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Early detection of Non-alcoholic Liver Disease in type 2 Diabetes mellitusIraqi patients by assessment of Transthyretin, clusterin, and retinol binding protein4 with liver of enzymes by Ultrasound imaging

Ghasak Hashim Sakban[1*], Sandbaker Mohammed[2], Tawfeeq F. R. Al-Auqbi[3]

[1]Department Chemistry, University of Baghdad College of Science for Women Al-Jadriya Baghdad Iraq.
E.mail: ghasak.kasaq1105a@csw.uobaghdad.edu.iq
[2]Department Chemistry, University of Baghdad College of Science for Women Al-Jadriya Baghdad Iraq.
E.mail: dr.sanad55@yahoo.com
[3]National Diabetic Center for Treatment and Research, Mustansiriyah University, Baghdad, Iraq
E.mail: Tawfeeqauqbi@yahoo.co.uk

***Correspondence author:** Ghasak Hashim Sakban (ghasak.kasaq1105a@csw.uobaghdad.edu.iq)

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Abstract

Background Study role Retinol, Clusterin, and TTR important in understand and treat the pathology of obesity and NAFLD to prevent metabolic syndrome. Treatments for obesity and NAFLD commonly include lifestyle management through weight loss, dietary restriction, exercise, etc. Objective This study was aim to investigate the role of Retinol, Clusterin, and TTR,in the non-alcoholic fatty liver disease and nonalcoholic fatty liver disease NAFLD among the patients with type 2 diabetes mellitus (T2DM). The study will be, done at the National Diabetes Center of Mustansiriyah University. One hundred thirty five subjects with age ranged from (35-65) years were in the serum Retinol, Clusterin, and TTR; concentrations were measured by radioimmunoassay in 150 patients. Diabetic patients were chosen from almost diagnosed patients on follow up visits to the center. The NAFLD diabetic patients were chosen according to clinical features of liver diseases had liver ultrasonic appearance and abnormal liver function tests with Anthropometric characteristics, BMI, HC, and WC, Patients were distributed; three study groups as following: Group one: 50 patients, T2DM with NAFLD Group two: 50 patients, T2DM without NAFLD;Group three 50 healthy controls. Results Retinol, Clusterin, and TTR there was a significant increase it was discovered that there is apositive relationship between transthyretin and that this relationship is statistically significant Conclusions:This study found that transthyretin levels were negatively correlated with the severity of NAFLD in patients with type 2 diabetes. Retinol and Clusterin had a highly significant positive correlation, with Retinol and BMI having a correlation of indicating a substantial effect size. Clusterin had a moderately positive correlation with the protein Clustrin.

Keywords

Retinol, Clusterin, and Transthyretin (TTR), Nonalcoholic Fatty Liver Disease (NAFLD); Type 2 Diabetes Mellitus (T2DM); Metaboliciated Fatty Liver Disease (MAFLD) treatment Diabetes mellitus;sonographerfeatures Anthropometric characteristics.

DM affects more than 463 million people globally, and this number is supposed to increase to 700 million by 2045[1]. Diabetes is one of the worst chronic diseases that is caused either by the lack of producing enough insulin inthe pancreas or by the lack of insulin in the cells. Someone is diagnosed to have diabetes when the level of plasma glucose density is more than 6.1 mmol/L[2] Retinol binding protein 4 (RBP4) is a member of the lipocalin family and the major transport protein of the hydrophobic

