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Relation of retinol binding protein4, visfatin and vitamin a in obese and non obese Iraqi patients with non alcoholic fatty liver disease

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***Abstract*---**One of the most common public liver diseases over the world is fatty liver which contain alcoholic and non-alcoholic fatty liver. One-fourth among general population are impact Non-Alcoholic Fatty Liver Disease (NAFLD) in the worldwide. Retinol binding protein 4 (RBP4) is known as an adipokine, mainly synthesized and secreted from the liver and form adipose tissues. RBP4 acts as a transporter and specifically bound to retinol from liver to others tissues. Visfatin is an adipocytokine and mainly produced from visceral fat tissue, skeletal muscles as well as liver. Vitamin A absorbed, transported as retinyl esters to the liver then hydrolyzed to the retinol form and storage in hepatic stellate cells (HSCs) after reesterified with riglycerides. Hepatic retinyl esters are hydrolyzed to retinol and binds to its carrier RBP4 then transport to the target tissues. Methods: Ninety serum blood samples were collected and distributed into three groups: First group (G1) contained of (34) patients of obese NAFLD. Second group (G2) contained of (26) non obese NAFLD and control patients (G3) contained of (30) patients. Ultrasound was used to confirm the diagnosis of nonalcoholic fatty liver disease by examining the abdomen. Adiponectin and visfatin kits were used Sandwich ELISA principle. Vitamin A was measured by competitive inhibition enzyme immunoassay. Results: There was a high significant increase in RBP4 and visfatin levels (58.43±18.04) and (7.79±2.26) ng/ml respectively in obese NAFLD (G1) according to the control (G1) (10.07±3.40) and (2.37±0.20) ng/ml. While there was a significant decrease in vitamin A level (28.24 ±1.39) ng/ml in G1 compared to G3

(54.65±3.70) ng/ml. A non significant increase in RBP4, visfatin levels and vitamin A levels (58.43±18.04), (7.79±2.26) and (28.24 ±1.39) ng/ml respectively for obese NAFLD (G1) according to non obese NAFLD (G2) (52.4±3.49), (6.88± 0.62) and (28.06±6.95) ng /ml respectively. Conclusion: Serum RBP4, visfatin and vitamin A associated with obese NAFLD Iraqi patients.

***Keywords*---**Non alcoholic, fatty liver disease, RBP4, visfatin, body max index.

Introduction

One of the most common public liver diseases over the world is fatty liver which contain alcoholic and non-alcoholic fatty liver. One-fourth among general population are impact Non-Alcoholic Fatty Liver Disease (NAFLD) in the worldwide (1). NAFLD is known as a commutation of excessive fat (> 5%) as triglycerides form (steatosis) in the liver by excluding alcohol consuming (2). The pathogenesis of NAFLD is known as a multi factorial disease correlated with metabolic dysregulation, unhealthy lifestyle, diets, oxidative stress, inflammation, insulin resistance and altered gut-liver axis addition to the genetic factor (3). The increased levels of lipids result changing in lipolysis which liberate free fatty acids and these generate more reactive oxygen species (ROS) in respiratory chain reactions (β-oxidation), causing lipid peroxdation(4, 5).

Retinol binding protein 4 (RBP4) is known as an adipokine and one of lipocalin family, it possess a tertiary structure of protein known as "lipocalin fold"(6). It is mainly synthesized and secreted from the liver and form adipose tissues as secondary secreted in spite of other tissues such as adipose tissue, skeletal muscle, kidney, testis and ovary (7). RBP4 acts as a transporter and specifically bound to retinol (vitamin A /retinol acid (RA)) from liver to others tissues(8). Recently, RBP4 can transport fatty acids and has a significant role in lipid metabolism (6,9). RBP4 has role in production, regulation of cell proliferation and differenation addition to its role in immune system (10). The expression of RBP4 in adipose tissues is regulated by 17-estradiol, therefore the RBP4 levels will be elevated in women with poly cystic ovary syndrome and obesity (11).

Visfatin is an adipocytokine, known also as pre-β cell colony-enhancing factor (PBEF) as well as nicotinamide phosphoribosyl transferase (NAMPT)(12). Visfatin is mainly produced from visceral fat tissue, skeletal muscles as well as liver, and also expressed in other tissues like heart, activated lymphocytes bone marrow, lungs, kidney and placenta (13). Vitamin A absorbed, transported as retinyl esters to the liver then hydrolyzed to the retinol form and storage in hepatic stellate cells (HSCs) after reesterified with triglycerides. Hepatic retinyl esters are hydrolyzed to retinol and binds to its carrier RBP4 then transport to the target tissues (14, 15).
This research aimed to find the relation between RBP4, visfatin and vitamin A in obese and non obese NAFLD Iraqi patients.

