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C-reactive protein as a marker for insulin resistance in non-alcoholic fatty liver disease in non-obese South Indian population

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Abstract: Objective: *Non – alcoholic fatty liver disease (NAFLD) is a sonological entity without alteration of liver enzymes. The insulin resistance is measured from homeostasis model assessment (HOMA) as (HOMA-IR), calculated from estimations of fasting glucose(mg/dl) x fasting insulin (U/l) /405.The purpose of this case – control study was to corelate C-reactive protein to non-alcoholic fatty liver disease with and without insulin resistance.*

Method*: This study was done at the Department of Medicine,DR. PSIMS & RF Chinaoutpally, A.P. India between Jan 2012 to Dec 2012. 200 patients (100 cases, 100 controls) above 18 years of age were included in this study.*

Results*: The mean fasting insulin levels in cases were 14.71050±8.70280 statistically significant than controls 5.27556±3.65791 (p: 0.05). The mean HOMA IR levels in cases were 3.18 ± 0.24statistically significant than controls 1.09 ± 0.07 (p: < 0.05). The mean CRP levels in cases were 3.06 ± 1.61statistically significant than controls 1.03 ± 0.86 (p: 0.05).*

Conclusions: *Patients with Non – alcoholic fatty liver disease with high fasting insulin levels and high insulin resistance have higher serum C-reactive protein levels than controls.*

Key words*: NAFLD, insulin resistance, C-reactive protein,*

I. Introduction:

Non – alcoholic fatty liver disease (NAFLD), in which imageological changes of hepatic parenchyma without changes in biochemical parametres of liver function. Non – alcoholic fatty liver disease (NAFLD) is a rapidly growing health problem in India along with other metabolic disorders like diabetes, hypertension, dyslipidemia and obesity[1]. The relation of non – *alcoholic fatty liver disease* with the features of the metabolic syndrome has raised an interest in its role in the development and progression of coronary artery disease (CAD) and chronic kidney disease (CKD). The NAFLD and metabolic syndrome are commonly associated and presence of the metabolic syndrome frequently suspects development of NAFLD[3]. The carbohydrate and fatty metabolisms are mediated through insulin levels in the liver parenchyma, in the presence of NAFLD, the glucose disposal and glucose production mechanisms are altered by development of insulin resistance [4-6].The development of insulin resistance at adipocyte level by decreased action of insulin on *free fatty acids (FFA) suppression [7-9] .The mechanisms responsible for development of fatty liver are either excess caloric intake through dietary fat or alcohol consumption, leading to obesity and than development of insulin resistance.* The hepatic insulin resistance by increased lipid oxidation and enhanced hepatic expression of inflammatory cytokines or mediators are to be involved in the defective glucose regulation[2].The insulin resistance is measured from homeostasis model assessment (HOMA) as (HOMA-IR), calculated from estimations of fasting insulin (U/l) × fasting glucose(mg/dl)/405, as described by Matthews et al [10]. Cut-off value >2. 5 as positive and <2.5 as negative for assessment of insulin resistance in Homeostatic Model Assessment for Insulin Resistance (HOMA-IR)[11].Insulin resistance is a sheet anchor of metabolic syndrome, associated with chronic inflammatory response through abnormal synthesis of cytokines and activation of pro-inflammatory signaling pathways. This chronic inflammatory response is assessed by systemic inflammatory biomarker C-reactive protein (CRP), has been elevated in cardiovascular and cerebrovascular conditions[12,13]. The positive and strong association of C-reactive protein (CRP) and CVD risk has been studied in risk factors like alcohol consumption, blood pressure, hypercholesterolemia and smoking[14]. The inflammatory processes and impaired glucose metabolism are linked to NAFLD risk factor in non-obese men[15]. Insulin resistance, intern causes changes in lipid metabolism, particularly in increased synthesis of cholesterol and very low density lipoprotein (VLDL),[16] and increased clearance of high density lipoprotein (HDL) [17]. The changes of lipid profile patterns are triggered by insulin

resistance in *Non – alcoholic fatty liver disease* individuals and considered a hepatic manifestation of metabolic syndrome[18].

