In particular, colonic diverticular bleeding causes sudden, painless hematochezia, and with massive bleeding, becomes a condition with high morbidity and mortality rates, in which blood transfusion and urgent treatment are required [7]. Bleeding develops in 3–5% of patients with colonic diverticulosis, and surgical intervention is required in 10–30% of the cases [8, 9]. In addition, patients with colonic diverticular bleeding show a high rate of recurrence within a short period [10]. With the increase in colonic diverticulosis, an increase in diverticular bleeding is to be expected [11]. However, the pathogenesis of bleeding remains unclear [12, 13]. Hypertension and use of nonsteroidal anti-inflammatory drugs (NSAIDs) have been reported as risk factors [12–14], but these issues have yet to be extensively investigated.

Accurate accumulation of data is important to evaluate the risk factors. Previous reports have investigated medical records retrospectively and thus may have been influenced by recall bias. We prospectively evaluated data including comorbidities, medications, and locations of diverticula. The purpose of this study was to identify risk factors for colonic diverticular bleeding.

Methods

Study design

We prospectively collected information of habits, comorbidities, history of medications, type of diverticula, and symptoms. We performed a case (diverticular bleeding)–control (diverticulosis) study to identify the risk factors for colonic diverticular bleeding.

Patient selection

Eligibility criteria included patients who underwent colonoscopy at the National Center for Global Health and Medicine (NCGM) between November 2009 and February 2011. NCGM is a tertiary care academic center with 900 beds, located in metropolitan Tokyo. Exclusion criteria were as follows: patients who did not provide informed consent, patients who did not know what medications they were receiving, patients with whom communication was difficult, patients who could not understand written documents, patients who were unable to write, patients with impaired vision, patients with decreased activities of daily living, patients who were unable to respond to the questionnaire due to serious illness, patients without diverticula in the colon on colonoscopy, patients in whom total colonoscopy could not be performed, and patients with lower gastrointestinal (GI) bleeding due to causes other than colonic diverticular bleeding (Fig. 1).

Diagnosis

An electronic video endoscope (high-resolution scope, model CFH260; Olympus Optical, Tokyo, Japan) was used for the diagnosis of colonic diverticulosis and colonic diverticular bleeding (Fig. 2). Intestinal lavage for endoscopic examination was performed using 2 L of solution containing polyethylene glycol. Diagnosis of colonic diverticular

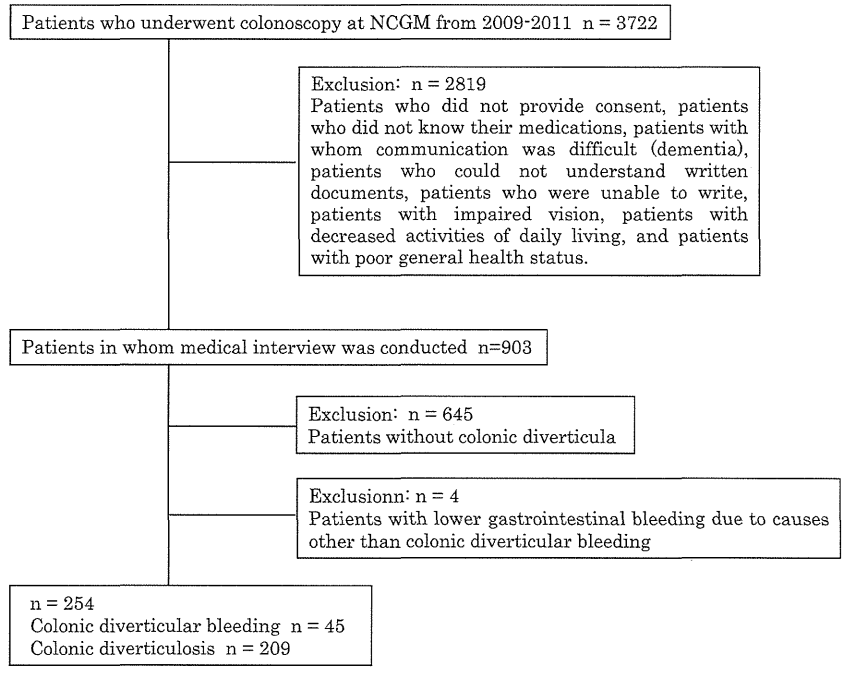

Fig. 1 Patient selection

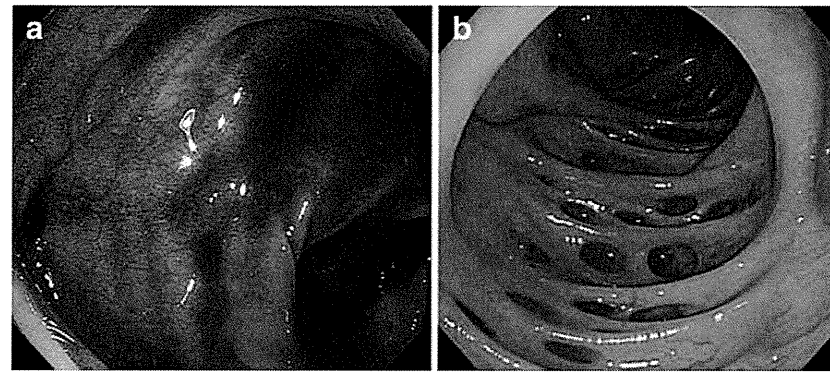

Fig. 2 **a** Colonic diverticular bleeding. **b** Colonic diverticulum

bleeding was based on the criteria reported by Jensen [15], including a chief complaint of painless hematochezia and the exclusion of hemorrhoidal bleeding on anal examination. We assessed the location and type of diverticula using colonoscopy. Location was defined as right-sided, involving the transverse or proximal colon; left-sided, involving the descending or distal colon; or bilateral, around the entire colon. Type was defined as sporadic type for 1 diverticulum, scatter type for 2–9 diverticula, and cluster type for ≥10 diverticula.

Questionnaire

The questionnaire included lifestyle habits, comorbid diseases, medications, and symptoms. Medical history was obtained in a face-to-face interview with medical staff. For the medication history, prescriptions and medical records were reviewed in addition to information from the patients themselves, in order to avoid omissions.

