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Relationship of Galectin-9 with Number of Biomarkers in Iraqi Chronic Hepatitis B Patients

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ABSTRACT

Chronic hepatitis B (CHB) is a global epidemic that can cause fibrosis, cirrhosis, and hepatocellular carcinoma. Galectins have the ability to regulate both intracellular and extracellular cell death. In this study, we attempted to see if there is any relationship between Gal-9 with ALT, AST, ALP, T. BIL, D. BIL, IN.BIL, TSP, Albumin, and Globulin. This research included 40 patients from Fallujah city, whoever was having CHB disease, with 40 there were also healthy controls registered. Serum ALT, AST, T. BIL, D. BIL, IN. BIL, ALP, TSP, Albumin, and Globulin Colorimetric methods were used to estimate concentrations. Gal-9 concentrations were estimated by, enzyme linked immunosorbent assay (ELISA) technique. Levels of in serum Gal-9, ALT, AST, ALP, T. BIL, D. BIL, IN. BIL and Albumin were higher in CHB patients (P< 0.0001) in comparison with healthy controls, but TSP , Globulin concentrations were lower in CHB patients in comparison to controls (P= 0.6843) and (P < 0.0001) respectively, serum Gal-9 concentrations related positively with serum ALT, AST, ALP, T. BIL, D. BIL, IN. BIL , Albumin and negatively correlated with Globulin concentrations (all P<0.01), the area under the receiver operating characteristic (AUROC) curve of ALT and AST[AUROC=1], T.BIL, Albumin , D.BIL , IN.BIL, globulins, ALP ,levels were, 0.9588 , 0.9222 , 0.9197, 0.9084, 0.8559, 0.7678 respectively, in conclusion serum levels of Gal-9 can use as in diagnosis CHB disease, also it may be use in manufacture treatment of CHB disease.

Key words: Galectin-9**,** Chronic Hepatitis B, ALT, AST.

INTRODUCTION

The hepatitis B virus (HBV) causes a life threatening infection of the liver.(1) In the 1970s, the prevalence of HBV infection was determined by weighting the data for each sample person collected from populations of first-time blood donors. (2) HBV is an important public health threat all over the world, with nearly 300 million people infected for CHB. (3) Liver disease at the end of its course, liver transplantation, hepatocellular carcinoma as well as death are all complications of CHB infection, with the risk of these health problems varying depending on the mode of transmission and disease duration.(4) In Iraq, 3–4.5% of the people are infected with HBV, including 2–3% of seemingly healthy blood donors.(5)

Galectins are a big protein family that mediates cell–cell interactions, cell–matrix cohesion, and transmembrane signaling. Its expression and secretion are tightly controlled, implying they may

be expressed at different stages of development.[6] Galectins are highly conserved lectins that belong to a family of pattern recognition receptors are found in a variety of tissues, cells, and immune cells.[7] Galectins are a type of lectin that has a specific affinity for b-galactosides and modulates a variety of biological processes.[8]

Galectin-9 was discovered to be involved in a variety of cellular processes, including differentiation, aggregation, adhesion, and death, as well as various phases of immune responses after being roneo and identified as a T cell-derived eosinophil-specific chemoattractant. [9] Gal-9 is abundant in the liver, also participates in a number of innate and adaptive biological processes that are important in maintaining hepatic homeostasis. Increased Gal-9 expression promotes viral persistence in the context of viral hepatitis by stimulating the increase in regulatory T cells and the decrease in effector T cells [10] Recent Iraqi study deals with many variables of oxidative stress in CHB disease. [11] While this study was the first study determine serum level of Gal-9 in Iraqi CHB patients, our research aims to estimate Gal-9 concentrations in Iraqi CHB patients, as well as to explore of any relationship between Gal-9 and a number of biochemical parameters.

MATERIALS AND METHODS

The study included 40 CHB patients ranging in age from 20 to 55 years, participants in the Al-Fallujah teaching hospital and numerous private laboratories were chosen at random and with care. The healthy control group consists of 40 people ranging in age from 20 to 55 years, with no medical signs of any illness. All cases and healthy controls had full histories and medical investigations that included determining ALT, AST, T. BIL, D.BIL, IN.BIL, Albumin, and Globulin through commercial available kits by colorimetric enzymatic methods, while serum level of Gal-9 was estimated by ELISA kits manufactured by Bioassay Technology Laboratory.

STATISTICS

A statistical analysis of data was carried out using “SPSS version 24” and ’GraphPad prism and version 7’, with statistical significance level set to less than 0.05. For each parameter, mean, standard deviation (SD), as well as standard error of mean (SEM) were analyzed separately. Pearson's correlation (r = 1 to 1) was used to investigate the relationships between ALT, AST, ALP, TSP, albumin, and bilirubin with characteristics of Gal-9. Receiver's operating characteristic (ROC) curve was developed to investigate the distinct ability of levels Gal-9 in CHB patients.

