

Original Article

Insulin Resistance in Nonalcoholic Fatty Liver Disease: Experience from Bangladesh

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Abstract

Background: Insulin resistance (IR) has largely been hypothesized as central in multifactorial pathogenesis of nonalcoholic fatty liver disease (NAFLD). This study was aimed to explore the association of IR with NAFLD and nonalcoholic steatohepatitis (NASH).

Methods: We enrolled 219 patients of NAFLD with sonographic evidence of fatty changes in liver excluding patients with alcohol intake and other causes of fatty change during June 2012 to July 2014. Liver biopsy was done for 110 patients with elevated ALT of >30 U/L for male and >18 U/L for female. We have measured IR by homeostatic model assessment of insulin resistance (HOMA-IR).

Results: Age of the study population was 40.6 ± 10.0 years, male and female was 83 (37.9%) and 136 (62.1%), ALT was 42.0 (16-861) U/L, AST was 32 (16-608) U/L and GGT was 39 (10-243) U/L. According to Asian criteria 54 (25.9%) were non-obese, 139 (64.1%) had metabolic syndrome, 163 (74.8%) were hypertriglyceridemic, 200 (91.3%) had low HDL and 170 (77.4%) had high waist. Hypertensive and diabetic were 58 (26.7%) and 57 (26.1%) respectively. IR was 1.9 ± 1.3 with the range of 0.4 to 9.3 and only 87 (39.7%) were above normal. Of the 110 biopsied, 65 (59.1%) had NASH. Normal and raised IR was associated with 32 (50.8%) and 33 (70.2%) NASH respectively ($p < 0.05$). Correlation between IR and steatosis, ballooning and fibrosis was not significant except lobular inflammation. IR was similar in NASH (2.2 ± 1.6) and non NASH (1.9 ± 1.6).

Conclusion: Large proportion of NAFLD patients had normal IR. IR had inconsistent association with histological activity.

Key Words: Insulin Resistance; Fatty Liver; NAFLD; NASH; HOMA IR; IR; Metabolic syndrome.

Introduction:

Non alcoholic fatty liver disease (NAFLD) encompasses spectrum of disorders ranging from simple steatosis to

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steatohepatitis, ultimately cirrhosis.¹ It is characterized by abnormal accumulation of triglyceride (TG), in the liver without significant alcohol consumption. Hepatic steatosis generally considered as a benign condition affecting 60-70% of diabetic and of obese person.² Evidence showed 25% individual with NAFLD progress to non alcoholic steatohepatitis (NASH) and 25% of NASH patients develop cirrhosis.^{3,4} Though most patients with NAFLD are asymptomatic, NASH may progress to end stage liver disease and hepatocellular carcinoma (HCC).^{5,6} NAFLD prevalence in general population of both Western and developing countries is rising. In developed countries like Western Europe and United States (US) prevalence ranges from 20-30% which is increasing day by day.^{7,8} In Korea, it is estimated that 20-25% of general population has documented NAFLD.⁹ In other Asian countries, incidence and prevalence of obesity related NAFLD are also increasing due to ongoing socio-economic transition and shift towards westernized diet.¹⁰ It was thought earlier that NAFLD was mostly associated with obesity but lean NAFLD or NASH is increasingly detected in Asian subcontinent, marked as third world NAFLD.¹¹

Pathogenesis of NAFLD is multifactorial. Generally accepted hypothesis is insulin resistance (IR) and increased free fatty acid (FFA) and NASH is developed by oxidative stress, mitochondrial dysfunction and cytokine release.^{12,13} But other factors such as genetic, environmental factors like exercise

and diet also found to interact to define NAFLD phenotype and determine progression. Insulin resistance largely been hypothesized as central in pathogenesis of hepatic steatosis.^{14,15} The pathogenesis is referred to adipose tissue, insulin resistance an increase flux of FFA to the liver. Increased lipogenesis induced by hyperinsulinemia, abnormal intrahepatic lipid metabolism and dietary factor. Hepatic fat accumulation in turn worsens IR and liver damage determining risk of both cardiovascular and liver related mortality.¹⁶ Now a days it is speculated that insulin resistance may not be the only factor in development of NAFLD and its further progression. A genome-wide association study revealed that the rs738409 single-nucleotide polymorphism (SNP) in patatin-like phospholipase domain-containing 3 (PNPLA3) is strongly associated with hepatic fat content.¹⁷ It is suggested that I148M polymorphism variant becomes a critical factor determining hepatocellular fat accumulation and further inflammation, when stressor factors such as increased influx of FFA related to adipose tissue IR in visceral obesity. Increased lipogenesis stimulated by hyperinsulinemia and carbohydrate or altered lipid metabolism intervene.¹⁸ But pathogenesis of metabolic complications associated with NAFLD as hepatic steatosis and the PNPLA3, I148M may be independent of insulin resistance.¹⁹

