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Prevalence of NAFLD and Its Association with Insulin Resistance in First Degree Relatives of Type 2 Diabetes

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Abstract **Original Research Article**

Non-alcoholic fatty liver disease (NAFLD) is one of the major risk factors in hepatic insulin resistance (IR). Though the association of NAFLD with type 2 diabetes (T2DM) is well studied, the prevalence of NAFLD and its association with IR in non-diabetic, first degree relatives (FDR) of T2DM is not explored. Therefore, the present study is aimed to estimate the prevalence of NAFLD and its association with IR in FDR of T2DM individuals. It is a cross-sectional study involving 165 FDR subjects who are non-diabetic were screened for NAFLD using abdominal ultrasound scanning, clinical assessment and biochemical parameters. The study found 22% prevalence of NAFLD and 43% prevalence of IR in the study population. Further, comparison was made between NAFLD and non NAFLD subgroups. The prevalence of IR, obesity, central obesity and hypertension were significantly high ($P<0.01$) in NAFLD subjects when compared to that of non NAFLD. Prevalence of elevated SGPT, cholesterol and triglyceride were also significantly high among NAFLD subjects. The results showed strong association of NAFLD with IR with odds ratio >13 in FDR individuals. Overall, the study indicated high prevalence of NAFLD and its association with insulin resistance among first degree relatives of diabetics even in normoglycemic status.

Keywords: Type 2 diabetes mellitus; NAFLD; Insulin resistance; First degree relatives.

INTRODUCTION

Non-alcoholic Fatty Liver Disease (NAFLD) is a major cause of illness and death in both developed and developing countries. Ectopic accumulation of triglycerides as cytoplasmic lipid droplets in more than 5% of hepatocytes in the absence of significant alcohol consumption and negative viral and autoimmune liver disease is defined as NAFLD[1].The clinical manifestations of NAFLD comes to medical attention incidentally when liver function tests show elevated levels of aminotransferase or radiographic report show fatty liver. Initially it was thought that simple fatty liver (steatosis) is a benign condition with no harmful effects, however, increasing evidences suggest that NAFLD is a potential pathologic condition leading to non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis and hepatocellular carcinoma.

Epidemiology reports show the prevalence of NAFLD to be nearly 90% among obese population when compared to 9-32% among general Indian population[2] though substantial proportion of NAFLD subjects is lean also. Increasing evidences support the association of NAFLD with metabolic syndromes such as obesity, insulin resistance (IR), glucose intolerance, hypertension and dyslipidemia[3,4]. It is well-known that there is an increasing tendency of overweight/obesity worldwide and since NAFLD has emerged as a frequent cause of chronic liver disease in many parts of the world, NAFLD has become a topic of public health importance. NAFLD patients die more frequently from non-hepatic complications and majority of them are attributed to cardiovascular disease[5]. Hence it is also important to determine the long term outcomes of patients with NAFLD particularly among population with high risk of IR or diabetes.

NAFLD has prevalence of over 70% in type 2 diabetics[6]. Although the underlying mechanisms of NAFLD are not completely understood, the contradictory reports indicate that NAFLD is probably a heterogeneous spectrum of disease arising from different etiologies. Increased visceral obesity, high fructose and fat intake and genetic risk factors, including congenital defects of metabolism might be

associated with NAFLD which also support the development of NAFLD in lean subjects. But the majority of lean subjects with NAFLD were less insulin sensitive[7] suggesting a link between NAFLD and IR, independent of obesity.

Impairment of insulin sensitivity in adipose tissue, hepatic cells and skeletal muscle; and metabolic flexibility in NAFLD individuals were comparable to those observed with diabetic individuals [8]. Also, progression of NAFLD to advanced hepatic complications is more common when the diabetes coexists[9]. Hence NAFLD should be viewed as a serious health threat for the development of diabetes and associated complications in population at risk of T2DM.