Materials and Methods

Ninety serum blood samples were collected for the period from November 2021 to March 2022 from the Gastroenterology and Liver Teaching Hospital and Baghdad Hospital Consultation within the Medical City Department. The samples distributed into three groups:

- First group (G1) contained of (34) patients of obese Non- Alcoholic Fatty liver disease (NAFLD), 18(52%) were female and 16(48%) were male patients with BMI (35 kg/m^2).
- Second group (G2) contained of (26) non obese NAFLD, 16 (61.53%) of them female and the rest (38.46) male, with BMI (23 kg/m^2).
- Third group (G3) control patients contained of (30) patients 12(40%) of them were male and 18 (60%) were female, with BMI (22 kg/m^2).

The NAFLD patients complicated with other disease were excluded from this study. Ultrasound was used to confirm the diagnosis of NAFLD by examining the abdomen. Adiponectin and visfatin kits were provided from Elabscince Company, USA. The Sandwich ELISA principle was applied on these kits. Vitamin A was provided from My Bio Sourse Company, USA and the competitive inhibition enzyme immunoassay was utilized. Body mass index (BMI) was measured according to the mathematical equation (BMI= weight (Kg)/High (m^2) and the correlation between RBP4 with visifatin and vitamin A was obtained in this study.

Statistical analysis

The data were statically analyzed by variance ANOVA using Computer Windows DESKTOP-83QUV4R Program Microsoft Office Excel 2010. For comparing between obese, and non-obese NAFLD patients with the control group, t-test was used at the probability level ($P<0.001$).

Results

The data obtained from this study was listed in the table(1). The (mean± SD) value for RBP4 was higher in G1 (58.435±18.043 ng/mL) than G2 (52.4±3.497 ng/mL) and it was five times greater than in G3 (10.071±3.400 ng/mL) .The *P* value of RBP4 of G1 vs. G2 (0.762), G1 vs. G3 ($P \leq 0.001$) and G2 vs.G3 (0.053).

Table 1
The (mean ± SD) of RBP4, visfatin and vitamin A

Parameters	Mean ±SD			*P* value		
	G1=No.34	G2=No.26	G3=No.30	G1 Vs. G2	G1 Vs.G3	G2 Vs.G3
RBP4 (ng/mL)	58.43±18.04	52.4±3.49	10.07±3.40	0.762	0.001	0.053
Visfatin (ng/mL)	7.79±2.26	6.88± 0.62	2.37±0.20	0.103	0.001	0.001
Vitamin A(ng/ml)	28.24 ±1.39	28.06±6.95	54.65±3.70	0.896	0.001	0.001

G1: Obese nonalcoholic fatty liver disease (NAFLD), G2: Non obese nonalcoholic fatty liver disease, G3: controls.

The results have been showed increase in concentration RBP4 serum in obese NAFLD patients and non obese NAFLD compared to G3: healthy or control group. This finding is agreement with a previous study that showed increased systemic RBP4 concentrations can be seen in conditions of obesity; glucose tolerance, and T2DM. In the liver, RBP4 increases fasting blood glucose by augmenting the action of phosphoenolpyruvate carboxykinase (PEPCK) enzyme increasing hepatic glucose liberation(16). The association of RBP4 levels with NAFLD is still conflicting. Many studies finding correlation between RBP4 with NAFLD in the general population, some of these studies supported that RBP4 levels increased and significantly correlated with NAFLD(17-19), while others found that RBP4 independently positively correlated with NAFLD(20,21). Other studied reported that RBP4 levels significantly decreased in NAFLD patient (22).

Other study suggested that visceral obesity might act a role by increasing RBP4 level and some studies were conducted to reduce weight of subjects by bariatric surgery or lifestyle alteration, these results showed that RBP4 level decreased markedly, accompanied by reduction in visceral fat mass(23). The visfatin levels (mean ± SD) were (7.794±2.265), (6.880±0.62) and (2.379±0.205) (ng/mL) for G1, G2 and G3 respectively. The *P* value of G1vs.G2 (0.103), G1 vs.G3 0.001 and G2 vs.G3 (0.001). Visfatin level increases through obesity development and correlated with different inflammatory conditions(24, 25). In our study we found serum visfatin increased in G1 and G2 compared with G3. This in agreement with another study that revealed visfatin level was significantly increased in NAFLD patients when compared with control group (26). Previous literature confirmed visfatin level increases with BMI in females with visceral obesity(27), unfortunately, this study did not adjust sex different. Visfatin levels showed increased in plasma overweight and obese. This elevation of visfatin comes from the increases of expression and secretion of pro-inflammatory and adipocytokines(28). George S. Riada and colleagues confirmed that increased serum vasfatin level in patients with NAFLD with subsequent decrease when NASH is diagnosed, so this protein can be used as a noninvasive marker for NAFLD and as an unfavorable prognostic indicator for development of NASH (29).

