JMMDC JOURNAL OF MULTAN MEDICAL AND DENTAL COLLEGE

ORIGINAL ARTICLE

CLINICAL IMPORTANCE OF ELEVATED LEVELS OF ALPHA-FETOPROTEIN IN EARLY DIAGNOSIS OF NON-ALCOHOLIC FATTY LIVER DISEASE IN NATIVE POPULATION OF SOUTHERN PUNJAB, PAKISTAN

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Abstract

Objective: To evaluate the relation between elevated levels of AFP (alpha-fetoprotein) and NAFLD (Non-Alcoholic Fatty Liver Disease) in the native population of Southern Punjab, Pakistan.

Place of Study: Ibn-e-Siena Hospital & research institute, Multan

Study Design: Descriptive Cross-Sectional Study

Duration of Study: December 2021 to July 2022

Methodology: 800 patient's samples were included in this study. All subjects showed elevated serum Alpha-fetoprotein (AFP) levels. To measure AFP sandwich immune-detection FIA (florescence immunoassay) technique was used. The tests were performed on ichroma for the quantitative analysis. Value above the cut off value was *10.9 ng/mL* further investigated for the clinical history, full clinical record of anthro-pometric and biochemical tests.

Results: Fatty Liver Disease (FLD) was diagnosed in 19.5% subjects. Most of the cases were of Non-Alcoholic Fatty Liver Disease (NAFLD) with 98.71%. More males were affected with NAFLD with 11.5%. NAFLD was more observed in >50years aged patients with 12.62% than patients <50years with 6.625%. There was a non-significant correlation between residence type of the patient with the disease incidence. There was a significant relationship between NAFLD and Blood Pressure, Fasting Blood Sugar, Triglycerides, Total Cholesterol, HBA1C and High-Density Lipids (HDL) with p value <0.01.

Conclusion: It is concluded that elevated levels of alpha-fetoproteins are clinically important and can be useful tool for the early diagnosis of fatty liver disease especially NAFLD.

Keywords: Alpha-fetoprotein, Fatty Liver Disease, Liver Cirrhosis, and Hepatocellular Carcinoma)

INTRODUCTION

FLD is commonly referred as accumulation/deposition of lipid fats inside the liver-parenchyma. There are two possible pathways for FLD Induction; Alcoholic pathway and metabolic Fatty-liver Disease/Non-Alcoholic Pathway. Metabolic Fatty-liver Disease/Non-Alcoholic type of disease is also more associated with MS (Metabolic-Syndrome) and Obesity (1). Moreover, ALD (Alcoholic-liver Disease) is closely associated with the alcohol utilization. ALD is also responsible for higher tendency of LC (Liver Cirrhosis) (2). In United States more than 30% adult population is diagnosed with Non-Alcoholic Fatty Liver disease (NAFLD); parallel with high prevalence of Obesity (3). Even 30% population is affected with Non-Alcoholic Fatty Liver disease (NAFLD)/Metabolic Fatty Liver Disease (MFLD) in China (4). Fatty Liver Disease (FLD) is a matter of great public concern worldwide and the prevalence is increasing significantly each year (5). Fatty Liver Disease (FLD) shows a wide variety of clinical and histological spectrum of disease. Steatosis, Steao-hepatitis, Fibrosis and LC (Liver Cirrhosis) showed extensively different spectrum of the disease. Steatosis is laterally diagnosed with benign form of tumors, while Steato-hepatitis leads to LC (Liver Cirrhosis) in around 20% patients (6). Fatty Liver Disease Especially Non-alcoholic /metabolic FLD leads to HCC (Hepato-cellular Carcinoma) in most of malignant cases. Liver-carcinoma is end-phase complication of Non-alcoholic /metabolic FLD. Other complications of Non-alcoholic /metabolic FLD includes Chronic-Liver Fibrosis (CLF). Sufficient documented evidences indicated Hepato-carcinogenesis could be correlated with initial stages of Non-alcoholic /metabolic FLD (7, 8). NASH (Non- alcoholic steato-hepatitis) investigated and documented by researchers as second-

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Received for publication: May 26, 2022

Revised : August 02, 2022

Accepted for Publication: September 23, 2022

DOI : https://doi.org/10.58380/jmmdc.v1i2.81

leading etiological agent of Hepato-cellular Carcinoma (9). There is prospective correlation between *a*-fetoprotein (AFP) and Fatty liver Disease (FLD); documented evidence in recent research findings. (10). There is a significant positive correlation between high levels of *a*-fetoprotein and disease-grade of hepatic-steatosis (11). While few studies did not observe any positive correlation between high levels o f *a*-fetoprotein and histo-pathological findings of patients with Non-alcoholic Fatty Liver Disease (12). Factors correlated with Fatty Liver Disease were significantly investigated in recent past.

