PREVALENCE OF NON-ALCOHOLIC FATTY LIVER IN A HYPERCHOLESTEROLEMIC POPULATION OF NORTHWESTERN PENINSULAR MALAYSIA

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Abstract. Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease worldwide and a frequent finding on ultrasound examination. NAFLD is considered as the liver component of metabolic syndrome and is linked to accelerated atherosclerosis and cardiovascular disease. No data from systematic studies regarding the prevalence of NAFLD are available for the Malaysian population. One hundred eighty untreated hypercholesterolemic volunteers underwent blood and ultrasound examinations to evaluate their livers. NAFLD was diagnosed in 102 subjects (56.7%) with similar prevalences between sexes. Of the 102 positive subjects 82 (80.4%) were graded as mild, 17 (16.7%) as moderate and 3 (2.9%) as severe fatty liver cases. Elevated fasting plasma glucose (FPG) levels were found in 13 of 180 subjects (7.2%), while elevated AST and ALT levels were seen in 30 (16.7%) and 22 (12.2%) of the180 subjects, respectively.

Key words: non-alcoholic fatty liver, NAFLD, steatosis, liver, ultrasound, prevalence, cholesterol, hypercholesterolemia

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a range of liver disorders which ranges from simple accumulation of fat in the hepatocytes (steatosis) to macrovescicular steatosis, periportal and lobular inflammation (steatohepatitis) (Medina *et al*, 2004). NAFLD is generally hinted at by the presence of cryptogenic elevation aminotransferases, such as alanine transaminase (ALT) and aspartate transaminase (AST), in absence of significant alcohol consumption, and non-invasively confirmed/graded by ultrasound (US) examination. However, the prevalence of NAFLD appears to be independent of liver function tests results (Bedogni *et al*, 2005). The gold standard to diagnose liver diseases is biopsy, however this has two major problems: it is dependent on the sampling area, which may cause an evaluation error in the case of mild fatty liver, and it is an invasive technique which carries a morbidity rate of 1-5% and a mortality rate of 0.01-0.1% (Miele *et al*, 2007). Non-invasive US scanning provides valuable information regarding the liver condition with a sensitivity of 93-

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100% (Saadeh *et al*, 2002) without any known concomitant risks, although US cannot differentiate between simple steatosis and steatohepatitis from fibrosis (Mathiesen *et al*, 2002). Frequently NAFLD is associated with dyslipidemia, obesity, insulin resistance and type II diabetes (Federico *et al*, 2006). It is widely accepted that NAFLD represents the liver component of metabolic syndrome (MetS) (Marchesini *et al*, 2001; Kim and Younossi, 2008). NAFLD patients have a significantly greater cardiovascular (CV) risk than those without NAFLD, thus linking NAFLD, MetS and accelerated atherosclerosis together (Targher and Arcaro, 2007). NAFLD is not the innocuous condition as it was considered to be in the past. In a small percentage of patients it can develop into (non-alcoholic) cirrhosis and hepatocellular carcinoma (HCC) (Collier, 2006). Matteoni *et al* (1999) found patients diagnosed with NAFLD have a higher incidence of CV related deaths, similar to liver related deaths, and second only to cancer.

The purpose of the present work was to evaluate the prevalence of NAFLD in a local hypercholesterolemic population.

MATERIALS AND METHODS

In the course of a clinical trial (approved by the Research Ethics Committee for Human Studies, Universiti Sains Malaysia) aimed at evaluating the hepatoprotective activity of palm vitamin E (tocotrienols) in subjects with non-clinically significant hypercholesterolemia and normal to slightly elevated AST, ALT and GGT, volunteers of both sexes underwent blood screening and US examinations to ascertain the presence of NAFLD. Volunteers, recruited from the North Seberang Perai District, provided written informed consent to participate in the study.