The values of of vitamin A (mean ±SD) were (28.245±1.399), (28.064±6.950) and (54.65±3.707) (ng/dl) for G1, G2 and G3 respectively. Retinoid is primary storage in the liver, about 80% of the total body retinoid stored as retinyl esters in triglyceride-rich lipid droplets in quiescent hepatic stellate cells (HSCs), specialized mesenchymal cells of the liver (30). The HSCs activated and rapidly loss their vitamin A contents in response to liver injury and this process linked to Chronic liver diseases including NAFLD(31, 32). It is unclear, the level of hepatic retinoid are reduced in high fat diet, NAFLD(33-36), and acute liver injury(37). The level of hepatic retinoid were inversely correlated with hepatic steatosis and NAFLD, this suggesting that ectopic hepatic lipid itself perhaps an early excite hepatic retinoid losing before the onset of HSC activation(38). It was found that reduces retinol combined with elevated level of RBP4 in metabolic syndrome and obesity (14).

Table 2 Listed the level of serum GPT/ALT, GOT/AST and ALP (mean±SD) in obese and non obese NAFLD and control groups.

Table 2
The level of GPT/ALT, GOT/AST, AST/ALT and ALP

Parameters	Mean± SD	Mean± SD		*P* value		
	G1,No.=34	G2,No.=26	G3,No.=30	G1Vs. G2	G1 Vs. G3	G2 Vs.G3
ALP (U/L)	95.52±38.88	188.86±36.84	63.56±15.75	0.109	0.001	0.031
SGPT/ALT (U/L)	40.21±17.00	130.97±56.65	27.88±6.88	0.373	0.044	0.250
SGOT/AST (U/L)	40.70±2.44	99.84±34.84	25.44±6.16	0.452	0.018	0.284
AST/ALT	1.012±1.43	0.76±0.61	0.912±0.89	1.211	0.409	1.136

G1: Obese nonalcoholic fatty liver disease (NAFLD), G2: Non obese NAFLD and G3:Control

The values of serum GPT/ALT (Mean±SD) were (40.213±17), (130.97±56.655) and (27.886±6.881) (U/L) for G1, G2 and G3 respectively. The *P* value were (0.373) for G1 vs. G2, (0.044) for G1vs. G3 and for G2 vs. G3 (0.250). Alanine aminotrasferase (ALT) is exists in the cytosol of hepatocytes, its level increased in blood stream during hepatic inflammation and liver injury. ALP consider as standard indicator for liver function assessment and monitoring the patients with liver diseases(39). The obtained data from this study referred to increase in GPT/ALT serum level in G2 compared with G1 and G3. Many studies revealed that ALT level increased with NAFLD (40-41) and the elevation is associated with NASH and advanced fibrosis(42). While other study referred that the patients with NAFLD have normal levels of ALT especially as the disease progresses and more than half of patients with NAFLD have normal liver enzyme concentrations(43,44, 45). The values of serum (GOT/AST) (mean ± SD) were (40.700±24.441), (99.844±34.845) and (25.443±6.169) (U/L) for G1, G2 and G3 respectively and *P* value was G1vs. G2 (0.452), G1vs. G3 (0.018) and G2 vs. G3 (0.284). The obtained data revealed GOT/AST increased in G2 as compared with G1 and G3. The high ratio of AST/ALT (1.012± 1.437) belong to G1 as compared with G2 and G1 (0.762±0.615) and (0.912±0.896) respectively.

During the last two decades, studies have been using the AST/ALT ratio in the recognition of NAFLD from alcoholic liver disease. The ratio of AST/ALT refers to the ratio between the concentrations of these two enzymes in the blood. The normal AST/ALT should be (<1) and this ratio elevated with hepatocytes injury (AST/ALT > 1) and in patients with NASH, alcoholic liver disease (AST/ALT > 2) and hepatitis C(46). The value of ALP (mean ± SD) was (95.520±38.888), (63.566±15.758) and (188.86±36.84) (U/L) for G1, G2 and G3 respectively and *P* value was G1vs.G2 (0.001) *P* value G1vs.G2 (0.109), G1 vs.G3 (0.001) and G2 vs.G3 (0.031). In this study there an increase in ALP level in non obese NAFLD patients this agree with a previous study showed that ALP in non obese NAFLD

patients higher than obese NAFLD patients (47). This increasing may be back to metabolic syndrome is a significant risk factor for NAFLD severity (48,49). Other study showed a slight increasing in Serum ALP level in NAFLD patients (50).