RESULTS

This research involved 80 samples (40 patients and same number of healthy persons as control group). The mean value of ALT, AST and ALP (U/L), of patients was 40.35, 37.03 and 238.6, which decreased in the healthy group of values 15.53, 16.7 and 185.5 (P <0.0001), as demonstrated in (**table 1, figs. 1, 2, 3),** respectively, while the mean level of T.BIL, D.BIL and IN.BIL (mg/dL) of patients was 1.143, 0.6125 and 0.53, which decreased in the healthy group to value 0.595, 0.3275 and 0.2675 (<0.0001) as shown in (**table 1, figs. 4, 5, 6),** but the average level of albumin (g/dL) was lower in patients with CHB as compared to healthy control 5.248 *vs* , 4.348 (<0.0001), as demonstrated in (**table 1, fig. 7),** while the mean level of TSP and Globulin (g/dL) was higher in patients with CHB as compared to healthy control 7.03 and 1.77 *vs* ,7.105 and 2.77,(p=0.6843) , (p<0.0001) as shown in **table** (**1), (fig. 8, 9)**

Serum levels of Gal-9 (**pg/mL**) was higher in CHB patients (618.6) than controls (136.3) with high significant differences ($p < 0.0001$), as shown in the **table (1), (fig. 10).**

Table 1: Distribution of Studied variables in CHB patients and controls

Parameter	Healthy controls			CHB Patients			p-value
	Mean	SD	SEM	Mean	SD	SEM	
ALT (U/L)	15.53	3.154	0.4987	40.35	10.75	1.699	<0.0001
AST (U/L)	16.7	1.937	0.3063	37.03	12.48	1.974	<0.0001
ALP (U/L)	185.5	48.07	7.6000	238.6	63.64	10.06	<0.0001
T.BIL (mg/dL)	0.595	0.171	0.0270	1.143	0.2836	0.0449	<0.0001
D.BIL (mg/dL)	0.3275	0.124	0.0196	0.6125	0.1682	0.0266	<0.0001
IN.BIL(mg/dL)	0.2675	0.089	0.0141	0.53	0.1911	0.0302	<0.0001
TSP (g/dL)	7.105	0.465	0.0735	7.03	1.065	0.1684	0.6843
Albumin (g/dL)	4.348	0.354	0.0560	5.248	0.6135	0.0970	<0.0001
Globulins (g/dL)	2.77	0.374	0.0591	1.77	0.7297	0.1154	<0.0001
Gal-9 (pg/mL)	136.3	35.48	5.61	618.6	171.7	27.15	<0.0001

