

Association of hypoadiponectinemia with non-alcoholic fatty liver disease in urban south Indians - (CURES - 81)

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Background & objective: Association between adiponectin and non-alcoholic fatty liver disease (NAFLD) has been reported in west. Studies in Indian population are lacking. This study was undertaken to assess the association of hypoadiponectinemia with NAFLD in Asian Indians.

Method: In this cross-sectional study, subjects were randomly selected from Phase 5 of the Chennai Urban Rural Epidemiology Study (CURES), an epidemiological study based on a representative population of Chennai in south India. One hundred twenty one subjects without NAFLD and 72 subjects with NAFLD were selected. NAFLD was diagnosed by ultrasonography. Serum adiponectin levels were measured using radioimmunoassay. Insulin resistance was calculated using Homeostasis Assessment model (HOMA-IR).

Results: Serum adiponectin values were significantly lower in subjects with NAFLD compared to those without [5.6 µg/ml (95% Confidence Interval (CI) 5.0 - 6.3 µg/ml] vs 7.4 µg/ml (95% CI: 6.7 - 8.1 µg/ml, $P<0.01$). Adiponectin levels decreased with increasing severity of NAFLD. Subjects with moderate to severe steatosis had significantly lower adiponectin levels (5.1 µg/ml, 95% CI: 4.1- 6.4 µg/ml) compared to subjects with mild steatosis (5.9 µg/ml, 95% CI: 5.0 - 6.9 µg/ml; $P<0.001$) and subjects without NAFLD (7.3 µg/ml, 95% CI: 6.6 - 8.0 µg/ml; $P<0.01$). Multiple logistic regression analysis revealed adiponectin to be negatively associated with NAFLD [Odds Ratio (OR): 0.865, 95% Confidence Interval (CI): 0.792- 0.944, $P=0.001$]. This remained statistically significant even after adjusting for confounding factors age, gender, body mass index, insulin resistance, waist circumference, total cholesterol, triglycerides and glucose intolerance (OR: 0.873, 95% CI: 0.793 - 0.961; $P=0.005$).

Interpretation & conclusion: NAFLD is associated with lower serum adiponectin levels independent of conventional cardiovascular risk factors in Asian Indians known to have high prevalence of diabetes and coronary artery disease.

Key words Adiponectin - Asian Indians - diabetes - fatty liver - non alcoholic fatty liver disease - South Asians

Non alcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic elevation of hepatic enzymes in the general population. This

clinicopathological syndrome has been increasingly recognized as a major cause of liver-related morbidity and mortality¹. NAFLD has been reported to be closely

associated with obesity, dyslipidaemia, and diabetes². In prospective studies, it has been shown that NAFLD is a risk factor for type 2 diabetes and cardiovascular disease independent of the classic risk factors³. NAFLD is considered to be a hepatic manifestation of the metabolic syndrome (MS)⁴. However, the pathophysiological link between NAFLD and the metabolic syndrome is not completely understood. We have shown that NAFLD is present in one third of urban Asian Indians and that its prevalence increases with increasing severity of glucose intolerance and also in those with MS⁵. Earlier studies have suggested that Asian Indian subjects with NAFLD had lower body mass index, lower prevalence of diabetes and metabolic syndrome compared to that reported in the west⁶ and that NAFLD is associated with multiple features of metabolic syndrome^{7,8}.

Adipose tissue secretes various biologically active substances, known as adipocytokines, which include adiponectin, leptin, resistin and tumour necrosis factor- α [TNF- α]. These adipocytokines may directly be responsible for various metabolic and vascular diseases⁹. Of the adipocytokines, adiponectin is of special interest. Unlike other adipocytokines, adiponectin is paradoxically reduced in those with obesity. Hypoadiponectinaemia has been observed in subjects with type 2 diabetes and coronary artery disease¹⁰. Our group has earlier shown that a lower adiponectin level was associated with the metabolic syndrome, diabetes, insulin resistance and dyslipidaemia¹¹. A specific role for adiponectin in the liver has also been suggested. Adiponectin levels correlate inversely with hepatic fat and hepatic insulin resistance in subjects with diabetes¹², and it decreases hepatic glucose production¹³, reduces free fatty acid turnover¹⁴ and may have a protective role during liver injury in mice¹⁵. Studies have also documented that hypoadiponectinaemia is associated with increased fat content and more extensive necroinflammation in subjects with NAFLD¹⁶.

