TNF- α is An Inflammatory Marker of Cardiovascular Risks in Non-Alcoholic Fatty Liver Disease

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ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is a common clinical condition with histological features resembling those of alcohol induced liver injury but occurs in patients who do not abuse alcohol. It is often associated with features of obesity, hypertension, dyslipidemia and type2 diabetes mellitus. NAFLD plays a central role in the pathway connecting the metabolic syndrome and cardiovascular disease.Overall, the current body of evidence strongly suggests that NAFLD is likely to be associated with increased cardiovascular disease risk, and raises the possibility that NAFLD may be not only a marker but also an early mediator of atherosclerosis.

Aims of the present study: Our study was an attempt to analyze the association between non-alcoholic fatty liver disease, and various cardiovascular risk factors & plasma biomarkers of inflammation like TNF-α in non-alcoholic subjects.

Methods: In this prospective, open label, observational study, 39 cases of non-alcoholic fatty liver diseasewere evaluated for inflammatory markers and cardiovascular risk factors. The data was analysed and the results were given in percentage in accordance with previous related studies.

Results: In patients of non-alcoholic fatty liver disease serum levels of inflammatory marker TNF-α are significantly elevated, supporting the view that inflammation plays an important role in the pathogenesis of NAFLD.

Conclusion: The study concludes that there is significant association of inflammatory markers and cardiovascular risk factors in patients of non-alcoholic fatty liver disease.

Key Words: Non-alcoholic fatty liver disease (NAFLD), Tumour necrosis factor-alpha (TNF-α).

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a common clinical condition with histological features which resemble those of alcohol induced liver injury, but it occurs in patients who do not abuse alcohol. NAFLD encompasses a histological spectrum which ranges from simple steatosis to steatohepatitis, advanced fibrosis and cirrhosis. The problem of this disease is not confined to its potential to cause serious liver related morbidity and mortality, but it is often associated with the features of obesity, hypertension, dyslipidaemia and type 2 diabetes mellitus. NAFLD plays a central role in the pathway which connects the metabolic syndrome and the cardiovascular disease. The biological mechanism by which NAFLD promotes atherosclerosis is not known. The possible mechanistic pathways include increased oxidative stress, subclinical inflammation, an adipocytokine profile, endothelial dysfunction and lipid abnormalities.

Recent evidence suggests that the severity of the liver histology in the NAFLD patients is closely associated with the markers of early atherosclerosis, such as a greater carotid artery wall thickness and a lower, endothelial, flow-mediated vasodilatation, which is independent of the classical risk factors and the components of the metabolic syndrome. Overall, the current body of evidence strongly suggests that NAFLD is likely to be associated with an increased cardiovascular disease risk and that it raises the possibility that NAFLD may not only be a marker but also an early mediator of atherosclerosis[1]. Patients with NAFLD appear to have an increased risk of death as compared to the general population, and it has been reported that the overall mortality among these patients was increased as compared to the general population; this was most commonly due to the cardiovascular disease and malignancy[2]. Abdominal obesity, type 2 diabetes, insulin resistance, hypertension and dyslipidaemia – the typical components of the metabolic syndrome are the co-existing pathological conditions which are frequently associated with NAFLD and their co-existence within the same individual increases the likelihood of having more advanced forms of NAFLD [3-4]. Truncal obesity is an important risk factor for NAFLD [5]. Hye Soon Parka et al. (2005) [6] conducted a study on NAFLD and showed that the CRP, TNF-α and IL-6 concentrations were higher in the obese than in the non-obese individuals. Adipose tissue is an important source of cytokines, and adiposity contributes to the proinflammatory milieu [7].

Tumour necrosis factor-α (TNF-α) has been considered to be a key player in the progression from simple fatty liver to non-alcohalic steato hepatitis (NASH). TNF-α is produced by macrophages in the adipose tissue and it is increased in obesity. Free fatty acids can induce the expression of TNF-α in hepatocytes through the activation of NF-κB, thereby linking the increased influx of free fatty acids which are seen in hepatic steatosis, to the progression of inflammation. In adipocytes, TNF-α down regulates the adiponectin production [8].

Our study was designed to check the association between non-alcoholic fatty liver disease, various cardiovascular risk factors and the plasma biomarkers of inflammation like TNF-α in non-alcoholic subjects (those who had less than 20 gms of alcohol per day).

MATERIALS AND METHODS

STUDY POPULATION

The present study was undertaken in patients who had non-alcoholic fatty liver disease which was diagnosed by ultrasound,

who attended the Medicine OPD and were admitted in Medicine Indoors, at Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh. Informed consent was obtained from all the patients at recruitment and the study was approved by the Institutional Ethics Committee.

