CORRELATION BETWEEN LIVER FUNCTION TESTS AND FATTY LIVER DISEASE/NON-ALCOHOLIC STEATOHEPATITIS

Jibran Umar Ayub Khan[1], Humera Fayaz[1], Ayesha Qaisar[2], Said Amin[3], Azhar Zahir Shah[1]

Abstract

Objective: This study aims to determine the correlation between liver Function Tests and fatty liver disease/non-alcoholic steatohepatitis

Methodology: This cross-sectional study was performed in two major tertiary care hospitals (Maqsood Medical complex and Hayatabad Medical Complex) in Peshawar, Pakistan, from September 2021 to February 2022. to The study was approved by the institutional review board of Khyber Girls Medical College/Hayatabad Medical Complex, Peshawar, Pakistan. After informed consent, all patients aged 18 years and above were included in the study. Ultrasound was performed for each patient to determine the stage of fatty liver disease. History alcoholism was ascertained in all patients. In addition, hepatitis-B surface antigen and Anti-HCV, Anti-Hepatitis E and anti-Hepatitis A were performed in all patients.

A sample size of 69 patients is calculated with a 90% confidence level, 7.1% margin of error and by taking an expected percentage of non-alcoholic fatty liver disease in Pakistan as 15%.
Data entry and analysis were done with SPSS version 25. Study variables were age, gender, height, weight, ALT, SGOT, and bilirubin.

Results: A total of 69 patients fulfilled the inclusion criteria, with predominant female patients at 75.36%. The mean age of participants was 39.71±14.8.

The mean ALT was 77.88±70.52 in the male group and 53.40±29.12 in the female group. The alkaline phosphatase was 243.29±65.27 in the male in comparison to the female group 249.36±115.07. The ALT was positively correlated with ALP (r=0.328, p-value=0.006) and bilirubin (r=0.363, p-value=0.002). However moderate positive correlation was observed between ALP and bilirubin (r=0.549, p-value=0.000).
Conclusion: The study shows alterations in liver enzyme levels. In addition, it highlights the importance of gender, BMI, and dyslipidemia in assessing the risk of individuals with NAFLD, reaffirming the association with the disease.

Introduction

Non-alcoholic fatty liver disease (AFLD) is part of the metabolic syndrome characterized by insulin resistance (diabetes or pre-diabetes), Body mass index (BMI) in the overweight or obese region, abnormal blood lipid levels, and hypertension. (1) Hemochromatosis, viral hepatitis, alcohol misuse, alcoholic hepatitis, and metabolic abnormalities are common causes of chronic liver disease. (2) These conditions cause hepatocellular damage, which leads to liver fibrosis, cirrhosis, and hepatocellular cancer.

1. Kabir Medical College Peshawar, Pakistan
2. Khyber Medical College Peshawar, Pakistan
4. Khyber Girls Medical College Peshawar, Pakistan

Address for Correspondence:
Dr. Said Amin
Associate Professor, Khyber Girls Medical College, Peshawar, Pakistan
saidamin@live.com

Therefore, an accurate assessment of the disease severity is important for treatment planning. (3) Patients with NFLD may have right upper quadrant pain, fatigue, pruritis, and hepatomegaly, but they are often asymptomatic, and stigmata of chronic liver disease are uncommon. (4) Risk factors for NASH include age > 45 years, an aspartate transaminase (AST) level > alanine transaminase (ALT) level, insulin resistance, obesity, and portal hypertension. (5) Liver fibrosis is due to repetitive injury to the liver with subsequent wound healing. (6) Parenchymal cells renew and replace cells undergoing apoptosis or necrosis after hepatocyte damage (such as acute viral hepatitis). (7) However, the process is accompanied by an inflammatory response and a wound-healing process involving the deposition of a limited amount of extracellular matrix (ECM) in the liver parenchyma.(8) If the hepatocellular injury persists or continues, this liver regeneration process is overwhelmed and fails, and the normal liver parenchyma is substituted with an abundant ECM rich in fibrillar collagen.(9) This

finally results in cirrhosis, which has a poor prognosis and a high fatality rate. In people with chronic liver disease, the progression to end-stage liver disease varies but is often slow, taking place over 2-4 decades.(10)