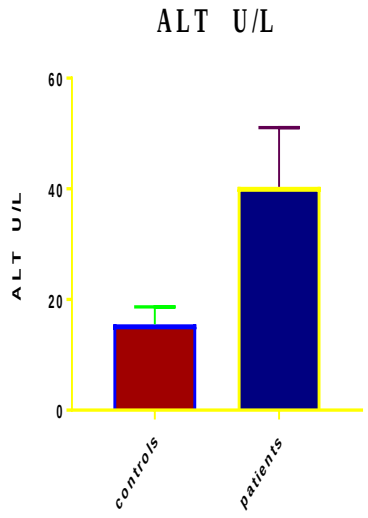


Fig. (1): Mean+ S.D for ALT in Control and Patients

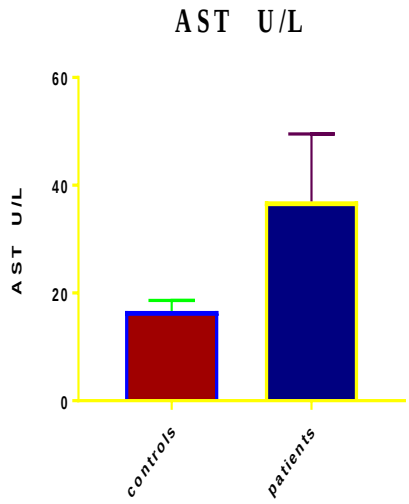


Fig. (2): Mean+ S.D for AST in Control and Patients

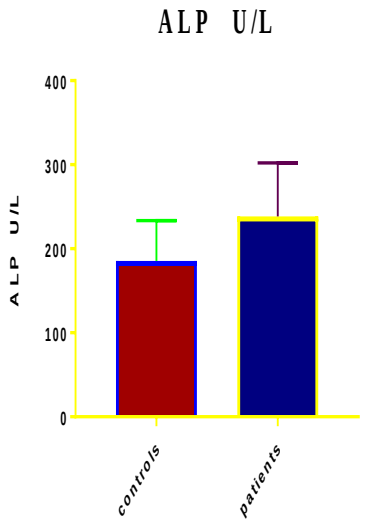


Fig. (3): Mean+ S.D for ALP in Control and Patients

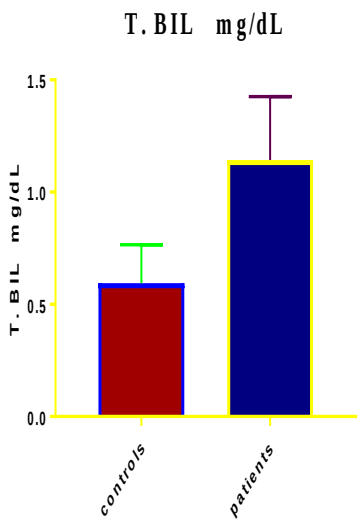


Fig. (4): Mean+ S.D for T. BIL in Control and Patients

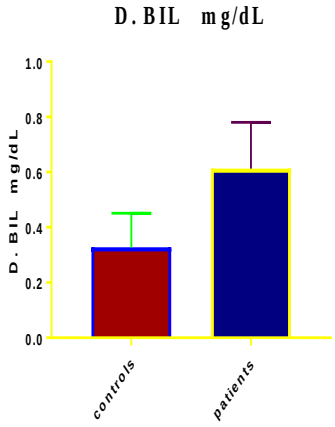


Fig. (5): Mean+ S.D for D. BIL in Control and Patients

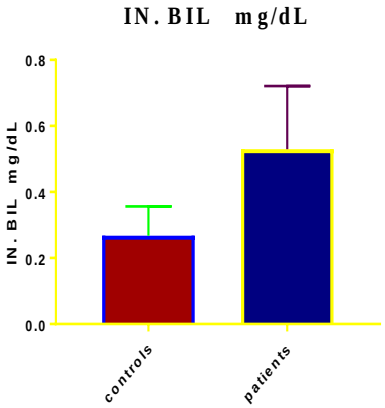


Fig. (6): Mean+ S.D for IN. BIL in Control and Patients

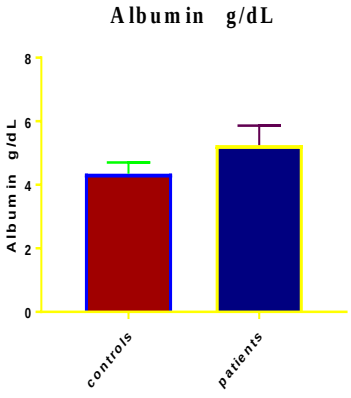


Fig. (7): Mean+ S.D for Albumin in Control and Patients

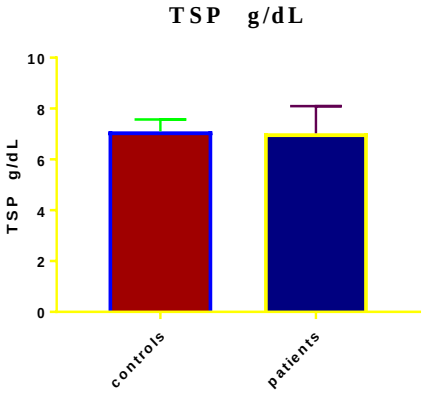


Fig. (8): Mean+ S.D forTSP in Control and Patients

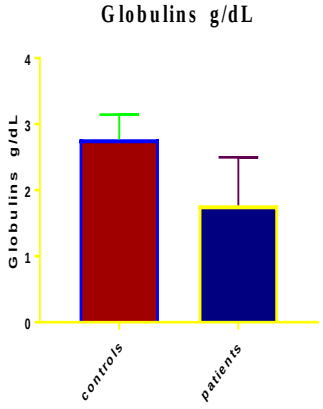


Fig. (9): Mean+ S.D for Globulins in Control and Patients

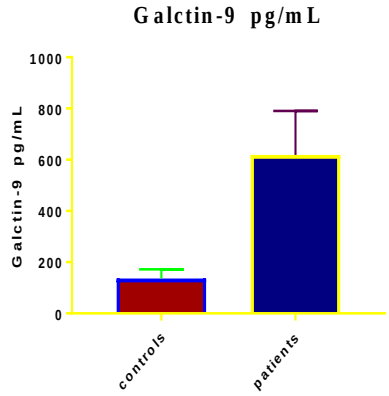


Fig. (10): Mean+ S.D for Galctin-9 in Control and Patients

The relationship between Gal-9 and other studied variables are investigated in this study, and the results are presented in a **table (2)** as shown a strong positive relationship between Gal-9 and serum levels of T.BIL (r = 0.731, p< 0.001), as demonstrated in **table (2) ,figure(11)**, ALT (r = 0.725, p< 0.001) illustrated in **table (2) ,figure(12)**, as well as a moderately strong positive relationship of Gal-9 with AST (r= 0.683, p< 0.001) as demonstrated in **table (2) ,figure(13)**, also moderately strong positive relationship of D.BIL (r= 0.656, P< 0.001, IN.BIL(r = 0.650, P< 0.001), and Albumin (r = 0.581, p< 0.001) were observed respectively in **table (2) ,figures 14, 15, 16,** also a weak positive relationship of GaL-9 with ALP serum levels (r= 0.468, P <0.001), **table (2) ,figure(17)** , but negative relationship of GaL-9 observed with Globulins (r= -0.563, P < 0.001) as shown in **table (2)** , **figure (18)**.

While non-significant relationship was observed between Gal-9 and TSP (r= -0.038, P= 0.739).

