Original Article

# Assessment of the insulin resistance- insulin sensitivity and β cell function in type 2 diabetes with NAFLD associated with HFE gene mutation

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## Abstract

**Introduction:** Diabetes mellitus has reached pandemic proportion and in 2030, there will be approximately 366 million diabetics; of these 79 million will be from India. Diabetes mellitus is a group of metabolic diseases that includes hyperglycemia resulting from defects in insulin secretion, insulin action or both. Type 2 diabetes is characterized by insulin resistance and insulin deficiency (often relative rather than absolute). **Aims and Objectives:** To study Assessment of the insulin resistance- insulin sensitivity and β cell function in type 2 diabetes with NAFLD associated with HFE gene mutation. **Materials and Methods:** The work embodied in this study was conducted at tertiary care Hospital. It is a hospital based case control study. All the type 2 diabetic patients attending the OPD irrespective of their sex and treatment protocol were taken; it includes both old and new cases of type 2 diabetes. All the type 2 diabetic patients were subjected to ultrasonography to rule out the fatty liver. Type 2 DM with NAFLD was taken as cases and Type 2 DM without NAFLD was taken as controls. Biochemical parameters analysed were glucose, insulin, serum iron and total iron binding capacity. DNA was extracted from frozen whole blood to detect Cys282Tyr mutation.**Result:** In our study population out of 100 type 2 diabetes, 54 patients had fatty liver. The prevalence of fatty liver in our study group was 54%.In our study population, majority of the patients were in the age group of 51- 60 years in both cases and controls. Age distribution between cases and controls were matched (Chi square value 2.45 degree of freedom (df) is 3, p value = 0.48).NAFLD cases had more female patients (59.2%) than controls and it was statistically significant (Chi square value $x^2$= 4.94 and p<0.02). In our study population, mean fasting glucose in cases and controls (147.8 ± 44.6 and 138.5 ± 33.9 respectively) were not significantly different (p<0.5). Whereas post prandial glucose, fasting insulin, HOMA-IR and HOMA-β were significantly(p<0.5) higher in NAFLD patients. In comparison to controls, Insulin sensitivity index (QUICK) was statistically low(p< 0.05) in NAFLD patients. **Conclusion:** We observed significant increase in fasting insulin, beta-cell function (HOMA-β) and insulin resistance along with decreased insulin sensitivity in NAFLD patients.

**Key Words:** Insulin resistance- insulin sensitivity, β cell function, Type 2 diabetes, NAFLD, HFE gene mutation.

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## INTRODUCTION

Diabetes mellitus has reached pandemic proportion and in 2030, there will be approximately 366 million diabetics; of these 79 million will be from India [1].

Diabetes mellitus is a group of metabolic diseases that includes hyperglycemia resulting from defects in insulin secretion, insulin action or both. Type 2 diabetes is characterized byinsulin resistance and insulin deficiency (often relative rather than absolute) [2]. Rapid rise of diabetes, obesity and metabolic syndrome, in

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parallel with NAFLD have been identified worldwide [3]. NAFLD is a common hepatic disorder characterized by fat accumulation in the liver, identical to that seen in alcoholic fatty liver disease, but in patients who do not drink excessive alcohol [4]. NAFLD is defined as fat accumulation in the liver exceeding 5% to 10% by weight, but it is estimated practically as the percentages of fat-laden hepatocytes observed by light microscope [5].

NAFLD consists of a wide spectrum of liver abnormalities, ranging from simple steatosis to Non-alcoholicsteatohepatitis (NASH), can progress to cirrhotic and Hepatocellular carcinoma [6]. NASH was first reported by Ludwig and associates, in a series of patients, who had no significant history of alcohol intake [7].NAFLD is the most common liver disease responsible for significant progression to liver cirrhosis, portal hypertension, and need for liver transplantation in adults [8].Recent studies indicate that substantial fibrosis or liver cirrhosis varies from 15 to 50% of patients with NAFLD and in the long term follow up study, 30% of patients with fibrosis had cirrhosis after 10 years [9,10].NAFLD is mostly seen in obesity (60-95%), type 2 diabetes mellitus (28-55%) and hyperlipidemia (27-92%)[11,12]. The HFE gene is located at chromosome 6p 21.3, approximately 4.6 mega bases telomeric from HLA-A, and covers approximately 10 kilo bases .The HFE protein is a 343 residue type 1 transmembrane protein that associates with class I light chain beta2 microglobulin. The HFE product binds to the transferring receptor and reduces its affinity for iron loaded transferrin by 5 to 10 fold [13].The mutations in the HFE gene alter the affinity of the Transferrin Receptor (TFR), for its ligand Transferrin. Thus it attenuate uptake of transferrin-bound iron from plasma. Hepcidin is responsible for the down regulation of basolateral iron carrier ferroportin. It has been demonstrated that hepcidin is up regulated by HFE gene [14].