Subjects

Walk-in volunteers were screened for inclusion and exclusion criteria. Inclusion criteria were age above 35-years, having a total cholesterol (TC) between 5.2-6.2 mMol/l, having a low-density lipoprotein (LDL) between 2.6-4.2 mMol/l, not being on anti-hypercholesterolemic and/or anti-hyperlipidemic treatment, having a daily ethanol intake <20 g. Normal levels for glucose, high-density lipoprotein (HDL), triglycerides (TG) and TC/HDL ratio were defined as 3.9-5.6 mMol/l, >1.04 mMol/l, <1.7 mMol/l and <5.0, respectively. Normal values were 39-117 IU/l, <40 IU/l, <53 IU/l and 11-49 IU/l in males and 7-32 IU/l in females for ALP, AST, ALT and GGT, respectively. Candidates were excluded from the study if the liver function tests were elevated, defined as more than thrice the normal value.

Fasting serum TC, LDL, HDL, TG, ALP, ALT, AST and GGT and glucose were analysed at an accreditated commercial laboratory.

Ultrasound examinations

Two experienced radiologists (MAA and ILS) performed all the high sensitivity B-mode ultrasound examinations on the same instrument, a Pentax-Hitachi EUB6500 (Tokyo, Japan) fitted with a EUP-C516 (3.5-5.0MHz) probe. The radiologists were blinded to the clinical and metabolic conditions of the subjects. The US evaluation consisted of a visual scoring system which assigned to focal hyperechoic hepatic areas of interest a value between 0 and 2. Hepatorenal echodiscrepancy, posterior echo penetration and portal vein wall clarity were the zones where echogenicity were evaluated. A total score >3 was interpreted as having a fatty liver, with grade 3 being mild, 4 moderate and 5-6 severe. The subjects were examined in the supine position.

Statistical analysis

The results of analytical test measurements are expressed as mean ± SD. The independent two-tailed Student's *t*-test, was used to compare quantitative data. The level of significance was set at $p<0.05$.

RESULTS

Eighty-four thousand five hundred individuals aged 35-74 years were in the district (State Department of Statistics, Penang, Malaysia). One hundred eighty volunteers met inclusion criteria and underwent US examination. Fatty liver was detected in 102 out of 180 (56.7%) (Table 1). The prevalences of NAFLD in male and female populations were similar, with 37 out of 66 (56.1%) males examined being diagnosed with NAFLD and 65 out of the 114 (57.0%) females examined being positive for NAFLD.

Of the 102 NAFLD positive subjects, 82 (80.4%) were graded as mild, 17 (16.7%) as moderate and 3 (2.9%) as severe fatty liver cases. Of the 37 NAFLD positive males, 29 (78.4%), 6 (16.2%) and 2 (5.4%) had mild, moderate and severe NAFLD, respectively. Of the 65 NAFLD positive females, 53 (81.5%), 11 (16.9%) and 1 (1.5%) had mild, moderate and severe NAFLD, respectively.

Overall, in our study we screened 21.3 people per 10,000 population, 15.7 males and 26.8 females per 10,000 population. The prevalence of NAFLD was 12.1 people per 10,000 population, equivalent to 8.8 males and 15.3 females per 10,000 population in the district.

The average values of the blood results in the present study for all subjects are shown in Table 2. The differences in

Table 1
Prevalence of NAFLD among subjects screened.

Total subjects	Male	Female
180	66 (36.7%)	114 (63.3%)
Total NAFLD+	Male NAFLD+	Female NAFLD+
102 (56.7%)	37 (56.1%)	65 (57.0%)

NAFLD+ , NAFLD positive

Table 2
Blood parameters values for all subjects screened.

	Total (*N*=180)	Male (*N*=66)	Female (*N*=114)
Age (years)	51 ± 9	51 ± 8	51 ± 9
Glucose[a]	5.6 ± 1.6	5.5 ± 1.0	5.6 ± 1.8
TC[a]	5.7 ± 0.5	5.7 ± 0.5	5.7 ± 0.4
HDL[a]	1.45 ± 0.34	1.31 ± 0.26	1.52 ± 0.35
LDL[a]	3.6 ± 0.4	3.6 ± 0.5	3.6 ± 0.4
TG[a]	1.4 ± 0.7	1.6 ± 0.7	1.3 ± 0.6
TC/HDL	4.1 ± 0.9	4.5 ± 0.9	3.9 ± 0.9
ALP[b]	72 ± 20	69 ± 17	74 ± 22
AST[b]	32 ± 12	34 ± 10	32 ± 13
ALT[b]	32 ± 17	33 ± 13	31 ± 19
GGT[b]	28 ± 18	32 ± 17	26 ± 18
AST/ALT	1.1 ± 0.4	1.1 ± 0.4	1.2 ± 0.4

[a]mMol/l; [b]UI/l

Table 3
Blood parameters values for NAFLD positive (+) and NAFLD negative (-) subjects.