Although role of significantly increased AFP levels cannot serve as independent factor for the diagnosis of Fatty Liver Disease (13). In Pakistani population drinking habits were less documented reason of Fatty Liver Disease in our studies. Therefore, these inconsistent observations enforced us to evaluate the relationship between elevated levels of *a*-fetoprotein and Non-Alcoholic Fatty liver Disease and its associated risk factors. These inconsistent observations might arise because of difference in sample size, population and diagnostic techniques to diagnose the disease. In current cross sectional non-probable study, we select native population visiting Health care Hospital to investigate the positive correlation of *a*-fetoprotein and FLD in Pakistani population.

Methodology:

Eight hundred patients were included in this study brought to the Pathology Laboratory Ibne-e-Seena Hospital Multan from December 2021 to July 2022. All 800 samples showed mildly elevated serum Alpha-fetoprotein (AFP) levels. To measure alpha-fetoprotein a sandwich immune-detection FIA (florescence immunoassay) technique was used. The test was performed on ichroma for the quantitative measurement of alpha-fetoprotein in human serum/plasma. The cut off value of AFP (*a*-fetoprotein) was ≤ 10.9 ng/dl Samples more than cut off value were further investigated for the clinical history, full clinical record of anthro-pometric and biochemical tests. BP (Blood pressure) was recorded. Serum biochemical FBS, Cholesterol, Triglycerides, High Density Lipids, and Liver function tests were measured by Selectra Promax 2000 auto-analyzer.All other supportive diagnostic records of hepatic ultrasound reports, CT scans were also analyzed for ultimate diagnosis. A designed questionnaire related to demographic and personal patient information was presented to all patients included in this study. Purpose of this information was to collect information related to personal, demographic and associated risk factors evaluation.

Obtained data was tabulated in MS Excel spread sheet and further analyzed by SPSS STATA 12. Bivariate-analysis was applied to evaluate relationship between NAFLD (Non-Alcoholic Fatty Liver Disease) and risk factors. 95% CI (Confidence Intervals) and p-value <0.05 were calculated to access the significance of the parameters.

Results:

In current study, we evaluate association between alpha-fetoprotein (AFP) and Non-Alcoholic Fatty Liver Disease (NAFLD). 800 samples were included in this study with more than cut off value ≤ 10.9 ng/dl. Fatty Liver Disease was diagnosed in 19.5% (156/800) subjects. Most of the cases were of Non-Alcoholic Fatty Liver Disease (NAFLD) with 98.71% (154/156). In Table 1 Clinical parameters were correlated between with Fatty Liver Disease (FLD) and without fatty Liver Disease (FLD). More males were affected with Non-Alcoholic Fatty Liver Disease (NAFLD) with 11.50% as compare to female with 7.75% but the p value is >0.05 so there is not significant correlation between NAFLD and gender. Age was a significant parameter-affecting course of disease. Patients >50years were more affected with 12.62% as compare to the patients <50years with 6.625%. p-value is 0.03 which indicated there is positive significant correlation between age and NAFLD. According to our findings Non-Alcoholic Fatty Liver Disease is the major reason of Fatty Liver Disease with 98.71% and p value was 0.000. Patients living in urban areas were more affected with NAFLD with 10.75% but there is non-significance association between NAFLD and locality of the patients with p value 0.716. Clinical parameters were correlated with and without Fatty Liver Disease (FLD) in Table 2. Significant positive correlation was observed between Non-Alcoholic Fatty Liver Disease (NAFLD) with Blood Pressure (BP), Fasting Blood Sugar (FBS), Triglycerides (TG), HBA1C Total Cholesterol (CH) and High-Density Lipids (HDL) because p-value was <0.005 as shown in in Table 2.

