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CLINICAL & BASIC RESEARCH

Non-Alcoholic Fatty Liver Disease (NAFLD) - Is it an Emerging Risk Factor for Coronary Artery Disease?

Preliminary study in a local Indian population

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مرض الكبد الدهني غير الكحولي - هل هو عامل اختطار لمرض الشريان التاجي؟

دراسة أولية لمجموعة من سكان الهند

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الملخص: الهدف: تحديد وجود مرض الكبد الدهني غير الكحولي عند مرضى الشريان التاجي . الطريقة: تم اختيار 149 مريضا أحيلوا إلى معهد القلب في منطقة باجلور بالهند. في الفترة ما بين يناير 2007 إلى يونيو 2009. وتم تشخيصهم بإصابتهم بمرض الشريان التاجي . أربعة مرضى لم يشاركوا في الدراسة. تم أخذ عينات دم وريدي من المرضى المشاركين ومن عينة ضابطة (100 شخصا) من الذين ترددوا لأداء الفحص الطبي الدوري .متوافقة أعمارهم مع المرضى. كذلك تم عمل فحص روتيني لوظائف الكبد شاملا أنزيمات ناقلات الأمين وإنزيم المقايسة المناعية لمثبط منشط البلازمينوجين (I) والبروتين المتفاعل (ج) وعامل نخر الورم (ألفا) .وباستخدام تَخْطيطُ الصَّدَى وقياس مستوى أنزيم ناقلة أمين الالانين في المصل تم تحديد وجود مرض الكبد الدهني غير الكحولي عند مرضى الشريان التاجي. النتائج: وجد لدى مرضى الشريان التاجي الذين يعانون من مرض الكبد الدهني غير الكحولي مستويات مرتفعة من أنزيمات الكبد مع ارتفاع طفيف بمستوى (AIC) مقارنة بمستويات العينة الضابطة. كانت مستويات عامل نخر الورم (ألفا) وكذلك إنزيم المقايسة المناعية لمثبط منشط البلازمينوجين (I) أعلى لدى مرضى الشريان التاجي والكبد الدهني غير الكحولي مقارنة بمستويات العينة الضابطة بدرجة معتدة إحصائيا ($P<0.1$,$P<0.05$) في كل من المرضى الإناث والذكور.كان مستوى البروتين المتفاعل (ج) ($P<0.01$) في المجموعتين) وكذلك حمض اليوريك أعلى في المجموعتين من المرضى ($P<0.05$,$P<0.01$) في المرضى الذكور والإناث على التوالي). كما كانت مستويات الاديبونكتين منخفضة بشكل كبير في المرضى مقارنة بالعينة الضابطة ($P<0.05$, $P<0.001$) في المرضى الذكور والإناث على التوالي). الخلاصة: زيادة مستوى كل من إنزيم المقايسة المناعية لمثبط منشط البلازمينوجين (I) وعامل نخر الورم (ألفا) في المصل عكس حالة الاستعداد للالتهاب في هؤلاء المرضى والذي قد يكون بسبب وجود مرض الكبد الدهني غير الكحولي. وهذا قد يساهم بشكل إضافي في نشوء أمراض القلب والأوعية الدموية.

مفتاح الكلمات: مرض الكبد الدهني غير الكحولي. اديبو نكتين. مقاومة الأنسولين. متلازمة الايض. مرض الشريان التاجي .

ABSTRACT: ***Objectives:*** The objective of this study was to identify the presence of non-alcoholic fatty liver disease (NAFLD) in patients with coronary artery disease (CAD). ***Methods:*** 149 patients were selected, who had been referred to the Institute of Cardiology, Banglore, India, between January 2007 and June 2009 and diagnosed with CAD. Four patients did not participate in the study. Venous blood samples were taken from these cases, and age-matched healthy controls who came for a master health check-up (N = 100). All were subjected to routine liver function tests including serum transaminases, enzyme immunoassays for plasminogen activator inhibitor I (PAI-I), C reactive protein (CRP), and tumour necrosis factor-alpha (TNF-α). Using ultrasonography and serum alanine aminotransferase (ALT) levels, the presence of NAFLD in CAD patients was reported. ***Results:*** CAD patients with NAFLD had significantly higher liver enzymes and marginally higher A1C levels compared to control subjects. Levels of TNF-α and PAI-I were higher in CAD patients with NAFLD compared to both female and male controls (P <0.1 and P <0.05). Levels of CRP (P <0.01 in both groups) and uric acid were increased in both group of patients (P <0.05 and P <0.01 in male and female patients, respectively). Levels of adiponectin were significantly reduced in the patients compared to the controls (P <0.05 and P <0.001) in male and female patients respectively. ***Conclusion:*** The increased serum levels of PAI-I and TNF-α reflected the proinflammatory status in these CAD patients which may be due to the presence of NAFLD. This could contribute additively to the development of cardiovascular events (CVD).

