Isolated low HDL-Cholesterol in Japanese patients with type 2 diabetes

（日本人２型糖尿病患者における単独型および複合型低 HDL-コレステロール血症の冠動脈疾患への関与について）

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Abstract

Background: The clinical features of diabetic dyslipidemia are low levels of high-density lipoprotein cholesterol (HDL-C) associated with hypertriglyceridemia. However, isolated low HDL-C, defined as low HDL-C in the absence of other lipid abnormalities, has not been well studied in patients with type 2 diabetes (T2DM).

Objective: We revealed the prevalence of isolated and combined (those with low HDL-C with other lipid abnormalities) low HDL-C in patients with T2DM and compared clinical features and studied the influence on coronary artery disease (CAD) risk of the patients between two types of low HDL-C.

Methods: The study population included 398 patients (238 men, 160 women) with T2DM. Their profiles of glucose and lipid metabolism and atherosclerosis were analyzed. Then, the prevalence of CAD concerning low HDL-C was studied in 173 male patients with T2DM.

Results: The prevalence of low HDL-C (HDL < 40 mg/dL, in both sexes) was 33.7% (n=134); 12.6% of the patients (n=50) had isolated low HDL-C and 21.1% of the patients (n=84) had combined low HDL-C. There was no difference in clinical parameters of diabetes and atherosclerosis between patients with isolated or combined low HDL-C. Among the 173 male patients who had evaluated CAD, it was reconfirmed that low HDL-C was the independent risk factor for CAD, which did not depend on triglycerides and other risk factors. Actually, similar risks for CAD were seen between those men with isolated versus combined low-HDL-C (34.1% vs. 32.4%, respectively; no significant difference).

Conclusions: The prevalence of isolated low HDL-C in T2DM was the same as that of the general population, and it was shown clearly that combined type increased. In the limited analysis in male patients, CAD risk of both was shown to be equal. Then, risk of the low HDL-C in the T2DM patients were presumed to be due to an increase in

combined type.

Keywords: HDL-C, isolated low HDL-C, type 2 diabetes, dyslipidemia, coronary artery disease, atherosclerosis

List of abbreviations used in the manuscript

LDL-C low-density lipoprotein cholesterol

CAD coronary artery disease

HDL-C high-density lipoprotein cholesterol

TG triglyceride

UKPDS United Kingdom Prospective Diabetes Study

T2DM type 2 diabetes mellitus

WHO World Health Organization

JDCS Japan Diabetes Complications Study

ABCA1 ATP-binding cassette transporter A1

JAS Japan Atherosclerosis Society

TC total cholesterol

CAG cardio-angiography

SD standard deviation

ANOVA analysis of variance

BMI body mass index

CPR C-peptide immunoreactivity

IRI immunoreactive insulin

IMT intima media thickness

H-CRP highly sensitive C-reactive protein

ABI ankle brachial index

PWV pulse wave velocity

HSL hormone-sensitive lipase

VLDL very low-density lipoprotein

SREBP-1c Sterol regulatory element-binding protein 1c

miRNA micro ribonucleic acid

Introduction

The use of statins to treat elevated low-density lipoprotein cholesterol (LDL-C) has been identified as the most effective therapy for avoiding cardiovascular events [1]. Statin therapy has been found to reduce coronary events by about up to 30%; however, 70% risk remains unresolved [2]. The Framingham large-scale study showed that a decreased high-density lipoprotein cholesterol (HDL-C) level was a major risk factor for coronary artery disease (CAD) [3]. The association between the incidence of CAD and HDL-C levels has been reported to be stronger than the tie between CAD and LDL-C levels; a recent meta-analysis including 302,430 subjects from 68 long-term prospective studies supported the importance of HDL-C measurement in the risk assessment for CAD [4]. It has been also reported by meta-analysis with 20 statin RCTs that statin therapy does not alter the association between low levels of HDL-C and increased cardiovascular risk induced [2]. The importance of HDL-C management is recognized as valuable therapy for a patient's residual risk for CAD [5,6].

Diabetic dyslipidemia is characterized as decreased plasma HDL-C, increased triglyceride (TG), and the existence of small dense LDL-C [7,8]. In United Kingdom Prospective Diabetes Study (UKPDS), HDL-C was seen as a significant risk factor for macroangiopathy in type 2 diabetes mellitus (T2DM) [9]. However, isolated low HDL-C, defined as low HDL-C in the absence of other lipid abnormalities, had not been well evaluated in patients with dyslipidemia including diabetes. It has been thought that a dyslipidemia diagnosis relied on elevated plasma cholesterol and/or triglyceride levels as "hyperlipidemia". The actual clinical phenotype of hyperlipidemia defined using the World Health Organization (WHO) classification overlooked low HDL-C completely and did not even identify isolated low HDL-C [10].

TG, but not HDL-C, has been nominated as risk factor of CAD in the Japan Diabetes Complications Study (JDCS) [11]. TG level is often discussed as a risk factor of

cardiovascular diseases, related to a remnant lipoprotein. Although HDL-C and TG show good negative correlation in regards CAD risk, low HDL-C is an independent negative risk factor for CAD which did not depend on the TG level in the diabetic patient [12]. Analysis of isolated low HDL-C may contribute to understanding to what extent low HDL-C levels are associated with plasma TG levels and CAD in Japanese patients with T2DM.

Recent advances in researches of ATP-binding cassette transporter A1(ABCA1) suggest that lipidation of apoA-I via interaction with ABCA1 was an essential initial step for the formation of HDL, and it ultimately determines plasma HDL-C levels [13,14]. However, little has been reported about the pathogenesis of isolated low HDL independent of TG formation.

We studied the prevalence of isolated low HDL-C in patients with T2DM, and compared those with low HDL-C with other lipid abnormalities (especially hypertriglyceridemia, or "combined" low HDL-C). We then evaluated the association of CAD risk of both type of low HDL-C separately in these male patients.

In this paper, the definition of low HDL-C was < 40 mg/dL in both men and women, according to the Guidelines for Prevention of Atherosclerotic Cardiovascular Diseases 2012 of the Japan Atherosclerosis Society (JAS) [15]. Further, to compare and discuss the prevalence of isolated low HDL-C with other reports, low HDL-C was also evaluated with a defined value < 40 mg/dL in men and < 50 mg/dL in women.

Methods

Study subjects

The study population included 398 patients with T2DM (238 men, 160 women) who had been seen at our university hospital from 2007 to 2013 in an educational

program regarding the control of blood glucose levels. Study protocol was demonstrated in Fig.1. Fasting blood lipid levels were evaluated on the first day of study participation. Clinical characteristics of the patients and treatment for diabetes and dyslipidemia were illustrated in Table 1. Those patients excluded had type 1 diabetes, endocrinological disease, or severe liver, kidney, or infectious diseases.

