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Evaluation of Adiponectin, Irisin, Vitamin D Levels and Their Relation in Iraqi Patients with Non alcoholic Fatty Liver Disease

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is one of chronic liver and defines by fat accumulation ≥5% in liver which can progresses to non-alcoholic steatohepatitis (NASH). NAFLD related to obesity as well as non obese individuals. Adiponectin is a cytokine secreted from adipose tissue involved NAFLD pathogenesis and liked with obesity. Irisin is a myokine, has a convenient effect against metabolic diseases such as obesity, disylipemia diabetes type 2 and reversed liver steatosis and may be related with NAFLD.

Vitamin D is one of the fat soluble vitamins and more precisely as a pro-hormone through its metabolite ($1,25(OH)_2$ cholecalciferol) the major steroid hormone. After the skin exposure to the light, vitamin D undergoes to activation through two successive hydroxylation reactions in liver and kidney. Several metabolic diseases such as dyslipidaemia, obesity, hypertension, T2D and metabolic syndrome (MS) have been related to vitamin D deficiency.

Keywords: Adiponectin, irisin, non alcoholic fatty liver disease.

1. Introduction

The presence of more than 5% hepatic steatosis without evidence of hepatocellular injury is defined as nonalcoholic fatty liver (NAFL), while the more active and aggressive form of NAFLD, nonalcoholic steatohepatitis (NASH) includes ballooning of hepato, steatosis and lobular inflammation resulting cirrhosis, fibrosis and hepatocellular carcinoma, affects around 6.5 of the population [1,2]. In most patients, liver disease is stable or slowly progressive and will not result in cirrhosis or liver-related death, but a small proportion of affected individuals developing to advanced fibrosis and complicated to the end-stage liver disease and hepatocellular carcinoma.

Adiponectin is a protein generally secreted by white adipose tissue (WAT) and existent at high levels in circulation [3,4]. Also this protein has been detected in cardio myocytes, skeletal muscle, lymphocytes, pituitary gland, adrenal gland osteoblasts, and liver tissue [5]. Scherer *et al*, in 1995 were distinguishing this protein from cell line 3T3-L1 by cDNA cloning and its coding gene located on the 3q27 chromosome [5].

Adiponectin consists of 244 amino acids with molecular weight 28 KDa showing similar structural to TNF-α and collagen. Adiponectin also known as ACRP30, AdipoQ, apM1 or GBP28 [6]. The biological function of adiponectin exert through two receptors, (AdipoR1) mainly expressed in skeletal and (AdipoR2) the liver [5].

Adiponectin also called "adipokines or adipocytokines", the regulation of adipocytokines is associated and altered in diseases like obesity, atherosclerosis, diabetes type2, and metabolic syndrome (MS) due to the increase in the mass of white adipose tissue [3].

Irisin is a glycosylated protein hormone with a molecular weight about 12 KDa, composed of 112 amino acids residues [7]. Irisin existing a homodimer form and stabilized by interaction between side chains. About 72% of the total amount irisin in plasma comes from skeletal muscle and others from white adipocyte cells [8]. Through physical exercise, the extracellular membrane protein fibronectin type III domain-containing protein 5 (FNDC5) undergoes proteolytic cleavage and resulting irisin secretion [9]. Irisin has a convenient effect against metabolic diseases such as obesity, dislipemia diabetes type 2 and reversed liver steatosis [10]but still the correlation between NAFLD and irisin in human an argumentative and unclear[11].

Vitamin D is one of the fat soluble vitamins and more precisely as a pro-hormone through its metabolite cholecalciferol ($1,25(OH)_2$ cholecalciferol the major steroid hormone) [12]. When the skin exposure to the sun light, vitamin D undergoes to activation through two successive hydroxylation reactions in liver and kidney [13]. Vitamin D has massive role in calcium regulation and homeostasis which aids calcium level to maintain in the body and bone health [14], anti-inflammatory effects, antifibrotic, and anti-proliferative in the liver [15]. Several metabolic diseases such as dyslipidaemia, obesity, hypertension, T2D and metabolic syndrome (MS) have been related to vitamin D deficiency [16-18].

