Original Article

Assessment of the risk of NASH with clinical predictor score associated with HFE gene mutation

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Abstract

Introduction: NAFLD is becoming a major public problem worldwide. NAFLD is an emerging problem in the Asia-Pacific region and its overall prevalence at present is broadly similar to west. Based on surveys using ultrasonography, the prevalence of NAFLD in the general population across Asia varies from 5% to 40%. **Aims and Objectives:** To Assess the risk of NASH with clinical predictor score associated with HFE gene mutation. **Materials and Methods:** The work embodied in this study was conducted at tertiary care Hospital. 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 parameter analyzed are fasting glucose, fasting insulin, serum iron, T.I.B.C and ALT.DNA was extracted from frozen whole blood to detect Cys282Tyr mutation. Based on HAIR score, the NASH was assessed in our NAFLD patients. **Result:** In our study population out of 100 type 2 diabetes patients, 54 of them had fatty liver. 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). Fasting insulin and insulin resistance were significantly higher in cases (p< 0.05).Serum Iron and transferrin saturation were significantly raised (p<0.01) in cases when compared to controls. NAFLD patients had significantly higher ALT levels. In our study, 27.8% of patients had NASH based on HAIR score. HFE gene C282Y mutation was not found in our study population. **Conclusion:** Based on HAIR score, 27.8% of NAFLD patients might have NASH. NAFLD patients had significantly increased serum iron and transferrin saturation when compared to controls. None of our patients had HFE mutation.

Keywords: Non-alcoholic steatohepatitis (NASH), HFE gene mutation, NAFLD (Non Alcoholic Fatty Liver Disease).

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INTRODUCTION

NAFLD is a clinicopathological condition that comprises a wide spectrum of conditions characterized by macrovesicular hepatic steatosis in the absence of significant alcohol intake (less than 20 gm/day for males and less than 10 gm/day for females. NAFLD is becoming a major public problem worldwide. Kareem *et al*[1] pointed out that NAFLD is an emerging problem in the Asia-Pacific region and its overall prevalence at present is broadly similar to west. Based on surveys using ultrasonography, the prevalence of NAFLD in the general population across Asia varies from 5% to 40%[2,3]. In India, the community prevalence of NAFLD varies from 5% to 28%[4,5]. Among the obese persons the prevalence of NAFLD rises from 57% to 74% and 25% to 75% among obese diabetics. Among all the subjects of NAFLD, features of NASH can be seen in 10-20%. It has been suggested that NASH accounts for more than 58% of cryptogenic cirrhosis[6]. Fatty liver is the earliest and most prevalent stage of NAFLD. Non-alcoholic steatohepatitis (NASH) is the most extreme form of NAFLD, which is regarded as a major cause of hepatic cirrhosis of unknown cause. However, the pathophysiology that leads to NAFLD is yet not well understood. The leading proposal for the pathogenesis of NASH is a "2-hit" hypothesis [7].In 1998, Day and James [8] first proposed the 'two hit' hypothesis for pathogenesis of NASH. Many studies have reported that oxidative stress phenomenon may induce a number of pathophysiological events in the liver.

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Oxidative stress induced Hepatotoxicity may be achieved through a direct attack of Reactive oxygen species (ROS) and Reactive nitrogen species (RNS)[9]. One of the potential cofactors suspected to enhance the oxidative stress is excessive hepatic iron accumulation. The Hemochromatosis (HFE) gene is one of the genetic modifiers of NAFLD. Homozygosity for the C282Y mutation of the HFE gene is associated with susceptibility to iron overload. HFE protein consists of extra cellular α_1 and α_2 domains that sit on top of the immunoglobulin like α_3 domain, which spans the cell membrane and binds a separate protein called $\beta_2$microglobulin[10]. The α_1 and α_2 domains interact with transferrin receptor, a transmembrane protein which plays a very important role in iron uptake regulation. Hepcidin is a master regulatory peptide, which plays an important role in the iron metabolism. It is secreted mainly by hepatocytes in response to iron perturbations, inflammation and hypoxia. HAMP is the gene that encodes for hepcidin. It is a master regulator of iron hemostasis. Hepcidin exerts its regulatory function on iron hemostasis via binding to FP-1, thereby leading to FP-1 phosphorylation, internalization, degradation and thus blockage of cellular iron export[11].Important upstream regulators of hepcidin expression include hemojuvenile (HJV)[21], a bone morphogenetic protein co-receptor, HFE[13], a non-classical MHC class 1 molecule and TFR-2[14], a liver specific iron uptake molecule. Mutations of these genes are associated with inappropriately low hepcidin formation and hereditary iron overload syndrome.

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 are taken as Cases and type 2 DM without NAFLD are taken as Controls. 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 surgeryand patient with alcohol consumption were excluded from the study. For all the patients detailed clinical history was elucidated, and general examination was doneand blood pressure was measured. The serum collected was analyzed for the estimation of following biochemical parameters using auto analyzer (Roche$_R$ – Cobasmira™ S). Glucose – Glucose oxidase- Peroxidase method. Insulin – Enzyme immunoassay. Iron-Direct method (Ferene) and T.I.B.C – direct method (ferene). Alanine aminotransferase (ALT) – IFCC Method. The primers for polymerase chain reaction (PCR) amplification of the Cys282Tyr mutation were [15]: Forward primer- 5' TGG CAA GGG TAA ACA GAT CC 3' Reverse primer- 5' CTC AGG CAC TCC TCT CAACC 3'. Insulin resistance was estimated by the homeostasis model assessment insulin resistance score (HOMA-IR score. HOMA-IR = fasting glucose(mmol/L) x fasting insulin(μIU/ml) /22.5. HAIR Score for prediction NASH was calculated [16]. HAIR Score: Hypertension, Alanine transaminase (ALT) > 40 IU/L, Insulin resistance (IR) index > 5. Presence of 2 or 3 factors predicts NASH.