The gold standard for diagnosis of NAFLD involves a thorough clinical history with pathological correlations. This is confirmed by detecting steatosis on liver biopsy and excluding all other causes, including alcohol consumption.(11) Liver biopsy is the most dependable and specific method of detecting and staging fibrosis, diagnosing the cause of fibrosis, and determining whether it has progressed to cirrhosis.(12) However, liver biopsy has many limitations, including high cost, and sampling error, as it only represents 1/50000 of the liver volume and therefore does not accurately reflect the entire liver's architecture and fibrotic changes.(13) Consequently, biopsies from different areas depict varying stages of fibrosis, and cirrhosis may be missed in up to 30% of patients, resulting in it not being an ideal prognostic indicator.(14)

Furthermore, variations of opinion among pathologists may lead to under-staging of cirrhosis, which correlates with recounts of inter and intra-observer discrepancies of up to 20% in assessing fibrotic changes. Given the prevalence of NAFLD and the invasive nature of a liver biopsy, it is not cost-effective or practical to conduct this procedure on all patients at risk of NASH (non-alcoholic steatohepatitis) or fibrosis.(15) Last but not least, a variety of hazards and issues could arise, with a 1% chance of post-biopsy severe problems like infection, bile leak, harm to nearby organs, or haemorrhage. (16) As a result of these limitations, the use of liver biopsies as a diagnostic tool has dramatically reduced and led to the development of novel alternative non-invasive imaging modalities and laboratory tests for assessing liver fibrosis in NAFLD and NASH. These methods include the AST/ALT ratio, AST platelet ratio index (APRI), Fibroscan, ultrasonography (US), and Fib-4 score, which might be capable of overcoming the limitations of liver biopsy.(17) They have been reported to be highly sensitive and specific in estimating liver fibrosis and predicting outcomes. In addition, they are liver-specific, easy to perform, reliable, inexpensive, and accurate tools for fibrosis staging and disease progression monitoring. (11) To assess the prevalence of fatty liver and detect moderate to severe heavy changes, non-invasive imaging modalities, such as the US, are preferred. It is recommended that this is the first-line imaging technique, as it is a reliable method for detecting moderate to severe steatosis in the liver. It is inexpensive, non-invasive, and readily available. (18) However, ultrasound is imperfect when staging fibrotic changes in the liver because of a lack of accuracy and reliability. Although a variety of parameters, including the size of the liver, the coarseness of the parenchyma, and the nodularity of the liver surface, have been evaluated using ultrasound scoring systems to define the stage of fibrosis, these results are technology primarily reliant on the equipment used. (14) Previously, clear correlations were not seen between the grayscale ultrasound findings and histological findings. However, recent developments in ultrasound have resulted in increased diagnostic accuracy when measuring hepatic fibrosis with the aforementioned ultrasound scoring system.

Materials And Methods:

This cross-sectional study was performed in two major tertiary care hospitals (Maqsood Medical complex and Hayatabad Medical Complex) in Peshawar, Pakistan, from September 2021 to February 2022.The study was approved by the institutional review board of Khyber Girls Medical College/Hayatabad Medical Complex, Peshawar, Pakistan. After informed consent, all patients aged 18 years and above were included in the study. Ultrasound was performed for each patient to determine the stage of fatty liver disease. History alcoholism was ascertained in all patients. In addition, hepatitis-B surface antigen and Anti-HCV, Anti-Hepatitis E and anti-Hepatitis A were performed in all patients.

A sample size of 69 patients is calculated with a 90% confidence level, 7.1% margin of error and by taking an expected percentage of non-alcoholic fatty liver disease in Pakistan as 15%.(19)

Data entry and analysis were done with SPSS version 25. Study variables were age, gender, height, weight, ALT, SGOT, and bilirubin.

Quantitative variables were presented with mean±SD and qualitative variables with frequency and percentage. A Scatter plot was made to see the linear association between lipid profile parameters. Pearson correlation was applied to see the correlation between ALT, AST and Bilirubin. A p-value $\leq$0.05 was considered statistically significant.