Keywords: Non-alcoholic fatty liver disease; Adiponectin; Insulin resistance; Metabolic syndrome; Coronary artery disease.

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Advances in knowledge

1. *This study clarifies and contributes to the hypothesis that non-alcoholic fatty liver disease (NAFLD) could be a risk factor for coronary artery disease (CAD).*
2. *The release of proinflammatory markers like tumour necrosis factor alpha (TNF-alpha) and plasminogen activator inhibitor- I (PI-I) may be causative factors that may initiate or contribute to the precipitation of coronary vascular events.*
3. *The study confirms this supposition reflected by the increased serum levels of serum TNF-alpha and PAI-I in CAD patients with NAFLD.*
4. *The study points out that every region and race must have their physical as well as biochemical parameters standardised for early detection and prevention of obesity related disorders*

Application to patient care

1. *The study indicates that it would be prudent to look for markers other than lipid or diabetic profiles in patients with marked visceral obesity susceptible to coronary artery disease.*
2. *It is shown that in India the prevalence of diabetes is very high due to obesity. It is known that a body mass index (BMI) of >25 is considered to be high for the Indian population, but considered normal for Western populations. Therefore, it is necessary to have a standardised record of BMI for all populations.*
3. *It may be helpful to estimate serum aminotransferases to identify the presence of NAFLD in obese patients along with impaired glucose tolerance and hypertriglyceridemia (excluding other causes of fatty liver disease).*

Metabolic syndrome is frequently associated with a hypercoagulable condition, in that the coagulation system is switched towards a prothrombotic state, involving increased plasmatic coagulation, reduced fibrinolysis, decreased endothelial thromboresistance and predominantly platelet hyperactivity.[1-6] All of these abnormalities in the coagulation and fibrinolytic systems may contribute to the development of cardiovascular complications in patients with metabolic syndrome.

Now, there is growing evidence that non-alcoholic fatty liver disease (NAFLD), a hepatic manifestation of the metabolic syndrome,[7-8] is strongly associated with a systemic pro-inflammatory/procoagulant state, independently of shared cardiometabolic risk factors. Recently, we have shown that in non-alcoholic steatohepatitis (NASH) patients there is an increase in the levels of acute inflammatory markers, including C-reactive protein (CRP).[9] There is evidence from another study that NASH is not simply a marker of the prothrombotic state in the metabolic syndrome, but is directly involved in its pathogenesis, possibly through the systemic release of proinflammatory and procoagulant factors from the steatotic/inflamed liver.[10]

Therefore, the present study was undertaken to evaluate the levels of plasminogen activator inhibitor-I (PAI-I), tumour necrosis factor-α (TNF-α), along with CRP in NAFLD patients in a local population in India.