It is now becoming clearer that insulin resistance is not the only contributor in the development of NAFLD and its consequences. IR possibly plays part in development of NAFLD but other factors may play role in sustaining the change and further damage. So our study purpose is to find out the actual correlation of IR with NAFLD and NASH.

Materials and Methods:

Study population: We have prospectively enrolled patients with fatty change in liver attending in the department of Hepatology of Bangabandhu Sheikh Mujib Medical University (BSMMU) between June 2012 and July 2014. The university hospital is the apex referral institute of the country. The protocol was approved by the departmental technical committee and research was carried out in accordance with the Helsinki Declaration and informed written consent was taken from every patient. Diagnosis of fatty liver by ultrasonography was defined by the presence of at least two of three abnormal findings including i) diffusely increased echogenicity (“bright”) of liver with liver echogenicity stronger than kidney or spleen and either ii) deep attenuation of ultrasound signal or iii) vascular blurring. In particular, all patients were negative for hepatitis B surface antigen and antibodies against hepatitis C virus (anti-HCV), they reported no alcohol use or a weekly alcohol use <210 gm for male patients and <140 gm for female patients. None of them were on hepatotoxic drugs or agent that could give rise to elevated enzymes or fatty change. Autoimmune hepatitis, Wilson’s disease, haemochromatosis and hypothyroidism were excluded by clinical evaluation and relevant laboratory investigations.

Clinical and Laboratory Data: Body mass index (BMI), ALT, AST, GGT, total serum cholesterol, triglycerides, low

density lipoprotein (LDL), high density lipoprotein (HDL), and fasting blood sugar (FBS) were estimated. Serum samples were obtained after an overnight fast of at least 12 hour & immediately frozen at -20° Celsius. We have measured the levels of immunoreactive insulin by a chemiluminescence immunoassay and insulin resistance (IR) was calculated by homeostasis model assessment insulin resistance (HOMA-IR)²⁰ and IR index of > 1.8 was considered as raised IR.^{21,22}

Metabolic syndrome was defined according to Asian criteria,²³ and three of the five listed criteria were considered: waist circumference ≥ 80 cm for women and ≥ 90 cm for men, serum triglyceride ≥ 150 mg/dL (1.7 mmol/L), serum high-density lipoproteins (HDL) cholesterol <50 mg/dL (1.3 mmol/L) for women and < 40 mg/dL (1 mmol/L) for men, elevated blood pressure (systolic blood pressure ≥ 130 and or diastolic blood pressure ≥ 85 mmHg or drug treatment for hypertension) and fasting plasma glucose concentration ≥ 100 mg/dL (5.6 mmol/L) or drug treatment for diabetes. Obesity was considered if BMI ≥ 25 kg/m² and BMI < 25 kg/m² was considered as non-obese.

Histological assessment: Liver biopsy was done in patient with ALT of >30 U/L for male and >18 U/L for female. The diagnosis of NASH was based on the criteria of Brunt et al,²⁴ as modified by Kleiner et al.²⁵ In this scoring system, the degree of disease activity in NAFLD was evaluated using the NAFLD Activity Score (NAS), which was calculated as the unweighted sum of the scores for steatosis (0-3), lobular inflammation (0-3), and hepatocyte ballooning (0-2); therefore, the score ranged from 0 to 8. A NAS of 5 or more was diagnosed as “definitive NASH”, a NAS of <5 considered as non-NASH fatty liver (NNFL). The hepatic fibrosis staging was as follows: 0 = no fibrosis; 1 = zone 3 fibrosis only; 2 = zone 3 and portal/ periportal fibrosis; 3 = bridging fibrosis; and 4 = cirrhosis.