Habits

Smoking and alcohol drinking habits were inquired about. A smoker was defined as someone who smoked at the time of, or anytime prior to, the interview. A drinker was defined as someone who consumed alcohol at least 1 day/week.

Table 1 Demographic of patient's characteristics

	Patients
Median age (IQR)	67 (60–73)
Gender	
Male	178
Female	76
Location of diverticulum[a]	
Right side	125
Left side	59
Bilateral	70
Type of diverticulum[b]	
Sporadic/scatter type	135
Cluster type	119

[a]Right side, transverse and proximal colon; left side, descending and distal colon; bilateral, around the entire colon

[b]Sporadic type, 1; scatter type, 2–9; and cluster type, ≥10

Comorbidities

Comorbidities that were evaluated included hypertension, diabetes mellitus, hyperlipidemia, ischemic heart disease, chronic liver dysfunction, and chronic renal failure. Hypertension was considered present in patients taking any antihypertensive drugs. Diabetes mellitus was defined based on the diagnostic criteria of the American Diabetes Association [16]. Hyperlipidemia was considered present in patients taking any antihyperlipidemic drugs. Ischemic heart disease was considered present in any patient with a history of myocardial infarction or angina pectoris. Chronic liver dysfunction included chronic viral hepatitis and alcoholic liver disease. Chronic renal failure was considered present in patients on hemodialysis or peritoneal dialysis, or with serum creatinine ≥2.0 mg/dL.

History of medications

Patients were asked about the use of antiplatelet drugs (aspirin, clopidogrel, cilostazol, ticlopidine), anticoagulants, acetaminophen, NSAIDs, steroids, and antihypertensive drugs. The survey form included photographs of all these oral drugs, which are approved in Japan. Use of a medication was defined as oral administration starting at least 1 month before the interview.

Symptoms of constipation

Symptoms of constipation were evaluated in 7 grades using the subscale of Gastrointestinal Symptom Rating Scale [25]: 1, no impediment to daily activities; and 7, symptoms so severe as to be intolerable. Positive symptoms were defined as a score ≥3.

Table 2 Univariate analysis: risk factors for colonic diverticular bleeding

Factor	Cases (n=45)	Controls (n=209)	Odds ratio	95% CI	p value
Age					
<65	13	87	1 (referent)		
≥65	32	122	1.8	0.84–3.9	0.11
Gender					
Female	12	64	1 (referent)		
Male	33	145	1.2	0.57–2.8	0.60
Location of diverticulum[a]					
Left side	8	51	1 (referent)		
Right side	13	112	1.4	0.53–3.5	
Bilateral	24	46	4.5	2.1–9.6	<0.01
Type of diverticulum[b]					
Sporadic/scatter type	10	125	1 (referent)		
Cluster type	35	83	5.3	2.4–13	<0.01
Habits					
Alcohol drinking					
No	27	96	1 (referent)		
Yes	18	113	0.57	0.28–1.1	0.09
Smoking					
No	22	101	1 (referent)		
Yes	23	108	1.0	0.49–2.0	0.95
Comorbidities					
Hypertension					
No	13	119	1 (referent)		
Yes	32	90	3.3	1.5–7.1	<0.01
Hyperlipidemia					
No	34	175	1 (referent)		
Yes	11	33	1.7	0.71–3.9	0.17
Diabetes mellitus					
No	39	172	1 (referent)		
Yes	6	37	0.72	0.23–1.9	0.48
Ischemic heart disease					
No	28	182	1 (referent)		
Yes	17	27	4.1	1.8–8.9	<0.01
Chronic liver dysfunction					
No	43	195	1 (referent)		
Yes	2	14	0.65	0.069–3.0	0.75
Chronic renal failure					
No	39	206	1 (referent)		
Yes	6	3	11.0	2.1–67	<0.01
Medication					
Antiplatelet drugs[c]					
No	26	159	1 (referent)		
Yes	19	50	2.3	1.1–4.8	0.01
Aspirin	16	38			
Clopidogrel	2	1			
Cilostazol	1	1			
Ticlopidine	3	2			
Anticoagulants					
No	42	195	1 (referent)		

Table 2 (continued)

Factor	Cases (n=45)	Controls (n=209)	Odds ratio	95% CI	p value
Yes	3	14	0.99	0.18–3.8	1.0
Acetaminophen					
No	45	205	1 (referent)		
Yes	0	4	0	0–4.5	1.0
NSAIDs					
No	36	191	1 (referent)		
Yes	9	18	2.7	1.0–6.8	0.02
Steroids					
No	44	201	1 (referent)		
Yes	1	8	0.57	0.013–4.5	1.0
Symptom					
Constipation					
No	32	164	1 (referent)		
Yes	13	45	1.5	0.66–3.2	0.29

CI confidence interval, *NSAIDs* nonsteroidal anti-inflammatory drugs

[a]Right side, transverse and proximal colon; left side, descending and distal colon; and bilateral, around the entire colon

[b]Sporadic type, 1; scatter type, 2–9; and cluster type, ≥10

[c]Antiplatelet drugs, including aspirin and other antiplatelet drugs

Ethics

The study protocol was approved by the NCGM Ethics Committee. Written informed consent was obtained from all patients prior to starting the study. The study protocol was registered with UMIN 000004533.

Statistics

Patients with colonic diverticular bleeding were defined as cases, patients with colonic diverticulosis were defined as controls, and the relationships with clinical findings were examined. To determine the risk factors for colonic diverticular bleeding, we estimated the odds ratio (OR) and 95% confidence interval (CI). Age was compared between groups using the Mann–Whitney U test, while frequency distributions were compared using the chi-square test or Fisher's exact test. In multivariate analysis, we used a multiple logistic regression model with factors that had p values <0.2 on univariate analysis. A value of $p<0.05$ was considered statistically significant. All statistical analyses were performed using Stata version 10 software (StataCorp, College Station, TX, USA).

