Kallistatin and Glypican-3 as Reliable Biomarkers for the Prediction of Liver Cirrhosis

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KEYWORDS
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ABSTRACT
Background: Cirrhosis is a condition in which the liver does not function properly due to long-term damage, this damage is characterized by the replacement of normal liver tissue by scar tissue. Objective: In the present study, an attempt was made to explore the role of kallistatin (KAL) and glypican3 (GPC3) as a predictor of liver cirrhosis (LC) in patients with hepatitis, non-alcoholic fatty acid and autoimmune diseases. Materials and Methods: This case–control study included 98 cirrhotic patients (34 patients with hepatitis B & A, 31 patients with non-alcoholic fatty liver disease and 33 patients with autoimmune) and 30 apparently healthy individuals. The laboratory investigations were performed, and the serum KAL and GPC3 were measured in all volunteers. Results: Serum levels of KAL, GPC3, and TAC were significantly decreased and MDA increased ($p<0.01$) in patients with LC as compared to control. The significant difference can also be indicatly seen in patients with hepatitis > non-alcoholic fatty acid > autoimmune diseases, respectively. The area under the curve (AUC) results obtained indicate that Kal and GPC3 could potentially be used as greater predictive biomarkers in LC with hepatitis > NAFLD > autoimmune diseases (Kal: AUC= -0.97, -0.91, -0.88; Gyp3: AUC= 0.89, 0.84, 0.79, respectively). Conclusion: Incorporating KAL and GPC3 screening into routine check-ups for patients with hepatitis, non-alcoholic fatty liver, and autoimmune diseases could aid in the early detection and prevention of LC-associated complications.

1. Introduction

Liver cirrhosis (LC), the ultimate consequence of chronic liver diseases, is a pathological phenomenon distinguished by widespread hepatic fibrosis, wherein the customary liver structure becomes substituted by regenerating nodules, which can arise from any prolonged hepatic ailment (1). The majority of prevalent incidents of cirrhosis stem from alcohol use disorder (approximately 45% of all cirrhosis cases), hepatitis C (41%), and nonalcoholic fatty liver disease (26%), with a substantial number of patients exhibiting overlapping causes. Nevertheless, hepatitis C can now be remedied through direct-acting antiviral agents, and the majority of recently diagnosed cirrhosis cases are attributable to nonalcoholic fatty liver disease (NAFLD) (accounting for 61.8% of incident cases) and alcohol use disorder (20.0%) (2). The diagnosis of LC patients in resource-limited environments is frequently postponed due to the unavailability of liver biopsy and elastography. The majority of these individuals manifest critical complications that pose a substantial burden on their well-being and lead to increased levels of mortality (3). Kallistatin is a protein of the serpin superfamily of serine protease inhibitors. It is predominantly produced and secreted by the liver. Low concentrations are also secreted by the kidneys, pancreas, heart, arteries, veins, atheroma, blood cells, and bodily fluids. It is an anti-angiogenic, anti-inflammatory, antioxidant, vasodilator, and anti-tumor growth protein. Also, collagen fraction volume, type I and type II collagen expression, and deposition are all decreased by kallistatin (4). Additionally, it prevents the expression of collagen and fibronectin by altering the expression of growth factor-β1 in cultured mesangial cells and reducing the production of reactive oxygen species caused by angiotensin II. Furthermore, due to its ability to block VEGF and bFGF-induced endothelial cell proliferation, migration, and adhesion, kallistatin contributes to the control of tumor growth and angiogenesis (5). Glypican-3 (GPC3), a proteoglycan consisting of heparin sulfate, is strongly expressed in the liver of fetuses but not adults. It is attached to the cell surface by glycosylphosphatidylinositol anchors (GPI). It has been demonstrated that GPC3 and hepatocellular carcinoma (HCC) are tightly connected. According to reports, GPC3 expression can be detected up to 90% of the time in patients with α-fetoprotein (AFP)-negative HCC, indicating that it may be useful for HCC diagnosis (6). To date, a limited number of studies on kallistatin and Glypican-3 in liver diseases of various etiologies has been published, but little attention has been paid to liver cirrhosis. Hence, in the present study, an attempt was made to investigate the relation of kallistatin, glypican3

and oxidant/antioxidant status as a noninvasive predictor of liver cirrhosis (LC) in patients with hepatitis (H), non-alcoholic fatty acid (NAFLD) and autoimmune diseases.