Classification of dyslipidemia in patients with T2DM

Participating patients were classified following the Guidelines 2012 in JAS [15]: those with normolipidemia, those with hyper-LDL cholesterolemia (LDL-C >140 mg/dL), those with hypertriglyceridemia (TG >150 mg/dL), and those with low-HDL cholesterolemia (HDL-C<40 mg/dL in both men and women). The low-HDL-C group was subdivided into two groups: isolated and combined low HDL-C. The absolute frequency of each hyperlipemia was calculated.

Comparison of clinical characteristics between isolated and combined low HDL-C

We then compared clinical features between isolated and combined low HDL-C. Parameters concerning diabetes were fasting plasma glucose (FPG, mg/dL), HbA1c (%), serum C-peptide immunoreactivity (CPR, ng/mL) and urinary CPR (ug/day) and IRI (uU/mL). As to atherosclerosis, hypersensitive-CRP (H-CRP, ug/dL), ABI (ankle brachial index), PWV (pulse wave velocity, cm/sec) and mean IMT (intima media thickness, mm) were compaired between the subgroups. All blood measurements were performed by the clinical labolatory in Hirosaki University Hospital with routine automated laboratory methods. HbA1c was measured by HPLC and expressed as the National Glycohemoglobin Standardization Program (NGSP) value. Non-HDL cholesterol was calculated by subtracting HDL cholesterol from total cholesterol. ABI was calculated using the formula: ABI = ankle systolic BP / brachial systolic BP. Measurements of

brachial-ankle PWV were carried out using an automatic waveform analyzer (Colin Medical Technology Corporation, Japan). IMT using carotid ultrasound were investigated. Carotid IMT was measured using high-resolution B-mode ultrasonography with 7.5-MHz scanner probe. Three determinations of IMT were conducted, at the site of the greatest thickness and two other points, 1 cm upstream and 1 cm downstream from this site. These three determinations were averaged. The greatest value among the two averaged IMTs (from left and right common carotid artery) was used as the representative mean IMT.

Odds ratio of low HDL-C for CAD in male patients with T2DM

290 of 398 diabetics (173 men, 117 women) had undergone detailed cardiovascular examinations using cardio-angiography (CAG), coronary CT angiography, and exercise treadmill ECG in the Division of Cardiovascular Disease at our hospital.

To clarify any association between low HDL-C to CAD, we investigated using 173 male patients (Fig.1).

Of 173 male patients, odds ratios were evaluated for CAD according to low HDL-C and several major CAD risk factors in logistic-regression models. Odds ratios for continuous variables are its 95% confidence interval.

Association of isolated and combined low HDL-C with CAD in male patients with T2DM

The prevalence of CAD in isolated and combined low HDL-C was compared. The population of 173 male patients was further divided into four groups, using levels of HDL-C (cut-off value of 40 mg/dL) and TG (cut-off value of 150 mg/dL); isolated low HDL-C, combined low HDL-C, normal HDL-C and normal TG (defined as the n-HDL/n-TG and normal HDL-C and high TG (defined as the n-HDL/h-TG) groups (Fig.1).

Statistical analysis

Values are presented as mean ± standard deviation (SD). Differences between two groups were evaluated by two-tailed unpaired Student's t-test. Differences among groups were determined using analysis of variance (ANOVA) with Bonferroni/Dunn post-hoc correction. Statistical analyses were performed using StatView 5.0 (SAS Institute, Cary, NC, USA). Statistical significance was defined as $p < 0.05$. Odds ratios were evaluated for CAD according to low HDL-C and several major CAD risk factors in logistic-regression models. Odds ratios for continuous variables are its 95% confidence interval.

The protocol was made in accordance with the ethical guigeline of Hirosaki University Graduate School of Medicine and with the Declaration of Helsinki.

Results

Classification and absolute prevalence of diabetic dyslipidemia in patients with type 2 diabetes

It is noteworthy that mean values of the lipid parameters (total cholesterol [TC], LDL-C, HDL-C, TG, and non-HDL-C) were within normal ranges when averaged all the participated patients with T2DM (Table 1).

As illustrated inTable 2 and Fig.1, 2, of the 398 patients included in the study, 112 patients (28.1%; 80 men, 32 women) presented with normolipidemia and received no anti-hyperlipidemic agents. The remaining 286 patients (71.9%) with dyslipidemia were classified into three groups: those with elevated LDL-C ("hyper LDL-C", n=154, 38.7%; 74 men, 80 women, 86 with statin and 13 with fibrates), those with elevated TG

("hyper TG", n=123, 30.9%, 80 men, 43 women, 58 with statin and 25 with fibrates), and those with decreased HDL-C ("low HDL-C", n=134, 33.7%; 94 men and 40 women, 46 with statin and 12 with fibrates). Low HDL-C was seen in one-third of the patients with T2DM.

Prevalence of isolated and combined low HDL-C in type 2 diabetes

The low HDL-C group (n=134) was further sorted into isolated and combined types (Table 3). They were divided into 50 isolated low HDL-C patients (12.6%; 38 men, 12 women, 19 with statin and 2 with fibrates) and 84 combined low HDL-C patients (21.1%; 56 men, 28 women, 27 with statin and 10 with fibrates). The isolated low HDL-C was seen at about half of the rate of combined low HDL-C.

When low HDL-C was defined as <40 mg/dL in men and <50 mg/dL in women, numbers of female patients with low HDL-C increased by 45 persons; isolated type by 18 persons and combined one by 27 persons. Then, the prevalence of low HDL-C patients adjusted to 179 patients (45.0%; 94 men, 85 women). (old version of Table 2 was omitted)

Clinical features of isolated low HDL-C versus combined low HDL-C

A comparison of the two HDL-C groups (isolated vs. combined) with clinical features and laboratory parameters related to diabetes and atherosclerosis is shown in Table 3. As to lipid profile, mean plasma levels of TC, TG, LDL-C and non HDL-C in the combined HDL-C group were higher than those in the isolated HDL-C group. As to diabetic profile, no significant differences were noted in body mass index (BMI, kg/m2), HbA1c, FPG, S-CPR and U-CPR, and IRI between the groups. When only male patients were evaluated, the mean S-CPR level was higher in the combined HDL-C group (Appendix 1). When the following parameters of atherosclerosis were evaluated,

there were no significant differences between the two low HDL-C groups in IMT, H-CRP, and PWV, but ABI (Table 3).