Results

During the study period, 3,722 patients underwent colonoscopy (Fig. 1). Of these, 903 patients participated in medical interviews (Fig. 1). Of the 903 patients, 645 patients had no colonic diverticula. Ultimately, 254 patients were included for analysis, comprising 45 patients with colonic diverticular bleeding and 209 patients with colonic diverticulosis. Table 1 shows the patient characteristics. Many of the patients were elderly men. Diverticulum location was predominantly right-sided or bilateral. Diverticulum type was cluster type in about half of the cases.

Bilateral diverticula (OR, 4.5; 95% CI, 2.1–9.6), cluster type (OR, 5.3; 95% CI, 2.4–13), hypertension (OR, 3.3; 95% CI, 1.5–7.1), ischemic heart disease (OR, 4.1; 95% CI, 1.8–8.9), chronic renal failure (OR, 11; 95% CI, 2.1–67), antiplatelet drugs (OR, 2.3; 95% CI, 1.1–4.8), and NSAIDs (OR, 2.7; 95% CI, 1.0–6.8) were significant risk factors for diverticular bleeding (Table 2). On multivariate analysis, cluster type (OR, 4.0; 95% CI, 1.8–8.9; $p<0.01$), hypertension (OR, 2.2; 95% CI, 1.0–4.6; $p=0.05$), ischemic heart disease (OR, 2.4; 95% CI, 1.1–5.4; $p=0.03$), and chronic renal failure (OR, 6.4; 95% CI, 1.3–32; $p=0.02$) were identified as independent risk factors for diverticular bleeding (Table 3).

Discussion

The present detailed prospective survey identified cluster-type diverticula, hypertension, ischemic heart disease, and

Table 3 Multivariate analysis: risk factors for colonic diverticular bleeding

	Odds ratio	95% CI	p value
Cluster type	4.0	1.8–8.9	<0.01
Hypertension	2.2	1.0–4.6	0.05
Ischemic heart disease	2.4	1.1–5.4	0.03
Chronic renal failure	6.4	1.3–32	0.02

chronic renal failure as risk factors for colonic diverticular bleeding. Typical diverticular locations differ between the Asian and Western populations [6]. In our study, diverticula were predominantly right-sided or bilateral, unlike the predominantly left-sided distribution seen in the Western countries. Bilateral [13] and left-sided [14] predominance have not been reported as risk factors for diverticular bleeding, and location was not found to be a significant risk factor for diverticular bleeding with predominantly right-sided bleeding in this study. On the other hand, the number of diverticula has not previously been noted as a risk factor, but as the number of diverticula increased, the risk of bleeding increased significantly in our study. This represents a new finding. We speculate that diverticular bleeding occurs due to rupture of exposed blood vessels inside the diverticulum. As the number of diverticula increases, the number of potentially exposed blood vessels may also increase, resulting in a greater likelihood of bleeding.

Under conditions of hypertension, increased pressure within exposed blood vessels may elevate the risk for bleeding [12]. Vascular endothelial injury and atheroma formation occur, leading to arteriosclerosis. This arteriosclerosis causes fragility of exposed blood vessels in the diverticula, which then may lead to bleeding.

In our study, ischemic heart disease was newly identified as a risk factor for diverticular bleeding. Ischemic heart disease is a condition that reflects arteriosclerosis [24].

A case report found that patients with chronic renal failure may develop diverticular bleeding [17]. Our case–control study is the first to identify chronic renal failure as a risk factor for diverticular bleeding. In chronic renal failure, blood vessels throughout the body, including the intestine, are affected by arteriosclerosis [18–20]. In addition, heparin, which is used in dialysis, and platelet function also influence bleeding. Renal failure in association with arteriosclerosis and a bleeding tendency would thus represent a risk factor for diverticular bleeding. Patients with hemodialysis and chronic renal failure are at an increased risk for bleeding, and in such patients, caution should be exercised.

As with hypertension, ischemic heart disease, and chronic renal failure, rupture of exposed blood vessels in diverticula may occur due to arteriosclerosis. Use of antiplatelet drugs has often been reported as a risk factor for lower GI bleeding [21]. However, in reports on diverticulosis and diverticular bleeding, as in the present study, antiplatelet drugs have not been identified as an independent risk factor on multivariate analysis [12, 13]. Antiplatelet drugs, including aspirin, often are reported to cause mucosal injury of the upper GI tract and the small intestine [22, 23], but to the best of our knowledge, mucosal injury, such as ulcer formation in the colon, has not been reported. These drugs may thus also have few effects on blood vessels within diverticula. The influence of antiplatelet drugs on colonic diverticulosis should further be investigated in studies with a suitable sample size and study design.

By inhibiting PG synthesis through blocking of cyclooxygenase-1 activity, NSAIDs decrease mucosal protective function. NSAID injury of the lower GI tract mucosa has often been reported [21], and NSAIDs are also thought to be a risk factor in colonic diverticular bleeding [12, 14]. However, NSAIDs were not a risk factor for bleeding in our study. This may be because we excluded patients with decreased daily activities, due to low back pain or arthritic pain, and patients who were unable to undergo colonoscopy. Analysis therefore could not be performed in patients taking large doses of NSAIDs over a long period for chronic low back pain, which may have led to some bias. To investigate whether NSAIDs represent a risk factor for diverticular bleeding, a study that includes patients with decreased activities of daily living should be conducted.

No studies on the relationship between constipation and diverticular bleeding have previously been reported. As constipation is a condition that causes increased intestinal pressure [6], we hypothesized it as a potential risk factor. In our study, constipation was not identified as a risk factor for bleeding. This also represents a new finding. Hard stools alone, with constipation, may not have influence on mucosal injury within diverticula.

Although endoscopic therapy, angiography, and surgery were performed for colonic diverticular bleeding, it was not an established standard therapy to prevent bleeding [7]. Recently, a case report of barium enema therapy [26] and a case series of endoscopic band ligation therapy [27] were reported to effectively prevent colonic diverticular bleeding. Hypertension and chronic renal failure, which we determined as risk factors in this study, were also risk factors for rebleeding [10]. Therefore, our findings helped in the selection of high-risk patients who required these novel therapies in order to prevent bleeding. However, a limited number of studies on therapies for rebleeding have been reported. The effect of these therapies on colonic diverticular bleeding should be further investigated.