2. Materials and Methods

2.1. Subjects

The current examination is a case-control clinical trial. Samples were gathered from the Gastroenterology and liver specialized hospital in Basra-Iraq, during the period from August 2023 until February 2024. One hundred twenty-eight individuals (68 men and 60 women) with ages ranging between (20-45) years were included in this study. They were divided into two main groups: The first group consisted of 98 cirrhotic patients and was divided into three subgroups; group one (G1) consisted of 33 patients (68 men and 60 women) with autoimmune. Group two (G2) consists of 34 patients (68 men and 60 women) with hepatitis B & A. Group three (G3) consists of 31 patients (68 men and 60 women) with Non-alcoholic fatty liver disease. The second group (G4) included 30 apparently healthy individuals (68 men and 60 women) as a control group. All the volunteers were from the Basra province. All subjects enrolled in the study were evaluated by detailed history regarding chronic liver disease and physical examination. Grading of fibrosis was done using transient elastography (FibroScan). FibroScan scoring card is used to convert FibroScan results (measured in kPa) into the Metavir scale F1–F4. None of the control group had a history of acute and chronic diseases, such as diabetes, myocardial infarction, acute and chronic renal failure, cancer, and pneumonia. None of the participating women were pregnant or breastfeeding. Moreover, the control group included only participants who tested negative for liver diseases 6-12 months before the blood draw. Assent for the examination was acquired from all enlisted patients through a basic meeting that was directed during the visit, clinical history, and current prescriptions. Also, it gave informed written consent before participating in the study, which was approved by the Ethics Committee of the Hospital.

2.2. Inclusion criteria

Patients aged >18 years who had been clinically diagnosed with LC, encompassing both males and females were included in the study. All patients were previously diagnosed with liver cirrhosis based on clinical examination, biochemical parameters, and radiological evidence.

2.3. Exclusion criteria

Patients with hepatocellular carcinoma (HCC), exudative ascites, neurological diseases, hepatic encephalopathy, chronic renal failure, chronic pancreatitis, diabetes mellitus, chronic diarrhea, inflammatory bowel diseases, the human immunodeficiency virus (HIV), and patients with cancer in the advanced stage, as well as patients less than 18 years of age or more than 60 years of age, and patients who did not give informed consent to participate in the study, were excluded from the study.

2.4. Samples

After giving the information about the study, written consent was obtained from the study subject. Fasting blood samples were taken from each patient and controlled in the morning between 09:00 and 10:00 am following 12 hours fasting time and 30 minutes of rest in the prostrate position. Five milliliters of venous blood were drawn by medical syringes and 2mL of blood was put into an EDTA tube to use for prothrombin time. Other remaining 3mL of blood was transferred into gel tubes. Hemolysis samples were rejected. After 10-15 minutes, the clotted blood samples were centrifuged for five minutes at 3,000 Xg, and then serum was separated and transported into four new Eppendorf tubes and stored at -20C until assay.

2.5. Methods of Biochemical Estimation

The control and patient's blood samples were analyzed for biochemical parameters by standard procedures as follows: Body mass index was calculated as the following formula [BMI (kg/m^2) = Wt in kg/Ht in m^2] (7) and serum AST, ALT, ALP, and GGT levels were determined using the COBAS CIII analyzer. Serum urea and creatinine levels were determined by UV-Vis Spectrophotometer (UV-

EMC-LAB, Duisburg, Germany) by using the following kits (Linear, Barcelona, Spain, Cat. No.: 1156015; Randox, County Antrim, UK, Cat. No.: CR 511/S). Levels of serum Alb, MDA, TAC, Kal, and GPC3 markers were assayed by human ELISA kits (Creative Diagnostics, New York, USA, Cat. No. DEIA2299; Elabscience, USA, Cat. No. E-EL-0060; Elabscience, USA, Cat. No. E-E-BC-K136-M, Elabscience, USA, Cat. No. E-EL-H5550 and Elabscience, USA, Cat. No. E-EL-H1712), respectively.

2.6. Statistical analysis

Statistical analysis was performed using SPSS software version 26 (IBM Corporation, Armonk, NY, USA). The data were distributed normally and the comparison between groups was analyzed using the analysis of variance followed by Dunnett's t-test to find the statistical significance. The receiver operating characteristics (ROC) curve, which is formed by graphing sensitivity (y-axis) against 1-specificity (x-axis) and calculating the area under the ROC curve (AUC), was used to calculate the sensitivities and specificities, as well as the 95% confidence interval. A $p<0.05$ was considered statistically significant, $p<0.01$ highly significant, and an AUC value near 0 (or 1) implies a strong diagnostic value, the values of one group were mainly greater (or lower) than the values of the comparison group in this circumstance.