Odds ratio of low HDL-C with CAD in male patients with T2DM

Among 290 of 398 diabetics (173 men, 117 women) who had undergone detailed cardiovascular examinations, non-fatal myocardial infarction and significant coronary-artery stenosis were identified in 55 patients (40 men, 15 women). The association between low HDL-C to CAD was investigated using 173 male patients (Fig.1). The reasons are that there is a difference to the normal range of HDL-C levels in men and women, that the age of menopause of women did not know, and that there had been a large difference in the incidence of CAD in men(40/173; 23.1%) and women (15/117; 12.8%). The 40 male patients with CAD included 22 with myocardial infarction and 18 with coronary artery ischemic changes.

Of 173 male patients who had examined the presence of CAD, HDL-C level was inversely associated with the risk of coronary disease (odds ratio, 0.86; 95%CI, 0.5-0.9; $p<0.01$) as illustrated in Fig. 3. However, each of other risk factor, i.e. BMI, smoking status, presence or absence of hypertension, HbA1c, LDL-C levels and TG levels showed no significant levels. Then, it was reconfirmed that low HDL-C is the independent risk factor for CAD which did not depend on triglycerides and other risk factors.

Association of isolated and combined low HDL-C with CAD in male patients with T2DM

The prevalence CAD was compared among four groups of 173 male patients as defined in method; 44 patients with isolated low HDL-C" group, 34 patients with combined low HDL-C" group, 61 patients with n-HDL/n-TG group, and 34 with n-HDL/h-TG group (Fig. 1 and Appendix 2).

There was no difference in HbA1c, smoking rate, plasma LDL-C levels, medical

treatment for dyslipidemia, or use of anti-hypertensive agents (Appendix 2). Thus, we could ignore all but low HDL-C in evaluating major risk factors for CAD. The prevalence of CAD among the four subgroups was the lowest in the n-HDL/n-TG group (normolipidemia: 13.1%); it was statistically lower than the isolated low-HDL-C (34.1%) and the combined low HDL-C (32.4%) groups (Fig. 4). The CAD onset rate was highest in the isolated low HDL-C group (34.1%); however, there was no significant difference between the onset rate for the isolated and combined low HDL-C groups. As a result, the isolated and combined low-HDL-C groups presented with equally negative risk profiles.

Discussion

Type 2 diabetes mellitus is one of the very common diseases which combine low HDL-C with hypertriglyceridemia, and HDL-C levels are known to be negatively associated with plasma TG levels. Actually, an inverse relationship was demonstrated with linear regression between plasma HDL-C and TG levels ($r=-0.269$, $p<0.0001$, $y=-0.047x + 54$, x; TG (mg/dL), y; HDL-C (mg/dL)) in our 398 patients (238 men, 160 women) with T2DM.

In our study, the frequency of low HDL-C in T2DM was found to be as high as 33.7%. Among these patients, there were more individuals with combined-type low HDL-C (21.1%) than isolated-type low HDL-C (12.6%) in patients with T2DM (Fig.2). Approximately one-third of the patients with T2DM presented with low HDL-C, and isolated low HDL-C was seen at about half of the rate of combined low HDL-C.

To discuss the prevalence of isolated low HDL-C in T2DM, we used the definition of HDL-C <40 mg/dL in men and <50 mg/dL in women. Isolated low HDL-C was corrected to 17.1% (16.0% of men, 18.8% of women) and combined one to 27.9% (23.5%

men and 34.4% women) of the diabetic patients in our study. Huxiey et al., using the same definition of isolated low HDL-C, performed a meta-analysis of 23 studies of patients (220,060 participants) in the Asia-Pacific region. They reported that the prevalence of isolated low HDL-C was higher in Asians (22.4%) compared with non-Asians (14.5%) [16]. They also demonstrated 18,779 Japanese subjects (including 4.3% of diabetics) had 16.3% of isolated- low HDL-C and 7.7% of combined one in the supplemental table in the report [16]. Then, the prevalence of combined low HDL-C in diabetic patients (27.9%) was about 3.6 times as much as that in the general Japanese subjects (7.7%). On the other hand, isolated low HDL-C was especially worth noting to be about the same frequency together (17.1% in diabetic patients and 16.3% in general Japanese).

The etiology of low HDL-C accompanied with high TG in diabetic dyslipidemia has been explained to be intertwined with insulin resistance as follows: reduced hormone-sensitive lipase (HSL) activity retards catabolism of TG-rich very low-density lipoprotein (VLDL) [17] and this directly or through the formation of TG-rich HDL leads to decreased HDL genesis [18]. Increased plasma insulin activates transcription factor, sterol regulatory element-binding protein 1c (SREBP-1c), which participates in the biosynthesis of TG, and simultaneously, micro ribonucleic acid(miRNA)33b restricts transcription of ABCA1 [19], with subsequent decrease in formation of HDL. Hyperglycemia or hyperinsulinemia in diabetes have been reported to reduce expression of ABCA1,mRNA and protein in human [20] and animal models [21].

On the other hand, little has been reported about the pathogenesis of isolated low HDL independent of TG formation. Several papers have reported the direct formation mechanism of isolated low HDL-C, although HDL-C levels are under considerable genetic control with heritability estimates of up to 80% [22]. A positive correlation between plasma adiponectin and HDL-C has been found in several studies that have

included non-diabetic [23] and diabetic [24] subjects, independent of plasma TG level. Adiponectin may have a direct role in HDL metabolism through plasma hepatic lipase activity [25]. Serum unacylated ghrelin levels were reported to be positively associated with isolated low HDL-cholesterol in obese patients, independent of insulin resistance and CRP level [26]. Serum ghrelin could act more directly in HDL metabolism by interacting with a species of HDL associated with paraoxonase and apolipoprotein J [27]. Elucidation of the mechanism behind isolated low HDL-C is clinically important to developing new strategies for dealing with vascular disorder with low HDL-C.

LDL-C lowering is well supported by many lipid intervention trials demonstrating that statin therapy significantly reduces risk for cardiovascular events [1]. Then, current national guidelines for CVD risk reduction are primarily focused on strategies to reduce levels of LCL-C. However, meta-analysis of large-scale statin trials demonstrated that the independent and significant inverse association between HDL-C levels and cardiovascular outcomes is not altered by statin therapy [2]. In addition to not being affected by statin therapy, the association of HDL-C level with cardiovascular risk persists after controlling for on-treatment LDL-C level, age, hypertention, diabetes mellitus and smorking [2].

In this study, the existence of and classification of dyslipidemia was based on fasting plasma lipid levels measured on the first day of the hospitalization, because the treatment regimen and length of stay in the hospital varied according to each patient. This research did not consider the influence of lifestyle on patients, such as their amounts of exercise and alcohol intake. Generally, dyslipidemia improved during hospitalization, and the improvement of TG was larger compared with the changes in HDL-C values. The frequency of isolated low HDL-C in T2DM may be higher than seen in our study results. Postprandial TG levels should be also investigated, because the mean postprandial TG level, but not fasting TG level, has been reported to be an

independent risk factor for carotid atherosclerosis in type 2 diabetic patients [28].