Table (2): Relationship of Galectin9 with Studied Parameters

Gal-9 (pg/mL)	r	p-value
ALT (U/L)	0.725	<0.001
AST (U/L)	0.683	<0.001
ALP (U/L)	0.468	<0.001
T.BIL (mg/dL)	0.731	<0.001
D.BIL (mg/dL)	0.656	<0.001
IN.BIL (mg/dL)	0.650	<0.001
TSP (g/dL)	-0.038	0.739
Albumin (g/dL)	0.581	<0.001
Globulins (g/dL)	-0.563	<0.001

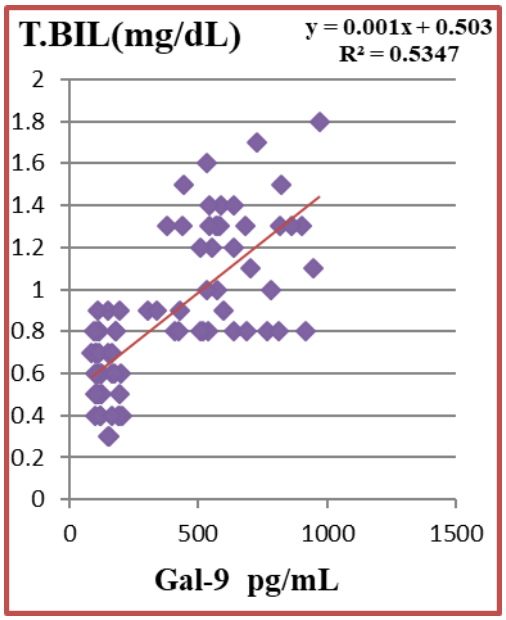


Figure (11): Relationship of Gal-9 with

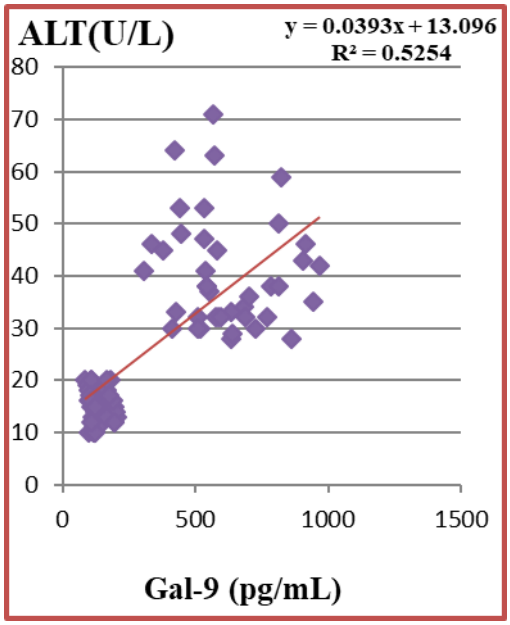


Figure (12): Relationship of Gal-9 with

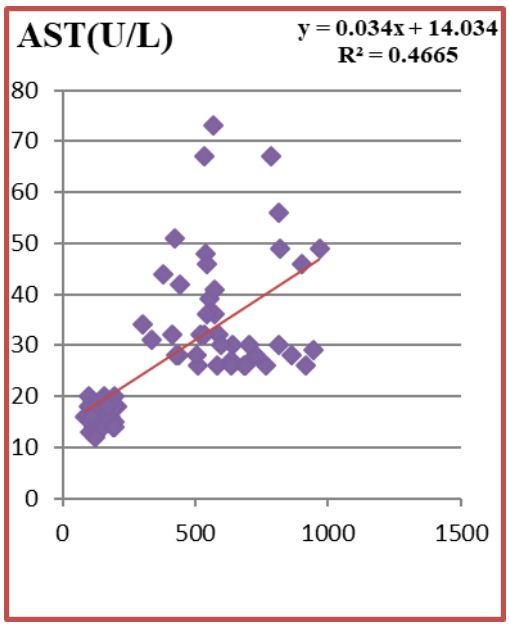


Figure (13): Relationship of Gal-9 with AST

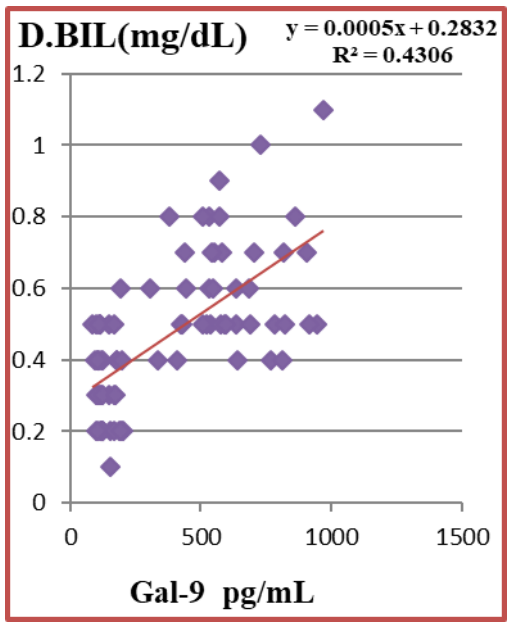


Figure (14): Relationship of Gal-9 with D.BIL

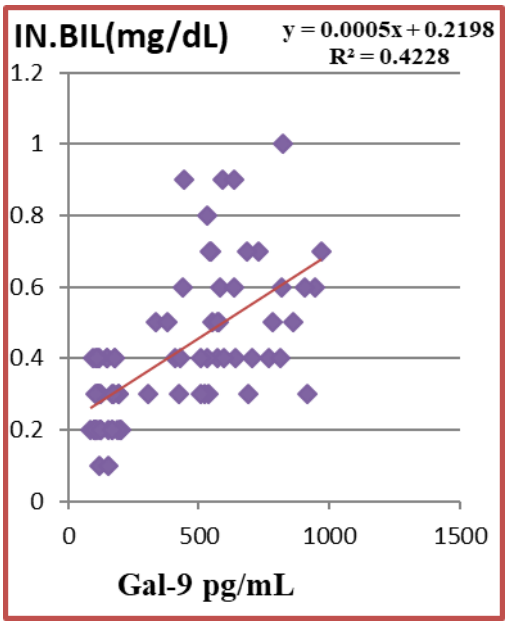
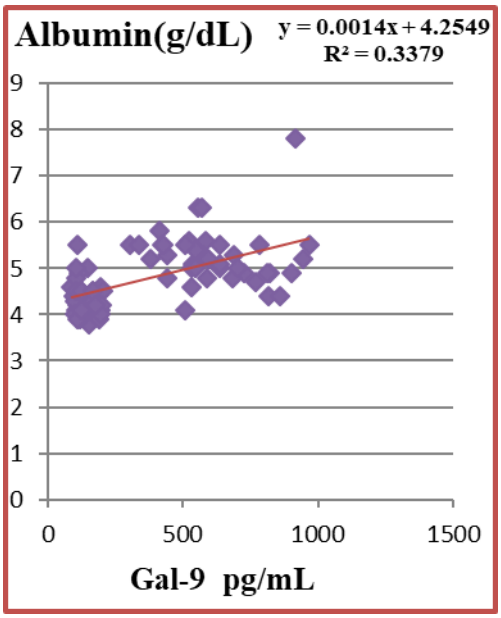


Figure (15): Relationship Gal-9 with IN.BIL



Figure(16): Relationship of Gal-9 with Albumin

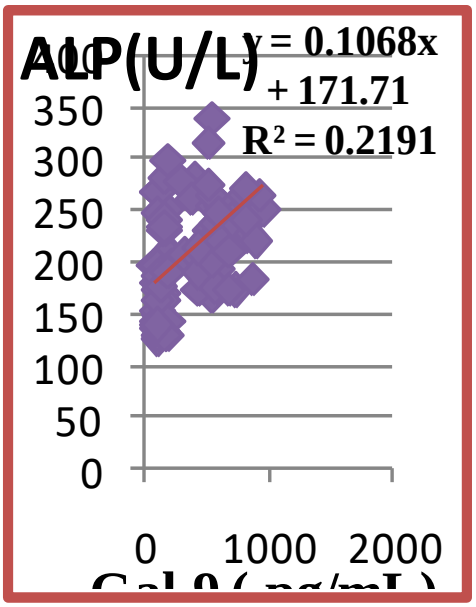