3. Result and Discussion

The general characteristics of all subjects participated in the present study were presented in Table 1.

Table 1. The demographic characteristics of the present study

The characteristics		**Control**	**Liver cirrhosis**
Total (No.)		30	**98**
Age (mean ± SD)		31.19 ± 1.24	**32.24 ± 1.27**
Sex	Men	17	**54**
	Women	14	**44**
Demographic Area	Urban	27	**89**
	Rural	4	**9**
Educational Background	Learned	25	**90**
	Illiterate	6	**8**
Smoking habit	Negative	28	**91**
	Positive	2	**7**
Drinking Alcohol	Negative	30	**98**
	Positive	0	**0**
Food Habits	Vegetarian	5	**28**
	Non - Vegetarian	25	**70**
Employment Status	Employed	20	**69**
	Non - Employed	**10**	**29**

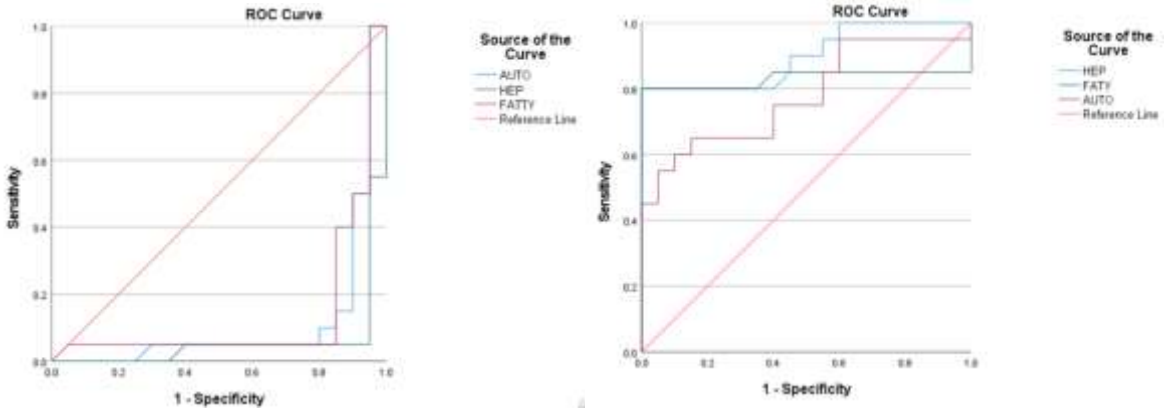
Table 2 clearly showed that highly significant ($p < 0.01$) increases were seen in the levels of Gpc3, MDA, AST, ALT, ALP, bilirubin, GGT, PT, and INR in LC patients with hepatitis > NAFLD > autoimmune diseases, respectively, as compared to the healthy control. On the other hand, levels of serum Kal, TAC, and Alb were significantly ($p<0.01$) decreased in LC patients with hepatitis < NAFLD < autoimmune diseases, respectively, as compared to the healthy control. The BMI, urea, and creatinine levels all showed non-significant changes ($p>0.05$), according to the same table 2.

Table 2. The levels of total parameters measured in the present study

Parameters	**Liver Cirrhosis with (mean ±SD)**			**Control (mean ±SD)**
	Hepatitis (H)	**non-alcoholic fatty acid (NAFLD)**	**Autoimmune (AI)**	
BMI (Kg/m$^{2)}$	21.32 ± 0.76	22.15 ± 0.98	21.25 ± 1.33	22.63 ± 0.14