This research aimed to evaluate the levels of adiponectin, irsin and vitamin D_3 in obese and non-obese NAFLD Iraqi patients. The correlation between adiponectin with Irisin and vitamin D_3 in NAFLD obese and non-obese Iraqi patients were also included in this research.

2-Materials and Methods

Between November 2021 and March 2022, we collected blood samples of 90 patients who visited Gastroenterology and Liver Teaching Hospital and Baghdad Hospital Consultation within the Medical City Department. From them, 60 examinees were diagnosed with NAFLD and 30 examinees were fixed as control group (G3). The NAFLD patients group was based on abdominal ultrasound imaging examine and divided to obese NAFLD group 34 as (G1) and non-obese NAFLD group 26 as (G2) according to the body mass index (BMI) by mathematical equation, BMI=weight (kg)/high (m^2) [19]. The patient suffering from other complications were excluded from this research.

Several parameters were assessed like age, sex, and clinical laboratory tests. We evaluated serum levels of adiponectin and irisin by Sandwich-ELISA technique (ELISA Kit, Elabscince, USA). A vitamin D level was evaluated as 25-hydroxyvitamin D (25(OH) D) by ELISA works on

the competitive binding concept (vitamin D kit, SIGMA-ALDRIGH, USA). Liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were evaluated photometrically (ALT and AST kits supplied from LINEAR company, Spain).

Statistical analysis: Descriptive analysis for variables was statically analyzed by variance ANOVA using Computer Windows DESKTOP-83QUV4R Program Microsoft Office Excel 2010 and expressed as (mean± SD). For comparison study between NAFLD and control groups we used t-test and $P \leq 0.001$ was considered as significant [20].

2.Results and Discussion

Table (1) displays the obtained levels of adiponectin, irisin and vitamin D_3 in obese and non-obese Iraqi patients with NAFLD and control group.

The body mass index (BMI) was found (35.18±5.44), (23.72±0.52) and (24.43± 2.30) for the three groups respectively. 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 [21], but still there are many NAFLA patients with normal BMI [23]. Recent studies have shown that non-obese people with a BMI (< 25 kg/m^2) and lean (< 23kg/m^2) also have NAFLD [23]. Obesity affects NAFLD, and NAFLD may also affect obesity via multiple mechanisms, including hepatic dysfunction, hepatic IR, oxidative stress, glucotoxicity and lipotoxicity.

The data in this study showed a significant increase in adiponectin and irisin in G1 as compared with G2 and G3 as showed in table (1).

Table (1): The (mean ± SD) of Adiponectinm, irisin and vitamin D_3.

Parameters	Mean ±SD			*P*.value		
	G1=No.34	G2=No.26	G3=No.30	G1 vs. G2	G1 vs.G3	G2 vs.G3
Age	42.89±8.36	38.6±13.68	41.36±11.96	0.529	0.570	0.68
BMI(Kg/m^2)	35.18±5.44	23.72±0.52	22.431±2.30	0.001	0.001	0.15
Adiponectin (ng/mL)	3.53±1.26	3.19±0.57	1.806±0.09	0.396	0.001	0.002
Irisin (pg/mL)	429.87± 92.97	404.68±86.49	62.08±18.32	0.627	0.001	0.001
Vitamin D3(ng/ml)	14.23±1.43	15.97±3.87	33.4±2.22	0.035	0.001	0.001
SGPT/ALT (U/L)	40.21±17.00	130.97±56.65	27.88±6.88	0.373	0.044	0.250

G1: Obese NAFLD, G2: Non obese NAFLD, G3: controls Iraqi patients.

The expansion of NAFLD is 15-30 of the common population and 50-90% in obese. The important risk factor to beginning NAFLD is visceral obesity [24]. Adipose tissue dysfunction results from visceral obesity and fatty liver leads to increase pro-inflammatory adiponectines and decrease anti-inflammatory adipokines, therefore NAFLD increases with body mass index (BMI) increase [25]. Adiponectin expression of NAFLD is decreased by 20%-40% during the development of NAFLD and it lower by more than 50% in NASH patients, from simple steatosis to NASH. The previous study reveled the low level of adiponectin in hepatic steatosis, NASH and in obese especially those with visceral obesity and correlate inversely with insulin resistance [26].

