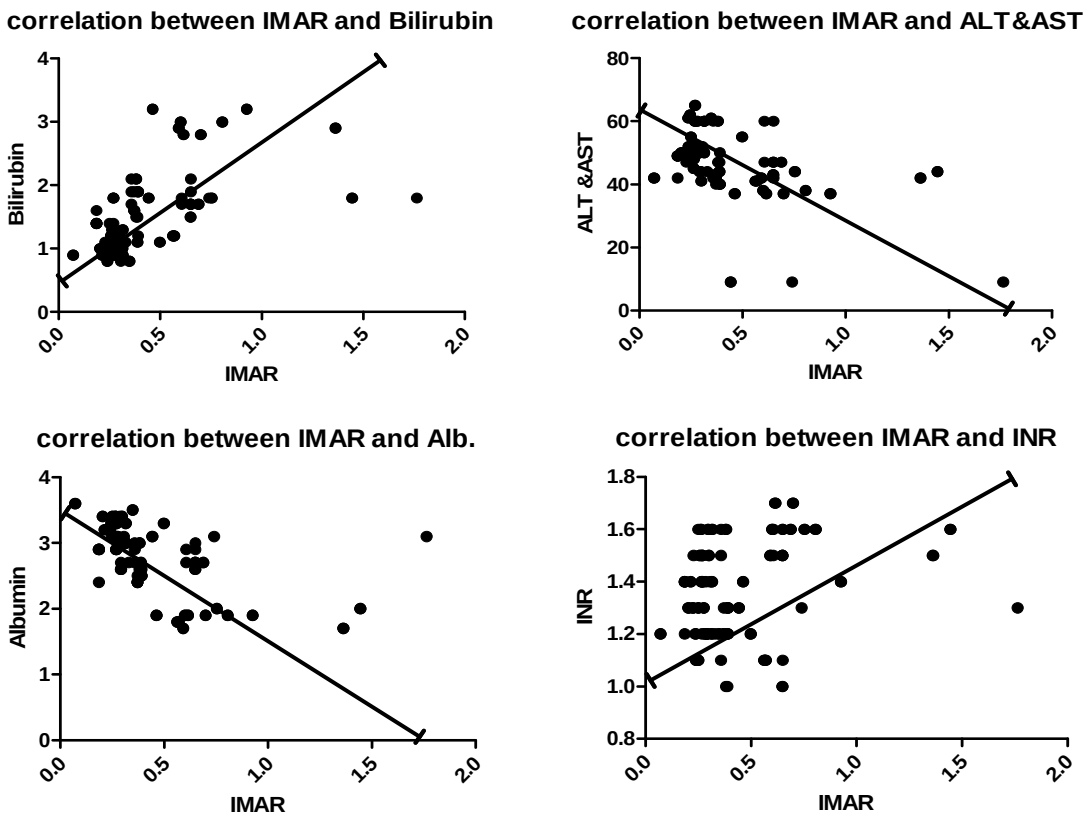
ALT	-0.3033	**0.0007***	-0.4499	**< 0.0001***
AST	-0.2940	**0.0011***	-0.4849	**< 0.0001***
T. bilirubin	0.4357	**< 0.0001***	0.5831	**< 0.0001***
Albumin	-0.2559	**0.0046***	-0.5987	**< 0.0001***
INR	0.4368	**< 0.0001***	0.2891	**< 0.0013***
Hb	-0.5987	**< 0.0001***	-0.5015	**< 0.0001***
WBCs	-0.1527	0.0945	-0.1952	**0.0319***
PLT	-0.2792	**0.0019***	-0.3875	**< 0.0001***



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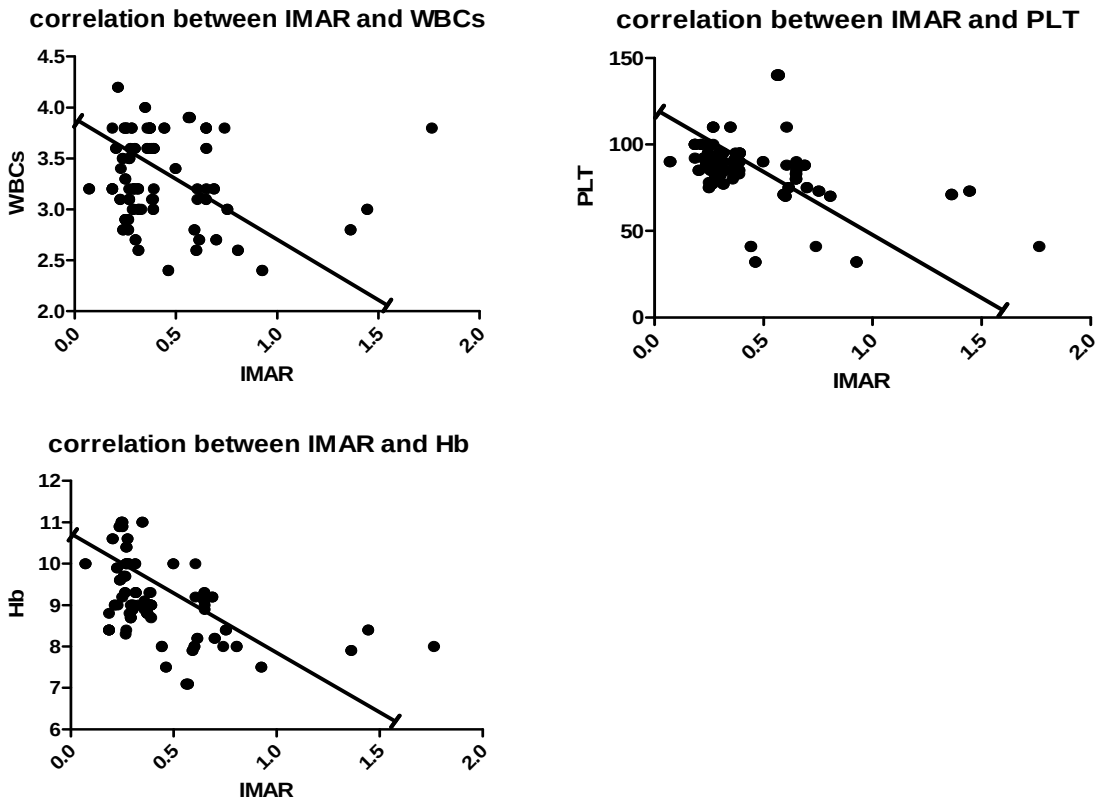