**Materials and Methods:** The work embodied in this study was conducted at tertiary care Hospital. It is a hospital based case control study. All the type 2 diabetic patients attending the OPD irrespective of their sex and treatment protocol were taken; it includes both old and new cases of type 2 diabetes. Patients with history of jaundice/ drug induced hepatitis, Patients receiving steroid, amiodarone, valporic acid or anti-epileptic drugs, iron etc., history of jejuno-ileal by-pass surgery and patient with alcohol consumption were excluded from the study. For all the patients detailed clinical history was elucidated, and general examination was done. All the type 2 diabetic patients were subjected to ultrasonography to rule out the fatty liver. Type 2 DM with NAFLD are taken as Casesand type 2 DM without NAFLD are taken as Controls. The serum collected was analyzed for the estimation of following biochemical parameters using auto analyzer ($Roche_R$ – Cobasmira™ S).Glucose – Glucose oxidase- Peroxidase method[15].Iron-Direct method (Ferene) and T.I.B.C – direct method (ferene). Insulin – Enzyme immunoassay [16].Insulin resistance was estimated by the homeostasis model assessment insulin resistance score (HOMA-IR score [17].HOMA-IR = fasting glucose(mmol/L) x fasting insulin(µIU/ml) /22.5 .Insulin sensitivity was calculated by using Quantitative Insulin.Sensitivity Check Index [18]; QUICK Index = 1 / log ($I_0$) + log ($G_0$))*i.e.* fasting insulin ($I_0$) and glucose ($G_0$); Beta-cell function using HOMA-β formula [19].HOMA-β = 20 x fasting insulin (µIU/ml) / fasting Glucose − 3.5 %. The primers for polymerase chain reaction (PCR) amplification of the Cys282Tyr mutation were [20]:Forward primer- 5' TGG CAA GGG TAA ACA GAT CC 3' Reverse primer- 5' CTC AGG CAC TCC TCT CAACC 3'.

## RESULT AND OBSERVATION

For present study, total of 100 consecutive type 2 diabetes mellitus patients without history of alcoholic intake were chosen. Informed verbal consent was taken from each patient and pre-designed pro-forma was filled in. The study was approved by ethical committee.All patients were submitted to an ultrasound scan of the liver to detect the fatty deposition. Biochemical parameters were analyzed. All patients were genotyped by polymerase chain reaction of the region that contained the C282Y mutation and digestion with Rsa 1 enzyme and 10% polyacrylamide gel electrophoresis was done. All quantitative data are presented as mean ± SD and qualitative data are presented as percentage. Statistical analysis was done by using SPSS (Version 17.0). Student's'2t' test for unpaired data were used for the comparison of mean values.p value less than 0.05 was considered statistically significant.

**Table 1:** Distribution of NAFLD in Type 2 Diabetes mellitus

| Type 2 DM with NAFLD (Cases) | Type 2 DM without NAFLD (Controls) |
|---|---|
| 54 | 46 |

In our study population out of 100 type 2 diabetes, 54 patients had fatty liver.

**Table 2:** Age distribution in cases and controls

| Age in years | Cases | Controls | Total |
|---|---|---|---|
| 30-40 | 5(9.3%) | 9(19.5%) | 14 |
| 41-50 | 17(31.4%) | 14(30.4%) | 31 |
| 51-60 | 25(46.3%) | 19(41.3%) | 44 |
| > 60 | 7(12.9%) | 4(8.7%) | 11 |
| **Total** | **54** | **46** | **100** |

In our study population, majority of the patients were in the age group of 51- 60 years in both cases and controls. Age distribution between cases and controls

were matched (Chi square value 2.45 degree of freedom (df) is 3, p value = 0.48).

**Table 3:** Sex distribution in Cases and Controls

| Sex | Cases | Controls | Total |
|---|---|---|---|
| Male | 22(40.7%) | 29(63.1%) | 51 |
| Female | 32(59.2%) | 17(36.9%) | 49 |
| **Total** | **54** | **46** | **100** |

In our study population, cases had more female patients (59.2%) than controls (36.9%) and it was statistically significant (Chi square value $x^2$= 4.94 and p<0.02).