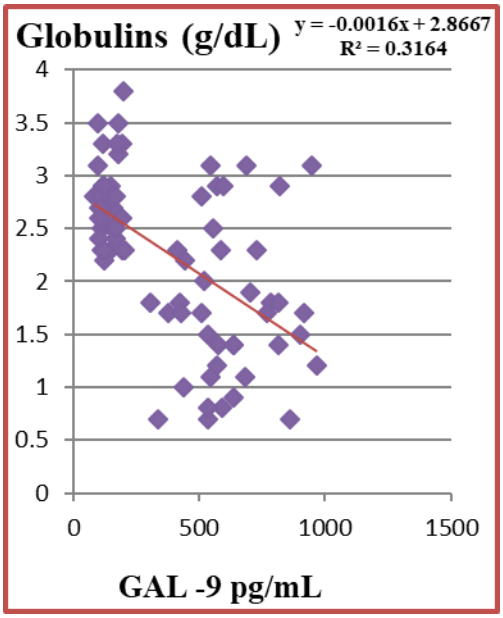


Figure (17): Relationship of Gal-9 with ALP



Figure(18): Relationship of Gal-9 with Globulins

The ROC curve evaluation for the outcomes obtained from the experiments and assessments carried out within the scope of the current research was once carried out, and the details are provided in **table (3)** and **fig.19. (A - J).**

Table 3: The ROC curve of Studied Variables

Parameter	AUC	Std. Error	95% confidence interval	p-value
ALT (U/L)	1	0	1 - 1	<0.0001
AST (U/L)	1	0	1 - 1	<0.0001
ALP (U/L)	0.768	0.0537	0.6627- 0.873	<0.0001
T. BIL (mg/dL)	0.959	0.0184	0.9228 -0.9947	<0.0001
D.BIL (mg/dL)	0.920	0.0285	0.8638 _0.9756	<0.0001
IN.BIL (mg/dL)	0.908	0.0304	0.8489 - 0.968	<0.0001
TSP (mg/dL)	0.564	0.0682	0.4308- 0.6979	0.3216
Albumin (mg/dL)	0.922	0.0313	0.8608- 0.9836	<0.0001
Globulin (mg/dL)	0.856	0.0469	0.764 - 0.9479	<0.0001
Gal-9 (pg/mL)	1	0	1 - 1	<0.0001