RESULTS

Sixty-nine patients fulfilled the inclusion criteria, with predominant female patients at 75.36%. The mean age of participants was 39.71±14.8.

The mean ALT was 77.88±70.52 in the male group and 53.40±29.12 in the female group. (Table 1) The alkaline phosphatase was 243.29±65.27 in the male compared to the female group 249.36±115.07. (Figure-1) ALT was positively correlated with ALP (r=0.328, p-value=0.006) and bilirubin (r=0.363, p-value=0.002). However moderate positive correlation was observed between ALP and bilirubin (r=0.549, p-value=0.000). The scatters plots show a linear association between ALT, ALP and bilirubin. (Figure: II-IV)

Table-1: Patients characteristics

	Male	Female	Total	p-value*
	17	**52**	**69**	
Age (Years)	41.11±17.04	39.25±13.27	39.71±14.81	
ALT	77.88±70.52	53.40±29.12	59.43±43.81	**0.181**
ALP	243.29±65.27	249.36±115.07	247.86±104.59	**0.837**
Bilirubin	1.32±1.24	0.95±0.78	1.04±0.92	**0.160**

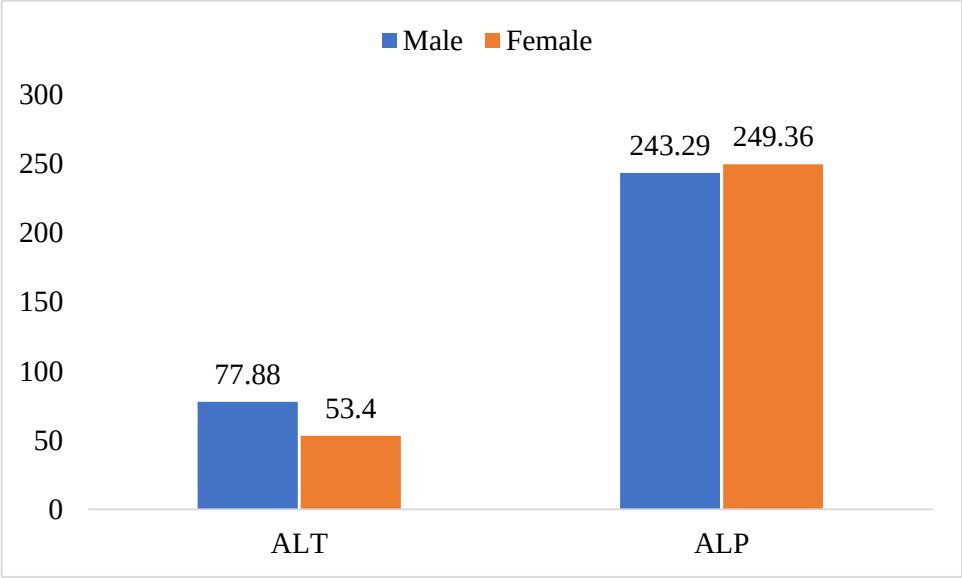

Figure-1: ALT and ALP levels among male and female patients

Table-3: Correlation between ALT, ALP and Bilirubin levels

		ALT	ALP	Bilirubin
ALT	***Pearson Correlation (r)***	1	.328**	.363**
	p-value		.006	.002
ALP	***Pearson Correlation(r)***		1	.549**
	p-value			.000
**. Correlation is significant at the 0.01 level (2-tailed).				