FibroScan (kPa))	28.31 ± 2.45	27.62 ± 2.71	26.19 ± 2.81	4.83 ± 0.15
Kallistatin (ng/mL)	151.37 ± 0.45**	187.52 ± 0.61**	216.87 ± 0.81**	388.51 += 0.21
GPC3 (ng/mL)	5.75 ± 0.06**	4.83 ± 0.03**	4.17 ± 0.02**	1.94 ± 0.08
MDA (nmol/mL)	18.72 ± 0.24**	15.34 ± 0.09**	12.27 ± 0.21**	7.27 ± 0.35
TAC (U/mL)	1.72 ± 0.06**	2.93 ± 0.09**	4.16 ± 0.08**	7.32 ± 0.05
AST (U/L)	47.54 ± 1.78**	44.68 ± 1.89**	40.18 ± 2.09**	17.11± 1.14
ALT (U/L)	56.24± 1.22**	53.23 ± 3.32**	51.31± 2.09**	17.36 ± 1.43
ALP (U/L)	99.74 ± 0.36**	83.62 ± 2.27**	68.74 ± 1.74**	46.51 ± 0.09
Bilirubin (mg/dL)	1.73 ± 0.24**	1.48 ± 0.17**	1.39 ± 0.09**	0.72 ± 0.05
GGT (U/L)	63.46 ± 2.17**	58.23 ± 0.68**	52.57 ± 1.73**	13.69 ± 2.71
Albumin (µg/mL)	2.12 ± 0.17**	2.63 ± 0.41**	2.95 ± 0.06**	4.48 ± 0.28
PT (s)	16.94 ± 0.12**	15.14 ± 0.93**	14.26 ± 0.07**	11.16 ± 0.07
INR	1.86 ± 0.08**	1.64 ± 0.50**	1.32 ± 0.05**	0.94 ± 0.03
Urea (mg/dL)	15.87 ± 0.05	15.76 ± 0.19	15.25 ± 0.24	14.12 ± 0.08
Creatinine (mg/dL)	0.86 ± 0.23	0.84 ± 0.23	0.83 ± 0.20	0.79 ± 0.07

Data are presented as mean ± SD, p-value: N.S ($p > 0.05$), *S ($p < 0.05$), **HS ($p < 0.01$) indicate the level of significance in comparison with the corresponding control value. The area under the curve (AUC) results obtained indicate that KAL and GPC3 could potentially be used as greater predictive biomarkers in LC with hepatitis > NAFLD > autoimmune diseases (Kal: AUC= -0.97, -0.91, -0.88; Gyp3: AUC= 0.89, 0.84, 0.79, respectively), as illustrated in Figure 1.



(A) Kallistatin **(B) Glypican 3**

Figure 1. Receiver operating characteristic curve (ROC) for kallistatin, glypican3 levels in LC patients with hepatitis, non-alcoholic fatty acids and autoimmune disease.

One of the leading causes of illness and death worldwide is liver cirrhosis (LC). Most of these patients with liver cirrhosis have potentially fatal side effects when they first appear. Therefore, the first step in lowering death rates in people with chronic liver disease is early detection and treatment of LC. In the present study, data showed that most volunteer patients with LC were smokers. There are some recent studies reported that smoking has hazardous effects on lifestyle for both active and passive smokers. Also, these studies were directed to illustrate that tobacco cigarette smoke able to create a carcinogen in many organs of the human body like the lungs and urinary bladder, as well as their direct effects on liver health (8). Moreover, Table 3.1 shows that most of the volunteers from the LC patients and healthy controls were from the Basra province. Therefore, our results cannot represent the actual state of the entire patient group in Iraq due to the low number of patients and also depends on the cooperatively of patients and they are willing to participate in the present study. The major differences between urban and rural areas are the differences in pollution, environment, social, psychological, genetic, food factors, and others, which are increasing dramatically in urban areas (9). Furthermore, our data (Table