molecule retinol, also known as vitamin A, in the circulation. Expression of RBP4 is highest in the liver, where most of the body's vitamin A reserves are stored as retinyl esters. For the mobilization of vitamin A from the liver, retinyl esters are hydrolyzed to retinol, which then binds to RBP4 in the hepatocyte. After associating with transthyretin (TTR), the retinol (RBP4), TTR complex is released into the bloodstream and delivers retinol to tissues via binding to specific membrane receptors. So far, two distinct RBP4 receptors have been identified that mediate the uptake of retinol across the cell membrane and, under specific conditions, bi-directional retinol transport. Although most of RBP4's actions depend on its role in retinoid homeostasis, functions independent of retinol transport have been description the structure, regulation, and functions of RBP4 and lay out the biological relevance of this lipocalin for human diseases. [2-3]. several studies have revealed that RBP4 increases the synthesis of the gluconeogenic enzyme, phosphoenolpyruvate carboxykinase, and inhibits insulin singling in the muscle [4]. Moreover, the deletion of the RBP4 gene can elevate insulin sensitivity [5]. Recent clinical studies in adults have demonstrated that RBP4 levels were associated with metabolic syndrome, obesity, insulin resistance, and type 2 DM (T2DM) [5].Furthermore, there is some evidence that serum or plasma RBP4 levels were increased in patients with advanced renal impairment of T2DM [6]. Non-alcoholic fatty liver disease is common among type 2 diabetic patients with obesity and insulin resistance. Nonalcoholic fatty liver disease is a chronic liver condition characterized by insulin resistance and hepatic fat accumulation, in the absence of other identifiable causes of fat accumulation, such as alcohol abuse, viral hepatitis, autoimmune hepatitis, alpha-1 antitrypsin deficiency, medications like corticosteroids and estrogens, and other conditions[7-8] Hepatic steatosis may range from a 'benign' indolent deposition of fat to severe lip toxicity-induced steatohepatitis with necroinflammation [known as nonalcoholic steatohepatitis (NASH)] .NASH is an overlooked complication of type 2 diabetes mellitus (T2DM) that if missed may carry serious long-term consequences. NASH is frequently associated with fibrosis and approximately 10% of patients develop cirrhosis. The risk of hepatocellular carcinoma is also increased in patients with T2DM and NASH [9]. Diabetes, dyslipidemia, hypertension, and cardiovascular disease (CVD) occur more frequently in individuals with NAFLD [10]. Clusterin is a heterodimer glycoprotein expressed in various mammalian tissues and is present in most biological fluids, including plasma, urine, and milk, at low levels under normal physiological conditions.[11] However, clusterin levels are up regulated in pathophysiological conditions, such as oxidative stress and inflammation. [12].In particular, many studies have shown that clusterin is associated with various metabolic diseases. For example, the plasma Concentration of clusterin is closely related to obesity and T2D, and clusterin levels are elevated in patients with metabolic syndrome. Additionally, clusterin reduces hepatic lipogenesis by down regulating sterol regulatory binding protein1c (SREBP-1C), a master regulator of lipogenesis. Clusterin deficiency also exacerbates high-fat diet (HFD)-induced insulin resistance (HFD) [13].Non-Alcoholic Fatty Liver Disease (NAFLD): NAFLD itself can affect liver enzymes due to the accumulation of fat and inflammation in the liver. The enzyme most commonly affected in NAFLD is ALT, followed by AST. The severity of liver enzyme abnormalities may correlate with the degree of liver fat accumulation and inflammation.[14].

Patients and Methods

The study will be done at the National Diabetes Center of Mustansiriyah University and Al-Yarmook Teaching Hospital during the period from January 2022until the end of May 2022. One hundred fifty-five subjects with age ranged from (35-65) years were in the serum RBP4, TTR, and Clustrin concentrations were measured by radioimmunoassay in 150 patients. Diabetic patients were chosen from almost diagnosed patients on follow up visits to the center. The NAFLD diabetic patients were chosen according to clinical features of liver diseases that had liver ultrasonic appearance and abnormal liver function tests. Patients were distributed; three study groups as following: Group one; 50 patients, T2DM with NAFLD Group two;50 patients, T2DM without NAFLD;Group three 50; healthy controls.

Anthropometric Measurement

Anthropometric parameters, involved, waist circumferences (WC), High conference (HC), and BMI have been measured. Body mass index was calculated as weight in kilograms divided by height in meters square (kg/m2).

Assessment Retinol, Clusterin, and TTR, by, Elisa. With assessment Anthropometric characteristics, body mass index (BMI), Hip circumference (HC), Waist circumference (WC).

Biochemical Assessment

Blood samples were collected after 12 hours of fast. Fasting serum glucose and lipid profile were estimated by enzymatic colorimetric methods using

Auto-analyzer.Serum insulin concentrations were assessed by the ELISA kit. Blood samples were collected after 12 hours of fast. Done in this study

Liver function tests serum (Albumin, ALT, AST, and Alk.phosphetase ALP, Total serum bilirubin TSB).were estimated by enzymatic colorimetric methods using auto-analyzer(Spectrophotometer, Kenza 240 TX, France). By assessment Retinol, Clusterin, and TTR, by, ELISA.