Figure (3): correlation coefficient between IMA and Some laboratory parameters

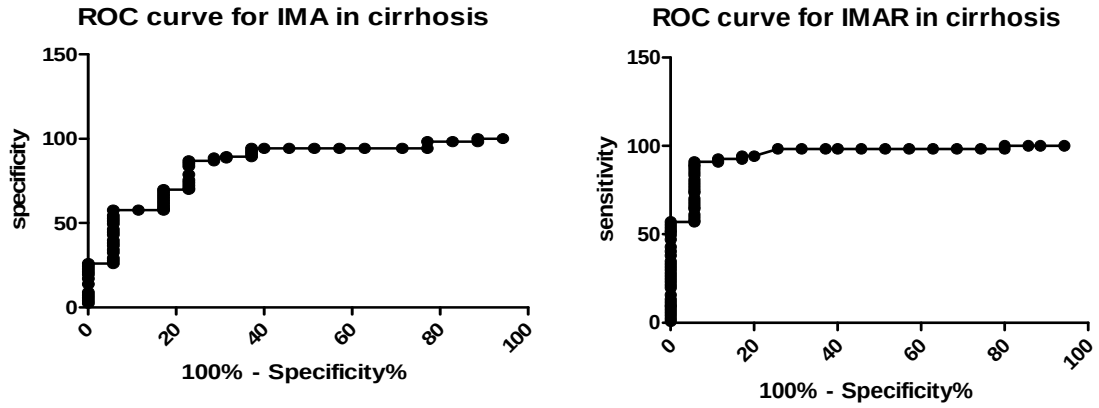

Figure (4): ROC curve for sensitivity and Specificity of IMA & IMAR in liver cirrhosis

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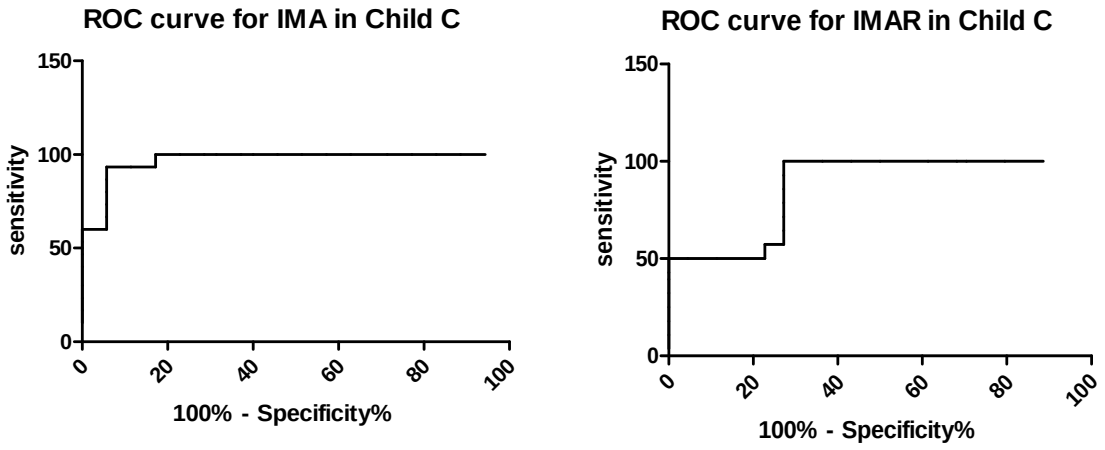

Figure (5): ROC curve for sensitivity and Specificity of IMA & IMAR in Child C cirrhosis

4. CONCLUSION

IMA , IMAR levels were investigated for the first time in Egyptian patients with CLD, and it has been shown that IMA, IMAR levels were elevated in CLD. This was more pronounced in more advanced disease. Thus, they could be useful markers of assessment of liver disease severity, but this needs further investigation with wider studies using multiple serial measurements rather than a single measurement.

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