There are several studies reported on the association of NAFLD with either T2DM or IR. However, to our knowledge, there are limited studies reported on association of NAFLD and IR in first degree relatives of T2DM. Most of these studies have been done either in general population, diabetics or NAFLD individuals[10–13]. Therefore the present study was designed to estimate the prevalence of NAFLD and IR in non-diabetic first degree relatives of T2DM. The outcome of the study may help to predict the risk of NAFLD, IR and associated risk factors of diabetes in first degree relatives of T2DM even in normoglycemic status.

METHODS

Study population and study design

It is a cross sectional study. Participants for the study were selected from first degree relatives of type 2 diabetic subjects who visited endocrinology OPD of K.R. Hospital, Mysuru, during July 2016 to March 2018. The study enrolled a total of 165 subjects from 151 families in the age group of 18 to 60 years. Participants who consumed alcohol (≥20g/day), diabetic, pre-existing liver diseases other than NAFLD or those who are under medications which affect liver function were excluded. The study was approved by institutional ethics committee at Mysore medical college & research institute, Mysuru (EC REG ECR/134/Inst/KA/2013). A written informed consent was taken from all the participants before enrolment.

Data regarding age, gender, height, weight, waist and hip circumferences, blood pressure, family history of diabetes and systemic examination results were recorded.

Diagnosis of NAFLD

All the patients included in the study underwent abdominal ultrasonography using – 5.0 MHZ high frequency curvilinear transducer (C5-1) in Philips affinity 70 ultrasound machine (PHILIPS medical systems, Bothell, WA) by experienced radiologists in the department of Radio diagnosis [14].

Biochemical measurements

5 ml of fasting blood and 2 ml of post prandial blood were collected in plain vacutainer and allowed to clot for 20 min at room temperature. The serum was separated by centrifugation at 1500 rpm for 10 min. and serum aliquots were stored at -80^{0}C till further use. Another 2 ml of post prandial blood was collected in EDTA vacutainer for glycosylated haemoglobin (HbA1c) analysis is stored at -20^{0}C till further use.

Fasting and post prandial blood glucose, serum lipids (total cholesterol, triglycerides, HDL-c, LDL-c), SGOT, SGPT, bilirubin total and direct, total protein, albumin, globulin and ALP were measured in serum samples using Cobas C311 fully automated chemistry analyser (Roche Diagnostics). HbA1c was determined in whole blood samples by turbidimetric immunoassay using Cobas C311 analyzer. Fasting serum insulin was determined using Cobas E411 automated immunoanalyser (Roche Diagnostics).

Insulin sensitivity was calculated using homeostasis model assessment - insulin resistance [HOMA-IR] formula:

$$HOMA\text{-}IR = [Fasting\ insulin\ (\mu U/ml) \times Fasting\ glucose\ (mg/dl)] / 405$$

Definitions

Diabetes was defined as fasting serum glucose ≥126 mg/dL, post prandial blood glucose ≥200 mg/dl, HbA1c ≥6.5%or use of oral anti-diabetic agents (after diagnosis of T2DM).Pre-diabetes was defined as fasting serum glucose 100-125 mg/dL or post prandial serum glucose 140–199 mg/dL or HbA1c 5.7-6.4%. HOMA-IR score ≥3 is considered as insulin resistant. Obesity was defined as BMI ≥25 kg/m^2 and central obesity was defined as waist circumference ≥90 cm for males and ≥80 cm for females according to the proposed cut-off for the diagnosis in Asians [15]. Hypertension was defined as systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg, or the use of antihypertensive medication. SGPT ≥34 U/L for females, ≥45 U/L for males and SGOT ≥31 for female, ≥35 for males are considered as elevated enzyme levels. Triglycerides ≥150 mg/dL and cholesterol ≥200 mg/dL, LDL-c ≥100 mg/dL, and HDL-c ≤50 mg/dL for females and ≤40 mg/dL for males are considered as abnormal levels[16].

STATISTICAL ANALYSIS

Mean differences were analysed by student's t-test and ANOVA. Chi-square and odds ratio were used to analyse the association between NAFLD and IR. Statistical tests were performed using SPSS software. Z-test in *EpiTools epidemiological calculators* was used to analyse the difference in proportions (http://epitools.ausvet.com.au/content.php?page=z-test-2).