Other study showed that low serum adiponectin level in NAFLD and considered adiponectin as a non-classical biomarker of metabolic complications with NAFLD [27].

Irisin synthesized in muscle and a higher amount in adipose tissues thus irisin level correlation positively with obesity and BMI [28-29]. Several studies cleared the relation between obesity and disorder of serum irisin, since irisin plays a protective role in obesity, it might be expect that levels of irisin would be lower in populations with obesity. Also clinical studies have been described a positive association between circulating levels of irisin and BMI [30]. Irisin level was significantly lower in NAFLD compared with control[31] , and lower in obese NAFLD in adolescents[32] while another study revealed that irisin level increasing with NAFLD patients[33] . Other study showed that irisin levels were higher in the NAFLD patient group than in healthy group in Asians and the level was higher in mild NAFLD group than in moderate NAFLD group[34] . In early stage of NAFLD, irisin level was increased temporarily then stabilized with aggravation of NAFLD.

In addition the data in table (1) showed a significant decrease in Vitamin D_3 in G1 as compared with G2 and G3. Different studies have referred that Vitamin D deficiency (VDD) is associated with the seriousness and disease progression in NAFLD/NASH patients and hepatic steatosis [35-42]. Vitamin D_3 regulates free fatty acids metabolism via peroxisome proliferator-activated receptor gamma (PPAR-g) which induced IR in vitro, the increased free fatty acids levels in bloodstream enhance fat storage in the liver this will development of NAFLD [43]. This is in agreement with other studies that lean to an association between low levels of vitamin D_3 and NAFLD [44-46] . Resent epidemiological studies have demonstrated the existence of Vitamin D_3 deficiency and NAFLD shared with multiple risk factors and about 55% occurs with NAFLD patients [39].

Liver enzymes (ALT and AST) gave a significant increase in G2 when compared with G1 and G3. ALT is exists in the cytosol of hepatocytes, its level increased in blood stream during hepatic inflammation and liver injury. ALT consider as standard indicator for liver function assessment and monitoring the patients with liver diseases [47]. Many studies pointed to the ALT level increased with NAFLD[48-51] and the elevation is associated with NASH and advanced fibrosis[52] . 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 [53-55].

Table (2) illustrated the correlations between adiponectin with irisin, and vitamin D_3 in obese NAFLD Iraqi patients.

Table (2): The correlation of adiponectin with Irisin and vitamin D_3 in obese NAFLD Iraqi Patients.

	Parameters	*P*.value	R
Adiponectin	Irisin	0.001	0.404
	Vitamin D3	0.001	-0.068

This study revealed the correlation between adiponectin and irisin, which gave a non significant positive correlation while a non signifivant negative correlation was obtained between adiponectin and vitamin D_3 as in Figures (1) and (2).

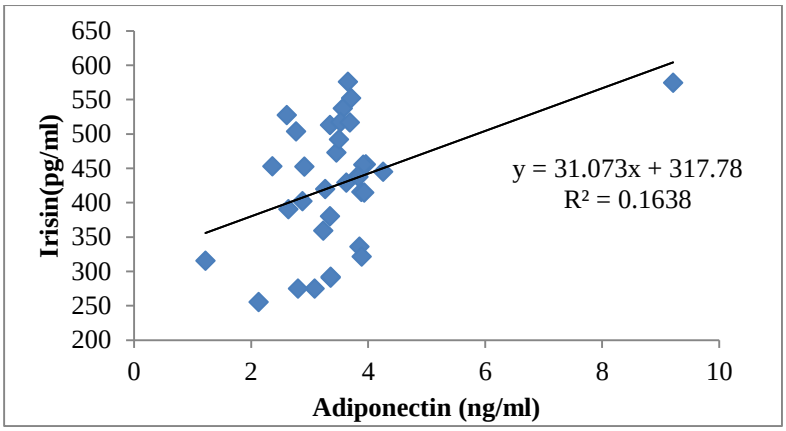

Figure (1): Correlation between adiponectin with irisin in obese NAFLD Iraqi patients.