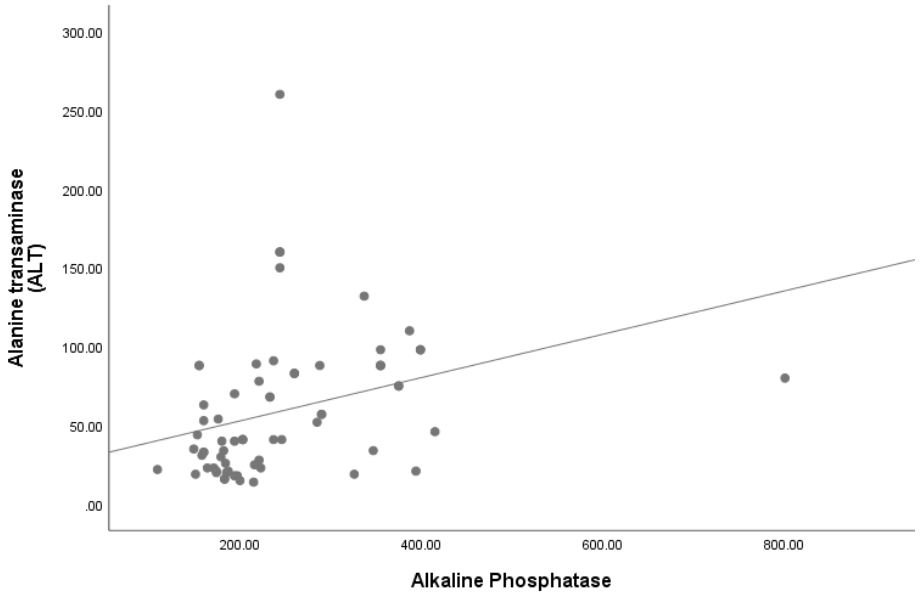

Figure-II: Scatter plot for correlation between ALT and ALP

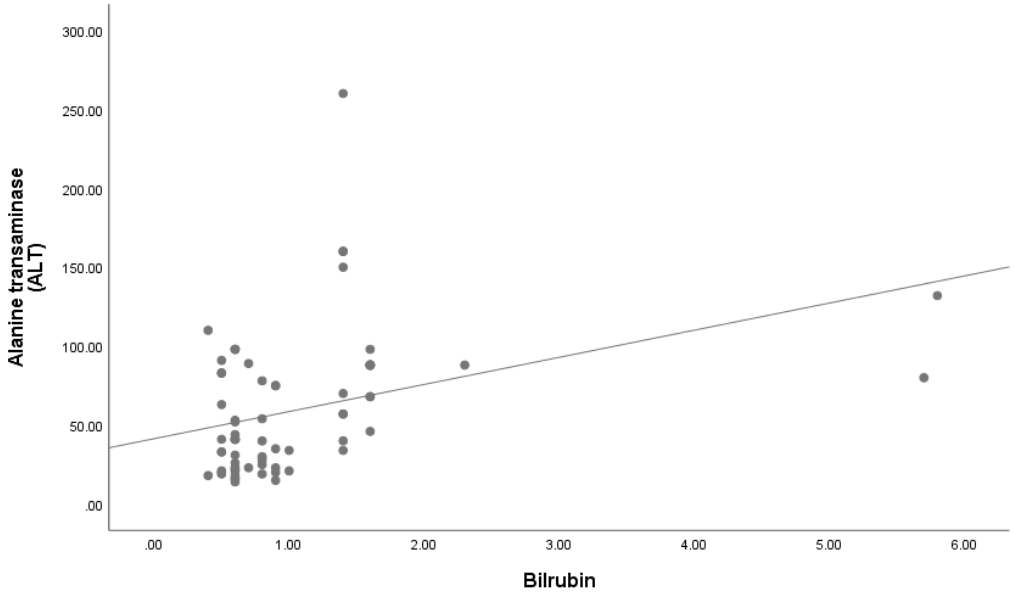

Figure-III: Scatter plot for correlation between ALT and Bilirubin

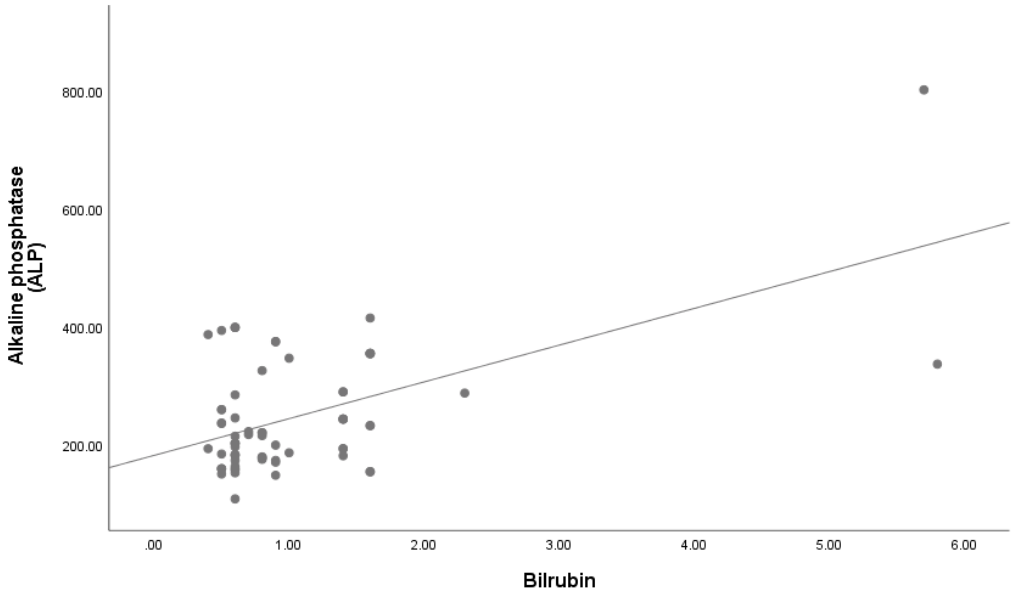

Figure-III: Scatter plot for correlation between ALP and Bilirubin

Discussions

Liver function enzyme elevation, particularly alanine aminotransferase, is often the first sign of NAFLD, an increase of one to three times its normal value being observed. (20) In this research, even though no statistical significance was found, the most frequent alteration was ALT level elevation (72.5% patients) with a higher level relative to AST level (25% patients), where similarly elevated values for both transaminases were not found. Contrary to this, Noé López-Amador Gaviria et al. found hypertransaminasemia AST levels (23.3%) more elevated in comparison with ALT (13.3%) in obese patients with NAFLD, which suggests that both enzymes are closely linked to the growing NAFLD associated obesity epidemic. (21) In addition, studies that have evaluated the aetiology of elevated levels of transaminases in the general population of the USA have proposed that elevated levels of ALT, as well as AST, are predictors of the presence of NAFLD. (22)

Another abnormality associated with the onset of NAFLD is the elevated alkaline phosphatase levels. (23) In this regard, a slight increase in levels in 45% total population studied was found. Still, it was not enough to be statistically significant when levels between the study groups were compared. Nevertheless, these findings are compatible with the results of other authors.

There is evidence that patients with hypertriglyceridemia or dyslipidemia have a 5 to 6-fold increased risk of developing NAFLD with respect to the average population. Rousch et al. agree that the foremost factor in NAFLD prevalence is dyslipidemia. (24) In this study, the most characteristic lipid alterations were the significant elevation of triglyceride levels (85%) and cholesterol (82.5%), biochemical parameters that exhibited an important difference when compared between study groups. This finding