AIMS AND OBJECTIVES:

The present study was conducted to evaluate the cardiovascular profile and the hepatic inflammatory markers like TNF-α in patients who had NAFLD and to establish the correlation, if any, between the cardiovascular risk factors and the inflammatory markers in patients with NAFLD.

INCLUSION CRITERIA:

The patients who were included in this study were those who had NAFLD, which was diagnosed by ultrasound on the basis of following criteria: Echo contrast, liver brightness, deep attenuation and vascular blurring.

EXCLUSION CRITERIA:

Patients with alcoholic hepatitis, acute or chronic viral hepatitis, cirrhotic portal hypertension, acute infection (sepsis), decompensated cardiac disease and severe renal dysfunction were excluded from the study.

STUDY DESIGN:

The sample size was 39 cases and it was a prospective, non randomised and observational study.

STATISTICAL ANALYSIS:

All the statistical data were analyzed by using the SPSS Statistical Package for Windows (Chicago Inc.) software, version 15.0. The continuous variables were expressed as mean ± standard deviation (Gaussian distribution) and the range and qualitative data was expressed as percentages. Depending on the normality distribution, the unpaired t test for independent samples was used for comparing the continuous variables between the two groups. The linear relationship between the variables was analyzed by using the Pearson's correlation coefficient and the significance of 'r' was tested. All the p values were two tailed and the values of p which were <0.05 were considered to be statistically significant. All the confidence intervals were calculated at a 95% level.

RESULTS

Age and Sex distribution:

The study group comprised of 39 patients with non-alcoholic fatty liver disease, out of which 25 were males and 14 were females. The ages of the patients in this study group ranged from 31 to 70 years (the mean age was 48.5±9.5 years). Among the males, the ages of patients ranged from 31 to 70 years (the mean age 48.1±9.5years), while among the females, the ages ranged from 31 to 60 years (the mean age 49.1±11.0 years).

TNF-α Levels:

TNF-α estimation was done by an antigen capture ELISA (Enzyme Linked Immunosorbent Assay) for its quantitative measurement in the serum.

As is evident in [Table/Fig 1], the levels of the inflammatory marker, TNF-α in patients with non alcoholic fatty liver disease were studied and any correlation with the cardiovascular risk factors was evaluated. The mean value of TNF-α in the study group was found to be 68.9±32.9 pg/ml [Table/Fig 1].

Sex distribution: The mean value of TNF-α was 65.8±35.8 pg/ml in the female group and it was 70.7±31.8 pg/ml in the male group. No significant difference was found between the two groups.

Smoking: There were 22(56.4%) smokers and all of them were males. The mean value of TNF-α in smokers was 78.9±31.8 pg/ml and it was 70.7±31.8 pg/ml in the non-smokers. The TNF-α levels were found to be significantly higher in the smokers, with the p-value being less than 0.05.

Hypertension: The study comprised 23 hypertensive patients. The mean value of TNF-α in the hypertensive patients was 84.1±17.8 pg/ml and it was 55.9±38.0 pg/ml in the normotensive group. In our study, the TNF-α levels were found to be significantly higher in the hypertensive group, as compared to those in normotensive group, with the p-value being less than 0.05. Thus, hypertension was found to significantly affect the levels of TNF-α.

Diabetes Mellitus: There were 27 (69.2%) diabetic patients, with a sex distribution of 17 (62.9%) males and 10 (37.1%) females. The mean value of TNF-α in the patients with diabetes mellitus was 87.6±7.4 pg/ml and it was 26.6±34.3pg/ml in the non-diabetic group. Diabetes mellitus was found to significantly affect the levels of TNF-α, with the p-value being less than 0.01.

Obesity: There were 32 (82.1%) obese patients, with a sex distribution of being 20 (62.5%) males and 12 (37.5%) females. The mean value of TNF-α in the non- obese patients was 77.5±25.9 pg/ml and it was 29.6±34.3 pg/ml in the non-obese patients. Obesity was found to significantly affect the levels of TNF-α, with the p-value being less than 0.01.

Dyslipidemia: The study comprised of 36 (92.3%) patients with dyslipidaemia, with a sex distribution being of 23 (63.9%) males and 13 (34.8%) females. The mean value of TNF-α in the patients with dyslipidaemia was 73.9±29.0pg/ml and it was 8.9±0.7 pg/ml in patients without dyslipidaemia. Dyslipidaemia was found to significantly affect the levels of TNF-α, with the p-value being less than 0.01.