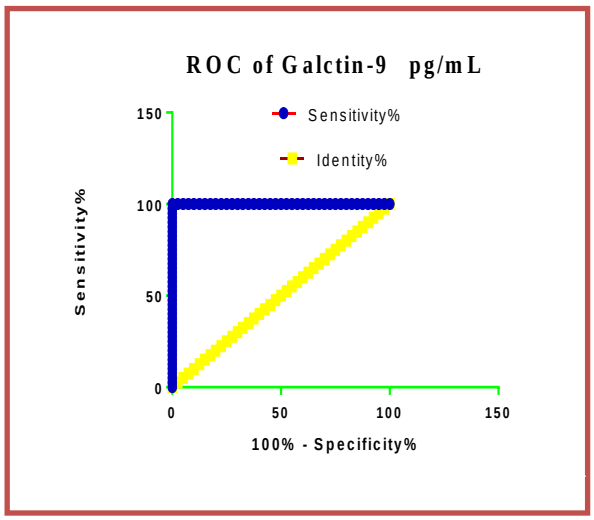
In this regard, taking the AUC values together with the various parameters listed in table (3), it is possible to make the following assessment for parameters, it was divided into five groups in descending order, the first of which included the values that yielded the result [AUS=1], which represents an ideal value for diagnosing the disease, the ROC curve revealed that the serum Gal-9, ALT, AST, of the standards with the highest validity and provides an perfect test with an excellent and fantastic method for discriminating' between healthy control and CHB patients [AUS=1; P <0.0001; 95% confidence interval (CI): 1- 1 with Std. Error (SE) :0] **(fig.19 A, B, C)** as a result, it is possible to state that the serum GAL-9, ALT, AST, are definitely functional for the diagnosis of CHB disease.

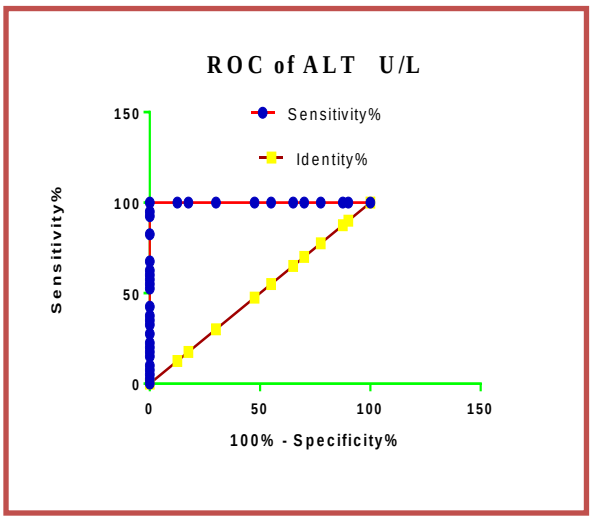
While the parameters in the second group, which represents a very strong value in diagnosing the disease were included T.BIL [AUC = 0.9588; P < 0.0001; 95% CI: 0.9228 - 0.9947 , SE: 0.01836] **(fig.19 D),** and also we found Albumin [AUC =0.9222; P < 0.0001; 95% CI: 0.8608 - 0.9836 , SE: 0.03131] (**fig.19 E**), as well D.BIL [AUC = 0.9197; P = < 0.0001; 95% CI: 0.8638 - 0.9756 with SE: 0.02853] (**fig.19 F**) and IN.BIL [AUC = 0.9084; P = < 0.0001; 95% CI: 0.8489 - 0.968 , SE: 0.0304] (**fig.19 G**), respectively.

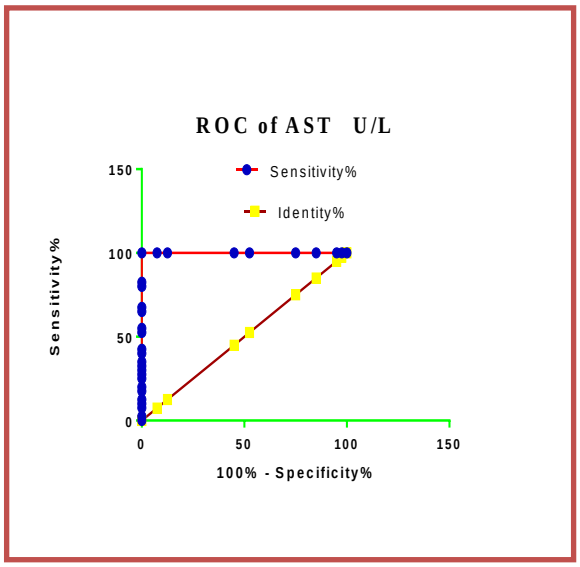
The third group in which represent the strong variables in disease diagnosis or prediction which was represented by globulins [AUC = 0.8559; P ≤ 0.0001; 95% CI: 0.764 - 0.9479 with SE: 0.04692] **(fig. 19 H**).