3.1) revealed that most of the patients with LC were non-vegetarian and not -drinking alcohol. Oxidative stress is an imbalance between free radicals and antioxidants in the. This can cause damage to organs and tissues and result in various diseases including liver diseases. So, malondialdehyde (MDA) is a compound that is derived from the peroxidation of polyunsaturated fatty acids (10). It has been used as a biomarker to measure oxidative stress in various biological samples in patients who are affected by a wide range of diseases such as liver cirrhosis. The liver is one important organ that is attacked by reactive oxygen species (ROS) (11). Primary cells in the liver injured by oxidative stress are called parenchymal cells. Parenchymal cells can produce ROS through their peroxisomes, microsomes, and mitochondrion. This helps to regulate PPARα, which is primarily linked to the expression of the liver fatty acid oxidation gene (12). Furthermore, endothelial cells, Kupffer cells, and hepatic stellate cells may be more exposed to or sensitive to chemicals linked to oxidative stress. Oxidative stress can cause Kupffer cells to generate a range of cytokines, including TNF-α, which may lead to an increase in apoptosis and inflammation. Regarding hepatic stellate cells, oxidative stress-induced lipid peroxidation is what initiates the cells' proliferation and collagen formation. Mammals have evolved a complex antioxidant mechanism to keep their livers in a state of redox equilibrium (13). Excessive ROS can upset homeostasis and lead to oxidative stress, which is a major factor in liver illnesses and other chronic and degenerative conditions. In addition to causing irreversible changes in the contents of lipids, proteins, and DNA, oxidative stress also damages the liver by altering pathways that regulate regular biological processes. The initiation and progression of various liver diseases, including chronic viral hepatitis, alcoholic liver diseases, and non-alcoholic steatohepatitis, are thought to be caused by pathological mechanisms such as oxidative stress (14). These pathways regulate gene transcription, protein expression, cell apoptosis, and activation of hepatic stellate cells. Furthermore, complex interactions between pathogenic variables, inflammation, free radicals, and immunological responses have been proposed (15). Moreover, extra-hepatic organ damage like renal failure and mental dysfunction can result from systemic oxidative stress brought on by liver illness. It was proposed that in cases of chronic liver failure, systemic oxidative stress could be a major "first hit," working in concert with ammonia to cause cerebral edema. Systemic oxidative stress is thought to be a key player in the pathophysiology of various kidney disorders, including renal failure (16). The total antioxidant assay provides a global assessment of aqueous and lipid phase antioxidant reserves, reflecting a complex array of factors, such as tissue antioxidant turnover rate, ascorbic acid and tocopherol recycling, and bilirubin production. The scientific reports indicated that a higher decrease in the level of total antioxidant capacity in whole serum was observed, and a significant depletion was found in protein-free serum, suggesting that the factors involved are not protein-bound antioxidants (-SH groups on proteins, vitamin E residing on the lipoprotein moiety, and bilirubin-bound to albumin) (17). The antioxidant defense system includes a wide range of enzymatic and nonenzymatic components several of which were reduced in this study. Selenium is an essential cofactor of glutathione peroxidase, the enzyme responsible for catalyzing hydroperoxide reduction by glutathione (18). In a previous study, serum selenium showed a small but significant reduction.

Selenium levels have been shown to be low in liver disease generally, irrespective of etiology, suggesting a relationship to overall nutritional status rather than dietary intake. Moreover, the plasma selenium in a variety of liver diseases declined in proportion to the severity of the cirrhotic condition (19). Among of blood plasma proteins there is been a protein (kallistatin) which belongs to the serine protease inhibitor family (a tissue-kallikrein selective 427 amino acid 58-60 kD glycoprotein serpin). Kallistatin attaches firmly to tissue kallikrein however it's binding to other serine proteinases like chymotrypsin and elastase is weakly (20). It has been recently revealed that kallistatin is new and dependable biomarker for the diagnosis of hepatic diseases such LC, due to close connection between the reduction in its serum amounts and development of early liver disease. Therefore kallistatin serum blood concentrations could help to detection of liver diseases as well as detect possible increase loss of liver function during therapy (4). Kallistatin has known as modulator and vasodilator of vascular development, and it has anti-inflammatory, anti-oxidant and antiangiogenic affects. Kallistatin is present in a large among of fluids and tissues, including blood vessels, plasma, urine, liver, kidney and

myocardium (21). This serine protease inhibitor connects specifically to tissue kallikrein and inhibits its proteolytic effects. But, heparin can neutralized this inhibitory effect. The main place of kallistatin generate is the liver, and to a lesser extent the pancreas, lung, heart, kidney, large intestine, and other tissues. Kallistatin takes part in the anti-inflammatory reactions and cellular conformity against oxidative stress. As mentioned before kallistatin is present in the blood cells and endothelial cells and takes part in cardiovascular function (22).