CARDIOVASCULAR RISK FACTOR		TNF-α Mean value(pg/ml)	t-value	p-value
SEX	F	65.8±35.8	0.44	0.663
	M	70.7±31.8		
SMOKING	Present	78.9±31.8	2.14	0.04
	Absent	70.7±31.8		
HYPERTENSION	Present	84.1±17.8	3.6	0.002
	Absent	55.9±38.0		
DIABETES MELLITUS	Present	87.6±7.4	7.2	<0.01
	Absent	26.6±34.3		
OBESITY (BMI>25Kg/m2)	Present	77.5±25.9	4.18	<0.01
	Absent	29.6±34.3		
DYSLIPIDEMIA	Present	73.9±29.0	3.8	<0.01
	Absent	8.9±0.7		

[Table/Fig 1]: Mean TNF-α value and its association with cardiovascular risk factors

Inflammatory markers in patients of metabolic syndrome and NAFLD:

In the present study, 24 patients were found to have metabolic syndrome according to the International Diabetes Foundation (IDF) criteria, out of which 8 (33.3%) were females and 16(66.7%) were males. As is evident in [Tables/Fig 2 and 3], the mean values of TNF-α in patients with metabolic syndrome were 86.4±7.2 pg/ml and 88.3±7.7 pg/ml, ie. in the females and males respectively. Thus, the TNF-α levels were found to be significantly higher in patients with metabolic syndrome (both in males and females) as compared to those in patients without metabolic syndrome.

Pearson's Correlation of TNF-α and C-Reactive Protein with metabolic syndrome :

By using Pearson's correlation, a significant correlation of TNF-α was found with metabolic syndrome (r=0.7), as compared to those without metabolic syndrome [Tables/Fig-4].

	METABOLIC SYNDROME	MEAN±S.D	t-value	P-value
TNF-α (pg/ml)	ABSENT N=6	38.3±40.9	-5.2	<0.01
	Present N=8	86.4±7.2		

[Table/Fig 2]: Association of TNF-α with metabolic syndrome in females using t-test

	METABOLIC SYNDROME	MEAN±S.D	t-value	P-value
TNF-α (pg/ml)	ABSENT	39.4±34.7	-4.2	<0.01
	Present	88.3±7.7		

[Table/Fig 3]: Association of TNF-α with metabolic syndrome in males using t-test analysis

		TNF-α (pg/ml)
Metabolic syndrome	Pearson Correlation	0.7
	p-value	<0.01
	N	39

[Table/Fig 4]: Correlations between metabolic syndrome and inflammatory markerTNF-α in patients of NAFLD

DISCUSSION

NAFLD is a very common disease and it occurs in persons of all ages and ethnic groups [9]. Although NAFLD was originally described to be more prevalent in women, no significant difference was found in the prevalence of NAFLD among men and women in the Indian population [10]. The available reports confirm that non-alcoholic steato hepatitis (NASH) is prevalent in the Indian population. Moreover, a recent, clinic-based study suggests the differences in the clinico-pathological profile of Indian patients with NAFLD [11]. In India, most of the studies on the prevalence of NAFLD have been performed in highly selected clinic populations [12-13] and the generalizability of the results to the community remains uncertain. India has the largest number of people with diabetes in the world. Moreover, Asian Indians are more prone to insulin resistance and have increased waist circumference and body fat : the Asian Indian phenotype [14].

In a majority of the cases, NAFLD has been found to be a benign condition with respect to the local liver damage; it may rarely progress to non-alcoholic steato hepatitis (NASH), cirrhosis, and hepatocellular carcinoma [15-16]. However, previous research has unravelled a number of systemic effects of NAFLD. Thus, NAFLD is associated with several atherosclerotic risk factors such as hypertension, diabetes and hypertriglyceridaemia [17].

As has been shown in a number of previous studies, Tumour Necrosis Factor-α (TNF-α) is considered to be a key player in the progression from simple fatty liver to NASH [18]. Our study also showed that the serum levels of inflammatory markers like TNF-α were significantly elevated in patients with non-alcoholic fatty liver disease.

CONCLUSION

This study concluded that the serum levels of inflammatory markers like TNF-α were significantly elevated in patients with non-alcoholic fatty liver disease, thus supporting the view that inflammation plays an important role in the pathogenesis of NAFLD. Also, there was a significant association of the TNF-α levels with various grades of fatty liver, thus suggesting an inflammatory cytokine cascade in NAFLD.

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