RESULTS

The study population involved 165 non diabetic, FDR subjects from 151 families. Majority of the study participants were females (67%) with mean age 31±10 (95 CI; 29.0-32.2) (Table 1). The frequency of NAFLD was found to be 22%. IR, obesity, central obesity and hypertension was present in >40% of study subjects (Fig 1). Central obesity was found to be significantly higher in females than males (61% Vs 35%). However prevalence of other parameters was not significantly different across gender.

Further, subjects were made into NAFLD and non NAFLD groups for comparison. The prevalence of IR, prediabetes, obesity, central obesity, hypertension, elevated SGPT, elevated TG and elevated cholesterol was high among NAFLD group (Fig 2). More than 90% of participants in NAFLD group was either having obesity or central obesity. Significant increase in blood glucose level, HbA1c, insulin, HOMA-IR, lipid profile and liver enzymes were observed in NAFLD group (Table 2). A decrease in HDL and SGOT/SGPT ratio was significant in NAFLD group. Also, the IR groups, made based on HOMA-IR score revealed that the odds of having NAFLD was increased from 3 to 13.8 with increasing severity of IR (Fig-3).

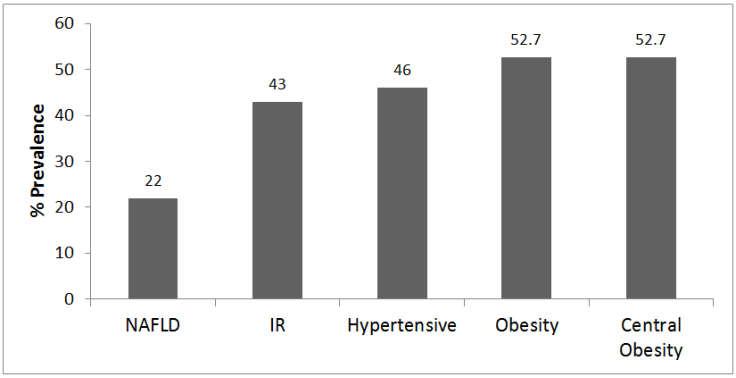

Fig-1: Prevalence of NAFLD, IR, hypertension, obesity and central obesity

Central obesity was found to be significantly higher in females than males (61% Vs 35%). However prevalence of other parameters was not significantly different across gender.

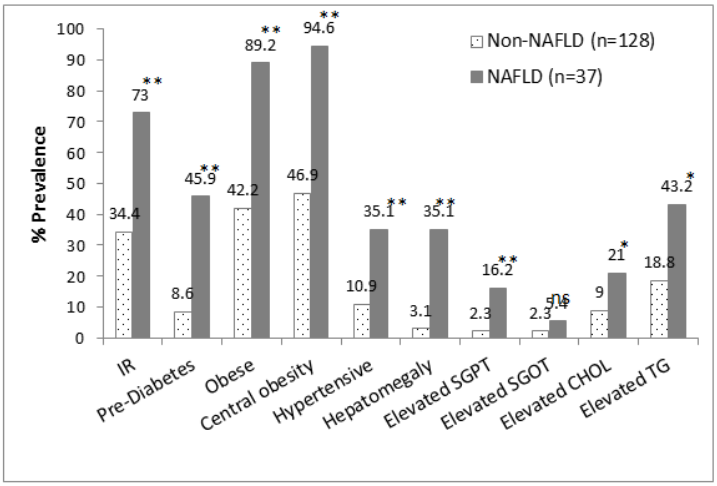

Fig-2: Prevalence of IR, Pre-diabetes, obesity, central obesity, hypertension, hepatomegaly, elevated SGPT, SGOT, cholesterol and triglycerides in NAFLD and non-NAFLD subjects