The limitation of this study was that patients in poor general health, for whom obtaining a medical history or performing colonoscopy was difficult, were not included. This may have introduced selection bias. To generalize the results of our study, a future multicenter cohort study would be desirable.

Hypertension, ischemic heart disease, and chronic renal failure are common diseases in daily clinical practice. In patients with colonic diverticulosis, the presence of these comorbidities must be considered in the risk for bleeding. Colonic diverticulosis is primarily a disease of the elderly and is a common disease encountered not only by gastroenterologists but also by physicians in general clinical practice (including cardiologists and nephrologists). Our results

thus provide useful information for general medical care settings. Cluster-type diverticula, hypertension, ischemic heart disease, and chronic renal failure represent independent risk factors for colonic diverticular bleeding. This study identified new risk factors for colonic diverticular bleeding.

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Conflicts of interest No conflicts of interest were declared.

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Circulating Levels of Adiponectin, Leptin, Fetuin-A and Retinol-Binding Protein in Patients with Tuberculosis: Markers of Metabolism and Inflammation

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Abstract

Background: Wasting is known as a prominent feature of tuberculosis (TB). To monitor the disease state, markers of metabolism and inflammation are potentially useful. We thus analyzed two major adipokines, adiponectin and leptin, and two other metabolic markers, fetuin-A and retinol-binding protein 4 (RBP4).

Methods: The plasma levels of these markers were measured using enzyme-linked immunosorbent assays in 84 apparently healthy individuals (=no-symptom group) and 46 patients with active pulmonary TB around the time of treatment, including at the midpoint evaluation (=active-disease group) and compared them with body mass index (BMI), C-reactive protein (CRP), chest radiographs and TB-antigen specific response by interferon-γ release assay (IGRA).

Results: In the no-symptom group, adiponectin and leptin showed negative and positive correlation with BMI respectively. In the active-disease group, at the time of diagnosis, leptin, fetuin-A and RBP4 levels were lower than in the no-symptom group [adjusted means 2.01 versus 4.50 ng/ml, $P<0.0001$; 185.58 versus 252.27 μg/ml, $P<0.0001$; 23.88 versus 43.79 μg/ml, $P<0.0001$, respectively]. High adiponectin and low leptin levels were associated with large infiltrates on chest radiographs even after adjustment for BMI and other covariates ($P=0.0033$ and $P=0.0020$). During treatment, adiponectin levels increased further and then decreased. Leptin levels remained low. Initial low levels of fetuin-A and RBP4 almost returned to the normal reference range in concert with reduced CRP.

Conclusions: Our data and recent literature suggest that low fat store and underlying inflammation may regulate these metabolic markers in TB in a different way. Decreased leptin, increased adiponectin, or this ratio may be a promising marker for severity of the disease independent of BMI. We should further investigate pathological roles of the balance between these adipokines.

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Introduction

Tuberculosis (TB) is a major infectious cause of death around the world, with most of the 1.5 million deaths per year attributable to the disease occurring in developing countries. Negative energy balance in chronic inflammation has been recognized as a prominent feature of TB and one of the major obstacles to manage the patients [1,2]. Recent emergence of drug resistant TB is assumed to be driven by poorly implemented drug regimens, but malnutrition as well as HIV co-infection might worsen the condition: Inflammatory responses evoked by infection increase the demand for anabolic energy, leading to a synergistic vicious circle and further deterioration of the clinical condition [3].

It is generally believed that undernourishment diminishes protective immunity against *Mycobacterium tuberculosis*. [4]. A series of animal experiments, particularly aerosol-infected guinea pig models have demonstrated that chronic protein-energy malnutrition reduces secretion of T-helper 1 (Th1) cytokines [5]. It is rapidly reversed with alimentary supplement, indicating a pivotal role of nutrition, although it remains unclear what the optimal nutritional interventions are for improving the human disease in an effective manner [4].

On the other hand, in many countries today, rapid industrialization and urbanization are accompanied by changing patterns of diet and physical activity and this results in overnutrition [6]. Consequently, a combination of these two unfavor-

Table 1. Characteristics of study population.

	no-symptom group (N = 84)	active-disease group (N = 46)	*P* values
Male/Female (n)	41/43	42/4	<0.0001
Age (year)*	40.0 (28.1–48.6)	47.2 (34.7–55.0)	0.0064
BMI (kg/m^2)*	21.8 (20.0–23.7)	18.3 (17.1–19.5)	<0.0001
BCG history (yes/no/unknown)	33/28/23	10/3/33	<0.0001
positive/negative results of IGRA (n)	55/29	41/4**	0.0015

*Median and 25-to-75 percentiles in parenthesis are shown.
**One indeterminate case is not shown here.
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able conditions, a slow decline of infectious diseases associated with undernutrition and a rapid increase in obesity and diabetes are a serious double burden to public health and clinical medicine in resource limited settings [7].

Mainly in studies carried out in industrialized countries, fat-cell-derived hormones/cytokines designated as adipokines and relevant mediators have been investigated extensively and proposed as markers of obesity and diabetes [8]. Of these adipokines, adiponectin is a unique insulin sensitizer with atheroprotective role [9,10]. Plasma levels of adiponectin are inversely correlated with body weight and visceral fat mass [11,12]. Leptin is another major adipokine in proportion to fat stores [13,14] and one of the key mediators of energy metabolism [2] Even mild weight loss induced by dietary restriction is known to reduce leptin levels [11]. These markers supposedly shift towards the opposite in lean patients with wasting diseases. However, the significance of these metabolic markers in chronic infectious diseases like TB has not been fully understood [2].

We have recently conducted a proteomic research and demonstrated that plasma levels of fetuin-A and retinol-binding protein 4 (RBP4), also closely linked to the metabolic and inflammatory state, were significantly lower in patients with active pulmonary TB than in control subjects [15]. Fetuin-A, also known as α2-Heremans-Schmid glycoprotein, is an abundant plasma component of hepatic origin [16] and a negative regulator of insulin signaling [17,18]. Elevation of plasma fetuin-A is strongly associated with atherogenic lipid profile as well as fatty liver in obese patients [18]. Lipid components in the liver presumably upregulate fetuin-A expression, which may in turn repress adiponectin and impair adipocyte function [19,20]. Fetuin-A is also downregulated in acute inflammation as a negative acute-phase protein [21]. RBP4, synthesized in the liver and adipose tissue, has recently been identified as another adipokine involved in the development of insulin resistance [22]. In humans, similar to leptin, circulating RBP4 levels are high in obesity and decreased after calorie-restriction induced weight loss [11,23]. RBP4 is also known as a specific transporter protein for retinol (vitamin A) and can be used to assess the short-term fluctuation of nutritional states as a rapid turnover protein [24].