	Pooled NAFLD+ (*N*=102)	Pooled NAFLD- (*N*=78)	Male NAFLD+ (*N*=37)	Male NAFLD- (*N*=29)	Female NAFLD+ (*N*=66)	Female NAFLD- (*N*=48)
Age (years)	51 ± 9	50 ± 9	50 ± 8	51 ± 8	52 ± 9	49 ± 9
Glucose[a]	5.8 ± 1.9	5.3 ± 1.0	5.6 ± 1.1	5.4 ± 0.8	5.8 ± 2.2	5.3 ± 1.0
TC[a]	5.7 ± 0.4	5.7 ± 0.5	5.5 ± 0.5	5.7 ± 0.5	5.7 ± 0.4	5.7 ± 0.5
HDL[a]	1.42 ± 0.29	1.49 ± 0.39	1.26 ± 0.24	1.34 ± 0.29	1.49 ± 0.29	1.58 ± 0.41
LDL[a]	3.6 ± 0.4	3.6 ± 0.5	3.5 ± 0.5	3.7 ± 0.5	3.6 ± 0.4	3.5 ± 0.5
TG[a]	1.5 ± 0.6	1.3 ± 0.7	1.7 ± 0.8	1.5 ± 0.6	1.4 ± 0.4	1.2 ± 0.8
TC/HDL	4.2 ± 0.8	4.1 ± 1.0	4.4 ± 0.9	4.4 ± 0.9	4.0 ± 0.7	3.8 ± 1.0
ALP[b]	73 ± 19	71 ± 21	68 ± 14	67 ± 20	74 ± 21	74 ± 22
AST[b]	35 ± 12	29 ± 10	35 ± 9	31 ± 11	34 ± 14	28 ± 10
ALT[b]	38 ± 18	24 ± 11	38 ± 12	26 ± 10	37 ± 21	23 ± 12
GGT[b]	33 ± 19	21 ± 14	36 ± 18	24 ± 13	30 ± 20	20 ± 14
AST/ALT	1.0 ± 0.4	1.3 ± 0.4	1.0 ± 0.4	1.3 ± 0.4	1.0 ± 0.4	1.3 ± 0.3

[a]mMol/l; [b] UI/l

Table 4
Significant differences between NAFLD positive (+) and NAFLD negative (-) subjects and by gender.

	Pooled NAFLD+ *vs* NAFLD-	Male NAFLD+ *vs* NAFLD-	Female NAFLD+ *vs* NAFLD-
Age	N.S.	N.S.	N.S.
Glucose	N.S.	N.S.	N.S.
TC	N.S.	N.S.	N.S.
HDL	N.S.	N.S.	N.S.
LDL	N.S.	N.S.	N.S.
TC/HDL	N.S.	N.S.	N.S.
TG	N.S.	N.S.	N.S.
ALP	N.S.	N.S.	N.S.
AST	$p<0.002$	N.S.	$p<0.01$
ALT	$p<0.001$	$p<0.001$	$p<0.001$
GGT	$p<0.001$[a]	$p<0.01$	$p<0.01$
AST/ALT	$p<0.001$	$p<0.01$	$p<0.001$

[a]Males and females had different normal values.

blood results by disease and gender are shown in Table 3. The significant differences are shown in Table 4. There were no significant differences in regard to inclusion criteria set for hypercholesterolemia as indicated by TC and LDL levels. No significant differences were detected in age, glucose, HDL, TG, TC/HDL ratio and ALP. A significant difference was seen between disease and non-disease states in all three

Table 5
Frequencies of elevated glucose, AST and ALT levels for each subgroup.