Although studies have reported on the association of adiponectin with NAFLD in the west³, such studies are not available in Asian Indians. Asian Indians are known to have very high prevalence of diabetes and premature CAD compared to Europeans. This is mainly attributed to the so-called "Asian Indian Phenotype"¹⁷ characterized by relatively lower prevalence rates of obesity but larger waist measurements indicating abdominal obesity and increased insulin resistance. This study was, therefore, undertaken to look at the

association of hypoadiponectinaemia with NAFLD in a representative population in south India.

Material & Methods

The Chennai Urban Rural Epidemiology Study (CURES) is a large cross-sectional study done on a representative population of the metropolitan city of Chennai (formerly Madras) in southern India with a population of about 5 million people. The detailed study design of CURES is described elsewhere¹⁸ and the sampling frame is shown in our website [www.drmoahansdiabetes.com]. The institutional ethical committee approval was obtained and informed consent was obtained from all study subjects.

Phase 1 of CURES was conducted in the field, and involved a door-to-door survey of 26,001 individuals ≥ 20 yr of age. A detailed questionnaire was administered to all study subjects to collect information regarding demographic, socio-economic, and behavioural and health status. A fasting capillary blood sugar, blood pressure and basic anthropometric measures were done in all eligible individuals. Phase 2 of CURES dealt with studies of the prevalence of microvascular and macrovascular complications of diabetes among those identified with diabetes in Phase 1. In Phase 3 of CURES, every tenth subject recruited in Phase 1 ($n=2600$) was invited to Dr Mohan's Diabetes Specialities Centre for detailed anthropometric measurements and biochemical tests. Of these, 2350 participated in the study (response rate: 90.4%). In Phase 4 of CURES, every second subject recruited in Phase 3 ($n=1,175$) was invited to our Centre for studies on cognitive function.

In Phase 5 of CURES, every fourth subject recruited in Phase 3 ($n=588$) was invited to our Center to undergo ultrasonography of the abdomen thus maintaining the representativeness of the original CURES sampling frame. Of these, 541 subjects participated (response rate: 92%). Of these, NAFLD was diagnosed in 173 subjects and the overall prevalence of NAFLD was 32 per cent⁵. For the present study, 121 subjects were randomly selected without NAFLD and 72 subjects with NAFLD from 541 subjects (Fig. 1).

All study subjects underwent an oral glucose tolerance test (OGTT) using 75gm glucose load, except self-reported diabetic subjects, for whom fasting venous plasma glucose was measured. Diagnosis of diabetes was based on WHO Consulting Group criteria, *i.e.*, 2 h post load plasma glucose (2 h PG) ≥ 11.1 mmol/l or 200 mg/dl or self reported diabetic subjects on treatment by a physician. Impaired glucose tolerance (IGT) was