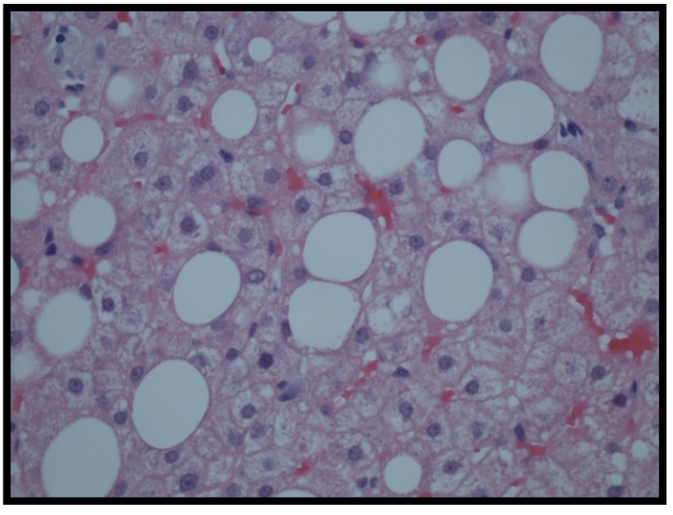

Figure 1: Shows microscopic appearance of pericentral fatty change in Hepatocytes at (400X)

Table 1: Prevalence of Non-Alcoholic Fatty Liver Disease (NAFLD) among the patients, according to demographic characteristics

Demographic characters		n=800	Positive NAFLD %	P-value	Odds ration	95% confidence interval
Male		410	92(11.50%)	0.085	1.38	(0.981-2.589)
Female		390	62(7.75%)	0.085		
Age (n=800)	<50 years	253	53(6.625%)	0.031	1.14	(0.557-2.563)
	>50 years	547	101(12.62%)			
Drinking Alcohol	Yes	15	2(0.25%)	0.000	6.407	(4.029-10.841)
	No	785	154(19.25%)			

Table 2: Biochemical analysis among the patients with elevated alpha-fetoprotein with or without Non-Alcoholic Fatty Liver Disease (NAFLD)

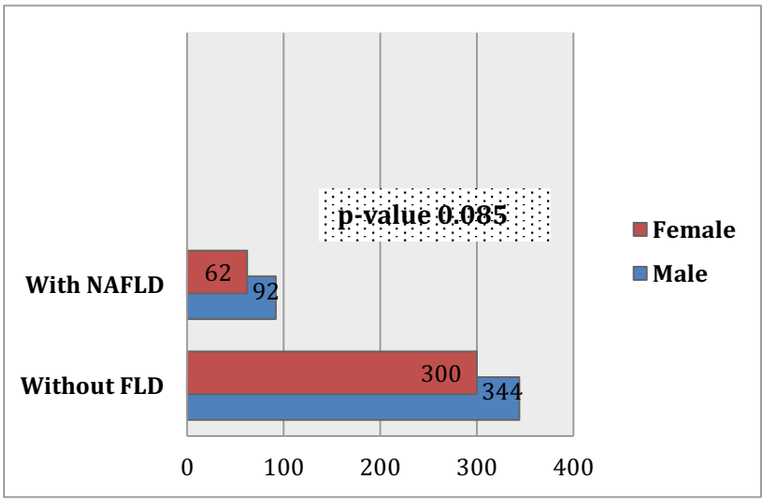

Figure 2: Prevalence of Non-Alcoholic Fatty Liver Disease (NAFLD) in male and female patients

It can be visualized from the figure 2, that males are more affected by NAFLD but this was a non-significant association between the gender because p value was 0.085.