suggests that lipid regulation, synthesis, and metabolism are altered in this group of patients. This abnormality may be due to an adjustment to the rise of protein binding to the sterol regulatory element SREP- 1 c, a transcription factor of some genes involved in the de novo synthesis of fatty acids; this element inhibits the oxidation of free fatty acids and the stimulation of the fat content in the liver. Similarly, the sterol regulatory element SREBP-2 and low-density lipoprotein (LDL) receptors are regulated downwards in subjects with NAFLD, thus inhibiting cholesterol absorption and the synthesis of very high-density lipoprotein (VLDL) in hepatocytes and resulting in high triglyceride content in the liver. On the other hand, elevated TG levels can alter the lipid profile even more by reducing the cholesterol in lipoproteins of high density (HDL-C) and increasing dense LDL particles. (25) In addition, a comparison of BMI between study groups revealed a significant difference among them (Group D>C>B>A). Therefore, BMI is a factor that predisposes people to NAFLD as it is directly associated with obesity. Data from the NHANES-III study on dyslipidemia also reflect a higher prevalence as BMI increases, especially in men. (26)

In clinical practice, it has been reported that serum bilirubin level is related to various diseases. (27) In this study group, total bilirubin levels were within the normal range, without statistical significance. So far, the literature has excluded bilirubin as a guiding test for NAFLD. However, a study by Chang et al. revealed that serum conjugated bilirubin levels were significantly associated with a lower incidence of NAFLD. Nevertheless, some authors found that total bilirubin levels are negatively associated, and others observed that unconjugated bilirubin levels are rigorously related to NAFLD. (28) Therefore, it is not clear which bilirubin influences NAFLD presence. Unfortunately, in this study, both bilirubins were not measured. Consequently, more research is needed to elucidate the mechanisms underlying this association and establish bilirubin's role as a risk marker of non-alcoholic fatty liver disease.