Statistical Analysis

Statistical analysis of data were done using SPSS software version 17, USA. Statistical tests used ANOVA way for analysis of variance. Data were expressed as means (±SD); statistical significance was agreed at $P<0.05$. In this study that Retinol, Transthyretin, Clustrin, and all the other variables in the study were subjected to a Pearson correlation analysis. Coefficients between.10 and.29 were considered to indicate a weak relationship, coefficients between.30 and.49 were considered to indicate a moderate effect size, and coefficients above.50 were considered to indicate a strong effect size using Cohen's standard.

Results

All of the groups had an equal number of male and female members (n = 25, or 50%) x2 0.00, P = 0.001. The results of the ANOVA were significant, The results in this study showed that BMI relationship between BMI and NAFLD higher in patients with type 2 diabetes by used ultrasound imaging to assess the degree of fatty infiltration in the liver and found that patients with a higher BMI had more severe NAFLD compared to those with a lower BMI [15]. A results showed of BMI in diabetic patients more than control healthy group, Also a significant increase in WC, BMI, in diabetic compared to control as showedin table 1[16]. Type2 diabetes mellitus normally exists together with different components of metabolic syndrome such as, hypertension, high BMI, hypertriglyceridemia, hypercholesterolemia, and fatty liver and raises the risk of all-cause and cardiovascular mortality [17]. Also study found that a larger hip circumference was associated with a lower risk of advanced in patients NAFLD with T2D. The relationship between hip circumference and NAFLD is still not fully understood. Furthermore, other factors such as overall body composition and fat distribution may play a role in the development NAFLD [18]. This study showed that lower albumin levels were significantly associated with the severity of NAFLD in patients with type 2 diabetes compar to T2DM group, lower albumin levels can be a marker of liver function and increased risk of NAFLD in patients with T2DM[19]. The results of the present study showed elevated ALT levels were related with an increased risk of NAFLD in patients with type 2 diabetes compar to T2DM group alone, elevated ALT can be a marker of liver damage or inflammation. Also, and increased risk of NAFLD in patients with type 2 diabetes [20]. Elevated (AST) levels were independently associated in patients with T2DM, more compar with an increased in risk of NAFLD in patients with T2DM [21].This results in this study showed ALP levels were higher in patients with type 2 diabetes compared to healthy controls. However, the same study found no significant difference in ALP levels between patients with type 2 diabetes with and without NAFLD as showed in table 2 [22]. In this study showed that role treatment with inhibitors, as class of medications used to treat T2D, was associated with an increase in retinol levels in patients with T2D and NAFLD compar to T2D alone or healthy controls. This study suggested that this increase may be due to greater improved glucose control and decreased fatty liver, as showed in table 2[23]. Also another study confirmed to the results in this study found that transthyretin levels were negatively correlated with the severity of NAFLD in patients with T2D, also suggested that low transthyretin levels useful as marker for identifying T2D patients with more severe forms of NAFLD [24]. The results in this study showed that role that treatment with the inhibitor empagliflozin was associated with an increase in clusterin levels in patients with T2D and NAFLD compar to T2D alone or healthy controls. The study suggested that this increase may be due to improved glucose control and decreased liver fat content, also this study agreement to results our study as showed in table 3[25]. Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) are normal in most patients with NASH. Another limiting diagnostic factor is that the distinction between benign steatosis or active NASH can only be done by performing a liver biopsy, a procedure that both patients and doctors are reluctant to pursue. However, suggesting that it will not be long before screening for fatty liver disease, either noninvasively or in selected cases with a liver biopsy, will be incorporated into our routine evaluation of patients in the same way that we currently do for other chronic complications of diabetes [26]. On ultrasonography, fatty infiltration of the liver produces a diffuse increase in echogenicity as compared with that of the kidneys. Regardless of the cause, cirrhosis has a similar appearance on ultrasonography. Ultrasonography has a sensitivity of percent and a specificity of percent in detecting steatosis and a sensitivity and specificity of percent and percent, respectively, in detecting increased fibrosis [27]. The diagnosis of nonalcoholic fatty liver disease is usually suspected in persons with