Methods

The patients for the present study were among those referred between January 2007 and June 2009 to the Institute of Cardiology, Banglore, India. A total of 149 patients with coronary artery disease (CAD) and NAFLD and 124 age-matched healthy control subjects who came for a master health check-up (N = 124) were selected for the present study. The presence of NAFLD in CAD cases was identified by serum alanine transaminase levels, serum aspartate aminotransferase/serum alanine aminotransferase (AST/ALT) ratio <1 and ultrasonographic findings. These cases were subjected to the following analyses. Venous blood was drawn in the morning after an overnight fast. Plasma liver function tests and other biochemical blood measurements were determined by standard laboratory procedures including enzyme immunoassays for PAI-I and TNF-α. All participants had negative serology for hepatitis B or C. Levels of low density lipoprotein (LDL) cholesterol was calculated by Friedwald's equation. HbA1c (A1C) was measured by a high-performance liquid chromatography analyser (HA-8140, Menarini Diagnostics, Florence, Italy); the upper limit of normal for the laboratory was 5.9%. The respective intra- and inter-assay coefficients of variation (CVs) for the assays were: fasting plasma glucose (FPG) 1.4 and 2.0%; total cholesterol (TC) 1.2% and 2.1%; triglyceride (TG) 1.5% and 3.6%; HDL-C 2.8% and 3.5%; PAI-I 2.5% and 6.5%; TNF-alpha 3.5% and 6.65%; HbA1C 1.9% and 2.1%.

The BMI was calculated by dividing weight in kilogrammes by height in metres squared. Waist

Table 1: Basic characteristics of males with NAFLD

	Normal (N = 63)	CAD Patients with NAFLD (N = 69)	*P*
Age (years)	47.7 ± 3.6	48.1 ± 14.6	0.982
Weight kg	66.1 ± 7.8	79 ± 11.9	<0.0001
BMI (kg/m²)	22.7 ± 2.4	27.8 ± 3.4	<0.0001
Waist-to-hip ratio (cm/cm),	0.87 ± 0.04	0.94 ± 0.04	<0.0001
Body fat mass (kg)	12.5 ± 3.4	21.3 ± 6.3	<0.0001
Systolic blood pressure (mmHg)	113.6 ± 13.4	127.9 ± 16.2	<0.0001
Diastolic blood pressure (mmHg)	69.6 ± 11.4	78.8 ± 11.8	0.002
Triglyceride (mg/dl)	123.6 ± 63.9	181.8 ± 92.1	0.004
HDL cholesterol (mg/dl)	47.1 ± 9.1	41.0 ± 7.9	0.005
HOMA	1.49 ± 0.71	3.04 ± 1.6	<0.0001
Uric acid (mg/dl)	5.6 ± 1.2	7.4 ± 1.7	0.05
ALT (IU/I)	20.7 ± 9.3	42.9 ± 29.1 ,	<0.0001
AST (IU/I)	19.7 ± 4.3	28.4 ± 14.2	<0.001
AST/ALT ratio	>1	<1	
CRP (mg/l)	3.72 ± 2.11	8.48 ± 2.75	<0.01
PAI-I ng%	3.6 ± 1.2	7.9 ± 2.3	<0.01
TNF- α pg/ml%	6.66 ± 0.26	8.92 ± 0.44	<0.05
Adiponectin (ug/ml)	6.2 ± 3.4	4.5 ± 2.8	<0.05

Legend: NAFLD = Non-alcoholic fatty liver disease; CAD = coronary artery disease; BMI = body mass index; HDL = high density lipoproteins; HOMA = homeostasis model assessment; AST = serum aspartate aminotransferase; ALT = serum alanine aminotransferase; CRP = C reactive protein; PAI-I =plasminogen activator inhibitor I; TNF-α = tumor necrosis factor-alpha.

circumference was measured in a standing position at the level of the umbilicus. Blood pressure was assessed with a standard mercury manometer. Metabolic syndrome was defined according to criteria proposed by the National Cholesterol Education Program Adult Treatment Panel III (ATP III).[11]

Hepatic ultrasound scanning was performed in all participants by a trained operator who was blind to all clinical and laboratory characteristics of participants, using an Acuson 128-XP/10 scanner with a 3.5-MHz linear transducer. Hepatic steatosis was diagnosed by characteristic echo patterns, according to conventional criteria, i.e. evidence of diffuse hyperechogenicity of liver relative to kidneys, ultrasound beam attenuation, and poor visualisation of intrahepatic structures.[12-14] Repeated measurements (that were performed on a subgroup of 100 patients) on the same subjects gave intra- and inter observer coefficients of variation within 5%.9 The Ethics Review Committee of the Institute of Cardiology, Banglore, India approved the study project.