RESULT AND OBSERVATION

For our 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 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. All patients were genotyped by polymerase chain reaction of the region that contained the C282Y mutation and digestion with Rsa 1 enzymeand 10% polyacrylamide gel electrophoresis was done.

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. The prevalence of fatty liver in our study group was 54%.In our study population, cases had more female patients (59.2%) than controls (36.9%) and it was statistically significant (Chi square value x2= 4.94 and p<0.02).

Table: 2. Distribution of blood pressure in cases and controls

	Cases (n = 54) Mean±SD	Controls (n = 46) Mean±SD	p value
Systolic blood pressure (mmHg)	127.2 ± 6.7	127 ± 1.7	> 0.5
Diastolic Blood pressure (mmHg)	79.5 ± 8.9	78.5 ± 9.3	> 0.5

Mean Systolic and diastolic blood pressure were also similar in both cases and controls (p> 0.5).

Table 3: Fasting glucose and fasting insulin 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
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*

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). Fasting insulin and HOMA-IR were significantly higher in cases (p< 0.05) when compared to controls.

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 when compared to controls. Total iron binding capacity values were not significantly different (p<0.5) between cases and controls.

Table 5: ALT in case and control

	Control	Cases	p value
ALT (U/L) Mean±SD	26.2 ± 16.0	34.8 ± 23.6	< 0.05

NAFLD patients had significantly higher ALT levels in comparison to controls.

Table 4: Assessing the risk of NASH by using HAIR score

Fatty liver patients	NASH patients
54	15(27.8%)

Based on HAIR score, the NASH was assessed in our NAFLD patients. The presence of at least 2 parameters predicted NASH with high sensitivity and specificity. Out of 54 patients, 15 of them had at least two parameters of HAIR score and it denotes 27.8% might have NASH.

DISCUSSION

Non-alcoholic fatty liver disease is an emerging problem in Asians plausibly due to the higher prevalence of type 2 diabetes in Asia-pacific region. In this present study the prevalence of NAFLD in type 2 diabetes was 54%. The various studies have been reported that prevalence of NAFLD in type 2 diabetes varies from 35 to 75%. Gupte *et al* [17] from India had reported 49% of NAFLD in type 2 diabetes. Similarly, Luxmi *et al* [18] in Pakistan and Akberand Kawther *et al* [19] in Saudi Arabia had reported 60% and 55% respectively. Ludwig *et al*[20], a pioneer in this field had noted that females are more frequently affected by NASH. In our study, females had higher prevalence of NAFLD i.e. 59% in females and it was statistically significant (p<0.05). The recent hypothesis for NAFLD pathogenesis considers that insulin resistance may be involved in both onset of fatty liver and in disease progression[21] and there are Studies that have shown the inextricable relationship between NAFLD and central obesity, insulin resistance and metabolic syndrome[22,23],which favors our study results. Significant increase in fasting insulin and insulin resistance were observed in our NAFLD patients. Studies have shown that elevated liver enzymes are the most common laboratory findings in NAFL[24], with often mild increases of both alanine aminotransferase and aspartate aminotransferase, although occasionally elevation can be up to 15 times the upper normal limit. But significantly an elevated level of ALT was found in our NAFLD patients. In present study, both NAFLD and controls had serum iron and transferrin saturation within in the normal reference range. But interestingly, NAFLD patients had significantly increased serum iron and transferrin saturation when compared to controls. Number of studies revealed HFE C282Y mutation is almost always associated with increased iron indices [25]. Whereas in our study group, none of them had HFE C282Y mutation and primary iron overload. There are few studies, which result does not favour iron overload and HFE gene mutations as major factors in the pathogenesis of NASH in Asian Indians. Hanson *et al* had reported that among the majority of Asian, Indian subcontinent, African, Australian and Amerindian populations, frequencies of C282Y mutation are close to zero[26]. Based on HAIR score [16], the NASH was assessed in our NAFLD patients. HAIR score was described in early work of Dixon and colleagues in a group of 105 severely obese patients undergoing gastric bypass surgery. The presence of at least two parameters predicted NASH with high sensitivity and specificity. In our study, out of 54 fatty liver patients, 15 of them had at least two parameters of HAIR score i.e. 27.8% of patients might have NASH in our study population.

STUDY LIMITATIONS

The diagnosis of NAFLD in our study was based on ultrasonography. No imaging modality can distinguish NASH from simple steatosis. However, ultrasonography is by for the commonest way of diagnosing NAFL and sensitivity of ultrasonography for diagnosing steatosis was 89% with specificity of 93% [27, 28]. Liver biopsy is considered the gold standard for the definitive diagnosis

of NAFLD. It is the only way to confirm the presence or absence of NASH in a person with features of NAFLD[29].

CONCLUSION

Based on HAIR score 27.8% of NAFLD patients might have NASH among our study population. NAFLD patients had significantly increased serum iron and transferrin saturation when compared to controls. None of our patients had HFE (C282Y) mutation.

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