In conclusion, approximately one-third of the patients with T2DM presented with low HDL-C, and the prevalence of combined low HDL-C, but isolated type in diabetic patients was shown to be increased than the general subjects. In the limited analysis in male patients, isolated low HDL-C had a risk of CAD that was equal to combined low HDL-C independent of TG levels. Then, the CAD risk in the low HDL in the T2DM patients was presumed to be due to an increase in combined type. Elevated TG values may contribute to lower HDL-C values; however, this was not shown to contribute directly to the onset of CAD. Although the metabolic pathogenesis of low HDL-C has not been completely explained, this data may lead to new strategies for the residual risk that remains after statin treatment.

Conflict of Interest Statement

All authors, Matsumura K, Kimura Y, Hiroshi Murakami-1 H, Yamashita M, Matsuki K, Tanabe J, Murakami-2 H, Matsui J, Tamasawa N and Makoto Daimon, declare that they have no conflict of interest.

Human rights statement and informed consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (Hirosaki University Graduate School of Medicine) and with the Helsinki Declaration of 1964 and later revision study.

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Table 1. Clinical features and laboratory data related to diabetes and atherosclerosis.in patients with T2DM

	Total	Men	Wemen
N	398	238	160
BMI (kg/m2)	26.1±5.4	25.7±5.1	26.8±5.7
Age (yrs)	57.1±14.5	56.1±14.9	58.6±13.8
TC (mg/dL)	186.9±44.2	183.6±43.6	191.9±44.7
TG (mg/dL)	135.2±76.6	143.0±85.1	123.5±60.3
HDL-C (mg/dL)	47.2±14.0	45.4±14.1	49.9±13.5
LDL-C (mg/dL)	112.7±36.3	109.6±35.2	117.3±37.6
Non HDL-C (mg/dL)	139.7±41.7	138.2±41.1	142.0±42.5
statin/fibrate (N)	175/40	82/33	93/7
HbA1c (%)	8.7±1.9	8.8±1.9	8.6±1.9
FPG (mg/dL)	137±40	136±58	140±62
IRI (uU/mL)	8.2±7.3	8.2±6.8	8.3±7.9
S-CPR (ng/mL)	1.8±1.1	1.8±1.0	1.8±1.3
U-CPR (ug/day)	58.7±48.1	68.2±53.5	42.5±32.3
DM therapy (N) Diet/OHA/insulin	50/113/235	31/72/135	19/41/100
H-CRP (ug/dL)	92.8±112.5	87.7±109.6	92.8±112.5
ABI	1.11±0.12	1.11±0.12	1.10±0.10
PWV (cm/sec)	1576±397	1586±421	1559±358
mean IMT (mm)	0.82±0.30	0.85±0.28	0.74±0.21

Clinical characteristics of participating patients with T2DM are summarized. Table included lipid profiles and numbers of patients who were treated with statin or fibrate derivatives, parameters of diabetes and their treatment and parameters of atherosclerosis.

FPG fasting plasma glucose, IRI immunoreactive insulin, S-CPR serum-C-peptide immunoreactivity, U-CPP urinary C-peptide immunoreactivity, H-CRP highly sensitive C-reactive protein, ABI ankle brachial index, PWV pulse wave velocity, IMT intima media thickness

Table 2. Classification of diabetic dyslipidemia and laboratory data related to diabetes and atherosclerosis

	normolipidemia	hyper LDL-Cnemia	hyperTGnemia	hypoHDL-Cnemia
N (M/W)	112 (80/32)	154 (74/80)	123 (80/43)	134 (94/40)
BMI (kg/m2)	23.9 ± 5.6	26.2 ± 5.4	27.8 ± 5.0	27.2 ± 5.4
Age (yrs)	58.8 ± 13.4	59.4 ± 13.4	54.1 ± 13.4	55.5 ± 16.3
TC (mg/dL)	174.9 ± 27.7	206.9 ± 53.5	208.8 ± 43.2	173.7 ± 41.5
TG (mg/dL)	89.3 ± 29.6	146.2 ± 73.9	219.5 ± 82.3	159.9 ± 80.7
HDL-C (mg/dL)	54.7 ± 12.8	48.4 ± 13.9	42.9 ± 12.6	34.4 ± 4.1
LDL-C (mg/dL)	102.4 ± 22.8	129.2 ± 45.6	122.0 ± 39.4	107.3 ± 34.5
Non HDL-C (mg/dL)	120.2 ± 14.9	158.8 ±39.6	165.9 ± 30.6	139.9 ± 37.4
statin/fibrate (N)	0/0	86/13	58/25	46 / 12
HbA1c (%)	8.8 ± 2.1	8.8 ± 1.8	8.9 ± 1.9	8.5 ± 1.7
FPG (mg/dL)				
IRI (uU/mL)	5.6 ± 3.9	8.2 ± 6.7	9.7 ± 6.4	10.2 ± 8.8
S-CPR (ng/mL)	1.4 ± 0.8	1.8 ± 1.2	2.1 ± 1.1	2.2 ± 1.4
U-CPR (ug/day)	48.7 ± 38.3	52.4 ± 45.6	76.6 ± 58.3	72.1 ± 55.8
DM therapy (N) Diet/OHA/insulin	10/28/74	23/34/97	14/29/80	12/39/75
H-CRP (ug/dL)	84.8 ± 123.1	95.4 ± 117.3	85.7 ± 82.0	88.2 ± 91.3
ABI	1.13 ± 0.10	1.08 ± 0.14	1.08 ± 0.14	1.11 ± 0.13
PWV (cm/sec)	1630 ± 444	1612 ± 385	1516 ± 355	1508 ± 378
mean IMT (mm)	0.82 ± 0.25	0.86 ± 0.36	0.84 ± 0.38	0.81 ± 0.34

Participating patients were classified divided into four classifications following the Guidelines 2012 in the Japan Atherosclerosis Society (JAS). Low HDL-C was seen in one-third of the patients with T2DM.