Table (3) illustrated the correlation between RBP4 with visfatin and vitamin A in obese NAFLD Iraqi patients.

Table 3
RBP4 correlation in obese NAFLD

	Parameters	*P* value	R
RBP4	Visfatin	0.001	0.080
	Vitamin A	0.001	0.036

RBP4 gave a high significant positive correlation with visfatin and vitamin A, (*P*<0.001) as in figure (1) and (2).

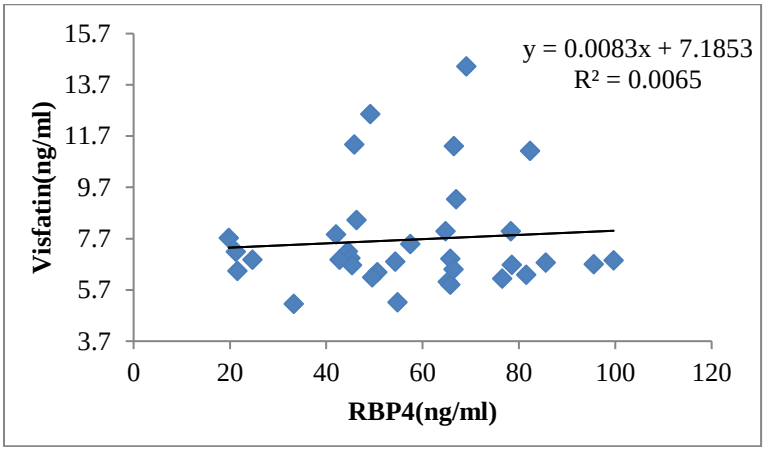

Figure 1. Correlation between Visfatin and RBP4 in obese NAFLD

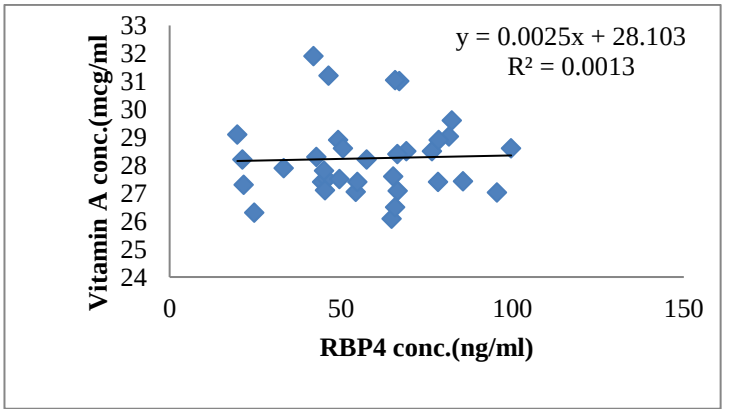

Figure 2. Correlation between RBP4 and Vitamin A in obese NAFLD

Table (4) illustrated the correlation between RBP4 with visfatin and vitamin A in non obese NAFLD Iraqi patients.

Table 4
RBP4 correlation in non obese NAFLD

RBP4	Parameters	*P* value	R
	Visfatin	0.001	-0.210
	Vitamin A	0.001	-0.291

A high significant negative correlation was obtained between RBP4 and visfatin and with Vitamin A in non obese NAFLD and (P<0.001) as in figures (3) and respectively (4).