On this background, our study aims at comparing the occurrence of C- Reactive protein in patients with *Non – alcoholic fatty liver disease* group in relation to fasting insulin resistance, thus, reflecting presence or absence of any additive effect of fasting insulin resistance in *Non – alcoholic fatty liver disease* in causing major cardiovascular or cerebro vascular complications and lipid abnormalities

II. Material and methods:

A case – control study was undertaken in the Department of General Medicine, Dr.PSIMS&RF, Chinoutpally, Gannavaram, Krishna (Dt), Andhra Pradesh.

100 diagnosed non-alcoholic fatty liver disease cases, who attended to our hospital and 100 age, sex matched controls, were included in our study group. All patients survive till the end of study period of one year duration i.e., Jan 2012 to Dec 2012. Diagnosis of non-alcoholic fatty liver disease cases was done as per the Ultra sound findings of fatty changes in liver in cases and normal echo pattern in controls Fasting Insulin levels and HOMA-IR index were also determined in cases and controls.

Lipid profile, and C – reactive proteins were also estimated in both cases and controls as for supporting parameters in non-alcoholic fatty liver disease. The study was approved by the Ethics committee of our college. After fulfilling the inclusion and exclusion criteria, prior consent was obtained from the subjects.

Inclusion criteria:

Patients with Ultra sound findings of fatty changes in liver in cases and normal echo pattern in controls Age 18 or older

Exclusion criteria:

Patients with diabetes, hypertension, alcohol, heart failure, acute febrile illness, renal, hepatic, malignant disorders, chronic illnesses, asymptomatic infections and smokers

Sample collection and analysis:

Both heparinised and plain blood samples were collected from each case and control. For analysis of FBS, lipid profile, CRP - serum was used. Serum glucose estimation was done by Trindler's GOD – POD method (commercial kit – ERBA – MANNHEIM), cholesterol estimation was done by CHOD – POD method (commercial kit – ERBA – MANNHEIM), Triglycerides estimation was done by GPO method (commercial kit – ERBA – MANNHEIM), HDL cholesterol estimation was done by APO protein precipitation or PTA method (ERBA – MANNHEIM), and HBA1c estimation was done by Ion exchange resin method (commercial kit – Randox Rx series). All these estimations were performed by Randox Daytona Autoanalyzer. VLDL-c or LDL-c levels of all cases and controls were calculated by using Friedwald's formula. CRP levels in all cases and controls were estimated by slide agglutination method in serial dilutions using serum.

Fasting serum insulin was measured on a Roche Elecsys 2010 analyser (Roche Diagnostics: Mannheim, Germany,coefficient of variation 3.8%) after polyethylyene glycol precipitation of interfering antibodies.

III. Results:

The mean age in cases and controls was 40.15 $\pm$ 7.67, among in males was 39.86 $\pm$ 8.17 and in females was 40.89$\pm$ 6.3, the percentage of males(72%) were higher than females (28%). The mean BMI in cases was 24.7 $\pm$ 0.9 and in controls was 23.5 $\pm$ 1.1. The mean FBS levels in cases were 87.76 $\pm$ 9.33statistically significant than controls 84.03 $\pm$ 8.83 (p: 0.0041). Mean FBS levels of non-alcoholic fatty liver disease males were 87.63 $\pm$10.06statistically not significant than controls 85.25 $\pm$ 9.03 (p 0.1381). Mean FBS levels of non-alcoholic fatty liver disease females were 88.11 $\pm$7.31statistically significant than controls 80.89 $\pm$7.6 (<0.0006). The mean fasting insulin levels in cases were 14.71050$\pm$ 8.70280 statistically significant than controls 5.27556$\pm$3.65791 (p: 0.05). Mean fasting insulin levels of non-alcoholic fatty liver disease males were 16.11208$\pm$7.15650 statistically significant than controls 6.69050$\pm$3.19267 (p 0.0002). Mean fasting insulin levels of non-alcoholic fatty liver disease females were 11.10643 $\pm$ 11.14997statistically significant than controls 4.72531$\pm$3.69922 (<0.0001).