Alteration of the circulating levels of these markers should be investigated in TB, since they are expected to provide a basis of a critical link among nutritional status, metabolism and immunity of the disease, and hopefully to consider efficient nutritional interventions. In the present study, we thus measured circulating adiponectin and leptin in addition to fetuin-A and RBP4 levels in patients with active pulmonary TB versus apparently healthy individuals and compared the levels with body mass index (BMI), a simple estimate of adiposity [25] and C-reactive protein (CRP),

Table 2. Correlation of tested marker levels with BMI, CRP and IGRA values in each of the no-symptom and active-disease groups.

	no-symptom group (N = 84)			active-disease group (N = 46)		
	Pearson's *r* (*P* values)[a]			Pearson's *r* (*P* values)[a]		
Variable	by BMI (kg/m^2)	by CRP (μg/ml)	by IFN-γ (IU/ml)[b]	by BMI (kg/m^2)	by CRP (μg/ml)	by IFN-γ (IU/ml)[b]
Adiponectin (μg/ml)	−0.4530	−0.2892	−0.2254	−0.4421	0.1477	−0.1092
	(<0.0001)*	(0.0076)	(0.0393)	(0.0021)	(0.3274)	(0.4700)
Leptin (ng/ml)	0.4518	0.1694	0.1179	0.2771	−0.0918	0.3568
	(<0.0001)*	(0.1234)	(0.2855)	(0.0623)	(0.5442)	(0.0149)
Leptin/adiponectin ratio	0.5820	0.2793	0.2067	0.4901	−0.1633	0.2804
	(<0.0001)*	(0.0101)	(0.0592)	(0.0005)*	(0.2783)	(0.0591)
Fetuin-A (μg/ml)	0.0309	0.0415	0.0322	0.1243	−0.1833	0.2402
	(0.7805)	(0.7079)	(0.7714)	(0.4105)	(0.2226)	(0.1078)
RBP4 (μg/ml)	0.1605	−0.0213	0.0716	0.1535	−0.3018	−0.0916
	(0.1447)	(0.8475)	(0.5173)	(0.3085)	(0.0415)	(0.5448)

[a]Pearson's correlation coefficients with *P* values were calculated. Plasma concentrations were analyzed after logarithmic transformation.
[b]TB-antigen stimulated IFN-γ response
*Statistically significant when the significance level is set as $P<0.002$ based on the Bonferrroni correction.
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Table 3. BMI, CRP and tested marker levels in IGRA-positive and -negative subgroups in the no-symptom group.

	IGRA-negative (N = 29)		IGRA-positive (N = 55)		
marker	adjusted mean[a]	(95%CI)	adjusted mean[a]	(95%CI)	*P* values (ANCOVA)
BMI (kg/m^2)	21.52	(20.58–22.46)	21.48	(20.74–22.22)	0.9392
CRP (μg/ml)	1.12	(0.60–2.08)	1.30	(0.80–2.12)	0.6663
Adiponectin (μg/ml)	7.19	(5.67–9.11)	6.39	(5.30–7.70)	0.3792
Leptin (ng/ml)	4.50	(3.34–6.05)	4.38	(3.47–5.54)	0.8783
Leptin/adiponectin ratio	0.63	(0.40–0.97)	0.69	(0.49–0.97)	0.7080
Fetuin-A (μg/ml)	234.22	(212.40–258.29)	263.88	(244.26–285.06)	0.0333
RBP4 (μg/ml)	39.64	(32.28–48.69)	42.88	(36.45–50.43)	0.4997

[a]Estimated means of plasma concentrations were compared after logarithmic transformation, being adjusted for gender and age as covariates. The data shown are transformed back to the original unit.
No *P* values were statistically significant when the significance level is set as $P<0.007$ based on the Bonferrroni correction.
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a representative positive acute phase protein [26]. We further characterized their relationship with disease severity and alterations during the course of treatment.

Methods

Study design

We randomly selected and used plasma samples and demographic information in 46 patients with active pulmonary TB (= active-disease group) without treatment history as a biomarker sub-study of a large cohort study [27]. All patients entered the study from July 2007 to March 2009. Diagnosis of active pulmonary TB was made clinically and radiologically and confirmed bacteriologically in Hanoi Lung Hospital. A sputum smear test showed positive results in all of the patients in the active disease group and all of them completed anti-TB treatment following the national standard regimen, 2 months of streptomycin, isoniazid, rifampicin, and pyrazinamide followed by 6 months of isoniazid and ethambutol (2SHRZ/6HE).

Chest radiographs were taken at the time of diagnosis and interpreted by two readers independently in a blind manner. The presence of cavitary lesions and the number of lung zones (zero to six corresponding to the upper, middle, and lower fields on the right and left sides of the lung) affected by infiltrates were recorded [28]. HIV status was examined before starting anti-TB treatment. The proportion of HIV co-infection is less than 10% in this study area and those with HIV positive were excluded from the drawing up of this sub-study.

As a reference, we also measured plasma samples derived from 84 apparently healthy men and women who may have chances of direct or indirect contacts with TB patients as health care staff (= no-symptom group). All participants were tested for TB-antigen specific interferon-γ response by the commercially available enzyme-linked immunosorbent assay (ELISA)-based interferon-γ release assay (IGRA), QuantiFERON-TB Gold In-Tube^{TM} (Cellestis, Victoria, Australia). In the no-symptom group, IGRA-positive individuals suspected of latent TB infection were recommended to take chest radiography and to confirm there were no active pulmonary lesions. Subsequently a chance of receiving isoniazid prophylactic therapy was given. The protocol was approved by ethical committees of the Ministry of Health, Viet Nam and National Center for Global Health and Medicine, Japan respectively and written informed consent was obtained from each participant.