Statistical analysis: Statistical analyses were performed by SPSS® for Windows® ver 16 (SPSS Inc., Chicago, IL, USA). The results are presented as the mean \pm SD for the quantitative data and as proportion or percentages for the categorical or qualitative data. The statistical differences in the quantitative data were assessed using a t test and Mann-Whitney U Test. The qualitative data were compared using the χ^2 test. Logistic regression analysis was performed for multivariate analysis. Probability value P< 0.05 was considered as significant.

Results:

Demographic, biochemical and anthropometrics of study subjects: Total 219 NAFLD patients were enrolled in this study; 83 (37.9%) of them were male and 136 (62.1%) were female. Mean age was 40.6 \pm 10.0 years. Occupation of male was student 22 (26.5%), service holder 26 (31.3%), businessman 34 (41%) and farmer 1 (1.2%). On the other hand occupation of female was house wife 116 (85.3%), student 12 (8.8%) and service-holder 8 (5.9%). Monthly income in USD was 18.75 to 1875.00 with median of 187.5 (Figure I).

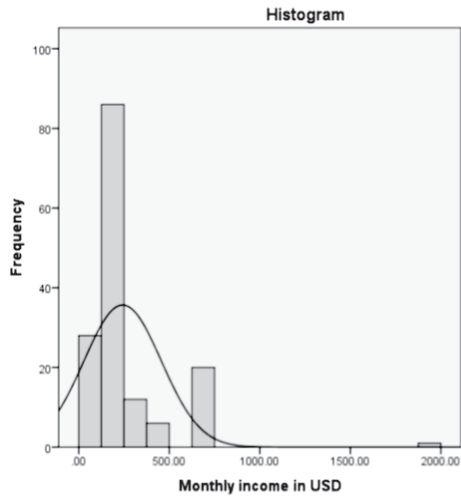
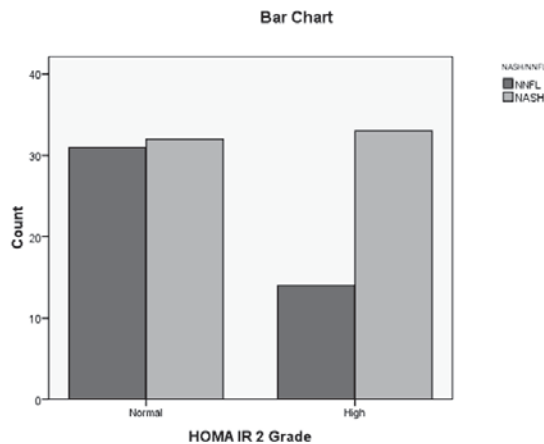


Figure I: Monthly income of the study population

ALT, AST and GGT was median (range) 42 (16-861) U/L, 32 (16-608) U/L and 39 (10-243) U/L respectively. In this study 58 patients (26.1%) were diabetic, 58 (26.1%) were hypertensive, 163 (74.8%) were hypertriglyceridemic, 139 (64.1%) had metabolic syndrome and non-obese (BMI < 25) were 54 (25.9%). Insulin resistance index (HOMA IR) was (1.9±1.3) ranging from 0.4 to 9.3 (Figure-II).



NASH; Nonalcoholic steatohepatitis
 NNFL; Non NASH fatty liver

Figure II: Association of insulin resistance with nonalcoholic steatohepatitis

IR was normal in 132 (60.3 %) and was raised in 87 (39.7%). Male were less commonly associated with high insulin resistance $\chi(1) = 3.939$, $p = 0.047$. Age, waist circumference in cm, serum cholesterol, serum LDL, triglyceride, hypertension, ALT, AST and GGT were similar in normal and high insulin resistance. Diabetes and metabolic syndrome was more prevailing with high insulin resistance $\chi(1) = 8.480$, $p =$

0.004 and $\chi(1) = 6.145$, $p = 0.013$. BMI was 26.4 ± 3.0 in normal insulin resistance and 27.7 ± 4.4 in high insulin resistance $t(217) = -2.265$, $p = 0.025$ (table I).