The mean HOMA IR levels in cases were 3.18 $\pm$ 0.24statistically significant than controls 1.09 $\pm$ 0.07 (p: < 0.05). Mean HOMA IR levels of non-alcoholic fatty liver disease males were 3.48 $\pm$ 0.17statistically significant than controls 1.40 $\pm$ 0.24 (p < 0.05). Mean HOMA IR levels of non-alcoholic fatty liver disease females were 2.41 $\pm$ 0.2 statistically significant than controls 0.94 $\pm$ 0.06 (P <0.05).The mean CRP levels in cases were 3.06 $\pm$ 1.61statistically significant than controls 1.03 $\pm$0.86 (p: 0.05). Mean CRP levels of non-alcoholic fatty liver disease females were 2.67 $\pm$0.75 statistically significant than controls 0.74 $\pm$0.6 (p 0.05). Mean CRP levels of non-alcoholic fatty liver disease males were 3.21 $\pm$ 1.82 statistically significant than

controls 1.16 ±0.97 (<0.05). The mean cholesterol in non-alcoholic fatty liver disease cases was 191.76 ± 27.32and in controls was 178.71 ± 37.45 (p 0.0054) which showed cholesterol is more significant elevation in non-alcoholic fatty liver disease cases, The mean triglyceride level in cases was 194.3 ± 94.87 and in controls was 138.67 ± 70.62 (p 0.0001) which showed mean triglyceride level is more significant elevation in non-alcoholic fatty liver disease cases, The mean LDL – cholesterol level in cases was 126.65 ± 42.8 and in controls was 114.64 ± 26.27 (p 0.0177) which showed mean LDL – cholesterol level is more significant elevation in non-alcoholic fatty liver disease cases, The mean HDL – cholesterol level in cases was 35.5 ± 8.33and in controls was 39.51 ± 11.79 (p 0.006) which showed mean HDL – cholesterol level is more significant reduction in non-alcoholic fatty liver disease cases.

Table 1
Mean age and BMI in cases and controls

Variable	Cases n 100	Controls n 100
Mean age	**40.15 ± 7.67**	**40.15 ± 7.67**
Males	**39.86 ± 8.17**	**39.86 ± 8.17**
Females	**40.89± 6.3**	**40.89± 6.3**
BMI	**24.7 ± 0.9**	**23.5 ± 1.1**

Table 2
Mean fasting blood sugar level in cases and controls

Variable	Cases	Controls	P value
Mean FBS	**87.76 ± 9.33**	**84.03 ± 8.83**	**0.0041**
Males	**87.63 ± 10.06**	**85.25 ± 9.03**	**0.1381**
Females	**88.11 ± 7.31**	**80.89 ± 7.6**	**0.0006**

Table 3
Mean fasting insulin levels in cases and controls

Variable	Cases	Controls	P value
Total	**14.71050± 8.70280**	**5.27556± 3.65791**	**<0.001**
Males n 72	**16.11208± 7.15650**	**6.69050±3.19267**	**0.0002**
Females n 28	**11.10643 ± 11.14997**	**4.72531± 3.69922**	**<0.0001**

Table 4
Mean HOMA IR levels in cases and controls

Variable	Cases	Controls	P value
Total	**3.18 ± 0.24**	**1.09 ± 0.07**	**< 0.05**
Males n 72	**3.48 ± 0.17**	**1.40 ± 0.24**	**< 0.05**
Females n 28	**2.41 ± 0.2**	**0.94 ± 0.06**	**< 0.05**

Table 5
HOMA IR levels in cases and controls

Variable	HOMA IR > 2.5	HOMA IR < 2.5	Chi squared value	P value
Cases	**29**	**71**	**18.746**	**0.0001**
Controls	**5**	**95**		

Chi squared equals 18.746 with 1 degrees of freedom. The two-tailed P value is less than 0.0001 The association between rows (groups) and columns (outcomes) is considered to be extremely statistically significant.

Table 6
HOMA IR and CRP levels in cases

Variable	HOMA IR	CRP > 3.0 mg/dl	Chi squared value	P value
HOMA IR > 2.5	**29**	**22**	**12.249**	**0.0005**
HOMA IR < 2.5	71	12		

Chi squared equals 12.249 with 1 degrees of freedom. The two-tailed P value equals 0.0005
The association between rows (groups) and columns (outcomes) is considered to be extremely statistically significant.