asymptomatic elevation of aminotransferase levels, radiologic findings of fatty liver, or unexplained persistent hepatomegaly [28]. The clinical diagnosis and liver tests have a poor predictive value with respect to histologic involvement. Imaging studies, although of help in determining the presence and amount of fatty infiltration of the liver, cannot be used to accurately determine the severity of liver damage. The clinical suspicion of nonalcoholic fatty liver disease and its severity can only be confirmed with a liver biopsy [29]. In persons with asymptomatic elevation of aminotransferase levels, radiologic findings of fatty liver, or unexplained persistent hepatomegaly. The clinical diagnosis and liver tests have a poor predictive value with respect to histologic involvement imaging studies, although of help in determining the presence and amount of fatty infiltration of the liver, cannot be used to accurately determine the severity of liver damage [30]. Also In the current study, high levels of HbA1c are due to poor glycemic control in type 2 diabetic group, Also in patient groups themselves there is significant level in diabetics on metformin immunotherapy compared to only DM (without therapy) [31]. A study other High BMI (obesity/excess weight) has a linear relationship with fatty liver disease. One of the risk factor in NAFLD development is obesity (NAFLD prevalence >23%) even in absence of T2D and insulin resistance although it is not a diagnostic criteria for NAFLD, but still there are many NAFLA patients with normal BMI [32].

Table (1) anthropometric characteristics comparison of the study groups

Variable	Groups	Mean ± SD	F-test	P value
BMI	Control	24.136± 1.354	30.884	< .001
	DM	27.795± 2.875		
	(DM+NAFLD)	27.234± 2.959		
Waist circumference	Control	66.194± 4.628	3.962	.021
	DM	67.990± 4.202		
	(DM+NAFLD)	68.864± 5.575		
Hip circumference	Control	83.720± 4.061	11.070	< .001
	DM	86.840± 4.812		

Table (2) liver function parameters comparisons between the groups

Variable	Groups	Mean ± SD	F-test	P value
Albumin	Control	4.274± 0.557	0.853	.428
	DM	4.310± 0.555		
	(DM+NAFLD)	4.412± 0.532		
ALT	Control	19.640± 5.623	34.422	< .001
	DM	23.880± 5.065		
	(DM+NAFLD)	29.240± 6.601		
AST	Control	23.800± 6.037	0.506	.604
	DM	24.960± 6.064		
	(DM+NAFLD)	24.860± 7.028		
ALP	Control	72.820± 23.406	4.148	.018
	DM	86.020± 24.188		
	(DM+NAFLD)	81.520± 22.258		

Table (3) Mean and standard deviation of the serum Retinol, transthyretin and Clusterin

Variable	Groups	Mean ± SD	F-test	P value
Retinol	Control	8.889± 0.729	1,067.933	< p0.001
	DM	12.107± 1.065		
	(DM+NAFLD)	21.822± 2.168		
Transthyretin	Control	1.702± 0.277	634.725	<p 0.001
	DM	2.750± 0.261		
	(DM+NAFLD)	3.821± 0.347		
Clustrin	Control	21.541± 1.724	1,044.950	<p0.001
	DM	37.824± 4.580		
	(DM+NAFLD)	63.825± 6.430		

Conclusions

In this study, there was an elevation inAnthropometric characteristics, BMI, WC, and HC, in diabetic patients as compared to the controls. These patients with a higher BMI had more severe NAFLD compared to those with a lower BMI. A result showed of weight, WHR, BMI

in diabetic patients more than control healthy group, Also a

Significant increase in WC, WHR, BMI, in diabetic compared to control. A study found that patients with NAFLD and type 2 DM had significantly higher waist circumference measurements than those without, also study found that a larger hip circumference was associated with a lower risk of advanced in patients NAFLD with T2D.

The relationship between hip circumference and NAFLD is still not fully understood.

This study showed that lower albumin levels were significantly associated with the severity of NAFLD in patients with type 2 diabetes compare to T2DM group, lower albumin levels can be a marker of liver function and increased risk of NAFLD in patients with T2DM. The results of the present study showed elevated ALT levels were related with an increased risk of NAFLD in patients with type 2 diabetes compare to T2DM group alone, elevated ALT can be a marker of liver damage or inflammation. Also, and increased risk of NAFLD in patients with type 2 diabetes. Elevated AST levels were independently associated in patients with T2DM, more compare with an increase in risk of NAFLD in patients with T2DM. These results in this study showed ALP levels were higher in patients with type 2 diabetes compared to healthy controls. However, the same study found no significant difference in ALP levels between patients with type 2 diabetes with and without NAFLD. Also another study confirmed to the results in this study found that transthyretin levels were negatively correlated with the severity of NAFLD in patients with T2D, also suggested that low transthyretin levels useful as marker for identifying T2D patients with more severe forms of NAFLD, this study showed that role that treatment with the inhibitor empagliflozin was associated with an increase in clusterin levels in patients with T2D and NAFLD compare to T2D alone or healthy controls. The study suggested that this increase may be due to improved glucose control and decreased liver fat content, also this study agreement to other results. In this study was found that Retinol Clusterin have a highly significant positive correlation.