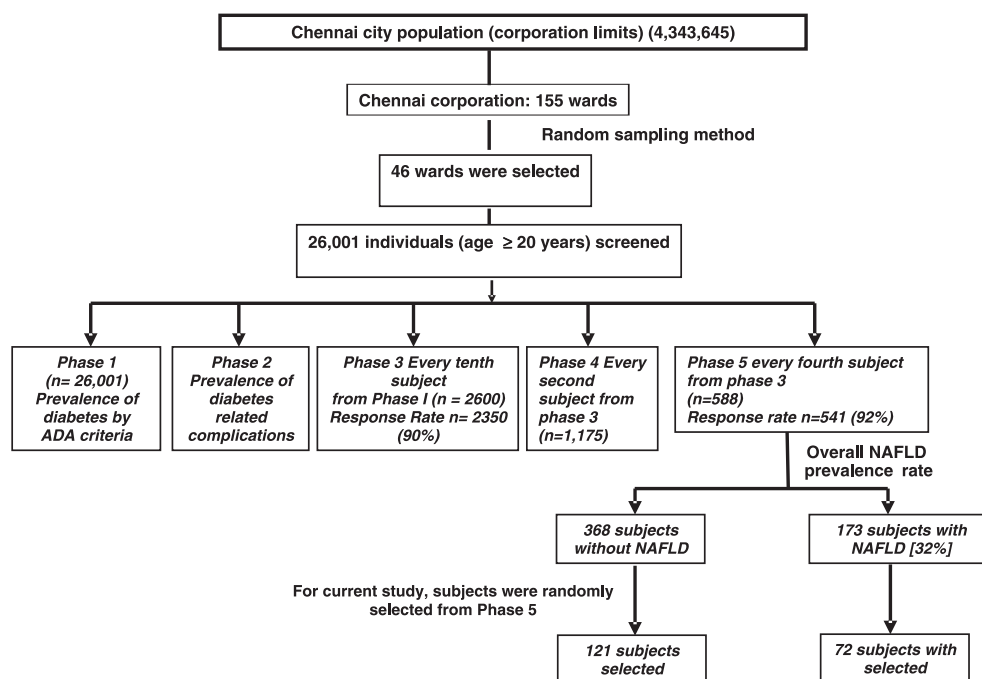


Fig. Chennai Urban Rural Epidemiology Study (CURES) – Methodology.

diagnosed if the 2 h PG was ≥ 7.8 mmol/l or 140 mg/dl and < 11.1 mmol/l or 200 mg/dl and normal glucose tolerance (NGT) if 2 h PG was < 7.8 mmol/l or 140 mg/dl¹⁹.

Anthropometric measurements: Anthropometric measurements including weight, height and waist measurements were obtained using standardized techniques as detailed elsewhere¹⁸. Height was measured with a tape to the nearest cm. Weight was measured with traditional spring balance that was kept on a firm horizontal surface. Waist was measured using a non-stretchable fibre measure tape. The body mass index (BMI) was calculated using the formula, weight (kg) / height (m²). Blood pressure was recorded in the sitting position in the right arm to the nearest 2mm Hg with a mercury sphygmomanometer (Diamond Deluxe BP apparatus, Pune, India). Two readings were taken 5 min apart and the mean of the two was taken as the blood pressure.

Biochemical parameters: Fasting plasma glucose (glucose oxidase-peroxidase method), serum cholesterol (cholesterol oxidase-peroxidase-amidopyrine method) serum triglycerides (glycerol phosphate oxidase-peroxidase-amidopyrine method) and HDL cholesterol (direct method-polyethylene glycol-pretreated enzymes) were measured using Hitachi-

912 Autoanalyser (Hitachi, Mannheim, Germany). The intra- and inter-assay co-efficient of variation for the biochemical assays ranged between 3.1 to 7.6 per cent. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula. Glycated haemoglobin (HbA1C) was estimated by high-pressure liquid chromatography using the Variant machine (Bio-Rad, Hercules, Calif., USA). The intra- and inter-assay co-efficient of variation of HbA1C was < 10 per cent.

Measurement of adiponectin: Fasting adiponectin levels were measured using radioimmunoassay (Cat. No. HADP-61HK, Linco Research, St Charles, MO, USA). The intra-assay and the inter-assay co-efficient of variation were 3.8 and 7.4 per cent respectively and the lower detection limit was 1 ng/ml.