Table 3. Comparison of clinical features and laboratory data related to diabetes and atherosclerosis in isolated and combined low HDL-C

TOTAL	isolated low HDL-C	combined low HDL-C	p value
N (M/W)	50 (38/12)	84 (56/28)	
BMI (kg/m2)	27.7±6.3	26.9±5.3	0.3774
Age (yrs)	57.2±14.8	57.2±14.8	0.1533
TC (mg/dL)	154.9±29.4	195.3±42.6	<0.0001
TG (mg/dL)	101.1±27.6	187.4±84.1	<0.0001
HDL-C (mg/dL)	37.4±6.0	36.8±6.0	0.472
LDL-C (mg/dL)	97.2±24.8	121.0±37.5	<0.0001
Non HDL-C (mg/dL)	117.5±23.4	158.5±36.6	<0.0001
statin / fibrate (N)	19 / 2	27 / 10	
HbA1c (%)	8.5±1.9	8.8±1.9	0.34469
FPG (mg/dL)	133±58	145±64	0.3268
IRI (uU/mL)	10.3±10.5	9.0±6.5	0.4378
S-CPR (ng/mL)	1.9±1.3	2.2±1.4	0.1937
U-CPR (ug/day)	63.9±48.7	65.7±54.8	0.8312
DM therapy Diet/OHA/insulin	7/19/24	16/25/43	
H-CRP (ug/dL)	114.5±211.9	108.9±108.2	0.4378
ABI	1.13±0.10	1.09±0.14	0.0334
PWV (cm/sec)	1464±413	1552±387	0.3445
mean IMT (mm)	0.731±0.205	0.796±0.209	0.0681

As to lipid profile, mean plasma levels of TC, TG, LDL-C and non HDL-C in the combined HDL-C group were higher than those in the isolated HDL-C group. As to diabetic profile, no significant differences were noted in BMI, HbA1c, FPG, S-CPR and U-CPR, and IRI between the groups. When the following parameters of atherosclerosis were evaluated, there were no significant differences between the two low HDL-C groups in IMT, H-CRP, and PWV, but ABI.

Appendix 1. Comparison of clinical features and laboratory data in isolated and combined low HDL-C according to the gender

MEN	isolated low HDL-C	combined low HDL-C	p value
N (94)	38	56	
BMI (kg/m2)	26.8±5.7	27.0±4.7	0.8571
Age (yrs)	52.8±16.7	56.5±15.5	0.2799
TC (mg/dL)	146.0±27.5	192.3±43.9	<0.0001
TG (mg/dL)	101.6±28.9	209.9±90.4	<0.0001
HDL-C (mg/dL)	34.4±4.0	34.0±4.3	0.6405
LDL-C (mg/dL)	91.2±23.2	116.3±40.5	0.0008
Non HDL-C (mg/dL)	111.6±23.5	158.3±39.6	<0.0001
statin / fibrate (N)	7 / 2	20 / 6	
HbA1c (%)	8.6±1.8	8.8±1.8	0.5522
FPG (mg/dL)	134±59	147±68	0.3345
IRI (uU/mL)	9.4±10.3	10.2±6.3	0.7202
S-CPR (ng/mL)	1.75±1.05	2.26±1.15	0.0369
U-CPR (ug/day)	67.8±50.3	90.5±61.0	0.0633
DM therapy Diet/OHA/insulin	4/16/24	11/19/26	
H-CRP (ug/dL)	37.4±37.6	119.0±103.9	0.0409
ABI	1.14±0.08	1.09±0.16	0.1282
PWV (cm/sec)	1520±414	1524±385	0.9672
mean IMT (mm)	0.756±0.23	0.84±0.21	0.1000

WOMEN	isolated low HDL-C	combined low HDL-C	p value
N (40)	12	28	
BMI (kg/m2)	28.8±6.8	26.8±5.9	0.1651
Age (yrs)	54.7±19.2	58.0±14.2	0.3797
TC (mg/dL)	166.1±28.2	198.3±41.4	0.0003
TG (mg/dL)	100.5±26.2	164.5±70.7	<0.0001
HDL-C (mg/dL)	41.3±6.0	39.6±6.3	0.2328
LDL-C (mg/dL)	104.7±25.0	125.8±33.8	0.0036
Non HDL-C (mg/dL)	124.8±22.2	158.7±35.1	0.0005
statin / fibrate (N)	12 / 0	7 / 4	
HbA1c (%)	8.4±2.1	8.7±1.9	0.4636
FPG (mg/dL)	131±53	145±62	0.3323
IRI (uU/mL)	11.5±11.0	7.6±6.5	0.1349
S-CPR (ng/mL)	2.05±1.46	2.07±1.67	0.9619
U-CPR (ug/day)	58.2±46.8	40.0±31.4	0.045
DM therapy Diet/OHA/insulin	3/3/6	5/6/17	
H-CRP (ug/dL)	183.0±278.2	93.7±116.6	0.2948
ABI	1.12±0.11	1.08±0.10	0.1516
PWV (cm/sec)	1455±417	1581±392	0.1847
mean IMT (mm)	0.70±0.17	0.77±0.25	0.1000

FPG fasting plasma glucose, IRI immunoreactive insulin, S-CPR serum-C-peptide immunoreactivity, U-CPP urinary C-peptide immunoreactivity, H-CRP highly sensitive C-reactive protein, ABI ankle brachial index, PWV pulse wave velocity, IMT intima media thickness

Appendix 2. Comparison of clinical features and laboratory data related to diabetes and atherosclerosis in four subgroups divided by levels of HDL-C and TG in 173 male patients with T2DM

MEN	isolated low HDL-C	combined low HDL-C	n-HDL/n-TG	n-HDL/h-TG
N	44	34	61	34
BMI	26.8±5.7	27.0±4.7	23.8±3.4	25.5±4.6
Age (yr)	52.8±16.7	56.5±15.5	58.3±11.7	54.0±12.8
smoking(%)	75	79.4	77	67.6
hypertension(%)	40.9	41.2	44.1	38.2
TC (mg/dL)	146.0±27.5	192.3±43.9	175.6±33.7	213.9±37.6
TG (mg/dL)	101.6±28.9	209.9±90.4	94.5±28.0	265.4±165.6
HDL-C (mg/dL)	34.4±4.0	34.0±4.3	57.2±12.7	52.5±16.1
LDL-C (mg/dL)	91.2±23.2	116.3±40.5	109.9±32.9	110.0±41.3
Non HDL-C (mg/dL)	111.6±23.5	158.3±39.6	118.4±32.2	161.4±31.9
statin / fibrate (N)	7 / 2	20 / 6	12 / 4	25 / 8
HbA1c (%)	8.6±1.8	8.8±1.8	9.0±1.9	9.3±3.0
FPG (mg/dL)	134±59	147±68	125±43	157±96
IRI (uU/mL)	9.4±10.3	10.2±6.3	6.1±2.3	7.8±5.1
S-CPR (uU/mL)	1.75±1.05	2.26±1.15	1.35±1.00	1.72±1.25
U-CPR (ug/day)	67.8±50.3	90.5±61.0	75.2±59.1	87.8±56.4
DM therapy (N) Diet/OHA/insulin	4/16/24	3/12/19	30/18/13	9/20/5
H-CRP (ug/dL)	37.4±37.6	119.0±103.9	151.8±147.2	245.1±252.9
ABI	1.14±0.08	1.09±0.16	1.17±0.25	1.09±0.13
PWV (cm/sec)	1520±414	1524±385	1532.9±322.7	1622.9±369.9
mean IMT (mm)	0.756±0.23	0.84±0.21	0.775±0.36	0.801±0.19