The fourth group in which represent the medium strength in diagnosing and detecting disease, this group include ALP [AUC = 0.7678; P ≤ 0.0001; 95% CI: 0.6627 - 0.873, SE: 0.05365] (**fig.19 I)**

Finally, Fifth group showed low validity in predicting validity this group include TSP [AUC =0.5644; P= 0.3216; 95% CI: 0.4308 - 0.6979 with SE: 0.06815] (**fig. 19 J)**


ROC of Galctin-9 pg/mL
Sensitivity%
Identity%
Sensitivity%
100% - Specificity%
0
50
100
150


ROC of ALT U/L
Sensitivity%
Identity%
Sensitivity%
100% - Specificity%
0
50
100
150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Sensitivity%
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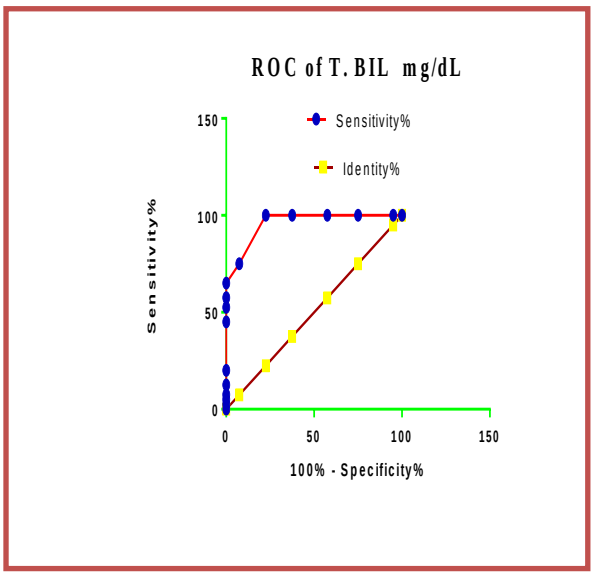
ROC of T. BIL mg/dL
Sensitivity%
Identity%
Sensitivity%
100% - Specificity%
0
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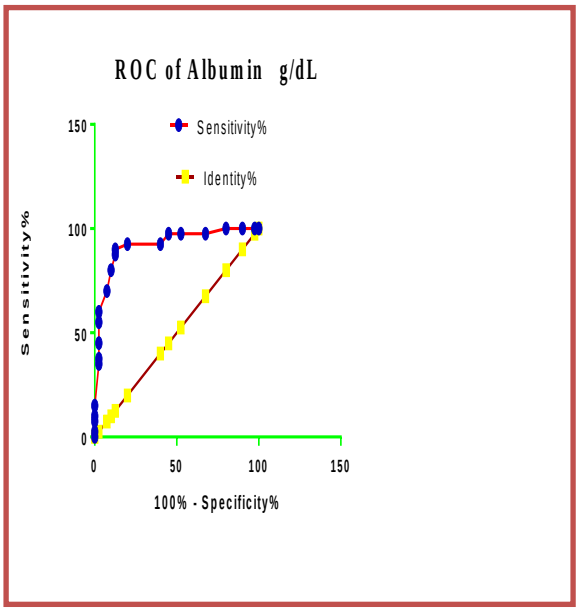
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Sensitivity%
Identity%
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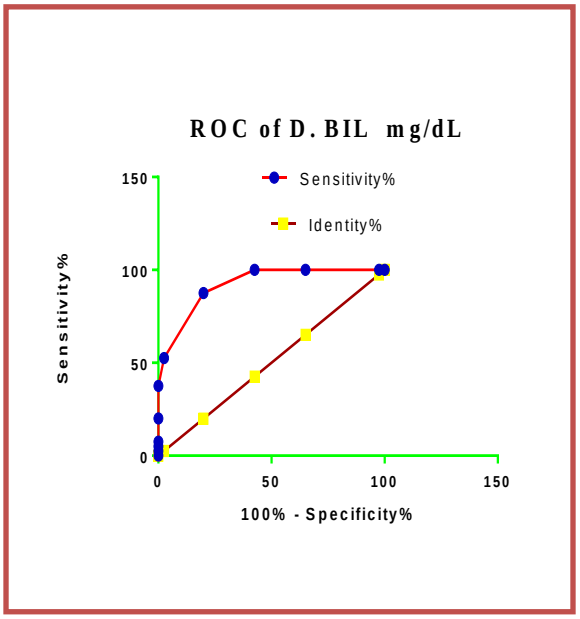
ROC of D. BIL mg/dL
Sensitivity%
Identity%
Sensitivity%
100% - Specificity%
0
50
100
150