Oxidative stress can cause a decrease in kallistatin serum level in blood vessels and kidney; reduction of endogenic kallistatin with anti-kallistatin antibody worsens renal and cardiovascular oxidative stress and result in inflammation (23). This serine protease inhibitor (serpin) has specifically inhibits tissue kallikrein. This serine protease inhibitor (serpin) is known as a negative acute-phase protein, since its generation in the liver is quickly decreased after induction inflammation by lipopolysaccharide (24). Kallistatin protecting organs and cells against inflammation, fibrosis, and oxidative stress. Therefore, demonstrated change in the level of serum kallistatin in LC patients with hepatitis, NAFLD, and AI as well as other hepatic disorders could lead to finding out the severity of disease or might be help to early diagosis of LC (5). As a well-known co-receptor, GPC3 is a membrane-bound heparin sulfate proteoglycan that controls the signaling activity of several growth factors, including Wnt, fibroblast growth factor, and bone morphogenic protein. The likely involvement of GPC3-mediated Wnt/β-catenin signaling in the genesis of LC is established by the suggestion that the Wnt/β-catenin signaling pathway may regulate biliary tract development. In chronic liver disease, particularly LC and HCC, hepatic pathology is physiologically caused by the biological activity of GPC3 (6). GPC3 enhances the growth of LC and HCC through the activation of canonical Wnt signaling, according to research done by Capurro and colleagues on the protein's influence on LC and HCC cell lines. This finding makes GPC-3-mediated Wnt signaling an appealing therapeutic target. Notably, Notum, a lipase that cleaves glycosyl-phosphatidylinositol (GPI) anchors, can release GPC3 into the circulation from the cell surface (25). This means that GPC3 may be useful as a circulating marker for human disorders. Remarkably, a recent investigation revealed that LC patients with hepatitis B and C had circulating GPC3 levels that were considerably greater than those of patients with other liver disorders and healthy controls. Additionally, it has been shown that in individuals with LC and HCC, the expression of GPC3 mRNA in the circulation is linked to the TNM stage, periportal malignant embolus, and extrahepatic metastases (26). These results corroborate our study's finding that LC patients have significantly greater circulating GPC3 levels than healthy controls. Subsequent investigation showed that circulating GPC3 levels were much higher in children with advanced LC, including jaundice and portal hypertension than in those in earlier stages (27).

Together, our data suggest that GPC3 may be involved in the severity of bile duct obliteration and may be a potential cause of hepatocellular injury and liver fibrogenesis in patients with hepatitis, NAFLD, and autoimmune. Hence, it makes sense to hypothesize that circulating GPC3 may one day be used as a non-invasive biomarker to track the development of LC illness. The finding by Kandil et al. that up-regulation of circulating GPC3 mRNA was a more specific and sensitive biomarker for tracking HCC metastasis lends credence to this theory (28). After taking all of this into account, it is clear that research into the potential regulatory role that GPC3 may play in hepatic injury is currently very important for the development of GPC3 as a biomarker for the hepatic dysfunction of post-operative LC patients (29). Nevertheless, the processes underlying the link between high levels of GPC3 in the bloodstream and LC disease remain unclear. One may be tempted to speculate that an imbalance between GPC3 synthesis and clearance is the cause of the elevated levels of GPC3 in the blood among LC patients, especially those who do not have a good prognosis (30). During episodes of LC flares, the immune system may release both inflammatory and anti-inflammatory cytokines as a mechanism to regulate and control inflammation. Therefore, it was shown that the levels of KAL were considerably significantly lower and GPC3 was significantly higher in patients with LC compared to the control group (31).

Through study, these biomarkers demonstrate a strong ability to differentiate between LC patients and

controls, as evidenced by the ROC curve, which is a commonly used method for summarising classifiers (32). A ROC curve is a graphical representation that demonstrates the accuracy and significance of predicting an event. These findings indicate an escalation of inflammation, which is commonly observed in an LC flare, but are insufficient to effectively control the total inflammation associated with the LC flares (33; 34).

2. Conclusion and future scope

Patients with hepatitis, nonalcoholic fatty liver disease (NAFLD), and autoimmune diseases had significantly lower serum levels of KAL and higher ($p<0.01$) levels of GPC3, which could indicate hepatic dysfunction or early imaging-invisible liver cirrhosis. It may also serve as a potent signal and a promising marker that can be used to forecast the emergence of problems leading to LC.

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Conflict of Interest

All authors declare that they have no conflict of interest.

Reference

[1] Tapper EB, Parikh ND. Diagnosis and Management of Cirrhosis and Its Complications: A Review. JAMA. 2023 May 9;329(18):1589-1602. doi: 10.1001/jama.2023.5997. PMID: 37159031; PMCID: PMC10843851.

[2] Liu YB, Chen MK. Epidemiology of liver cirrhosis and associated complications: Current knowledge and future directions. World J Gastroenterol. 2022 Nov 7;28(41):5910-5930. doi: 10.3748/wjg.v28.i41.5910.