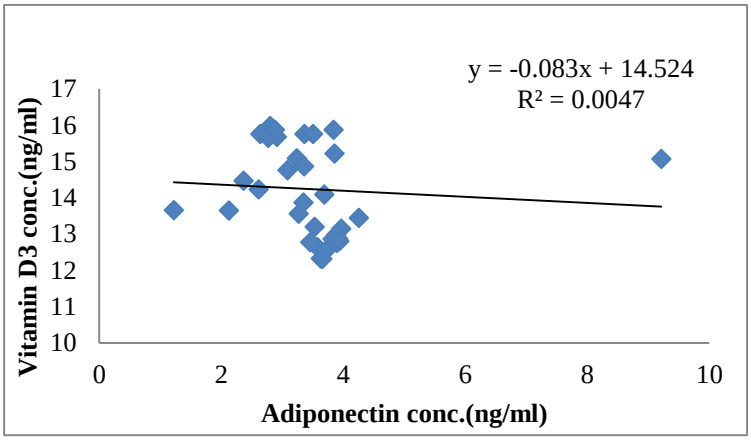

Figure (2): Correlation between irisin and vitamin D3 in obese NAFLD.

This study also investigated the correlation between adiponectin, with irisin and vitamin D_3 in non obese NAFLD Iraqi patients, the data were illustrated in the Table (3).

Table (3): The Correlation of adiponectin with irisin and vitamin D_3 in non obese NAFLD Patients.

Adiponectin	Parameters	*P*. value	R
	Irisin	0.001	-0.237
	Vitamin D3	0.001	-0.067

Adiponectin gave a high significant negative correlation with irisin as well as with vitamin D_3 as in **Figure (3) and (4).**

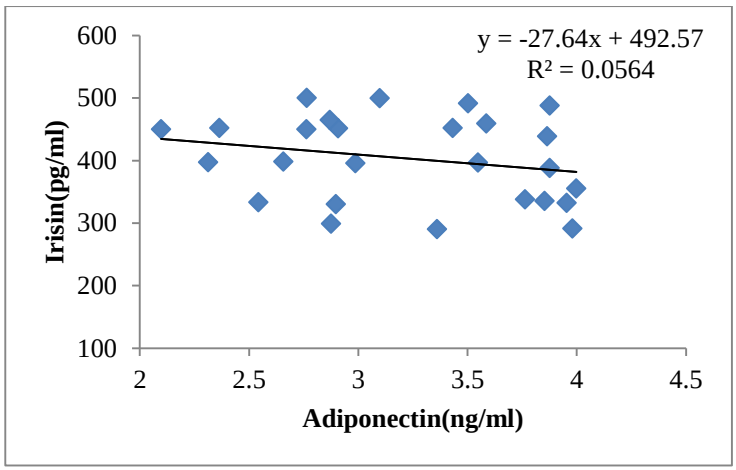

Figure (3): Correlation of adiponectin with irisin in non obese NAFLD Iraqi patients.

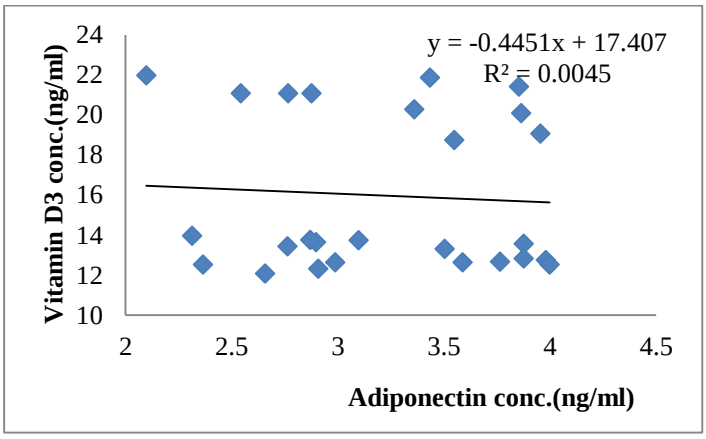

Figure (4): Correlation of adiponectin with vitamin D_3 in non obese NAFLD.

3.Conclusion

Adiponectin and irisin is a good predictor for NAFLD and may be used as a biomarker for the patients with NAFLD. While vitamin D_3 may be a risk factor in NAFLD.