	Pooled NAFLD+ (*N*=102)	Pooled NAFLD- (*N*=78)	Male NAFLD+ (*N*=37)	Male NAFLD- (*N*=29)	Female NAFLD+ (*N*=66)	Female NAFLD- (*N*=48)
Glucose >7.0 mMol/l	9 (8.8%)	4 (5.1%)	2 (5.4%)	1 (3.4%)	7 (10.6%)	3 (6.3%)
AST >40 IU/l	23 (22.5%)	7 (9.0%)	10 (27.0%)	4 (13.8%)	13 (19.7%)	3 (6.3%)
ALT >53 IU/l	20 (19.6%)	2 (2.6%)	7 (18.9%)	0 (0.0%)	13 (19.7%)	2 (4.2%)

populations (pooled, male and female) for ALT and GGT levles and the AST/ALT ratio. A significant difference in AST was seen in pooled and female populations, but not in males.

An eleveted fasting plasma glucose (FPG) was found in 13 out of 180 subjects (7.2%), while elevated AST and ALT levels (defined as >40 IU/l and >53 IU/l for AST and ALT, respectively) were seen in 30 and 22 out of 180 subjects (16.7% and 12.2%), respectively. Details of the frequencies of these occurrences by subgroup are shown in Table 5.

DISCUSSION

In recent years NAFLD has emerged as a not fully understood liver disorder, both in etiology and significance. NAFLD is reported to be the most common cause of chronic liver disease in the USA and other western countries with prevalence rates ranging from 15% to 30% of the adult population (Cave *et al,* 2007); about 10% of the adult population meets the current diagnostic criteria for non-alcoholic steatohepatitis (NASH) (Neuschwander-Tetri, 2001). Data for Asian populations have shown NAFLD prevalences of 21% in Shanghai (Fan *et al,* 2005), between 18% (Hamaguchi *et al,* 2005) and 31.7% (Omagari *et al,* 2009) in Japan and 13.5% in Thailand (Perera *et al,* 2008). No data from systematic studies are available for Malaysia, however Malik *et al* (2007) studied Malaysian patients with NAFLD, they found NAFLD, NASH and cirrhosis in 3 (4.3%), 59 (84.3%) and 8 (11.4%) of 75 subjects, respectively. However, these subjects were patients referred to the hospital for liver biopsy due to suspected liver disorder (SLD), which explains the high prevalence of NASH compared to NAFLD.

Elevated aminotransferases are considered a primary sign of NAFLD (Cave *et al,* 2007), however our study found elevated AST and ALT levels in <30% and 20% of diseased males, respectively, versus <14% and nil in non-diseased males. In diseased males, only three subjects had both enzymes elevated, and two cases with elevated FPG had no abnormalities in their aminotransferase levels. Amongst non-diseased males 4 had elevated AST levels, none had an elevated ALT level and 1 had an elevated FPG but normal aminotransferase levels.

In the NAFLD positive female group, 3 cases had elevated AST and ALT levels along with an elevated FPG level, 6 cases had elevation of both aminotransferases and 8 subjects had elevation of either AST or ALT levels. Amongst NAFLD negative females, 2 subjects had elevated AST and ALT level and 1 subject had an elevated AST level. None of the 3 subjects with el-

evated FPG levels had raised aminotransferase levels.

The American Diabetes Association states a FPG level above 7.0 mMol/l is considered as "impaired fasting glucose" (IFG) which is not sufficient to meet the diagnostic criteria for diabetes (American Diabetes Association, 2006). Tolman *et al* (2007) estimated the prevalence of diabetes in patients with NAFLD as 34-74%. We found the incidence of IFG was 69.2% in patients with NAFLD, which is in agreement with Tolman *et al* (2007). The present study showed even though the liver function test values were within the normal range, the values of the NAFLD positive group were significantly higher than those of the negative group.

Our study showed there is high prevalence of NAFLD amongst subjects with non-clinically significant hypercholesterolemia in a district of northwestern peninsular Malaysia.

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