FPG fasting plasma glucose, IRI immunoreactive insulin, S-CPR serum-C-peptide immunoreactivity, U-CPP urinary C-peptide immunoreactivity, H-CRP highly sensitive C-reactive protein, ABI ankle brachial index, PWV pulse wave velocity, IMT intima media thickness

Figure legend

Figure 1. Study protocol

Study subjects were 398 patients with type 2 diabetes (T2DM). They were divided into normolipidemia and diabetic dyslipidemia following the Guidelines 2012 in Japan Atherosclerosis Society (JAS). 134 patients with low HDL-C were further divided into 50 isolated and 84 combined type. With 173 male patients who had evaluated the presence of cardiovascular disease (CAD), association of low HDL-C with CAD and the prevalence of CAD in isolated low HDL-C were compared. The number in a parenthesis expresses the number of (Men/Women).

Figure 2. Absolute prevalence of normolipemia and dyslipidemia in participating patients with T2DM.

Of the 398 patients included in the study, 112 patients (28.1%; 80 men, 32 women) presented with normolipidemia. The remaining 286 patients (71.9%) with dyslipidemia were divided into hyper LDL-C (n=154, 38.7%; 74 men, 80 women), hyper TG (n=123, 30.9%, 80 men, 43 women), and low HDL-C (n=134, 33.7%; 94 men and 40 women). The low HDL-C group (n=134) was further sorted into isolated and combined types. They were divided into 50 isolated low HDL-C patients (12.6%; 38 men, 12

women) and 84 combined low HDL-C patients (21.1%; 56 men, 28 women). Shaded bar presents men and open bar presents women.

Figure 3. Odds ratios for coronary artery disease according to low HDL-C and several risk factors in male patients with T2DM

HDL-C level was inversely associated with the risk of coronary disease (odds ratio, 0.86; 95%CI, 0.5-0.9; $p<0.01$). However, each of other risk factor, i.e. BMI, smoking status, presence or absence of hypertension, HbA1c, LDL-C levels and TG levels showed no significant levels. Odds ratios for continuous variables are 95% confidence interval.

Figure 4. Coronary artery disease in male patients with isolated and combined low HDL-C in patients with T2DM

173 male patients were divided into four groups with cut-off levels with HDL-C with 40 mg/dL and TG with 150 mg/dL. The prevalence of CAD among the four low HDL-C subgroups was the lowest in the n-HDL/n-TG group (normolipidemia: 13.1%); it was statistically lower than the isolated low-HDL-C (34.1%) and the combined low HDL-C (32.4%) groups. There was no significant difference between the onset rate for the isolated and combined low HDL-C groups.

Figure 1

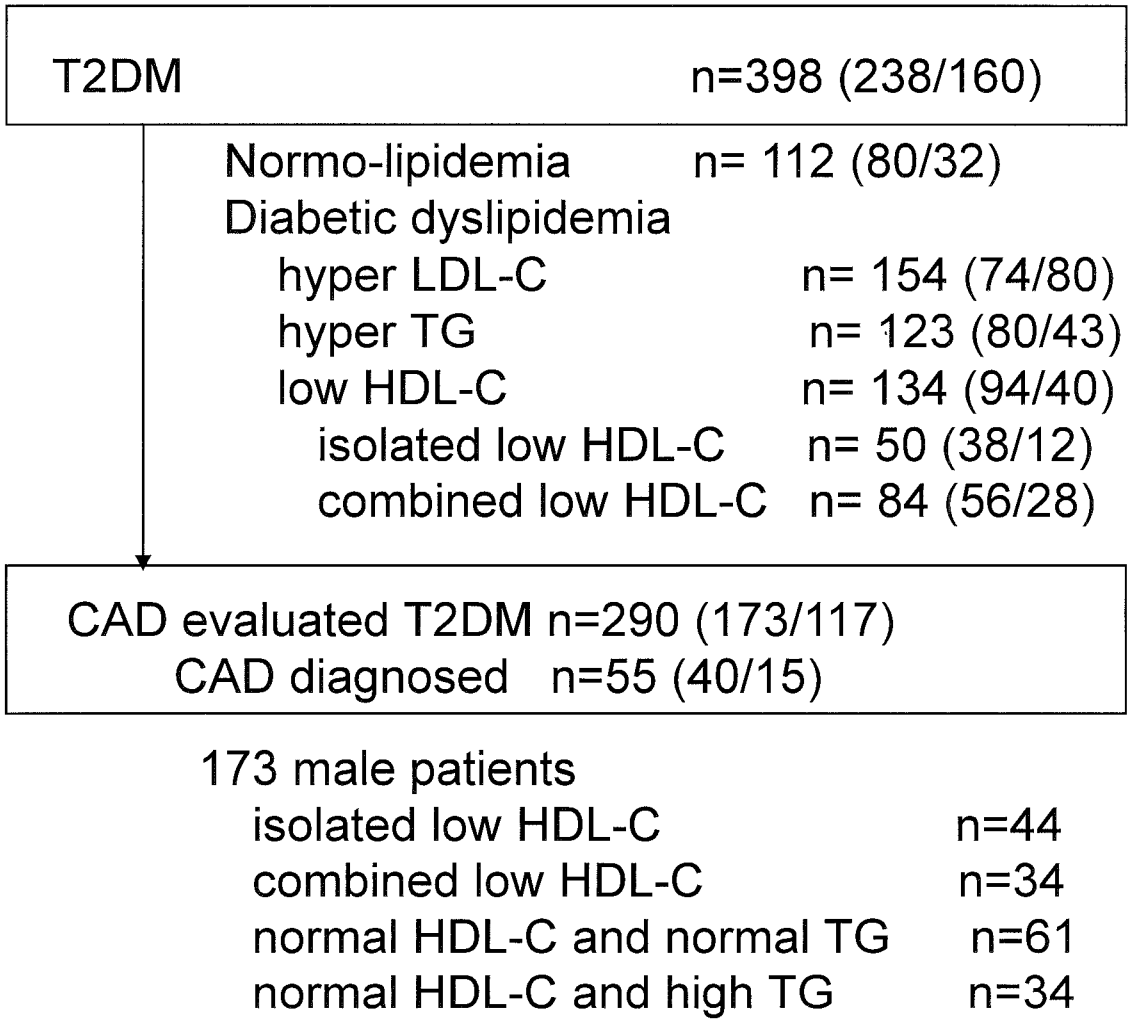

T2DM n=398 (238/160)
Normo-lipidemia n= 112 (80/32)
Diabetic dyslipidemia
hyper LDL-C n= 154 (74/80)
hyper TG n= 123 (80/43)
low HDL-C n= 134 (94/40)
isolated low HDL-C n= 50 (38/12)
combined low HDL-C n= 84 (56/28)
CAD evaluated T2DM n=290 (173/117)
CAD diagnosed n=55 (40/15)
173 male patients
isolated low HDL-C n=44
combined low HDL-C n=34
normal HDL-C and normal TG n=61
normal HDL-C and high TG n=34