Some limitations of our study deserve to be commented on. First, ultrasound diagnosed Non-alcoholic fatty liver disease without histological confirmation of a fatty liver. A more robust sample could allow other factors associated with NAFLD in obese adults to be identified. In addition, the study population had a moderate to severe prevalence of obesity and resistance to insulin was not determined; this has a significant influence on the physio-pathological process, where tumour necrosis factor (TNF) has been shown to increase in the serum of insulin resistant people, type 2 diabetics or android obesity carriers. (29) Finally, recent studies provide new evidence showing that exposure to tobacco smoke and indirect smoke aspiration can accelerate the development of experimental non-alcoholic fatty liver disease.(30) Therefore, future research is needed to establish the pathological condition of the liver precisely. However, our sample was representative, with evidence of similarities with results published in the international literature.

References

1. Youssef S, Nelder M, Sun G. The association of upper body obesity with insulin resistance in the Newfoundland population. Int J Environ Res Public Health. 2021;18(11).
2. Torruellas C, French SW, Medici V. Diagnosis of alcoholic liver disease. World J Gastroenterol. 2014;20(33):11684–99.
3. Nan Y, Xu X, Gao Y, Wang R, Li W, Yang M, et al. Consensus on the secondary prevention of primary liver cancer. Hepatol Int. 2021;1289–300.
4. Maurice J, Manousou P. CMJv18n3-CME-Manousou.indd. C Gastroenterol. 2018;18(3):245–50.
5. Alam S, Eslam M, SKM Hasan N, Anam K, Chowdhury MAB, Khan MAS, et al. Risk factors of non-alcoholic fatty liver disease in lean body mass population: A systematic review and meta-analysis. JGH Open. 2021;5(11):1236–49.
6. Engelmann C, Clària J, Szabo G, Bosch J, Bernardi M. Pathophysiology of decompensated cirrhosis: Portal hypertension, circulatory dysfunction, inflammation, metabolism and mitochondrial dysfunction. J Hepatol [Internet]. 2021;75:S49–66. Available from: https://doi.org/10.1016/j.jhep.2021.01.002
7. Zhou WC, Zhang QB, Qiao L. Pathogenesis of liver cirrhosis. World J Gastroenterol. 2014;20(23):7312–24.
8. Arriazu E, De Galarreta MR, Cubero FJ, Varela-Rey M, De Obanos MPP, Leung TM, et al. Extracellular matrix and liver disease. Antioxidants Redox Signal. 2014;21(7):1078–97.
9. Wu Y, Cao Y, Xu K, Zhu Y, Qiao Y, Wu Y, et al. Dynamically remodelled hepatic extracellular matrix predicts prognosis of early-stage cirrhosis. Cell Death Dis [Internet]. 2021;12(2). Available from: http://dx.doi.org/10.1038/s41419-021-03443-y
10. Tapper EB, Parikh ND. Mortality due to cirrhosis and liver cancer in the United States, 1999-2016: Observational study. BMJ. 2018;362.
11. Piazzolla VA, Mangia A. Noninvasive diagnosis of NAFLD and NASH. Cells. 2020;9(4).
12. Nalbantoglu I, Brunt EM. Role of liver biopsy in non-alcoholic fatty liver disease. World J Gastroenterol. 2014;20(27):9026–37.
13. Chowdhury AB, Mehta KJ. Liver biopsy for assessment of chronic liver diseases: a synopsis. Clin Exp Med [Internet]. 2022;(0123456789). Available from: https://doi.org/10.1007/s10238-022-00799-z
14. Sumida Y, Nakajima A, Itoh Y. Limitations of liver biopsy and non-invasive diagnostic tests for diagnosing non-alcoholic fatty liver disease/non-alcoholic steatohepatitis. World J Gastroenterol. 2014;20(2):475–85.
15. Kleiner DE, Makhlouf HR, Program D,