References

1. GRGUREVIC, Ivica; et al. Natural history of nonalcoholic fatty liver disease: implications for clinical practice and an individualized approach. *Canadian Journal of Gastroenterology and Hepatology*, **2020**, 2020.

2. Mikolasevic, Ivana; et al. "Nonalcoholic fatty liver disease and liver transplantation-where do we stand?" *World journal of gastroenterology* 24.14 **,2018:** 1491.

3. Yibby, María Luz Gunturiz Albarracína Ana, and Forero Torresb. "Adiponectin and Leptin Adipocytokines in Metabolic Syndrome: What Is Its Importance? *Dubai Diabetes Endocrinol J* **2020**;26:93–102.

4. CHOI, Hyung Muk; DOSS, Hari Madhuri; KIM, Kyoung Soo. Multifaceted physiological roles of adiponectin in inflammation and diseases. *International journal of molecular sciences*, **2020**, 21.4: 1219.

5. NGUYEN; Thi Mong Diep. Adiponectin: role in physiology and pathophysiology. *International journal of preventive medicine*, **2020**, 11.

6. Enrique, Z Fisman; Alexander, Tenenbaum. Adiponectin: a manifold therapeutic target for metabolic syndrome, diabetes, and coronary disease? *Cardiovascular Diabetology* **2014**, *13*:103
7. Leustean, Letitia; et al. Role of Irisin in Endocrine and Metabolic Disorders—Possible New Therapeutic Agent?. *Applied Sciences* 11.12 **,2021:** 5579.

8. Marrano, Nicola; et al. "Irisin and incretin hormones: Similarities, differences, and implications in type 2 diabetes and obesity." *Biomolecules* 11.2 ,**2021**: 286.
9. Ulualan, Gökçen, Z. E. Y. N. E. P. KÜSKÜ KİRAZ; BİRGÜL KIREL. Relation of serum irisin levels to obesity and non-alcoholic fatty liver disease. *Turkish Journal of Pediatrics 64.2* **2022**.
10. Polyzos, S.A.; Anastasilakis, A.D.; Efstathiadou, Z.A.; Makras, P.; Perakakis, N.; Kountouras, J.; Mantzoros, C.S. Irisin in metabolic diseases. *Endocrine* **2018**, 59, 260–274.
11. HU, Jie, et al. Circulating irisin levels in patients with nonalcoholic fatty liver disease: A systematic review and meta-analysis. *Gastroenterology research and practice*, **2020**, 8818191.
12. DAHIYA, Kiran; PAL, Sanghapriya; DHANKHAR, Rakesh.Vitamin D in Physiological and Pathological Conditions. *MedDocs eBooks, Resent Trends in Biochemistry.* **2020**.
13.Angle Gil; Julio Plaz-Diaz and Maria Dolores Mesa. Vitamin D: Classic and Novel Actions. Ann Nutr Metab. **2018**,72: 87-95.
14. Mary S. Matsui. Vitamin D update. *Current Dermatology Reports*: **2020,** 9: 323-330
15. Abramovitch S, Shirley, et al. Vitamin D inhibits proliferation and profibrotic marker expression in hepatic stellate cells and decreases thioacetamide-induced liver fibrosis in rats. *Gut*, **2011**, 60.12: 1728-1737.
16. BORGES-CANHA, Marta, et al. The impact of vitamin D in non-alcoholic fatty liver disease: a cross-sectional study in patients with morbid obesity. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, **2021**, 14: 487.
17. CIMINI, Flavia A., et al. Relationship between adipose tissue dysfunction, vitamin D deficiency and the pathogenesis of non-alcoholic fatty liver disease. *World journal of gastroenterology*, **2017**, 23.19: 3407.

18. Wimalawansa SJ. Associations of vitamin D with insulin resistance, obesity, type 2 diabetes, and metabolic syndrome. *J Steroid Biochem Mol Biol*, **2018**; 175: 177–189.