Figure 2

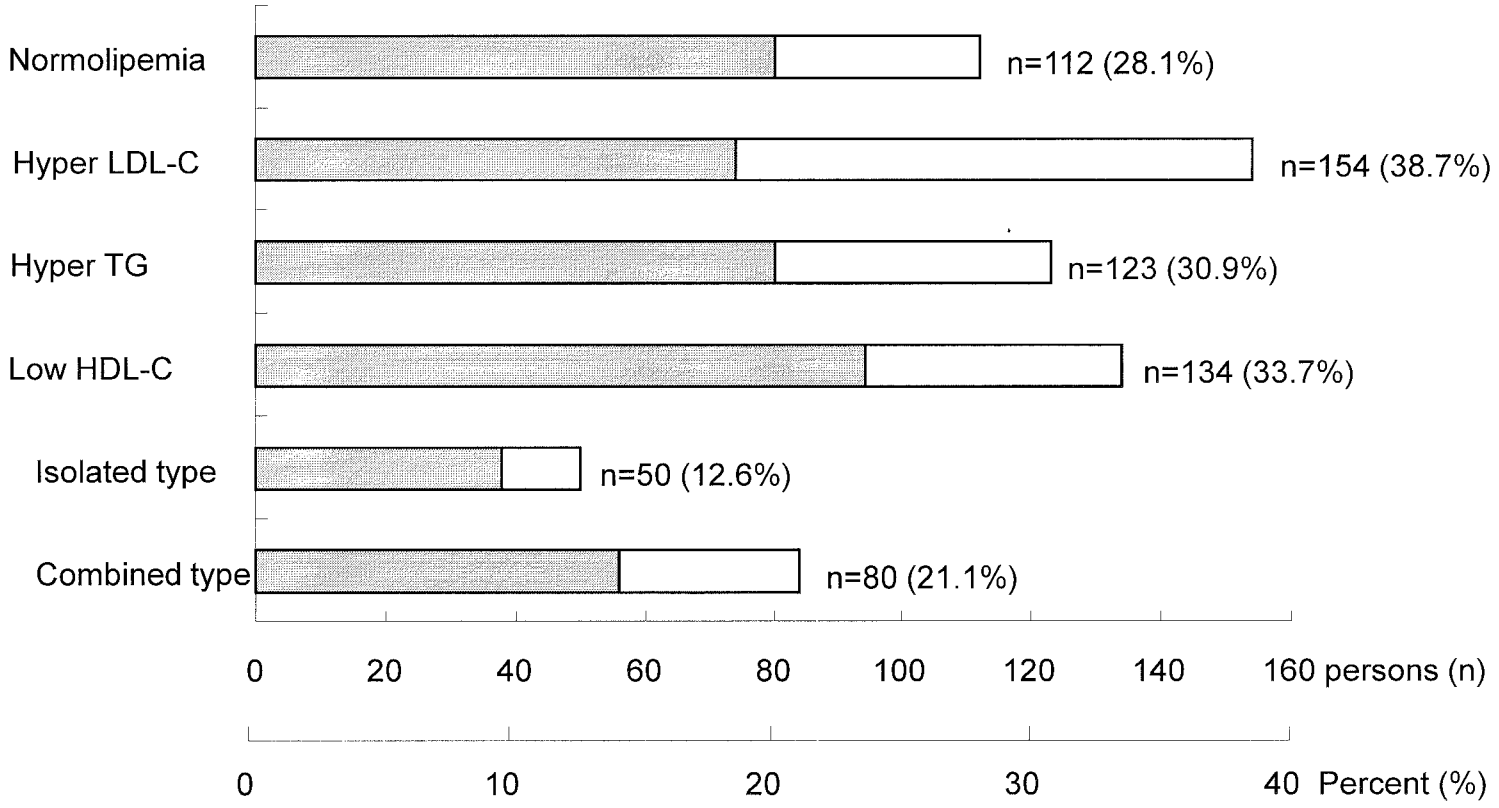

Normolipemia
n=112 (28.1%)
Hyper LDL-C
n=154 (38.7%)
Hyper TG
n=123 (30.9%)
Low HDL-C
n=134 (33.7%)
Isolated type
n=50 (12.6%)
Combined type
n=80 (21.1%)
0 20 40 60 80 100 120 140 160 persons (n)
0 10 20 30 40 Percent (%)