**Table 4:** Markers of carbohydrate metabolism in cases and controls

| Parameters | Cases Mean±SD | Controls Mean±SD | p value |
|---|---|---|---|
| Fasting Glucose(mg/dl) | 147.8 ± 44.6 | 138.5 ± 33.9 | < 0.5 |
| Post prandial Glucose(mg/dl) | 270.7 ± 53.1 | 244.6 ± 41.61 | < 0.01* |
| Fasting Insulin(µIU/ml) | 56.4 ± 23.3 | 45.85 ± 19.76 | < 0.05* |
| Homa-IR | 20.9 ± 11.7 | 16.23 ± 9.5 | < 0.05* |
| Quick index | 0.26 ± 0.02 | 0.27 ± 0.02 | < 0.05* |
| Homa-β | 298.3 193.8 | 160.7 ± 98.8 | <0.05* |

In our study population, mean fasting glucose in cases and controls (147.8 ± 44.6 and 138.5 ± 33.9 respectively) were not significantly different (p<0.5). In contrast post prandial glucose, fasting insulin, HOMA-IR and HOMA-β were significantly higher in cases (p< 0.05) when compared to controls. Insulin sensitivity index (QUICKI) was significantly low in NAFLD cases in comparison to controls (p< 0.05).

**Table 4:** Serum Iron homeostasis in Cases and Controls

| | Serum Ironµmol/l Mean±SD | TIBC Mean±SD | Transferrin saturation Mean±SD |
|---|---|---|---|
| Cases | 16.1 ± 2.5 | 63.4 ± 8.1 | 25.9 ± 5.0 |
| Controls | 14.5 ± 3.0 | 65.5 ± 7.1 | 22.4 ± 4.9 |
| p value | < 0.01 | < 0.5 | < 0.01 |

Serum Iron and transferrin saturation were significantly raised (p<0.01) in cases. Total iron binding capacity values were not significantly different (p<0.5) between cases and controls. None of patients (both cases and controls) revealed C282Y HFE gene mutation in our study population.

## DISCUSSION

The various studies have been reported that prevalence of NAFLD in type 2 diabetes varies from 35 to 75%[21,22]. In this present study the prevalence of NAFLD in type 2 diabetes was 54%. Gupte *et al* [23] from India had reported 49% of NAFLD in type 2 diabetes. Similarly, Luxmi *et al*[24] in Pakistan and Akber and Kawther *et al*[25] in Saudi Arabia had reported 60% and 55% respectively.

Ludwig *et al*[7], a pioneer in this field had noted that females are more frequently affected by NASH. More recent studies have shown that men and women are equally affected by it; in addition even higher involvement of men also been noted. In our study females had higher prevalence of NAFLD i.e. 59% in females and 41% in males and it was statistically significant (p<0.05). Significant increased beta-cell function (HOMA-β), fasting insulin and insulin resistance along with decreased insulin sensitivity were observe din NAFLD patients. There are many studies which go with these findings. A study done in 495 non-diabetics, non-alcoholic subjects by Mishra *et al* [26] in India showed that the prevalence of NAFLD increased with insulin resistance. A study report by Goritsas *et al* [27] on a group of Greek patients also confirms that NAFLD is strongly associated obesity, impaired glucose metabolism- hyperinsulinemia and insulin resistance.

In addition to other factors like insulin resistance, iron may also play a role in the progression of NASH by inducing oxidative stress. Although both serum iron and transferrin saturation were within in the normal reference range, NAFLD cases had significantly increased serum iron and transferrin saturation when compared to controls. Salonen *et al* [28] study supports the theory that increased iron stores, even in the range not considered to be associated with hemochromatosis, contribute to the development of type 2 diabetes.

More than 80% of hemochromatosis patients (HH) in populations of European origin are homozygotes for a single mutation C282Y or compound heterozygotes for C282Y and H63D mutations in the HFE gene. In contrast, none of our patients revealed HFE gene C282Y mutation. This finding was consistent with other studies. Ajay Duseja *et al*[29] result also do not favour iron overload and HFE gene mutations as major factors in the pathogenesis of NASH in Asian Indians.

## CONCLUSION

We observed significantly increased fasting insulin, beta-cell function (HOMA-β) and insulin resistance in addition todecreased insulin sensitivity in NAFLD patients.Among both cases and controls, none of them had HFE gene mutation.

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