Data are presented as the means ± standard devitaion (SD) or frequencies. Skewed variables (serum triglyceride and liver enzyme levels) were logarithmically transformed to improve normality before analysis. Statistical analyses included the unpaired T test and the $\chi 2$ test with Yates' correction for continuity (for categorical variables).

Results

The clinical and biochemical characteristics of participants are shown in Table 1 for male and Table 2 for female subjects respectively. Because of the study design, case and control subjects were almost identical in terms of gender and age. The CAD patients with NAFLD had significantly higher liver enzymes and marginally higher A1C levels compared to control subjects. The proportion using antihypertensive drugs was higher among case subjects (~72 versus ~58%), whereas the proportion taking lipid-lowering or antiplatelet drugs was essentially similar in both male and female groups (~35 and ~50%, respectively). CAD patients with NAFLD were older ($P < 0.001$), had higher liver enzymes ($P < 0.001$), and some tended to have a longer duration of diabetes (5.5 ± 2.5 years duration, $P = 0.064$). Gender, smoking status, LDL cholesterol, A1C, and diabetes treatment did not differ between the groups (not shown).

The levels of TNF-α and PAI-I were higher in CAD patients with NAFLD compared to normal control subjects (P <0.1 and P <0.05) in both female and male patients. The levels of CRP and uric acid were increased in both groups of patients (P <0.05

Table 2: Basic characteristics of females with NAFLD

	Normal (N = 61)	CAD patients with NAFLD (N = 76)	*P*
Age (years)	47.1 ± 11.5	55.6 ± 8.5	-
Weight kg	55.8 ± 6.4	67.2 ± 8.9	<0.0001
BMI (kg/m²)	22.6 ± 2.5	27.1 ± 2.9	<0.0001
Waist-to-hip ratio (cm/cm),	0.87 ± 0.05	0.94 ± 0.05	<0.0001
Body fat mass (kg)	16.3 ± 4.3	23.7 ± 4.3	<0.0001
Systolic blood pressure (mmHg)	116.8 ± 16.7	124.7 ± 13.6	0.0008
Diastolic blood pressure (mmHg)	71.5 ± 11.6	76.7 ± 10.4	0.015
Triglyceride (mg/dl)	98.7 ± 53.7	150.0 ± 73.2	<0.0001
HDL cholesterol (mg/dl)	57.2 ± 11.4	47.8 ± 9.0	<0.0001
HOMA	1.46 ± 0.8	2.56 ± 1.1	<0.0001
Uric acid (mg/dl)	4.55 ± 2.40	7.55 ± 2055	<0.001
ALT (IU/I)	16.6 ± 6.8	68.0 ± 19.7	<0.0001
AST (IU/I)	20.0 ± 5.9	45.0 ± 10.7	<0.001
AST/ALT ratio	-	<1	-
CRP (mg/l)	3.22 ± 1.11	7.80 ± 2.75	<0.01
PAI – I ng%	3.80 ± 1.2	6.90+2.3	<0.01
TNF- α pg/ml%	6.86+0.26	8.92+0.44	<0.05
Adiponectin (ug/ml	10.6 ± 4.8	7.2 ± 3.5	<0.0001

Legend: NAFLD = Non-alcoholic fatty liver disease; CAD = coronary artery disease; BMI = body mass index; HDL = high density lipoproteins; HOMA = homeostasis model assessment; AST = serum aspartate aminotransferase; ALT = serum alanine aminotransferase; CRP = C reactive protein; PAI-I = plasminogen activator inhibitor I; TNF-α = tumor necrosis factor-alpha.

and $P<0.01$ in male and female patients, respectively). The levels of adiponectin were significantly reduced in the patients compared to the control subjects ($P<0.05$ and $P<0.001$ in male and female patients respectively.)