Figure 3

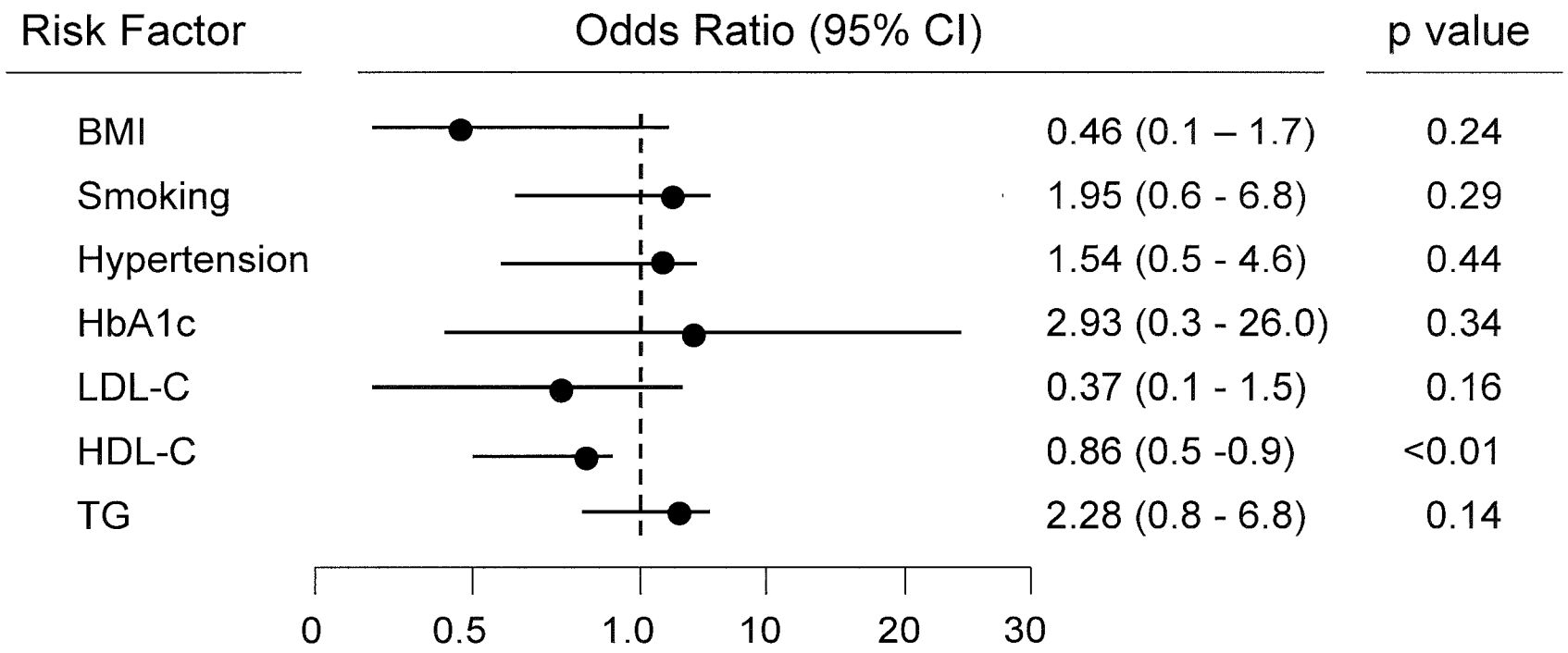
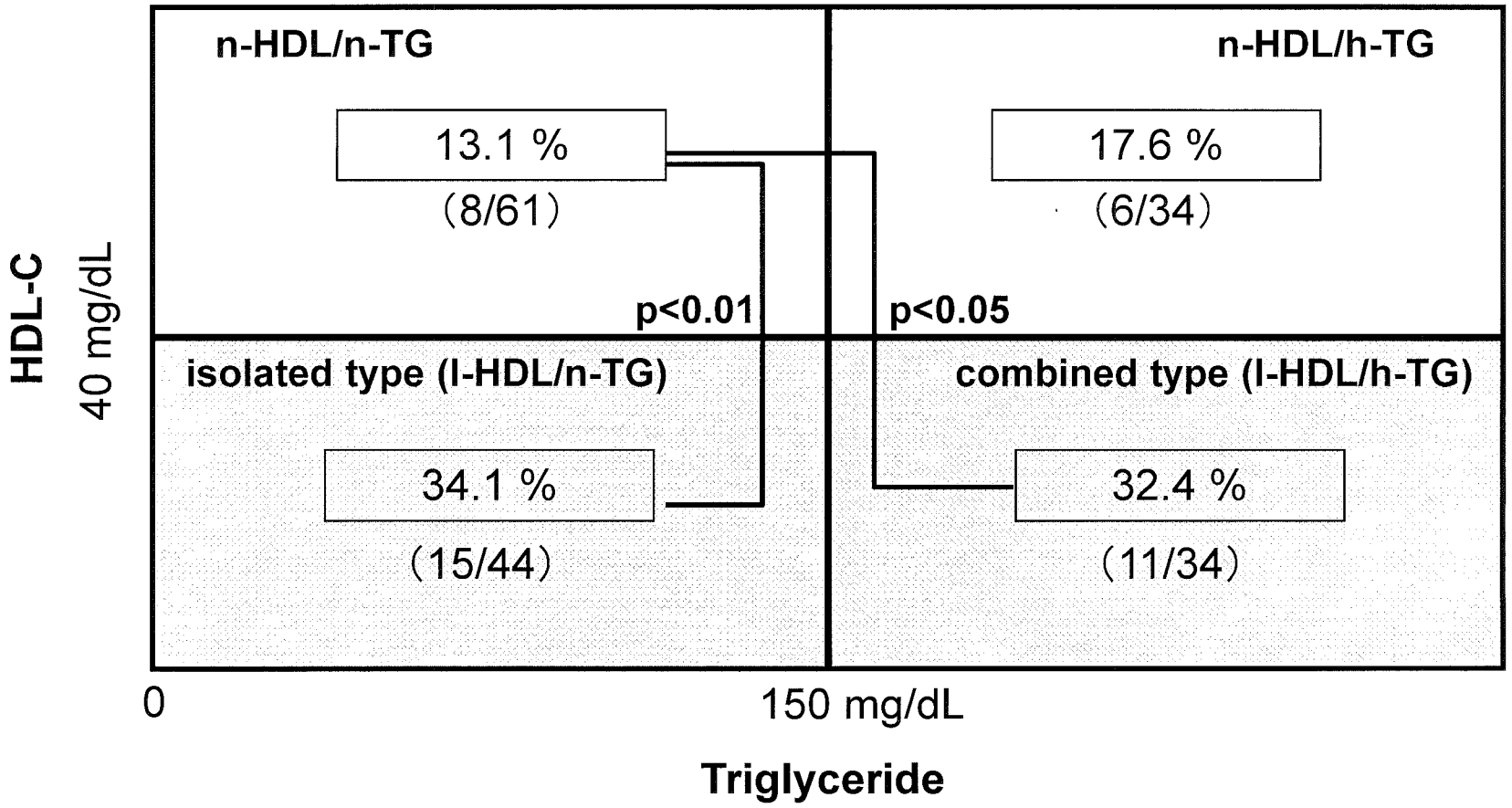

Risk Factor
Odds Ratio (95% CI)
p value
BMI
Smoking
Hypertension
HbA1c
LDL-C
HDL-C
TG
0.46 (0.1 – 1.7)
1.95 (0.6 - 6.8)
1.54 (0.5 - 4.6)
2.93 (0.3 - 26.0)
0.37 (0.1 - 1.5)
0.86 (0.5 -0.9)
2.28 (0.8 - 6.8)
0.24
0.29
0.44
0.34
0.16
<0.01
0.14
0
0.5
1.0
10
20
30

Figure 4


n-HDL/n-TG
n-HDL/h-TG
13.1 %
(8/61)
17.6 %
(6/34)
p<0.01
p<0.05
isolated type (I-HDL/n-TG)
combined type (I-HDL/h-TG)
34.1 %
(15/44)
32.4 %
(11/34)
HDL-C
40 mg/dL
0
150 mg/dL
Triglyceride