Assessment of NAFLD: Ultrasonographic examination of liver was performed by an experienced radiologist, using a high-resolution B-mode ultrasonography system (Logic 400; GE, Milwaukee, WI, USA) having an electric linear transducer mid frequency of 3-5 MHz. The radiologist was masked to all clinical and biochemical characteristics of subjects. The scan was done for an average of 20 min and the images obtained were recorded and photographed. Fatty liver was defined as the presence of an ultrasonographic pattern consistent with “bright liver”, with evidence of

increased ultrasonographic contrast between hepatic and renal parenchyma, vessel blurring, and narrowing of the lumen of the hepatic veins in the absence of findings suggestive of chronic liver disease²⁰. NAFLD was defined as any degree of fatty liver in the absence of any alcohol intake.

NAFLD, if present, was classified based on the severity of fatty liver based on standard criteria²⁰.

Grade 1 (mild steatosis) was defined as slightly increased liver echogenicity with normal vessels and absent posterior attenuation.

Grade 2 (moderate steatosis) was defined as moderately increased liver echogenicity with partial dimming of vessels and early posterior attenuation.

Grade 3 (severe steatosis) was defined as diffusely increased liver echogenicity with absence of visible vessels and heavy posterior attenuation.

Repeat measurements performed in a random subgroup of 20 subjects showed intra- observer coefficient of variation to be <5 per cent.

Statistical analysis: Student's t test or one-way ANOVA (with Tukey's HSD) as appropriate was used to compare groups for continuous variables and Chi-square test or Fisher's exact test as appropriate was used to compare proportions. Adiponectin was log transformed to obtain normal distribution. Logistic regression analysis was carried out using NAFLD as the dependent variable and other risk factors as independent variables. All analysis was done using Windows-based SPSS statistical package (Version 10.0, Chicago) and $P < 0.05$ was considered significant.

Results

Subjects (males - 41.7%) with NAFLD (n=72) and 121 subjects (males - 57.9%) without NAFLD were studied. Subjects with NAFLD were older ($P < 0.05$) and had significantly higher BMI ($P < 0.01$), systolic and diastolic blood pressure ($P < 0.01$), HOMA-IR ($P < 0.01$), fasting plasma glucose ($P < 0.05$), HbA1c ($P < 0.05$), total cholesterol ($P < 0.01$), serum triglycerides ($P < 0.01$), LDL cholesterol ($P < 0.01$) and lower HDL cholesterol ($P < 0.01$) values compared to subjects without NAFLD. Serum adiponectin values were significantly lower in subjects with NAFLD compared to those without [5.6 $\mu\text{g/ml}$, 95% Confidence Interval (CI) 5.0 - 6.3 $\mu\text{g/ml}$ vs 7.4 $\mu\text{g/ml}$, 95% CI: 6.7 - 8.1 $\mu\text{g/ml}$; $P < 0.01$] (Table I).

Among the total study subjects, the proportion of subjects with mild steatosis was 19.3 per cent, with

Table I. General characteristics of subjects with and without non-alcoholic fatty liver disease (NAFLD)

Variables	Subjects without NAFLD (n = 121)	Subjects with NAFLD (n = 72)
Age (yr)	42 \pm 13	46 \pm 12*
Male n (%)	53 (43.8)	42 (58.3)
BMI (kg/m ²)	22.9 \pm 3.6	25.2 \pm 4.0**
Systolic blood pressure (mm Hg)	121 \pm 15	128 \pm 19**
Diastolic blood pressure (mm Hg)	73 \pm 10	77 \pm 11*
HOMA - insulin resistance	2.0 \pm 1.5	2.6 \pm 1.7**
Fasting plasma glucose (mg/dl)	101 \pm 39	118 \pm 41*
Glycated haemoglobin (%)	6.3 \pm 1.4	6.8 \pm 1.6*
Total serum cholesterol (mg/dl)	171 \pm 37	189 \pm 36**
Serum triglycerides (mg/dl)	128 \pm 70	153 \pm 83**
LDL cholesterol (mg/dl)	107 \pm 31	119 \pm 33**
HDL cholesterol (mg/dl)	44 \pm 11	40 \pm 9**
Serum adiponectin ($\mu\text{g/ml}$) [Geometric mean and 95% Confidence Intervals (CI)]	7.4 (6.7-8.1)	5.6 (5.0-6.3)**
Males	6.5	5.2
Females	8.3	6.3**
Proportion of subjects with diabetes (%)	15.7	26.4