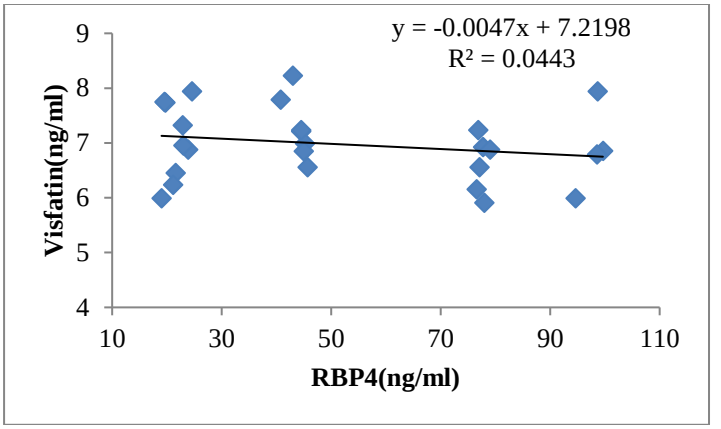

Figure 3. Correlation RBP4 with Visfatin in non obese NAFLD

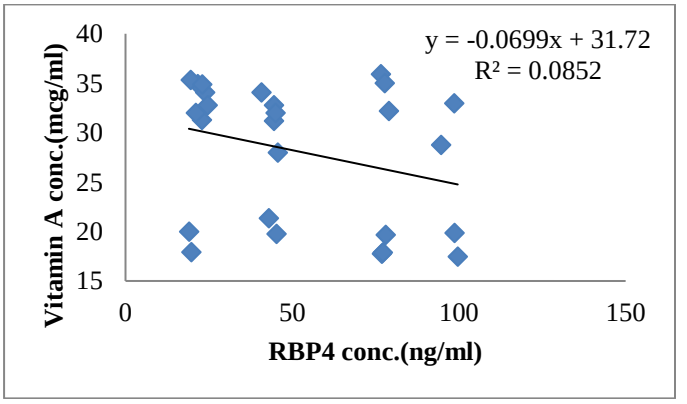

Figure 4. Correlation of RBP4 with Vitamin A in non obese NAFLD

In conclusion the high level of serum RBP4, visfatin and vitamin A is associated with obese NAFLD Iraqi patients. A significant correlation was found between RBP4, visfatin and vitamin A in the same patients. This elevation can be predicted

of NAFLD especially in obese besides routine diagnosis of ultrasound examination.

References

1. A.F. AL-Taie, Warka'a T. Saloum; and Forat Yahya Mohsin Shabil. Correlation Study of Retinol Binding Protein (-4), Nesfatin and Thyroid Hormones in Colorectal Cancer Iraqi Male Patients. Plant Archives. 2020, Vol. 20 No. 1, pp. 2841-2846
2. Ali Saeed, Robin P. F. Dullaart, Tim C. M. A. Schreuder, Hans Blokzijl; Klaas Nico Faber. Disturbed Vitamin A Metabolism in Non-Alcoholic Fatty Liver Disease (NAFLD). Nutrients. 2018, 10, 29. doi:10.3390/nu10010029
3. Anna Maria R., Marzena S. Is The Retionl Binding Protein 4 A possible Risk Factor for Cardiovascular Disease in Obesity. Int. J. Sci. 2020, 21,(15),5229.https:///doi.org/10.3390/ijms21155229
4. Ayşegül Sakin, Suleyman Sahin, Ahmet Behlul, Abdullah Sumnu, Meltem Gursu, Abdullah Sakin, Savas Ozturk. The Association of Visfatin Levels With Metabolic Parameters and Inflammation In Diabetic Nephropathy. East J Med. 2020, 25(2): 218-224.DOI: 10.5505/ejm.2020.27122
5. Bishnu Jwarchan, Subita Lalchan, Anil Dhakal, Ramesh R Acharya. Comparison of Liver Enzymes and Sonological Grading in Nonalcoholic Fatty Liver. Asian J Medical Sciences. 2020, Vol. 11, Issue 2.
6. Blaner, W.S.; O'Byrne, S.M.; Wongsiriroj, N.; Kluwe, J.; D'Ambrosio, D.M.; Jiang, H.; Schwabe, R.F.; Hillman, E.M.; Piantedosi, R.;Libien, J. Hepatic stellate cell lipid droplets: A specialized lipid droplet for retinoid storage. Biochim. Biophys. Acta 2009, 1791, 467–473.
7. Carmen Berasain, Matias A. Avila, Cima, IdiSNA. Vitamin A in Nonalcoholic Fatty Liver Disease: A Key Player in an Offside Position?. Cellular and Molecular Gastroenterology and Hepatology 2021, 11:291–293.
8. Chalasani N., Younossi Z., Lavine JE., Charlton M., Cui K., Rinella M., Harrison SA., 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: 328-357.
9. Chaves, G.V.; Pereira, S.E.; Saboya, C.J.; Spitz, D.; Rodrigues, C.S.; Ramalho, A. Association between Liver Vitamin A Reserves and Severity of Nonalcoholic Fatty Liver Disease in the Class III Obese Following Bariatric Surgery. Obes. Surg. 2014, 24, 219–224. [CrossRef] [PubMed]
10. Chen X, Shen T, Li Q, Chen X, Li Y, Li D, Chen G, Ling W, Chen YM. Retinol binding Protein-4 levels and non-alcoholic fatty liver disease: a communitybased cross-sectional study. Sci Rep. 2017, 7:45100.
11. Czuba, L.C.; Wu, X.; Huang, W.; Hollingshead, N.; Roberto, J.B.; Kenerson, H.L.; Yeung, R.S.; Crispe, I.N.; Isoherranen, N. Altered vitamin a metabolism in human liver slices corresponds to fibrogenesis. Clin. Transl. Sci. 2021, 14, 976–989. [CrossRef]
12. Davut Baltacı, Mert Can Tuncel , Mert Cetinkaya, Muhammet Talha Gunduz, Zemze Ozbey, Ozlem Admis, Ismail Hamdi Kara, Handan Ankarali. Evaluation of Visfatin in Patients with Obesity, Metabolic Syndrome, Insulin Resistance and Impaired Glucose Tolerance; Case-Control Study. Acta Medica Anatolia. 2016, Vol. 4 Issue 2.doi: 10.5505/actamedica.2016.00710