Investigation P, Branch R, Shams A. Histology of NAFLD and NASH in Adults and Children. 2017;20(2):293–312.
16. Thomaides-Brears HB, Alkhouri N, Allende D, Harisinghani M, Noureddin M, Reau NS, et al. Incidence of Complications from Percutaneous Biopsy in Chronic Liver Disease: A Systematic Review and Meta-Analysis. Dig Dis Sci [Internet]. 2022;67(7):3366–94. Available from: https://doi.org/10.1007/s10620-021-07089-w
17. Paul J. Recent advances in non-invasive diagnosis and medical management of non-alcoholic fatty liver disease in adult. Egypt Liver J. 2020;10(1).
18. Han A, Byra M, Heba E, Andre MP, Erdman JW, Loomba R, et al. Non-invasive diagnosis of non-alcoholic fatty liver disease and quantification of liver fat with radiofrequency ultrasound data using one-dimensional convolutional neural networks. Radiology. 2020;295(2):342–50.
19. Abbas Z, Zaheer R. Non-alcoholic fatty liver disease : A real threat in Pakistan. :2437–40.
20. Dyson JK, Anstee QM, McPherson S. Non-alcoholic fatty liver disease: a practical approach to diagnosis and staging. Frontline Gastroenterol. 2014;5(3):211–8.
21. diagnosed with non-alcoholic Veracruz : a comparative analysis. 2017;7:11–6.
22. Yadav D, Choi E, Ahn SV, Koh SB, Sung KC, Kim JY, et al. Fatty liver index as a simple predictor of incident diabetes from the KoGES-ARIRANG study. Med (United States). 2016;95(31):1–7.
23. Sheng G, Peng N, Hu C, Zhong L, Zhong M, Zou Y. The albumin-to-alkaline phosphatase ratio as an independent predictor of future non-alcoholic fatty liver disease in a 5-year longitudinal cohort study of a non-obese Chinese population. Lipids Health Dis. 2021;20(1):1–10.
24. Malaguarnera M, Vacante M, Russo C, Gargante MP, Giordano M, Bertino G, et al. Rosuvastatin reduces non-alcoholic fatty liver disease in patients with chronic hepatitis C treated with α-interferon and ribavirin. Hepat Mon. 2011;11(2):92–8.
25. Osborne TF. Sterol regulatory element-binding proteins (SREBPS): Key regulators of nutritional homeostasis and insulin action. J Biol Chem [Internet]. 2000;275(42):32379–82. Available from: http://dx.doi.org/10.1074/jbc.R000017200
26. Hudson SE, Feigenbaum MS, Patil N, Ding E, Ewing A, Trilk JL. Screening and socioeconomic associations of dyslipidemia in young adults. BMC Public Health. 2020;20(1):1–9.
27. Tian J, Zhong R, Liu C, Tang Y, Gong J, Chang J, et al. Association between bilirubin and risk of Non-Alcoholic Fatty Liver Disease based on a prospective cohort study. Sci Rep [Internet]. 2016;6(April):1–9. Available from: http://dx.doi.org/10.1038/srep31006
28. Chang Y, Ryu S, Zhang Y, Son HJ, Kim JY, Cho J, et al. A cohort study of serum bilirubin levels and incident non-alcoholic fatty liver disease in middle aged Korean workers. PLoS One. 2012;7(5):1–7.
29. Swaroop JJ, Rajarajeswari D, Naidu JN. Association of TNF-α with insulin resistance in type 2 diabetes mellitus. Indian J Med Res. 2012;135(1):127–30.
30. Akhavan Rezayat A, Dadgar Moghadam M, Ghasemi Nour M, Shirazinia M, Ghodsi H, Rouhbakhsh Zahmatkesh MR, et al. Association between smoking and non-alcoholic fatty liver disease: A systematic review and meta-analysis. SAGE Open Med. 2018;6:205031211774522.