Values are mean \pm SD
 $P < 0.05$, ** < 0.01 compared to subjects without NAFLD

moderate to severe steatosis was 13.7 per cent. Serum adiponectin levels decreased with increasing severity of NAFLD. Subjects with moderate to severe steatosis had significantly lower adiponectin levels (5.1 $\mu\text{g/ml}$, 95% CI: 4.1 - 6.4 $\mu\text{g/ml}$) compared to subjects with mild steatosis (5.9 $\mu\text{g/ml}$, 95% CI: 5.0 - 6.9 $\mu\text{g/ml}$; $P < 0.001$) and those without NAFLD (7.3 $\mu\text{g/ml}$, 95% CI: 6.6 - 8.0 $\mu\text{g/ml}$; $P < 0.01$).

Serum adiponectin levels were compared in those with and without NAFLD in relation to varying severity of glucose intolerance. Among subjects with NGT, adiponectin levels were significantly lower in those with NAFLD (6.0 $\mu\text{g/ml}$, 95% CI: 5.0 - 7.3 $\mu\text{g/ml}$) compared to those without (7.6 $\mu\text{g/ml}$, 95% CI: 6.8 - 8.6 $\mu\text{g/ml}$; $P < 0.05$). A similar trend was observed in subjects with IGT where those with NAFLD had significantly decreased adiponectin levels. Among diabetic subjects with and without NAFLD, fasting plasma glucose, HbA1c and adiponectin levels were not significantly different. Of the diabetic patients, 35 of 38 were on oral hyperglycaemic agents (OHA) and

Table II. Logistic regression analysis of association of adiponectin with NAFLD after adjusting for various factors

Parameter	Odds Ratio [OR]	95% Confidence Interval [CI]	<i>P</i> value
Adiponectin unadjusted	0.865	0.792 - 0.944	0.001
Adiponectin adjusted for age, gender, BMI, IR, waist circumference, total cholesterol, triglycerides and glucose intolerance	0.873	0.793 - 0.961	0.005

three had OHA plus insulin and four were on glitazone treatment.

Multiple logistic regression analysis (Table II) revealed adiponectin to be negatively associated with NAFLD [Odds Ratio (OR): 0.865, 95% Confidence Interval (CI): 0.792 - 0.944, *P*=0.001]. This remained statistically significant even after adjusting for confounding factors age, age, gender, BMI, IR, waist circumference, total cholesterol, triglycerides and glucose intolerance (OR: 0.873, 95% CI: 0.793 - 0.961, *P*=0.005).

Discussion

The main findings of this study were that, in Asian Indians adiponectin levels were lower in subjects with NAFLD than those without; adiponectin levels were inversely related to the degree of steatosis in NAFLD, with the lowest levels in more severe forms of steatosis; adiponectin levels were lower in subjects with NAFLD irrespective of the severity of glucose intolerance; and hypoadiponectinaemia was independently associated with NAFLD even after adjusting for age, gender, BMI, insulin resistance and glucose intolerance.

NAFLD is known to be associated with most cardiovascular risk factors, such as obesity, diabetes, dyslipidaemia and insulin resistance^{2,5}, all of which are features of the metabolic syndrome. In the present study, NAFLD was found to be associated with adiponectin independent of conventional cardio-metabolic risk factors. The results of the present study are also in accordance with earlier studies^{18,21}, which showed that hypoadiponectinaemia is a feature of NAFLD independent of insulin resistance and visceral adipose tissue content. Ethnic differences in adiponectin levels have been reported earlier, with African Americans having lower values compared to other populations²². In another study, Asian Indians were reported to have lower adiponectin values compared to white Caucasians²³. In the present study, levels of adiponectin were lower in males compared to females and also the proportion of male subjects with NAFLD was higher. Studies have shown that Asian Indian men may be

genetically predisposed to develop hepatic steatosis and hepatic insulin resistance at a lower BMI than other ethnic groups⁶. Further, increased prevalence of non-alcoholic fatty liver disease in the Asian Indian men has important implications for future health risks in these individuals because this condition is associated with steatohepatitis, which may progress to cirrhosis and end-stage liver disease.