19. Nuttall, Frank Q. Body mass index: obesity, BMI, and health: a critical review. *Nutrition today*, 2015, 50.3: 117.

20. Daniel, Wayne W., and Chad L. Cross. *Biostatistics: a foundation for analysis in the health sciences*. **2018**.
21. Johanna K. Disefano, Glenn S. Gerhard. NAFLD in Normal Weight Individuals. Diabetology and Metabolic Syndrome. (**2022**).14: 45

22. ZOU, Yang, et al. Association between the alanine aminotransferase/aspartate aminotransferase ratio and new-onset non-alcoholic fatty liver disease in a nonobese Chinese population: a population-based longitudinal study. *Lipids in Health and Disease*, **2020**, 19.1: 1-10.

23. Lee, So-Ryoung, et al. Nonalcoholic fatty liver disease and the risk of atrial fibrillation stratified by body mass index: a nationwide population-based study. *Scientific reports*, **2021**, 11.1: 1-9.

24. DIVELLA, Rosa, et al. Obesity, nonalcoholic fatty liver disease and adipocytokines network in promotion of cancer. *International journal of biological sciences*, **2019**, *15.3: 610.*

25. HOHENESTER, Simon, et al. Lifestyle intervention for morbid obesity: effects on liver steatosis, inflammation, and fibrosis. *American journal of physiology-gastrointestinal and liver physiology*, **2018,** *315.3: G329-G338.*

26. YAMAUCHI, Toshimasa; KADOWAKI, Takashi. Adiponectin receptor as a key player in healthy longevity and obesity-related diseases. *Cell metabolism*, **2013**, *17.2: 185-196.*

27. MOHAMED, Manal Sabry, et al. Correlation between adiponectin level and the degree of fibrosis in patients with non-alcoholic fatty liver disease. *Egyptian Liver Journal*, **2021**, *11.1: 1-10*.

28. PALACIOS-GONZÁLEZ, Berenice, et al. Irisin levels before and after physical activity among school-age children with different BMI: A direct relation with leptin. *Obesity*, **2015,** *23.4: 729-732*.

29. HU, Jie, et al. Circulating irisin levels in patients with nonalcoholic fatty liver disease: A systematic review and meta-analysis. *Gastroenterology research and practice*, **2020**, 2020.

30. WANG, Ya-Di, et al. New insight of obesity-associated NAFLD: Dysregulated "crosstalk" between multi-organ and the liver?. *Genes & Diseases*, **2022.**

31. SHANAKI, Mehrnoosh, et al. Lower circulating irisin is associated with nonalcoholic fatty liver disease and type 2 diabetes. *Diabetes & metabolic syndrome: clinical research & reviews*, **2017,** *11: S467-S472.*

32. ULUALAN, Gökçen; KÜSKÜ KİRAZ, ZEYNEP; KIREL, BİRGÜL. Relation of serum irisin levels to obesity and non-alcoholic fatty liver disease. *Turkish Journal of Pediatrics*, **2022**, *64.2.*

33. Jiang Q.; P.Yan; Y, Yang and S. Dai, Y. Luo. Correlation between Irisin Level and Insulin Resistance in Patients with Fatty Liver Disease. *Modem Practical Medicine*, **2017**. *29, 11, 1440-1441, 2017.*

34. HU, Jie, et al. Circulating irisin levels in patients with nonalcoholic fatty liver disease: A systematic review and meta-analysis. *Gastroenterology research and practice*, **2020**, 2020.

35. KARATAYLI, Ersin; STOKES, Caroline S.; LAMMERT, Frank. Vitamin D in preclinical models of fatty liver disease. *Anticancer research*, **2020,** *40.1: 527-534*.

36. BARCHETTA, Ilaria, et al. Strong association between non alcoholic fatty liver disease (NAFLD) and low 25 (OH) vitamin D levels in an adult population with normal serum liver enzymes. *BMC medicine*, **2011**, *9.1: 1-7.*

37. Dasarathy J; Periyalwar P;Allampati S; Bhinder V, Hawkins C, Brandt P, Khiyami A;McCullough AJ and Dasarathy S. Hypovitaminosis D is Associated with Increased Whole Body Fat Mass and Greater Severity of Non-Alcoholic Fatty liver Disease. *Liver Int* .**2014.** *34(6): 1*

38. Nelson JE; Roth CL;Wilson LA;Yates KP; Aouizerat B; Morgan- Stevenson V; Whalen E, Hoofnagle A, Mason M, Gersuk V, Yeh MM and Kowdley KV. Vitamin D Deficiency is Associated with Increased Risk of Non-Alcoholic Steatohepatitis in Adults with Nonalcoholic Fatty Liver Disease: Possible Role for Mapk and Nf-kappab? *Am J Gastroenterol***. 2016**.*111(6): 852-863*.