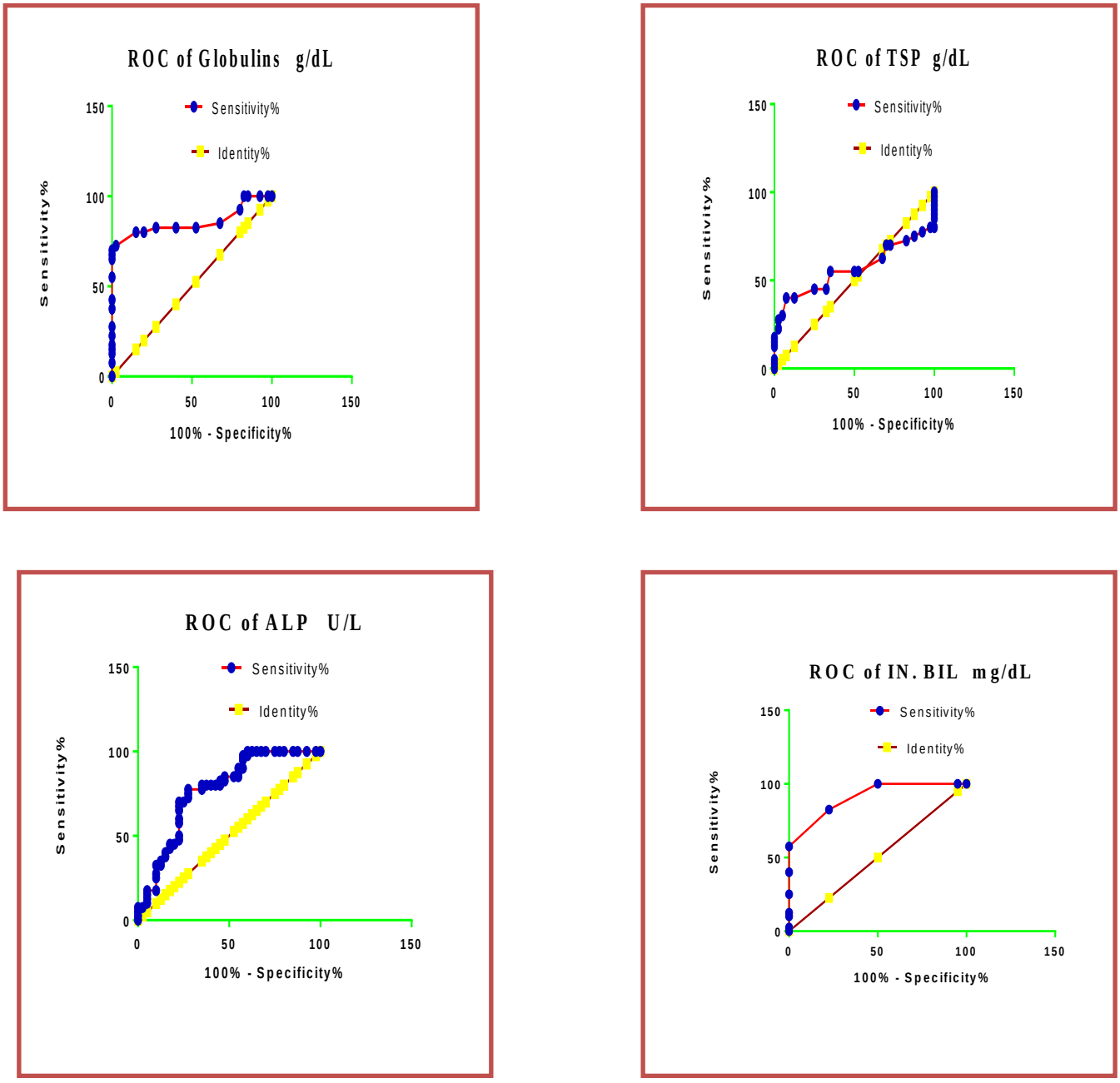

Fig.19. (A- J): The Receiver Operating Characteristic Curves

Discussion

According to the current study, elevated serum Gal-9 was detected in CHB patients as compared to healthy control. Moreover, Gal-9 concentration was found to be positively related to ALT, AST, T. BIL, D.BIL, IN.BIL and Albumin, while being negatively related to Globulin, Gal-9 is secreted by thymic epithelial cells and mediates T-cell cell death, the death of T-cell is also required after an immune response to eliminate activated and infected T cells. [12] Gal-9 abundant in the liver and plays a role through hepatic inflammatory processes, Gal-9 increased expression throughout chronic inflammation and fibrosis, and its immunoregulatory processes appear to be involved in hepatic illnesses.[13] Previous research found that Gal-9 levels in HBV-infected liver biopsies are high, and

serum levels are significantly higher than controls. [11] Results of this study were in agreement in recent Iraqi study. [14]

There was an increase in circulating Gal-9 in CHB patients, which associated with disease severity. [15] By binding to its ligand, Tim-3, Gal-9 controls T helper type 1 immunity in the presence of inflammation. Tim-3/Gal-9 axis contributes to T-cell exhaustion in CHB infections.[16] Gal-9 interaction of with T-cell immunoglobulin and Tim-3 ligand Gal-9/Tim-3 can result in terms of targeted inhibition of T helper cell immune responses, particularly Th1 with Th17.[17] That during inflammatory phase of HBV infection, regulatory T cells were found to be more active, and they were linked to higher serum ALT and Gal-9 levels.[18] Gal-9 has previously been shown to cause T cell apoptosis, most notably in CD4 + Th1 and Th17 cells, and this lectin also stimulates regulatory T cell activity .[19]

In this study, serum Gal-9 concentrations were higher in CHB patients and irregular the mean of serum ALT (40.35 U/L) or AST (37.03 U/L) concentrations than in those with regular serum ALT (15.53 U/L) or AST (16.7 U/L) concentrations. As a result, we assumed that increased Gal-9 concentrations would be associated with increased serum ALT concentrations in order to prevent severe inflammatory injury. Based on the current findings serum Gal-9 was correlated positively with all parameters with some exceptions, which suggest that are "low-grade inflammation" and Serum levels of Gal-9 may serve as a novel predictor of CHB progress in Iraqi patients.

In conclusion, this research established that serum concentrations of Gal-9, was beneficial indicators for CHB diagnosis. In CHB patients, moreover we detected great concentrations of AST, ALT, T.BIL, D.BIL, ID.BIL, ALP, albumin and low globulins. The above outcomes detect that Gal-9 can control immunity in CHB patients.

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