[3] Sharma B, John S. Hepatic Cirrhosis. [Updated 2022 Oct 31]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK482419/

[4] Kularaj SS, Verma SK, Kumar V, Patwa AK, Chaudhary SC, Sonkar SK, Gupta KK, Atam V, Verma SK, Bhosale V, Singh S. Serum kallistatin as a marker of severity of liver fibrosis in cirrhosis: A cross-sectional observational study. J Family Med Prim Care. 2022 May;11(5):2129-2133. doi: 10.4103/jfmpc.jfmpc_1922_21.

[5] Miao RQ, Agata J, Chao L, Chao J. Kallistatin is a new inhibitor of angiogenesis and tumor growth. Blood. 2002; 100: 3245–3325.

[6] Hayashi E, Motomura Y, Shirakawa H, Yoshikawa T, Oba N, Nishinakagawa S, Mizuguchi Y, Kojima T, Nomura K, Nakatsura T. Detection of glypican-3-specific CTLs in chronic hepatitis and liver cirrhosis. Oncol Rep. 2009 Jul;22(1):149-54.

[7] Al-Fartosy AJM, Awad NA, Mohammed AH. (2020). Intelectin-1 and Endocrinological Parameters in Women with Polycystic Ovary Syndrome: Effect of Insulin Resistance. Ewha Med J, 43(1): 1-11.

[8] Al-Shawi HM, Al-Fartosy AJM. (2022). Evaluating the clinical significance of insulin resistance, oxidant/ antioxidant status, some adipokines, and glycoproteins as monitoring indicators in Type 2 diabetic foot syndrome. Teikyo Medical Journal, 45(05): 6685-6697.

[9] Al-Fartosy AJM, Ati MH. (2021). A Predictive clinical markers to make prostate cancer and benign prostate hyperplasia easy diagnosis. Biochem. Cell. Arch, 21(2): 2939–2947.

[10] Faris ST, Al-Fartosy AJM, Al-Fregi AA. Interleukin-37 and interleukin-18 as prognostic biomarkers for end-stage renal disease. Azerbaijan Med J. 2022; 62(4): 1429-39.

[11] Abdualhay RA, Al-Fartosy AJM. (2022). Insulin Resistance and Other Adipokines as Clinical Predictors of Gestational Diabetes Mellitus among Pregnant Women. Indones Biomed J, 14(3): 243-51.

[12] Al-Fartosy AJM, Shanan SK, Awad NA. Biochemical study of the effects of some heavy metals on oxidant / antioxidant status in gasoline station workers /Basra-Iraq. Int J Sci Res Public 2017; 7(2): 83-94.

[13] Al-Fartosy AJM, Awad NA, Abdalemam DJ. Biochemical study of the effect of insulin resistance on adiponectin, lipid profile and some antioxidants elements with relation to obesity in type 2 diabetic patients /Basrah-Iraq. Amer J Biochem 2017; 7(4): 73-82. doi: 10.5923/j.ajb.20170704.03.

[14] Mohammed IM, Al-Fartosy AJM. Evaluating the clinical significance of Osteoprotegerin Serum Levels as a Predictive Marker in Rheumatoid Arthritis. Azerbaijan Medical Journal. 2022; 62(6): 1461- 1468.

[15] Al-Fartosy AJM, Awad NA, Alsalimi SA. Clinical markers and some trace elements in patients with type-2 diabetic nephropathy: Impact of insulin resistance. J Med Invest. 2021; 68(1): 76-84.

[16] Mohammed IM, Alsalimi SA, Al-Fartosy AJM. Trace Elements and Oxidant/Antioxidant Status in Beta-Thalassemia Patients. Bahrain Medical Bulletin. 2023; 45(4): 1772-78.

[17] Al-Fartosy AJM, Awad NA, Mahmood RA. A comparative study of leptin, oxidant/antioxidant status and some trace elements in women of healthy control and unexplained infertility in Basrah- Iraq. Indones Biomed J. 2019; 11(3): 327-37.

[18] Al-Fartosy AJM, Mohammed IM. Study the biochemical correlation of insulin resistance with HbA1c and sex hormones in NIDDM patients/Meisan-Iraq. J Diabetes Mellitus. 2017; 7(4): 302-15.

[19] Al-Fartosy AJM, Mohammed IM. Biochemical study of the effects of insulin resistance on sex hormones in men and women type-2 diabetic patients/Meisan-Iraq. Adv. Biochem. 2017; 5(5): 79-88.

[20] Osama M. Sobhey, Amal A. Jouda, Ashraf Metwally, Nagwa M. Shawky & Mohammad N. Elkhashab (2020) Evaluation of serum kallistatin level as a predictor of esophageal varices in cirrhotic patients, Alexandria Journal of Medicine, 56:1, 21-26.