Table I: Base line characteristics of study population

Variable	Total n=219	Normal IR n= 132	Raised IR n= 87	p
Age in years	40.6 ± 10.0	40.9 ± 9.6	40.1 ± 10.6	0.503
Sex male/female n (%)	83(37.9)/136(62.1)	57(68.7) / 75(55.1)	26(31.3)/ 61(44.9)	0.047
BMI (mean±SD)	27.0 ± 3.7	26.4 ± 3.0	27.7 ± 4.4	0.025
Waist circumference in cm (mean±SD)	94.2 ± 8.3	93.4 ± 8.6	95.4 ± 7.9	0.113
Serum cholesterol mg/dl (mean±SD)	207.1 ± 47.6	206.0 ± 50.0	208.8 ± 44.1	0.667
Low density lipoprotein mg/dl (mean±SD)	127.8 ± 46.6	128.0 ± 39.1	127.4 ± 56.6	0.932
High density lipoprotein mg/dl (mean±SD)	36.2 ± 9.1	35.2 ± 8.9	37.7 ± 9.2	0.052
Triglyceride mg/dl (mean±SD)	234.3 ± 128.9	227.7 ± 133.4	244.4 ± 121.8	0.342
ALT u/l (mean±SD)	54.3 ± 63.3	56.0 ± 77.5	51.6 ± 31.6	0.636
AST u/l (mean±SD)	41.5 ± 44.7	43.2 ± 54.9	39.0 ± 21.2	0.986
GGT u/l (mean±SD)	49.4 ± 34.5	46.0 ± 29.5	53.8 ± 39.8	0.162
Diabetes n (%)	58 (26.1)	25 (19.1)	33(26.1)	0.004
Hypertension n (%)	58 (26.1)	30 (23.6)	28 (31.2)	0.252
Metabolic syndrome n (%)	139 (64.1)	76 (57.6)	63 (74.1)	0.013

Liver histology and insulin resistance index:

We had liver biopsy reports of 110 cases with elevated ALT. Of these biopsied NAFLD patients IR was normal in 63 (57.3%) and was raised in 47 (42.7%), NNFL was 45 (40.9%) and NASH was 65 (57.3%).NASH was significantly associated with raised IR $\chi(1) = 4.1999$, $p = 0.040$. Steatosis score 1 was 34 (30.9%), 2 was 50 (45.5%), 3 was 26 (23.6%), ballooning score 0 was 4 (3.6%), 1 was 76 (69.1%), 2 was 30 (27.3%), lobular inflammation 0 was 4 (3.6%), 1 was 59 (53.6%), 2 was 47 (42.7%) and fibrosis stage 0 was 9 (8.2%), 1 was 77 (70.0%), 2 was 17(15.5%), 3 was 6 (5.5%) and 4 was 1 (0.9%) of the biopsied study subjects. Mean steatosis, ballooning, lobular inflammation and fibrosis were insignificantly differed with normal and high insulin resistance; $t(108) = -1.690$, $p= 0.904$, $t(108) =0.421$, $p=0.674$, $t(108) = 0.815$, $p= 0.417$ and $t(108) = 0.592$, $p=0.555$ respectively (table II).

Table II: Histological changes with insulin resistance index

Histopathology	Total N= 110	IR Normal n= 63	IR Raised n = 47	p
Steatosis score 1/2/3 (n)	34/50/24	24/26/13	10/24/13	0.166
Ballooning score 0/1/2 (n)	4/76/30	2/43/18	2/33/12	0.908
Lobular inflammation score 0/1/2/3 (n)	4/59/47/0	2/32/29/0	2/27/18/0	0.710
Fibrosis stage 0/1/2/3/4 (n)	9/77/17/6/1	4/47/9/3/0	5/30/8/3/1	0.618
Steatosis (mean ± SD)	1.9 ± 0.7	1.8 ± 0.8	2.1 ± 0.7	0.09
Ballooning (mean ± SD)	1.2 ± 0.5	1.3 ± 0.5	1.2 ± 0.5	0.67
Lobular inflammation (mean ± SD)	1.4 ± 0.6	1.4 ± 0.6	1.3 ± 0.6	0.41
NAS*	4.6 ± 1.2	4.5 ± 1.1	4.6 ± 1.2	0.67
Fibrosis (mean ± SD)	1.2 ± 0.7	1.2 ± 0.6	1.3 ± 0.8	0.55
NNFL/ NASH** n (%)	45(40.9)/65(59.1)	31(49.2)/32(50.8)	14(29.8)/33(70.2)	0.04