13. Elizabeth E Powell, Vincent Wai-Sun Wong, Mary Rinella. Non-alcoholic fatty liver disease. Lancet. 202, 397: 2212–24
14. Eloi Frepat; Maria Guillan-Fresco; Ana Alonso-Perez,\; Alberto Jorge-Mora; Vera Francisco; Oreste Gualillo; Rodolfo Gȯmez. Visfatin Connection: Present and Future in Osteoarthritis and Osteoporosis. J. Clin. Med. 2019, 8, 1178. DOI:10.3390/jcm8081178
15. Eri Ozaki, Hirotaka Ochiai, Takako shirasawa, et al. Eating Quickly is Associated with A Low Aspartate Aminotrasferase to Alanine aminotrasferase Ratio in Middle-Age Adults: A Large-Scale Cross-Sectional Survey in Japan. Arch. Pub Health. 2020, 78: 101.
16. Eunice Xiang-Xuan Tan, Jonathan Wei-Jie Lee, Nur Halisah Jumat, Wah-Kheong Chan, Sombat Treeprasertsuk, George Boon-Bee Goh, Jian-Gao Fan, Myeong Jun Song, Phunchai Charatcharoenwitthaya, Ajay Duseja, Kento Imajo, Atsushi Nakajima, Yosuke Seki, Kazunori Kasama, Satoru Kakizaki, Laurentius A. Lesmana, Kenneth I. Zheng, Ming-Hua Zheng, Calvin J. Koh, Khek-Yu Ho, Khean-Lee Goh, Vincent Wai-Sun Wong and Yock-Young Dan. Non-obese non-alcoholic fatty liver disease (NAFLD) in Asia: an international registry study. Metabolism Clinical and Experimental. 2022, 126: 154911. https://doi.org/10.1016/j.metabol.2021.154911 0026-0495.
17. George S. Riad, Hany A. Hussein, Ahmed Z. Abdel Ghafar. Study of Serum Vistafin Level in patients with Nonalcoholic Fatty Liver Disease and Its Role in Progression to Nonalcoholic Steatohepatitus. Egyptian Liver J. 2018, 8: 49-54
18. George S. Riada , Hany A. Husseina and Ahmed Z. Abdel Ghafar, Study of serum visfatin level in patients with nonalcoholic fatty liver disease and its role in progression to nonalcoholic steatohepatitis. 2018 Egyptian Liver J.DIO:10.1097/01.EXL.0000544522.99768.5b
19. Haaker MW, Vaandrager AB, Helms JB. Retinoids in health and disease: a role for hepatic stellate cells in affecting retinoid levels. Biochim Biophys Acta - Mol Cell Biol Lipids 2020:1865.
20. Hall P., Cash J. What is The Real Function of The Liver 'Function' Tests? Ulster Med J. 2012, 81, 1: 30-36.
21. Hayatul Najaa Miptah, Anis Safura Ramli, Mariam Mohamad, Hilwati Hashim and Zahirah Tharek. Non-alcoholic fatty liver disease (NAFLD) and the cardiovascular disease (CVD) risk categories in primary care: is there an association? BMC Family Practice. 2020, 21:238 https://doi.org/10.1186/s12875-020-01306-7
22. Imad Brema, The relationship between plasma Visfatin/Nampt and type 2 diabetes, obesity, insulin resistance and cardiovascular disease. Endocrinology and Metabolism International J. 2016, 3 (6): 00068.
23. Isaken VT. Larsen MA, Goll R, Florholmen JR; Paulssen EJ. Hepatic Steatosis, Detected by Hepatorenal Index in Ultrasonography, As A Predictor of Insulin Resistance in Obese Subjects. BMC Obes. 2016, 3:39.
24. Julia S. Steinhoff, Achim Lass and Michael Schupp. Biological Functions of RBP4 and Its Relevance for Human Diseases. Frontiers in Physiology. March 2021, Volume 12, Article 659977 DOI: 10.3389/fphys.2021.659977
25. K. Ugur, F. Erman, S. Turkoglu, Y. Aydin, A Aksoy, A. Lale, Z.K. Karagoz, I. Ugur, R.F. Akkoc , M. Yalniz. Asprosin, Visfatin and Subfatin as New Biomarker of Obesity and Metabolic Syndrome. European Review for Medical and Pharmacological Sciences. 2022, 26: 2124-2133.