Serial No	Demographic/Clinical Parameters		Normal value	Without Non-Alcoholic Fatty Liver Disease (NAFLD) (n=644) Mean	With Non-Alcoholic Fatty Liver Disease (NAFLD) (n=156) Mean	P-value
1	Alpha-fetoprotein (AFP) ng/dl		≤ 10.9 ng/dl	11.5±6.1	14.9±9.8	<0.003
2	TG (Triglycerides) mg/dl		≤ 150 mg/dl	145±59	209±131	<0.001
3	HDL (High Density Lipid) mg/dl		≥ 35 mg/dl	37±7	31±5	<0.001
4	Total CH (Cholesterol) mg/dl		≤ 200 mg/dl	165±76	198±96	<0.001
5	FBS (Fasting Blood Sugar) mg/dl		Up to 100	98.1±12	115.9±19.5	<0.001
6	HBA1C		≤ 5.7 %	5.6±1.9	7.2±4.1	<0.001
7	BP (Blood Pressure) mmHg	Systolic	<120mmHg	116±6.8	135.1±5.6	<0.001
		Diastolic	<80mmHg	75±7.1	83.1±6.9	<0.001
8	Bilirubin (Total) /dL		0.1-1.1mg/dl	1.2±1.1	1.5±1.9	0.0632
9	Bilirubin (Direct) mg/dL		0.1-0.9mg/dl	1.0±0.6	1.2±1.0	0.0617
10	ALT (Alanine Aminotransferase) U/L		10-40U/L	35±35	39±41	0.0592
11	AST (Aspartate aminotransferase) U/L		10-35U/L	28±23	42±39	0.610
12	GGT (Gama-glutamyltransferase) U/L		10-35U/L	25±28	39 ± 27	0.597
13	Alkaline Phospatase U/L		Adult<258, Child<727	179±86	241±91	0.615

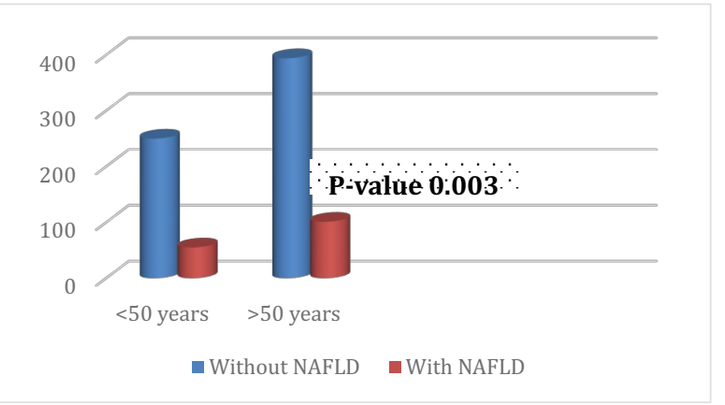

Figure 3: Prevalence of Non-Alcoholic Fatty Liver Disease (NAFLD) in different age groups

Patients >50years were more affected with 12.62% as compare to the patients <50years with 6.625%. there was a significant relation between prognosis of NAFLD and age of the patient as the p-value was 0.003.