References:

G.G.Nair,E.S.Tzanakakis,andM.Hebrok,"Emergingroutes to the generation of functional β-cells for diabetes mellitus cell therapy," Nature Reviews Endocrinology, vol. 16, no. 9, pp. 506–518, 2020.

Nasif, H. H., & Al-Nassir, S. (2023). Discrete Optimal Control Mathematical Model of Diabetes Population. Iraqi Journal of Science, 64(4), 1925–1934.

Fedders R., Muenzner M., Weber P., Sommerfeld M., Knauer M., Kedziora S., et al. (2018). Liver-secreted RBP4 does not impair glucose homeostasis in mice.J. Biol. Chem. 293 15269–15276. 10.1074/jbc.RA118.00429.

Fenzl A., Kulterer O. C., Spirk K., Mitulovic G., Marculescu R., Bilban M., et al. (2020). Intact vitamin A transport is critical for cold-mediated adipose tissue browning and thermogenesis. Mol. Metab. 42:101088. 10.1016/j.molmet.2020.101088

Cehajic-Kapetanovic J., Jasani K. M., Shanks M., Clouston P., MacLaren R. E. (2020). A novel homozygous c.67C>T variant in retinol binding protein 4 (RBP4) associated with retinitis pigmentosa and childhood acne vulgaris.Ophthalmic Genet. 41 288–292. 10.1080/13816810.2020.1755985

Fan, S. Yin, D. Lin et al., "Association of serum retinol- binding protein 4 levels and the risk of incident type 2 diabetes in subjects with prediabetes," Diabetes Care, vol. 42, no. 8, pp. 1574–1581, 2019.

S.Habashy, A. Adly, M. Kader, and S. Ali, "Predictors of future microalbuminuria in children and adolescents with type 1 diabetes mellitus in Egypt," Archives of Medical Science - Atherosclerotic Diseases, vol. 4, no. 1, pp. 286–297, 2019.

Day, E. A., Ford, R. J. & Steinberg, G. R. AMPK as a therapeutic target for treating metabolic diseases. Trends Endocrinol. Metab. 28, 545–560. (2017).

Klouckova, J. et al. Plasma concentrations and subcutaneous adipose tissue mRNA expression of clusterin in obesity and type 2 diabetes mellitus: the effect of short-term hyperinsulinemia, very-low-calorie diet and bariatric surgery. Physiol. Res. 65, 481–492 (2016).

Won, J. C. et al. Plasma clusterin (ApoJ) levels are associated with adiposity and systemic inflammation. PLoS ONE 9, e103351. (2014).

Targher G, Lonardo A, Byrne CD. Nonalcoholic fatty liver disease and chronic vascular complications of diabetes mellitus. Nat Rev Endocrinol. 2018;14:99–114.

Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, et al. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the study of liver diseases. Hepatology. 2018;67:328–57.

Park, J. S., Shim, Y. J., Kang, B. H., Lee, W. K. & Min, B. H. Hepatocytespecific clusterin overexpression attenuates diet-induced nonalcoholic steatohepatitis. Biochem. Biophys. Res. Commun.495, 1775–1781. (2018).

Bhatia, L. S., Curzen, N. P., Calder, P. C., & Byrne, C. D. (2020). Non-alcoholic fatty liver disease: a new and important cardiovascular risk factor?. European Heart Journal, 41(39), 3900-3908.

Eckel, R. H., Kahn, S. E., Ferrannini, E., Goldfine, A. B., Nathan, D. M., Schwartz, M. W., ... & Smith, R. J. (2011). Obesity and type 2 diabetes: what can be unified and what needs to be individualized?. Journal of Clinical Endocrinology & Metabolism, 96(6), 1654-1663.

Hu, F. B., Manson, J. E., Stampfer, M. J., Colditz, G., Liu, S., Solomon, C. G., & Willett, W. C. (2001). Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. New England Journal of Medicine, 345(11), 790-797.