Animal studies have indicated that adiponectin confers protection against alcoholic and non-alcoholic fatty liver disease¹⁶ and that administration of adiponectin alleviated non-alcoholic fatty liver disease in mice¹⁶. Another study reported that hypoadiponectinaemia might contribute to the development of necroinflammatory forms of NAFLD¹⁵. These data might also support the hypothesis that adiponectin has hepatoprotective effects in humans with NAFLD.

The metabolic syndrome is now proposed to reflect a failure of normal storage of surplus fat in adipose tissue. This leads to ectopic fat storage in the liver, muscle, and pancreatic beta cells, which in turn causes hepatic steatosis, dyslipidaemia, hepatic and peripheral insulin resistance²⁴. Adipocytokines, such as leptin and adiponectin, are proposed to play a pivotal role in preventing ectopic accumulation of lipids²⁵. It is suggested that, possibly as a result of a low adiponectin level, liver fat accumulation plays a key role in the development of NAFLD²⁶. However, this is speculative and can only be proved by prospective longitudinal studies.

It has been reported that raised serum leptin levels in non-alcoholic steatotic hepatitis (NASH) may be a reflection of the failure of leptin to stimulate hepatic lipid turnover, that is, hepatic leptin resistance²⁷. Further, it was shown that serum leptin was independently associated with the degree of hepatic steatosis but not hepatic inflammation or hepatic fibrosis²⁷. Leptin has been shown to have a prominent role in hepatic fibrosis in animal models of disease²⁸, mainly acting through the activation of hepatic stellate cells²⁹ and the enhanced release of osteopontin, a proinflammatory cytokine³⁰. Leptin knockout mice are protected from

hepatic inflammation and fibrosis in the methionine-choline-deficient diet-induced model of NASH³⁰.

Increased serum TNF- α levels have been demonstrated in several studies of fatty liver disease³¹. Strong evidence supports a key role for TNF- α and leptin among proinflammatory cytokines in the pathogenesis of the NASH as putative second hits in the 2-hit model proposed by Day and James³².

In recent years, it has been estimated that approximately 75 per cent of those with obesity or T2DM have NAFLD³³. The significance of adiponectin in protecting obesity-related NAFLD has been increasingly recognized. Relatively lower content of high molecular weight [HMW] adiponectin is closely associated with obesity-related metabolic complications³⁴ compared to total adiponectin. Epidemiological and genetic data suggest that the beneficial effects of adiponectin in humans might be mediated primarily by its HMW isoform, and the deficiency of this oligomer is an important aetiological factor that links obesity with its medical complications. HMW adiponectin has also been suggested to be the most potent isoform for alleviation of fatty liver disease in high fat diet-induced obese mice³⁵. Together, these data suggest that the beneficial effects of adiponectin in the hepatic tissue are mediated predominantly by its HMW form.

One of the limitations of this study is that it is a cross-sectional study, and hence the cause and effect relationship between adiponectin and NAFLD could not be assessed. However, the strengths of the study are that it is population based and done on a representative sample of the population of a large city in India.

In summary, we examined the association between adiponectin and NAFLD in an urban south Indian population, NAFLD was found to be associated with lower serum adiponectin levels independent of conventional cardiovascular risk factors. Timely detection of NAFLD through screening may help to plan appropriate therapeutic strategies or life style interventions.

Conflict of interest: None to declare for all authors.

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