**P<0.05, **P<0.001, [ns] no significant difference at P<0.05.*

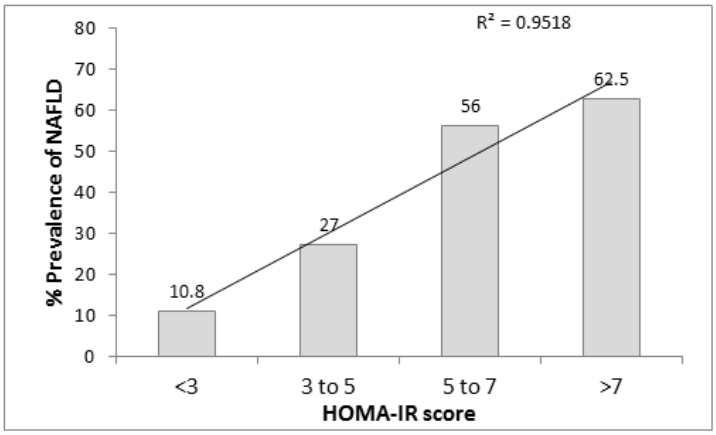

Fig-3: Prevalence of NAFLD by IR status.

HOMA-IR	<3	3 to 5	5 to 7	>7
Odds ratio	1	3	10.6	13.8

Prevalence of NAFLD increased significantly as the severity of IR increases. The values in the table indicate the increase in odds of having NAFLD as the HOMA-IR score increases.

Table-1: Anthropometric and serum biochemical data

Anthropometric data	FDR (n=165)	95% Confidence interval
Gender (count) – Male	54	
Female	111	
Age (years)	31±10	29.042-32.2
BMI (kg/m^2) – Male	24.3±3.96	23.2-25.3
Female	25.98±5.16	25.0-26.9
Waist circumference (cm) – Male	87.05±10.54	84.1-89.9
Female	84.16±12.18	81.7-86.5
Blood pressure (Hg)		
Systolic	119.10±14.05	116.9-121.2
Diastolic	78.87±8.42	77.5-80.1
Biochemical parameters		
FBS (mg/dL)	87.11±10.26	85.5-88.6
PPBS (mg/dL)	107.97±33.21	102.8-113.0
HbA1C (%)	5.28±0.97	5.1-5.4
Fasting Insulin (µU/mL)	14.60±9.25	13.1-16.0
HOMA- IR	3.21±2.30	2.8-3.5
Beta cell function (%)	240.61±146.67	218.0-263.1
Total Cholesterol (mg/dL)	162.01±33.84	156.8-167.2
Triglycerides (mg/L)	115.68±56.56	106.9-124.3
HDL-C (mg/L)	40.49±10.18	38.9-42.0
LDL-C (mg/dL)	104.91±28.81	100.4-109.3
VLDL (mg/dL)	16.71±9.77	15.2-18.2
Cholesterol/HDL ratio	4.24±1.39	4.0-4.4
Total Protein (g/dL)	7.46±0.52	7.3-7.5
Albumin (g/dL)	4.42±0.30	4.3-4.4
Globulin (g/dL)	3.07±0.53	2.9-3.1
Total Bilirubin (mg/dL)	0.55±0.26	0.50-0.58
Direct Bilirubin (mg/dL)	0.19±0.08	0.17-0.19
SGOT (U/L)	19.13±6.70	18.1-20.1
SGPT (U/L)	19.93±10.92	18.2-21.6
SGOT/SGPT ratio	1.09±0.33	1.0-1.1
ALP (U/L)	77.94±19.93	74.8-81.0