Bedaa Ahmed Abd. Evaluation of Some Novel Biomarkers as New Predictors in Patients with Thyroid Dysfunction in Type 2 Diabetes Mellitus. PhD thesis. College of Science for women: University of Baghdad; 2021.

Lee, S. B., Kim, M. K., Kim, J. M., Kang, S., Kim, J. H., Shin, D. H., & Nam, J. S. (2016). Visceral adiposity index is associated with significant fibrosis in patients with nonalcoholic fatty liver disease. Clinical and Molecular Hepatology, 22(2), 279-287.

Zhang, D., Jin, W., Yang, P., Zhang, R., & Huang, Z. (2019). Serum albumin correlates with severity of fibrosis in chronic hepatitis B: Combination of blood routine test and serum albumin level detections to screen liver fibrosis. Medicine, 98(15), e15163.

Bhatia, L. S., Curzen, N. P., Calder, P. C., & Byrne, C. D. (2020). Non-alcoholic fatty liver disease: a new and important cardiovascular risk factor? European Heart Journal, 41(39), 3900-3908.

Chen, X., Kong, H., Huang, K., Cai, H., Wu, W., Wu, D., &Gu, J. (2018). Relationship between serum liver enzymes and the risk factors/metabolic syndrome in the middle-aged and elderly Chinese population with normal liver function. Scientific Reports, 8(1), 1-7.

Kim, H., Kim, H. J., Lee, K., & Kim, D. J. (2017). Association of serum alkaline phosphatase and gamma-glutamyltransferase with risk of type 2 diabetes mellitus incidence in a Korean adult population: Kangbuk Samsung Health Study. Scientific Reports, 7(1), 1-7.

Hiroi, S., Sone, H., Nishio, Y., Kurokawa, M., Suzuki, H., Kawai, H.,& Yamamoto, S. (2018). Dapagliflozin as a treatment for nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus: A prospective case series. Canadian Journal of Diabetes, 42(1), 18-22.

Kuno, T., Takahashi, Y., Yasunaka, T., Matsuyama, T., Nakanishi, H., Tatsumi, C., & Nakamura, M. (2020). Lower serum transthyretin level as a marker for identifying type 2 diabetes patients with severe forms of non-alcoholic fatty liver disease. Journal of Diabetes Investigation, 11(3), 664-670.

Karatayli, S. C., Bozaci, E. A., Genc, H., Kume, T., Cetinkalp, S., Aydin, M.,&Uysal, A. R. (2018). The effect of empagliflozin treatment on hepatokines in patients with type 2 diabetes and non-alcoholic fatty liver disease. Experimental and Clinical Endocrinology & Diabetes, 126(10), 610-617.

Cusi, Kenneth. Nonalcoholic fatty liver disease in type 2 diabetes mellitus. Current Opinion in Endocrinology, Diabetes and Obesity 16(2):p 141-149, April 2009. | DOI: 10.1097/MED.0b013e3283293015.

Joseph AE, Saverymuttu SH, al-Sam S, Cook MG, Maxwell JD. Comparison of liver histology with ultrasonography in assessing diffuse parenchymal liver disease. Clin Radiol 1991; 43:26-31.

Bellentani S, Saccoccio G, Costa G, et al. Drinking habits as cofactors of risk for alcohol induced liver damage. Gut 1997; 41:845-850.

Bird GL, Williams R. Factors determining cirrhosis in alcoholic liver disease. Mol Aspects Med 1988; 10:97 105.

Van Ness MM, Diehl AM. Is liver biopsy useful in the evaluation of patients with chronically elevated liver enzymes? Ann Intern Med 1989; 111:473-478.

SuhaylaKhalied Mohammed Al-Obaidy.The link between serum Fetuin A, hepatic F box protein (FBXW7) and cardio-metabolic index in diabetes mellitus Type2 treated with metforminand not treated. PhD thesis. College of Science for women: University of Baghdad; 2020.

Alaa Abdul Sattar F. Warka'a T. AL-Sa'adi. Evaluation of Adiponectin, Irisin, Vitamin D Levels and Their Relation in Iraqi Patients with Nonalcoholic Fatty Liver Diseas. Journal homepage: jih.uobaghdad.edu.iq. Apr 2023, IHJPAS.36; (2), 241-250.