Table 4. BMI, CRP and tested marker levels in the no-symptom and active-disease groups after adjustment for gender and age.

	no-symptom group (N = 84)		active-disease group (N = 46)		
marker	adjusted mean[a]	(95%CI)	adjusted mean[a]	(95%CI)	*P* values (ANCOVA)
BMI (kg/m^2)	21.68	(21.06–22.30)	17.65	(16.66–18.65)	<0.0001*
CRP (μg/ml)	1.22	(0.86–1.74)	36.88	(20.94–64.94)	<0.0001*
Adiponectin (μg/ml)	6.82	(5.73–8.12)	9.29	(7.02–12.30)	0.0136
Leptin (ng/ml)	4.50	(3.78–5.35)	2.01	(1.52–2.66)	<0.0001*
Leptin/adiponectin ratio	0.66	(0.50–0.88)	0.22	(0.14–0.34)	<0.0001*
Fetuin-A (μg/ml)	252.27	(234.55–271.33)	185.58	(165.07–208.64)	<0.0001*
RBP4 (μg/ml)	43.79	(38.09–50.34)	23.88	(19.08–29.88)	<0.0001*

[a]Estimated means of plasma concentrations were compared after logarithmic transformation, being adjusted for gender and age as covariates. The data shown are transformed back to the original unit.
*Statistically significant when the significance level is set as $P<0.007$ based on the Bonferrroni correction.
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Table 5. CRP and tested marker levels in the no-symptom and active-disease groups after adjustment for gender, age and BMI.

	no-symptom group (N = 84)		active-disease group (N = 46)		
marker	adjusted mean[a]	(95%CI)	adjusted mean[a]	(95%CI)	*P* values (ANCOVA)
CRP (μg/ml)	1.11	(0.77–1.60)	47.80	(25.36–90.09)	<0.0001*
Adiponectin (μg/ml)	7.80	(6.63–9.19)	6.39	(4.81–8.49)	0.1671
Leptin (ng/ml)	3.77	(3.26–4.37)	3.28	(2.54–4.24)	0.2790
Leptin/adiponectin ratio	0.48	(0.38–0.61)	0.51	(0.35–0.76)	0.7704
Fetuin-A (μg/ml)	248.04	(229.95–267.57)	194.46	(170.48–221.80)	0.0004*
RBP4 (μg/ml)	42.90	(37.08–49.63)	25.27	(19.62–32.55)	0.0001*

[a]Estimated means of plasma concentrations were compared after logarithmic transformation, being adjusted for gender, age and BMI as covariates. The data shown are transformed back to the original unit.
*Statistically significant when the significance level is set as $P<0.008$ based on the Bonferrroni correction.
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Measurements of markers of metabolism and inflammation

Immediately after making the diagnosis of active TB disease, heparinized blood samples were drawn for IGRA before starting anti-TB treatment (0 month) and the remaining plasma without mixing any stimulants was reserved in a −80°C freezer until measurement. Samples were collected twice again, after the initial phase of treatment (2 months) and at the end of treatment (7 months) in the active disease group. This study was originally intended to identify a variety of biomarkers associated with TB phenotypes [15] and the participants were not obliged to keep fasting. The blood was collected in the daytime between 8 am and 4 pm at the outpatient clinic to avoid interference in dosing schedule of anti-TB drugs.

The AssayMax Human C-Reactive Protein ELISA kit was used for detection of human c-reactive protein (CRP) in plasma (Assaypro LLC. St. Charles, MO, USA). The minimum detectable dose was less than 0.25 ng/ml. The Quantikine® Human Total Adiponectin/Acrp30 Immunoassay kit was used to detect total (low, middle and high molecular weight) human adiponectin in plasma (R&D Systems, Inc.; Minneapolis, MN, USA). The mean minimum detectable dose was 0.246 ng/ml. The Quantikine® Human Leptin Immunoassay kit was used to detect human leptin in plasma (R&D Systems, Inc.). The mean minimum detectable dose was 7.8 pg/ml. The AHSG ELISA kit was used to detect fetuin-A in plasma (BioVender Laboratory Medicine Inc.; Modrice, Czech Republic). The detection limit was 0.35 ng/ml. A competitive ELISA for quantitative determination of RBP4 in human plasma was also applied (AdipoGen Inc.; Seoul, Korea) and the detection limit was 1 ng/ml. All were performed according to the manufacturer's instructions. Differences in measured concentrations between EDTA plasma samples as reference and these heparin samples were within a range of variation generally accepted in ELISA (coefficient of variance <15%) (data not shown)

Statistical analysis

Plasma protein levels were served for subsequent statistical analysis after logarithmic transformation of the measurements to minimize distortion of the data distribution. Means of demographic data between two groups were compared by analysis of variance (ANOVA) after testing for equal variances and

Table 6. BMI, CRP and tested marker levels in patients with small and large infiltrates on chest radiographs after adjustment for gender and age.

	small infiltrates[a] (N = 22)		large infiltrates[a] (N = 23)		
marker	adjusted mean[b]	(95%CI)	adjusted mean[b]	(95%CI)	*P* values (ANCOVA)
BMI (kg/m^2)	18.73	(16.74–20.71)	18.11	(15.95–20.27)	0.3065
CRP (μg/ml)	26.14	(12.63–54.10)	35.92	(16.29–79.21)	0.1520
Adiponectin (μg/ml)	10.28	(5.38–19.66)	18.83	(9.31–38.11)	0.0033*
Leptin (ng/ml)	2.42	(1.64–3.57)	1.65	(1.08–2.52)	0.0020*
Leptin/adiponectin ratio	0.24	(0.11–0.52)	0.09	(0.04–0.21)	0.0002*
Fetuin-A (μg/ml)	201.97	(149.87–272.18)	184.68	(133.52–255.46)	0.3222
RBP4 (μg/ml)	36.14	(21.76–60.03)	31.56	(18.17–54.79)	0.3770
IFN-γ (IU/ml)[c]	11.04	(2.13–57.16)	5.80	(0.97–34.82)	0.2039