26. Laurent Castera, Mireen Friedrich-Rust; Rohit Loomba. Noninvasive Assessment of Liver Disease in Patients with Nonalcoholic Fatty Liver Disease. Gastroenterology 2019;156:1264–1281
27. Marta Melis, Xiao-Han Tang, Steven E. Trasino; Lorraine J. Gudas. Retinoids in The Pathogenesis and Treatment of Liver Diseases. Nutrients 2022, 14, 1456.https://doi.org/10.3390/nu14071456
28. Martin-Rodriguez JL, Gonzalez-Cantero J, Gonzalez- Cantero A, Arrebola JP, Gonzalez- Calvin JL. Diagnostic Accuracy of Serum Alanin Aminotransferase as Biomarker for Nonalcoholic Fatty Liver Desaes and Insulin Resistance in Healthy Subjects Using 3T MR Spectroscopy. Medicine (Baltimore). 2017, 96: e6770
29. Milner KL, van der Poorten D, Xu A, Bugianesi E, Kench JG, Lam KS, Chisholm DJ, George J. Adipocyte fatty acid binding protein levels relate to inflammation and fibrosis in nonalcoholic fatty liver disease. Hepatology. 2009, 49:1926–34.
30. Mohammed Abdel-Hassiba , Abdelhaleem A. Hassaboa , Hossam Elashmawyb , Mostafa I.S. Mansour. Serum visfatin level in prediabetics and its relation to left ventricular function . The Egyptian Journal of Internal Medicine. 2019, 31:703-714
31. Noé López-Amador , Cirilo Nolasco-Hipolito , Macario de J. Rojas-Jimeno , Octavio CarvajalZarraba. Liver enzymes in patients diagnosed with non-alcoholic fatty liver disease (NAFLD) in Veracruz: a comparative analysis with the literature. Clin. Invest. (Lond.) 2017, 7(1): 011-016
32. Saeed, A.; Dullaart, R.P.F.; Schreuder, T.C.M.A.; Blokzijl, H.; Faber, K.N. Disturbed Vitamin A Metabolism in Non-Alcoholic Fatty Liver Disease (NAFLD). Nutrients 2017, 10, 29. [CrossRef]
33. Schina M, Koskinas J, Tiniakos D, Hadziyannis E, Savvas S, Karamanos B, Manesis E, Archimandritis A. Circulating and liver tissue levels of retinol-binding protein-4 in non-alcoholic fatty liver disease. Hepatol Res. 2009, 39: 972–8.
34. Seo JA, Kim NH, Park SY, Kim HY, Ryu OH, Lee KW, Lee J, Kim DL, Choi KM,Baik SH, Choi DS, Kim SG. Serum retinol-binding protein 4 levels are elevated in non-alcoholic fatty liver disease. Clin Endocrinol (Oxf). 2008, 68:555–60.
35. Suh JB, Kim SM, Cho GJ, Choi KM. Serum AFBP levels are elevated in patients with nonalcoholic fatty liver disease. Scand J Gastroenterol. 2014, 49: 979–85.
36. T. Basu; A. Doeve. Significance of Vitamin A (Retinol) in Ageing. The Open Nutraceuticals Journal, 2011, *4,* 65-68
37. Tan, B.K.; Chen, J.; Lehnert, H.; Kennedy, R.; Randeva, H.S. Raised serum, adipocyte, and adipose tissue retinol-binding protein 4 in overweight women with polycystic ovary syndrome: Effects of gonadal and adrenal steroids. J. Clin. Endocrinol. Metab. 2007, 92, 2764–2772.
38. Thair L. Jabbar and Ali A. Kasim, Association of Retinol Binding Protein- 4 (RBP4) with Glycemia, Dyslipidemia, Hypertension, and Obesity in Type 2 Diabetic Iraqi Patients. Iraqi J Pharm Sci. 2020, Vol. 29(2). https://doi.org/10.31351/vol29iss2pp263-270.