Discussion

The biological mechanisms by which NAFLD could contribute to accelerated atherosclerosis are still poorly known. Previous data suggest that the relationship between NAFLD and increased risk of CAD is mostly reflected by hypertension and dyslipidemia.[8,15,16] In this study, CAD patients with NAFLD show an increase in proinflammatory markers TNF-α, CRP and PAI-I which may have additively contributed to future cardiovascular disease (CVD) events. This may be independent of metabolic syndrome and classic risk factors.[10,11] Conversely, several studies have consistently documented that insulin resistance predicts the incidence of CVD events[10,11] and plays a pivotal role in the development of poor clinical outcomes in NAFLD patients.[12-14] Thus, NAFLD in its more advanced forms might act as a stimulus for further increased whole-body insulin resistance and dyslipidemia, leading to accelerated atherosclerosis. This hypothesis is also partly validated by recent prospective studies demonstrating that raised liver enzymes independently predict the development of type 2 diabetes and other metabolic syndrome features.[8,10,18]

Another possible atherogenic mechanism linking NAFLD and increased CVD risk is represented by increased oxidative stress and subclinical inflammation, which are thought to be causal factors in the progression from simple steatosis to more advanced forms of NAFLD.[1,12,24] Reactive oxygen species derived from steatosis-stimulated fatty acid oxidation, attendant hepatocyte injury and cytokine release, and the ensuing proinflammatory milieu are likely to perpetuate the liver damage of NAFLD and add further atherogenic stimuli to the already high oxidative/proinflammatory status that is closely related to the metabolic syndrome.[8,10] In the present study, significant associations of NAFLD with impaired fibrinolytic activity were evidenced by increased levels of PAI-I and elevated plasma C-reactive protein; these associations were independent of age, BMI, blood pressure, lipids, and insulin resistance.[20] Decreased plasma levels of adiponectin, which is an adipose-secreted cytokine with antiatherogenic properties,[21] may represent another possible mechanism linking NAFLD and CVD risk. It is shown that hypoadiponectinemia is closely correlated to NAFLD in obese individuals, independent of insulin resistance and other metabolic syndrome variables.[22] Finally, accumulating evidence also exists that NAFLD

could be linked to accelerated atherogenesis through the presence of abnormal lipoprotein metabolism. In NAFLD, hepatic apolipoprotein B-100 synthesis, a rate-determining step in hepatic very-low-density lipoprotein (VLDL) formation and in hepatocyte lipid export, is markedly reduced, and postprandial apolipoprotein B-100 responses are flat and strikingly dissociated from the concomitant increases of postprandial triglycerides.[23,24] Disturbances of VLDL assembly are an important factor in the natural history of NAFLD and can also result in increased levels of atherogenic triglyceride- and cholesterol-rich remnant particles.[1,10,11] Small dense LDL particles, which are thought to be more atherogenic,[8,10] could also be increased in NAFLD patients. However, detailed compositional lipoprotein studies should be performed in patients with NAFLD to prove this contention.

The diagnosis of NAFLD was based on ultrasonography and exclusion of known aetiologic factors of chronic liver disease and serum transaminases levels. Indeed, it has been reported that the presence of >33% fat on liver biopsy is optimal for ultrasound detection of steatosis, although ultrasonography is not completely sensitive, particularly when hepatic fat infiltration is <33%.[26]

Currently, it will be worthwhile to study whether improving NAFLD will ultimately prevent the development of CVD. However, it is notable that interventions that are known to be effective in preventing CVD among type 2 diabetic patients, including weight reduction and treatment with insulin-sensitising oral antidiabetic agents, may also improve NAFLD.[1,8,14]

The fact that the study was done on a representative sample of CAD patients with low reported alcohol intake and with no exposure to hepatotoxic chemical or viral agents, and that a liver ultrasound for the diagnosis of NAFLD was performed on all participants, could enhance the validity of the present findings.

Conclusion

In conclusion, the present study was done on proven CAD patients with NAFLD to show the presence of NAFLD in CAD patients. The increased levels of TNF-α, PAI-I coupled with reduced adiponectin levels in CAD patients with NAFLD, along with dyslipidemia, establishes the fact that NAFLD could be an additive risk factor for abnormal coronary vascular events. It is to be noted that the average age of CAD with NAFLD patients was below fifty years in the males and above fifty years in the females.[27] The lowered levels of adiponectin along with an increase in TNF-alpha may be aggravating insulin resistance in these patients. Further studies are being carried out with more patients to understand the possible link of NAFLD to atherogenic mechanisms.

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