[a]Small infiltrates = less than 3 of 6 zones in the lung affected, large infiltrates = 3 or more than 3 of 6 zones affected
[b]Estimated means of plasma concentrations were compared after logarithmic transformation, being adjusted for gender and age as covariates. The data shown are transformed back to the original unit.
[c]TB-antigen stimulated IFN-γ response
*Statistically significant when the significance level is set as $P<0.006$ based on the Bonferrroni correction.
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Table 7. CRP and tested marker levels in patients with small and large infiltrates on chest radiographs after adjustment for gender, age and BMI.

	small infiltrates[a] (N = 22)		large infiltrates[a] (N = 23)		
marker	**adjusted mean[b]**	**(95%CI)**	**adjusted mean[b]**	**(95%CI)**	***P* values (ANCOVA)**
CRP (μg/ml)	26.59	(12.78–55.28)	35.50	(16.02–78.63)	0.1991
Adiponectin (μg/ml)	10.84	(6.01–19.53)	18.15	(9.57–34.40)	0.0061*
Leptin (ng/ml)	2.37	(1.63–3.47)	1.67	(1.11–2.52)	0.0040*
Leptin/adiponectin ratio	0.22	(0.11–0.44)	0.09	(0.04–0.20)	0.0002*
Fetuin-A (μg/ml)	200.77	(148.59–271.28)	185.46	(133.74–257.18)	0.3886
RBP4 (μg/ml)	35.69	(21.43–59.46)	31.83	(18.29–55.42)	0.4626
IFN-γ (IU/ml)[c]	11.41	(2.17–59.90)	5.68	(0.94–34.53)	0.1760

[a]Small infiltrates = less than 3 of 6 zones in the lung affected, large infiltrates = 3 or more than 3 of 6 zones affected
[b]Estimated means of plasma concentrations were compared after logarithmic transformation, being adjusted for gender, age and BMI as covariates. The data shown are transformed back to the original unit.
[c]TB-antigen stimulated IFN-γ response
*Statistically significant when the significance level is set as $P<0.007$ based on the Bonferrroni correction.
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proportions between two groups were compared by the chi-squared test. Since it is well known that levels of adipokines such as leptin are influenced by gender and age, measurements of protein markers in any two groups were compared by analysis of covariance (ANCOVA) to allow for the covariates. The relationship between markers and other parameters were assessed by Pearson's correlation coefficients. Overall alterations of the measurements at three time points were initially analyzed by repeated-measures ANOVA and only when statistically significant, post-hoc comparisons were proceeded to: Difference of values between two time points was assessed by the paired-T test, under normal approximation based on the central limit theorem. P values<0.05 were considered to be statistically significant in general. When the Bonferroni correction was applied, however, a level of statistical significance was set as $0.05/n$ (n = the number of comparisons). Statistical analysis was performed using Stata version 11 (StataCorp, College Station, TX, USA).

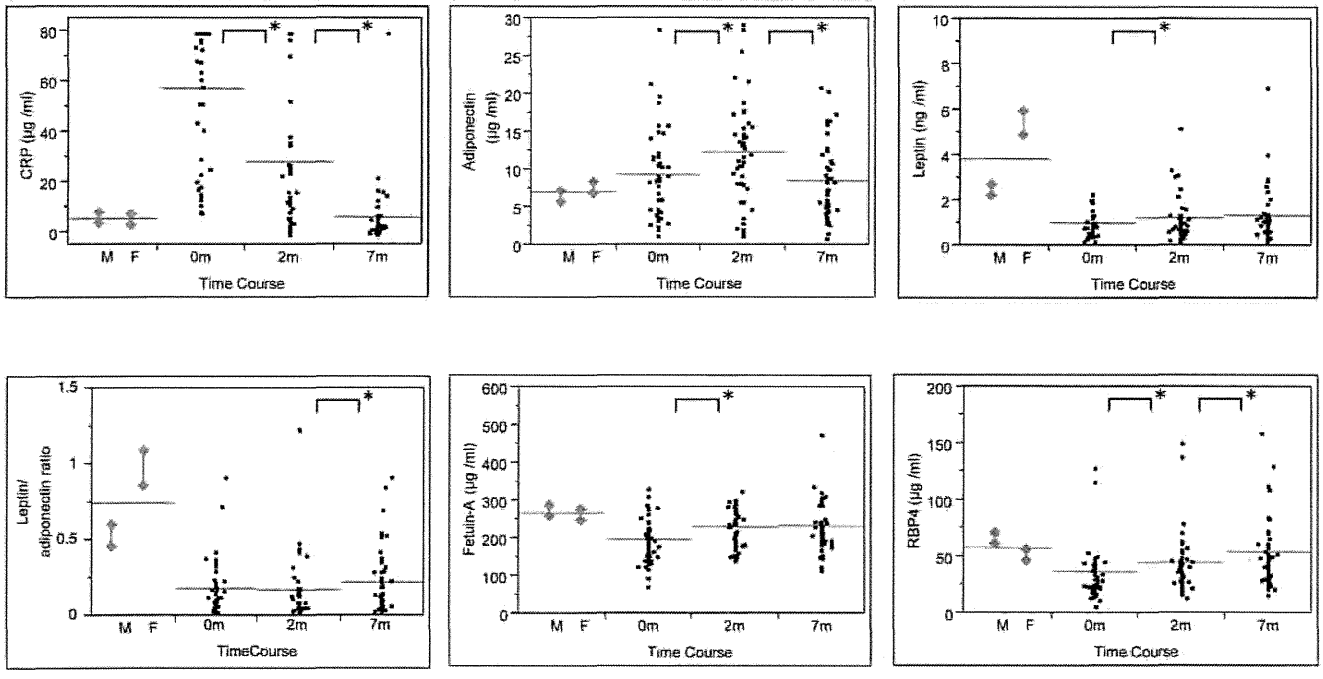

Figure 1. CRP and tested marker levels in patients with active TB before (0 month), during (2 months) and at the end (7 months) of anti-TB treatment (N = 46). Vertical bars with diamonds on the left side (M and F) indicate reference values, means ± SEM of the values in men (N = 41) and women (N = 43) of the no-symptom group. A horizontal bar indicates the grand mean of the values in each condition. * indicates $P<0.05$ by paired comparison between 0 month and 2 months. When significant, 2 months and 7 months were also compared.
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Results

Characteristics of study population

The no-symptom group consisted of 84 apparently healthy individuals, whose blood samples were used to obtain the standard values of markers in the study population. This group includes an approximately equal number of men and women with median age of 40, and more than half of the individuals had latent TB infection diagnosed by the IGRA method (Table 1). The active-disease group members were 46 patients with smear-positive active pulmonary TB. The majority of the patients were male with low body mass index (BMI$<$18.5 kg/m^2) and the median age was 47, slightly older than in the non-symptom group.