39. Nobili V; Giorgio V; Liccardo D; Bedogni G; Morino G; Alisi A and Cianfarani S. Vitamin D levels and liver histological alterations in children with nonalcoholic fatty liver disease. *Eur J Endocrinol*.**2014**,170(4): 547-553.

40. Targher G, Bertolini L, Scala L, Cigolini M, Zenari L, Falezza G and Arcaro G: Associations between serum 25-hydroxyvitamin d3 concentrations and liver histology in patients with non-alcoholic fatty liver disease. *Nutr Metab Cardiovasc Dis* .**2007**.*17(7): 517-524*.

41. Chung GE, Kim D, Kwak MS, Yang JI, Yim JY, Lim SH; Itani M: The serum vitamin d level is inversely correlated with nonalcoholic fatty liver disease. *Clin Mol Hepatol*.**2016** *22(1): 146-151*.

42. Bril F, Maximos M, Portillo-Sanchez P, Biernacki D, Lomonaco R, Subbarayan S, Correa M, Lo M, Suman A and Cusi K. Relationship of Vitamin D with Insulin Resistance and Disease Severity in Non-Alcoholic Steatohepatitis. *J Hepatol* , **2015**.*62(2): 405-411*.

43. Ilaria Barchetta, Francesco Angelico, Maria Del Ben, Marco Giorgio Baroni, Paolo Pozzilli, Sergio Morini;Maria Gisella Cavallo. Strong association between non alcoholic fatty liver disease (NAFLD) and low 25(OH) vitamin D levels in an adult population with normal serum liver enzymes. *BMC Medicine*. **2011**, *9:85*

44. Eliades M, Spyrou E, Agrawal N, et al. Meta-analysis: vitamin D and non-alcoholic fatty liver disease. *Aliment Pharmacol Ther***. 2013**;*38 (3):246–254.*

45. Liangpunsakul S and Chalasani N. Serum vitamin D concentrations and unexplained elevation in ALT among US adults. *Dig Dis Sci*. **2011**;*56 (7):2124–2129.*

46. Eliades M, Spyrou E. Vitamin D: a new player in non-alcoholic fatty liver disease? *World.J.Gastroenterol.***2015**;*21(6):1718–1727*.

47.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* .

48. Martin-Rodriguez JL, Gonzalez-Cantero J, Gonzalez- Cantero A, Arrebola JP and 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*

49. Isaken VT. Larsen MA, Goll R, Florholmen JR and Paulssen EJ. Hepatic Steatosis, Detected by Hepatorenal Index in Ultrasonography, As A Predictor of Insulin Resistance in Obese Subjects. *BMC Obes*. **2016**,*3:39.*

50. Bishnu Jwarchan, Subita Lalchan, Anil Dhakal and Ramesh R Acharya. Comparison of Liver Enzymes and Sonological Grading in Nonalcoholic Fatty Liver. *Asian J Medical Sciences*. **2020** ,*11: 2*

51. 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*.

52. Yu Z hang, He He, Yu-Ping Zeng, Li-Dan Yang, Dan Jia, Zhen-Mei An and 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.*

53. Elizabeth E Powell, Vincent Wai-Sun Wong, Mary Rinella. Non-alcoholic fatty liver disease. *Lancet* .**2021***; 397: 2212–24*

54. 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.*

55. Xuefeng Ma, Shousheng Liu, Jie Zhang , Mengzhen Dong, Yifen Wang, Mengke Wang and Yongning Xin. Proportion of NAFLD Patients with Normal ALT Value in Overall NAFLD Patients: A Systematic Review and Meta-Analysis. *BMC Gastroenterology*. 20, 10, **2020**.