Values are Mean±SD except Gender and 95% CI

Table-2: Comparison of subjects with and without NAFLD

	Non-NAFLD (n=128)	NAFLD (n-37)
Gender (count) – Male Female	44 84	10 27
Age (years)	29.27±9.56	35.32±11.34**
BMI (kg/m^2) – Male Female	23.76±3.75 24.51±4.43	26.66±4.23* 30.59±4.62**
Waist circumference (cm) – Male - Female	85.14±9.63 79.90±10.86	95.50±10.71** 97.43±8.44**
Blood pressure (Hg)		
Systolic	117.13±12.99	125.92±15.59**
Diastolic	77.91±8.12	82.16±8.73**
Biochemical parameters		
FBS (mg/dL)	85.55±9.07	92.52±12.24**
PPBS (mg/dL)	101.19±27.74	131.42±39.77**
HbA1C (%)	5.13±0.92	5.79±0.99**
Fasting Insulin (µU/mL)	12.69±6.15	21.22±14.07**
HOMA- IR	2.73±1.56	4.88±3.43**
Beta cell function (%)	224.66±126.79	295.79±193.03**
Total Cholesterol (mg/dL)	158.76±31.15	173.25±40.35*
Triglycerides (mg/L)	106.58±44.89	147.17±78.42**
HDL-C (mg/L)	41.23±10.61	37.91±8.14^{ns}
LDL-C (mg/dL)	102.46±26.93	113.38±33.59*
VLDL (mg/dL)	15.21±8.31	21.92±12.47**
Cholesterol/HDL ratio	4.08±1.22	4.82±1.76**
Total Protein (g/dL)	7.49±0.49	7.38±0.60 ^{ns}
Albumin (g/dL)	4.44±0.28	4.33±0.35 ^{ns}
Globulin (g/dL)	3.06±0.57	3.10±0.38 ^{ns}
Total Bilirubin (mg/dL)	0.55±0.26	0.52±0.27 ^{ns}
Direct Bilirubin (mg/dL)	0.19±0.07	0.18±0.09 ^{ns}
SGOT (U/L)	18.24±4.87	22.21±10.39*
SGPT (U/L)	17.70±8.55	27.64±14.35**
SGOT/SGPT ratio	1.15±0.34	0.87±0.21**
ALP (U/L)	77.50±19.39	79.46±21.92 ^{ns}

Independent samples t-test was performed between NAFLD and non-NAFLD groups.
**P<0.05, **P<0.01, ^{ns} No significant difference at p<0.05.*

DISCUSSION

NAFLD coexist with features of metabolic syndrome including obesity, T2DM, dyslipidemia and hypertension. In the current study an attempt was made to look for association between NAFLD and IR and other metabolic factors in non-diabetic first degree relatives of T2DM. The overall prevalence of NAFLD was 22% in FDR group. No significant difference in prevalence of NAFLD across the gender was found.

IR in our study was 43% in FDR, this was similar to the study done by Kumar *et al*. [17] who found 43% of IR in FDR compared to controls. Also we found IR is strongly associated with NAFLD as evidenced by increase in odds of having NAFLD as the severity of IR increases.

NAFLD is strongly associated with both hepatic and adipose IR [17,18]. In our study prevalence of IR was significantly high in NAFLD group compared to non-NAFLD group (73% Vs 34%). This result correlates with the study done by Jalal *et al*. [12].

Although muscle is the tissue i.e. mainly affected by IR, impairment in insulin action in liver and adipose tissue among NAFLD individuals may occur even before the onset of diabetes. The impairment in insulin action at the level of liver and adipose is proportional to the amount of hepatic and visceral fat[8, 19–21]. On the other hand, there are some reports showing fat causing IR. Increase in diacylglycerol content and inhibition of insulin signaling were found in lipid-infused rodents and rodents fed with high-fat diets [22]. Similar results were observed in studies involving human subjects also[23]. These reports suggest a link between IR and NAFLD though the cause and effect conundrum still exists.

Since FDR population are genetically exposed to risk of developing T2DM, the prevalence of other risk factors of diabetes such as IR, obesity, central obesity and dyslipidemia in the current study were found to be high among FDR subjects as expected. However, information about the influence of fatty liver on occurrence of these risk factors in FDR population is limited. Hence, the comparison was made between NAFLD and non-NAFLD subjects revealed that subjects with NAFLD were more prone to have risk factors of diabetes when compared to that of non-NAFLD as indicate din Fig. 2.

Therefore, the study suggests NAFLD as a therapeutic target for prevention and management of diabetes as clinicians concentrate more on micro and macro vascular complications while managing diabetes conditions. An increased risk of progression to NASH and cirrhosis in diabetic patients when coexistence of NAFLD [24] warrants the clinicians to emphasize on reducing ectopic fat in the liver in addition to maintaining balanced blood glucose level.

CONCLUSION

The current study showed high prevalence of NAFLD and its association with IR among first degree relatives of T2DM even in normoglycemic status.

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