39. Trasino, S.E.; Tang, X.-H.; Jessurun, J.; Gudas, L.J. Obesity Leads to Tissue, but not Serum Vitamin A Deficiency. Sci. Rep. 2015, 5,15893. [CrossRef] [PubMed]
40. Verónica Martín-Domínguez, Rosario González-Casas, JorgeMendoza-Jiménez-Ridruejo, Luisa García-Buey; Ricardo Moreno-Otero. Pathogenesis, Diagnosis and Treatment of Non-Alcoholic Fatty Liver Disease. Rev Esp Enferm Dig (Msdrid). 2013, Vol. 105, No. 7, 409-420.
41. Vicneswarry Dorairaj, Siti Aishah Sulaiman, Nadiah Abu; Nor Azian Abdul Murad. Nonalcoholic Fatty Liver Disease (NAFLD): Pathogenesis and Noninvasive Diagnosis. Biomedicines. 2022, 10,15.
42. Xiaoyan Li; Hua Wang. Cell and Bioscience. Multiple organs involved in the pathogenesis of Non-Alcoholic Fatty Liver Disease. 2020, 10:140 http://doi.org/10.1186/s13578-020-00507-y
43. Xinxia Chang, Hongmei Yan , Hua Bian , Mingfeng Xia , Linshan Zhang , Jian Gao and Xin Gao, Serum retinol binding protein 4 is associated with visceral fat in human with nonalcoholic fatty liver disease without known diabetes: a cross-sectional study. Lipids in Health and Disease. 2015, 14:28
44. Xuefeng Ma, Shousheng Liu, Jie Zhang, Mengzhen Dong, Yifen Wang, Mengke Wang; Yongning Xin. Proportion of NAFLD Patients with Normal ALT Value in Overall NAFLD Patients: A Systematic Review and Meta-Analysis. BMC Gastroenterology. 2020, 20, 10.
45. Yinuo Liu, Elke Albercht, Dirk Dannenberger, Harald M. Hammon, Christa Kuehn, Helga Sauerwein, Runjun Yang, Zhihui Zhao; Steffen Maak. Retinol Binding Protein4 Abundance in Plasma and Tissues is Related to Body Fat Deposition in Cattle. Scientific Reports. 2019, 9: 8056 https//doi.org/10.1038/s41598-019-44509-4
46. Yu Z hang, He He, Yu-Ping Zeng, Li-Dan Yang, Dan Jia, Zhen-Mei An; Wei-Guo Jia. Lipoprotein A Combined with Alanine Aminotransferase and Aspartate Aminotransferase, Contributes to Predicting The Occurrence of NASH: A Cross-Sectional Study. Lipids in Health and Disease. 2020, 19: 134.http://doi.org/10.1186/s12944-020-0130x
47. Yun Zhao, Jiahui Rao, Tong Qiu, Chunjin Li; Xu Zhou. The Effect of RBP4 on microRNA Expression Profiles in Porcine Granulosa Cells. Animals. 2021, 11, 1391.https://doi.org/10.3390/ani11051391
48. Yunkoo Kang., Sowon Park, Seung Kim; Hog Koh. Normal Serum Alanine Aminotrasferase and Non-Alcoholic Fatty Liver Disease Among Korean Adolescents: A Cross-Sectional study Using Data from KNHANES 2010-2015. BMC Pediatrics. 2018, 18: 215. https://doi.org/10.1186/s12887-018-1202-z
49. Zhi-Hui Zhang, Jiang-Feng Ke, Jun-Xi Lu, Yun Liu, Ai-Ping Wang, Lian-Xi Li. Serum Retinol-Binding Protein Levels Are Associated with Nonalcoholic Fatty Liver Disease in Chinese Patients with Type 2 Diabetes Mellitus: A Real-World Study. Diabetes Metab J. 2022, 46:129-139.https://doi.org/10.4093/dmj.2020.0222
50. Zhong, G.; Kirkwood, J.; Won, K.-J.; Tjota, N.; Jeong, H.-Y.; Isoherranen, N. Characterization of Vitamin A Metabolome in Human Livers with and without Nonalcoholic Fatty Liver Disease. J. Pharmacol. Exp. Ther. 2019, 370, 92–103. [CrossRef] [PubMed]