• *Nonalcoholic fatty liver disease activity score

• ** Non NASH fatty liver/ Non alcoholic steatohepatitis

• \$ IR; Insulin Resistance

Pearson’s correlation analysis showed no correlation between insulin resistance index and steatosis, ballooning and fibrosis except lobular inflammation ($r = .188$, $n = 110$, $p < .05$). ALT, AST were similar in NASH and NNFL but GGT was significantly differed $t(108) = -2.689$, $p = 0.008$. GGT was also positively correlated with fibrosis ($r = .283$, $n = 110$, $p < .005$). Presence of metabolic syndrome was similar in NASH and NNFL $\chi(1) = 1.382$, $p = 0.240$. A logistic regression was performed to ascertain the effects of insulin resistance index, metabolic syndrome, ALT and GGT on the likelihood that predict to have NASH. The logistic regression model was statistically insignificant; the model explained 14.3% of the NASH and correctly classified 61.1% of cases only. Insulin resistance index, metabolic syndrome, ALT and GGT had $p = 0.239$, $p = 0.482$, $p = 0.128$ and $p = 0.106$ in regression analysis for NASH respectively.

Discussion:

High insulin resistance was comprised 87 (39.7%) of 219 NAFLD patients attending to our tertiary care university hospital. It has been shown in previous report that up to 85% of subjects with NAFLD is insulin resistant and have abnormal glucose metabolism, i.e., prediabetes or T2DM, about which they may be unaware.²⁶ The dissimilarity of small number of high insulin resistance in our series is as because previous studies enrolled the patient with NAFLD who were diabetic and obese as well. Study from India

reported that the IR as indicated by $IR > 2$ was present in only 7.4% ($n = 02$) patients of lean NAFLD, which was significantly lower than that in overweight (40%, $P = 0.05$), or obese NAFLD (61%, $P = 0.001$).²⁷ Given that the degree of insulin resistance and the prevalence of metabolic syndrome may increase from lean to overweight and obese NAFLD patients.^{28,29,30} This is also supported by data suggesting that HOMA index, a marker of insulin resistance, is lower in NAFLD patients without than in those with metabolic syndrome.³¹ Our study was also in accordance with these studies where high insulin resistance was more prevailed with obesity, metabolic syndrome and diabetics, though over all percentage of high insulin resistance was lower than previous reports. Several reports from Bangladesh consistently elucidated that significant number of NAFLD is persisting without high insulin resistance.^{32,33,34} This is similar to study by Kumar et al from India.²⁷ Furthermore, various genetic factors are known to confer susceptibility to NAFLD in individuals without increasing the level of IR.^{35,36} Contribution of genetics and dietary habit above or along with insulin resistance in NAFLD of these populations are yet to be established.

Liver histopathology reports explored that 65 (57.3%) were NASH. Prevalence of NASH is much higher from previous study conducted in Bangladesh.³² This may be due to the selection criteria of raised ALT for liver biopsy. This high number of NASH in NAFLD is alarming for the country like Bangladesh which might be the main contributor of cirrhosis in future as hepatitis B virus will be eliminated with expanded programme of immunization. Though steatosis, ballooning, lobular inflammation, NAS and fibrosis were similar with different degree IR and had significant correlation with lobular inflammation only. There was significant association with raised IR and NASH by univariate analysis. But multivariate regression analysis failed to validate relation between IR and NASH. So the severity of NAFLD has got inconsistent relation with IR. This is supported by 2 hit hypothesis of NASH where IR is responsible for 1st hit and not for 2nd hit where simple steatosis progress to steatohepatitis.³⁷

Female preponderance in NAFLD of our series is dissimilar from reports from developed counties where male gender was described as a risk factor for fatty liver disease.^{1,38} This female preponderance 136 (62.1%) in our study may be due to sedentary life style which was supported by the statistics of occupation where house wife was 116 (85.3%) of female NAFLD. Similar female preponderance was observed in one population based study from India.³⁹ Median monthly income of our NAFLD patients was 187.5 USD. This indicates that NAFLD is not the morbidity of affluent only but affects low income group also. This study had several limitations; it was single center study and we could not confirm the influence of genetics and dietary habit on development and progress of NAFLD.

In conclusion, IR is not the only contributor in the development of NAFLD. It is common among female, in Bangladesh and low income group are also affected.

Prevalence of NASH is much high. IR has got inconsistent association with NASH. Contribution of genetics and diets on development of NAFLD and progression to NASH is recommended for further study.

Statement of informed consent and human right:

All procedures performed in this study were in accordance with the ethical standards of with the 1964 Helsinki declaration. Informed written consent was taken from every patient. The protocol was approved by the departmental technical committee.

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