[21] Ibrahim A.,A,, El Dahshan T.,A, Zakarya Z.,M. and Oadaa B.,N.,A. (2019) Kallistatin as a New and Reliable Biomarker for the Diagnosis of Liver Cirrhosis. The Egyptian Journal of Hospital Medicine. 75(6): 3027-3032.

[22] Cheng Z, Lv Y, Pang S, Bai R, Wang M, Lin S, Xu T, Spalding D, Habib N, Xu R. (2015) Kallistatin, a new and reliable biomarker for the diagnosis of liver cirrhosis. Acta Pharm Sin B. 5(3):194-200.

[23] Shen B, Hagiwara M, Yao YY, Chao L, Chao JL. Salutary effect of kallistatin in salt-induced renal injury, inflammation, and fibrosis via antioxidative stress. Hypertension. 2008; 51: 1358–1365.

[24] Fang Z, Shen G, Wang Y, Hong F, Tang X, Zeng Y, Zhang T, Liu H, Li Y, Wang J, Zhang J, Gao A, Qi W, Yang X, Zhou T, Gao G. Elevated Kallistatin promotes the occurrence and progression of non-alcoholic fatty liver disease. Signal Transduct Target Ther. 2024 Mar 12;9(1):66.

[25] Xing M, Wang X, Kiken RA, He L, Zhang JY. Immunodiagnostic Biomarkers for Hepatocellular Carcinoma (HCC): The First Step in Detection and Treatment. Int J Mol Sci. 2021 Jun 7;22(11):6139.

[26] Sirisomboonlarp K, Udomsinprasert W, McConachie E, Woraruthai T, Poovorawan Y, Honsawek S. Increased serum glypican-3 is associated with liver stiffness and hepatic dysfunction in children with biliary atresia. Clin Exp Hepatol. 2019 Mar;5(1):48-54.

[27] Al-Fartosy AJM, Awad NA, Alsalimi SA. Insulin resistance and specific biomarkers in blood and urine of type 2 diabetic patients with or without nephropathy in Basrah, Iraq. Afr J Biochem Res. 2020; 14(4): 125-134.

[28] Montalbano M, Georgiadis J, Masterson AL, McGuire JT, Prajapati J, Shirafkan A, Rastellini C, Cicalese L. Biology and function of glypican-3 as a candidate for early cancerous transformation of hepatocytes in hepatocellular carcinoma (Review). Oncol Rep. 2017 Mar;37(3):1291-1300.

[29] Beale G, Chattopadhyay D, Gray J, Stewart S, Hudson M, Day C, Trerotoli P, Giannelli G, Manas D, Reeves H. AFP, PIVKAII, GP3, SCCA-1 and follisatin as surveillance biomarkers for hepatocellular cancer in non-alcoholic and alcoholic fatty liver disease. BMC Cancer. 2008, 18;8:200.

[30] Ye L, Li D, Chen Y, Yu X. Evaluation for clinical and prognostic implications of glypican-3 and α-fetoprotein in hepatocellular carcinoma: a new subtype? Transl Cancer Res 2020;9(5):3443-3452.

[31] Alsalimi, S.A. and Al-Fartosy, A.J.M. (2024a). Association of Serum Programmed Cell Death Protein 1 (PD-1) and Gene Polymorphism with Some Valid Predictors for Systemic Lupus Erythematosus (SLE) Patients in Basra Province,

Iraq", IIUM Medical Journal Malaysia, 23(03).

[32] Alsalimi SA, Al-Mashkor IMA, Al-Fartosy AJM. (2023). Osteoprotegerin and Interleukin-37 are Correlated with Liver Diseases in Chronic Hepatitis B Virus (HBV)-infected Subjects. Indones Biomed J, 15(3): 222-30.

[33] Faris ST, Al-Fartosy AJM, Al-Fregi AA. Genetic polymorphism of vascular endothelial growth factor (VEGF) associated with hypothyroid in hemodialysis patients. Eur Chem Bull. 2022; 11(11): 1-9. doi:10.31838/ecb/2022.11.11.001.

[34] Alsalimi, S. A. and Al-Fartosy, A. J. M. (2024). Inflammatory cytokines and programmed death-1 correlation in subjects diagnosed with systemic lupus erythematosus in the province of Basra / Iraq. Obstetrics and Gynaecology Forum, 34(3s), 1610–1621