Table 7
Mean CRP levels in cases and controls

Variable	Cases	Controls	P value
Total	**3.06 ± 1.61**	**1.03 ± 0.86**	**< 0.05**
Males n 72	**3.21 ± 1.82**	**1.16 ± 0.97**	**< 0.05**
Females n 28	**2.67 ± 0.75**	**0.74 ± 0.6**	**< 0.05**

Table 8
Mean lipid levels in cases and controls

Variable	Cases	Controls	P value
Cholesterol	**191.76 ± 27.32**	**178.71 ± 37.45**	**0.0054**
Triglyceride	**194.3 ± 94.87**	**138.67 ± 70.62**	**0.0001**
LDL	**126.65 ± 42.8**	**114.64 ± 26.27**	**0.0177**
HDL	**35.5 ± 8.33**	**39.51 ± 11.79**	**0.006**

IV. Discussion:

In this case control study of the association between markers of systemic inflammation and fasting insulin level among patients, we observed an strong association between elevated levels of CRP and fasting hyperinsulinemia in *non – alcoholic fatty liver disease (NAFLD).*The mean age in *non – alcoholic fatty liver disease (NAFLD)* and control group was 40.15 ± 7.67, and 68% of these groups were noticed more between the age group of 31 – 40 years group. Mean fasting insulin levels were more in *non – alcoholic fatty liver disease (NAFLD) patients* 14.71050 ± 8.70280 than compared to controls (5.27556±3.65791), statistically significant elevations are compared with Salgado AL et. al study[19].The Insulin resistance by HOMA-IR method was seen in 29% of *non – alcoholic fatty liver disease (NAFLD) patients* when compared to controls 5%, statistically significant elevation in *non – alcoholic fatty liver disease (NAFLD)* group. Mean HOMA-IR levels in *non – alcoholic fatty liver disease (NAFLD)* group was 3.18 ± 0.24than compared to controls 1.09 ± 0.07,statistically significant elevation in *non – alcoholic fatty liver disease (NAFLD)* group, when compared to Salgado AL et. al study[19]our mean values are on higher side. Mean CRP levels in *non – alcoholic fatty liver disease (NAFLD)* group was 3.06 ±1.61 than compared to controls 1.03±0.86, statistically significant elevation in *non – alcoholic fatty liver disease (NAFLD)* group, when compared to 5.2 ± 2.5 versus 2.9 ± 0.5 mg/dl (NAFLD Vs. Controls), Oruc N et. Al [20] group, our mean values are on lower side.The elevated CRP levels in HOMA-IR more than 2.5 in *non – alcoholic fatty liver disease (NAFLD)* was 75.8% when compared to HOMA IR <2.5 in *non – alcoholic fatty liver disease (NAFLD)*cases was 16.9 statistically significant elevation of CRP was observed in *non – alcoholic fatty liver disease (NAFLD)*with insulin resistance. This findings were contradictory to study by Oruc N et. Al [20] group, they studied CRP levels in *non – alcoholic fatty liver disease (NAFLD) without insulin resistance. The* Oruc N et. Al [20] group *study* showed increase in mean serum CRP level was increased in NAFLD compared to controls. CRP can be used as an additional marker for diagnosis of NAFLD group. When compared to Andreas festa et al study [22], our study shows that CRP levels are significantly elevated in individuals having insulin resistance. The underlying mechanism responsible for

elevated CRP levels are associated with increased stimulation of adipose tissue to release cytokines which triggers haemodynamic hemostatic, and metabolic changes ultimately causing insulin resistance. Lipid levels are compared in in *non – alcoholic fatty liver disease (NAFLD)* group, Serum triglycerides and LDL were elevated than controls and HDL levels were decreased in *non – alcoholic fatty liver disease (NAFLD)* group compared to controls, these findings are correlated with Arnoldo Riquelme study[21].

V. Conclusions:

CRP levels were found to be significantly higher in individuals with insulin resistance by 34% in *Non – alcoholic fatty liver disease (NAFLD)*. 68% of *Non – alcoholic fatty liver disease (NAFLD)* patients belong to 31 – 40 years age group. Male to female ratio were found to be 2.57: 1.In *non – alcoholic fatty liver disease (NAFLD)* patients with elevated insulin resistance by HOMA IR method in 29%. Males with high insulin resistance with high mean CRP in *Non – alcoholic fatty liver disease (NAFLD)* patients had developed significantly elevated lipid levels. Larger studies are required to clarify the significance of development of early insulin resistance in NAFLD before the development of prediabetes and Prehypertension and to consider the fasting insulin levels and serum CRP as regular and routine screening markers to identify sub clinical target organ damage in *Non – alcoholic fatty liver disease (NAFLD)* patients.

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