DISCUSSION

In current study, we evaluate association between alpha-fetoprotein (AFP) and Non-Alcoholic Fatty Liver Disease (NAFLD). 19.5% patients were found with Fatty Liver Disease (FLD). Most of the cases were of Non-Alcoholic Fatty Liver Disease (NAFLD) with 98.71%. Significant positive correlation was observed between elevated levels of serum alpha-fetoprotein (AFP) with fasting blood sugar (FBS), Triglycerides (TG), HBA1C and High-Density Lipids (HDL) with p value < 0.001. Alpha-fetoprotein (AFP) was not the independent co-factor for the diagnosis of Fatty Liver Disease but cannot be ignored as important factor for ultimate diagnosis. *Xu P et al; (2014)* gave similar findings, they highlighted the significant association of high levels of alpha-fetoprotein (AFP) with Fatty Liver Disease. Prevalence of Fatty Liver Disease was 26.5% among patients had high levels of alpha-fetoprotein in Chinese population. They suggested considering the importance of high levels of serum alpha-fetoprotein levels for the ultimate diagnosis of Fatty Liver Disease as an important co-factor; but not an independent factor (13). Hanif H *et al; (2022)* highlighted the association of high levels of alpha-fetoprotein (AFP) and Fatty Liver Disease. They found higher levels of alpha-fetoproteins within the patients of Non-Alcoholic Fatty Liver Disease, while there was slight or no change observed in the concentration of alpha-fetoproteins without Fatty Liver Disease. Levels of alpha-fetoproteins were raised as grade of LS (Liver-Steatosis) disease increases. Non-Alcoholic Fatty Liver Disease should be considered as important diagnostic factor as raised alpha-fetoproteins level found; as correlated with the findings of our studies (10). Jang S *et al; (2022)* collected samples from both with Non-Alcoholic Fatty Liver Disease patients and without disease and compared the levels of alpha-fetoproteins. They did not find any marked significant correlation between elevated serum alpha-fetoproteins levels and Non-Alcoholic FLD (11). Chen Y *et al; (2016)* presented almost similar observations. They also did not observe any notable rise in the concentration of serum AFP in the patients of Non-Alcoholic FLD (12). This contradictory observation in studies might appeared because of variances in diagnostic techniques, region, geographic differences, different population sets, sample size and drinking habits. It is therefore interesting to understand why levels of alpha-fetoproteins raised in patients with Fatty Liver Disease. Hepatocytes-necrosis and regenerative changes in hepatocytes could be responsible for higher levels of alpha-fetoproteins (14). During Hepatic cells proliferation regenerative changes observed. In mature hepatic cells increased levels of alpha-fetoproteins were evaluated because of cellular differentiation (15). Increased levels of alpha-fetoproteins (AFP) could be the outcome of altered expression of hepatocellular self-interaction and alteration in normal cellular arrangement (16). These all are hypothesis and the actual phenomenon is still uncertain. One unfortunate reality is the prognosis of Hepato-cellular Carcinoma among the patients of Fatty Liver Disease. Levels of alpha-fetoproteins elevate in the patients of Fatty Liver Disease according to many investigations. Somehow the increased levels are still not proved as independent factor but still monitoring of the serum AFP levels can screen FLD patients to avoid later complications related with the disease. Regular monitoring of levels of AFPs in patients with Fatty Liver Disease is clinically significant to reduce the risk factor of Hepato-cellular Carcinoma and Liver Cirrhosis. A Clinical study reported alpha-fetoproteins unite with PT-induced (Prothrombin) complex without vitamin-K/Vitamin-K antagonist-II (PIVKA-II), might be significantly esteemed for prognosis of Hepato-Cellular Carcinoma in the patients of Fatty Liver Disease (17). Liver Biopsies are gold-standard method to diagnose HS (Hepatic-Steatosis) because its widely acceptable, highly sensitive and specific diagnostic technique (18). (Ultrasonography technique mostly used for the diagnosis of Fatty Liver Disease but it cannot detect mild forms of HS (Hepatic Steatosis); neither it is sensitive diagnostic method for the detection of SH (Steato-Hepatitis) nor Fibrosis. Therefore, alpha-fetoproteins level monitoring can be a crucial factor for the early diagnosis of Fatty Liver Disease as we analyzed in this study (19). Alcoholic or Non-Alcoholic pathways could instigate fatty Liver Disease. (20, 21) In this study we gathered the information related to alcohol consumption and a few patients were found with the history of alcohol consumption. The major reason of Fatty Liver Disease was Non-Alcoholic Fatty Liver Disease in our regions of Pakistan.

CONCLUSION

Our findings recommend that elevated levels of alpha-fetoproteins can be clinically important and helpful for the early diagnosis of fatty liver disease especially NAFLD (non-alcoholic fatty liver disease).

Conflict of Interest: None

Patient Consent: Inform Consent were taken

Ethical Approval: Ethical approval was taken from the IRB MMDC on Nov, 22, 2021 vide Letter No. **C-37-928**

Author's Contribution:

AN, AS & NF: Study design, data collection and analysis

IA, ASG & SJ: Manuscript preparation, drafting and revising

AN, IA & ASG: Review and final approval of manuscript

All the authors have approved the final version of the manuscript to be published

How to cite this article: Naz A, Samad A, Alam MI, Ghauri AS, Fayyaz N, Jabeen S. Clinical importance of elevated levels of alpha-fetoprotein in early Diagnosis of Non-Alcoholic Fatty Liver Disease in Native Population of Southern Punjab, Pakistan. J Multan Med & Den Coll 2022: *1* (2): Page no. 56-62 DOI: https://doi.org/10.58380/jmmdc.v1i2.81

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