Correlation of adiponectin, leptin, fetuin-A and RBP4 levels with BMI, CRP and IGRA values in the no-symptom and active-disease groups

Correlation coefficients (r) were calculated in the no-symptom and active-disease groups respectively (Table 2). Adiponectin and leptin showed negative and positive correlations with BMI respectively in the no-symptom group ($r=-0.4530$, $P<0.0001$; $r=0.4518$, $P<0.0001$). Leptin/adiponectin ratio showed a positive correlation with BMI in the active-disease group ($r=0.4901$, $P=0.0005$) as well as in the no-symptom group ($r=0.5820$, $P<0.0001$). These correlations were statistically significant even after Bonferroni correction for multiple comparisons. The other possible correlations including a pair of leptin and TB-antigen stimulated IFN-γ response did not reach significant levels in this study, when Bonferroni correction was applied.

Pairwise correlations between four tested markers

Pairwise correlation coefficients (r) between four tested metabolic markers were further calculated in the no-symptom and active-disease groups respectively (Table S1). A significant correlation was found only between fetuin-A and RBP4 levels ($r=0.4007$, $P=0.0058$) in the active disease group.

Adiponectin, leptin, fetuin-A and RBP4 levels with IGRA-positive and -negative subgroups in the no-symptom group

IGRA-positive values higher than the cutoff value, 0.35 IU/ml are regarded as latent TB infection after active disease is ruled out. We thus categorized the no-symptom group into IGRA-positive and -negative subgroups and compared plasma concentrations of the above markers. However, none of the marker levels including fetuin-A were significantly different between IGRA-positive and -negative subgroups after adjustment for gender and age, when considering the number of comparisons (Table 3).

Adiponectin, leptin, fetuin-A and RBP4 levels in the no-symptom and active-disease groups

The active-disease group had significantly low BMI and very high CRP levels at the time of diagnosis, when assessed by using ANCOVA with adjusted means (Table 4). In the disease group, leptin, leptin/adiponectin ratio, fetuin-A and RBP4 levels were remarkably lower than in the no-symptom group ($P<0.0001$ respectively) after adjustment for gender and age and these differences were statistically significant even after Bonferroni correction (Table 4).

Since BMI was strongly correlated with some of the adipokine values as shown in Table 2, we further analyzed levels of the four markers after adjustment for BMI as well as gender and age. Consequently, adiponectin and leptin levels were not significantly different between the two groups any more, whereas fetuin-A and RBP4 levels remained significant ($P=0.0004$ and $P=0.0001$) (Table 5)

Adiponectin, leptin, fetuin-A and RBP4 levels in patients with mild and severe disease

At the time of diagnosis, severity of the disease was assessed by spread of infiltrates on chest radiographs (Table 6). Small infiltrates affecting less than 3 of the 6 lung zones and large ones affecting more, categorized the patients into two subgroups (= mild and severe disease) half-and-half.

After adjustment for gender and age, adiponectin levels were higher and leptin levels were lower in patients with large infiltrates than in those with small infiltrates ($P=0.0033$ and $P=0.0020$). Interestingly, differences in the levels of these two adipokines between small and large infiltrates were significant respectively ($P=0.0061$ and $P=0.0040$), even after adjustment for BMI as well as gender and age (Table 7). Leptin/adiponectin ratio was lower, or adiponectin/leptin ratio was higher, in patients with large infiltrates than in those with small infiltrates independent of BMI ($P=0.0002$). None of the markers were associated with the presence of cavity on the chest radiographs (data not shown).

Adiponectin, leptin, fetuin-A and RBP4 levels in patients with active TB before, during and at the end of anti-TB treatment

Figure 1 shows plasma values at the time points before (0 month), during (2 months) and at the end (7 months) of anti-TB treatment. Mean values in men (N = 41) and women (N = 43) of the no-symptom group are shown as a reference, in which gender difference was observed in leptin levels and leptin/adiponectin ratio ($P<0.0001$).

Overall differences of the measurements during anti-TB treatment in all of these four markers were statistically significant by repeated-measures ANOVA ($P<0.01$). Post-hoc analysis showed that adiponectin levels increased transiently ($P=0.0004$; 0 month vs. 2 months) and then decreased close to the reference range by the end of treatment ($P<0.0001$; 2 months vs. 7 months). Leptin levels remained low throughout the treatment course, though gradually elevated ($P=0.0226$; 0 month vs. 2 months). Initial low levels of fetuin-A and RBP4 significantly improved during treatment ($P=0.0001$ and $P=0.0016$; 0 month vs. 2 months), almost reaching the reference range by the end in concert with reduced CRP levels.

Discussion

We assessed the clinical significance of four metabolic markers, adiponectin, leptin, fetuin-A and RBP4 in patients with active TB, analyzing them in relation to classical nutritional and inflammatory parameters, BMI and CRP, severity of disease and treatment course. BMI is known to be lower in patients with active TB than in control subjects [1,2]. After effective treatment, weight often increases but patients may remain underweight [11].

Plasma levels of adiponectin were inversely correlated with BMI in concordance with previous results [11,12]. The adiponectin levels tended to be elevated in the active-disease group characterized by low BMI, though it did not reach significant levels, which was also shown by others [29]. Interestingly in our study, adiponectin levels were significantly higher in severe disease with extensive pulmonary lesions than in mild disease, even after adjustment for BMI. Adiponectin as a modulator of inflammation in a variety of diseases has recently been highlighted [30]. For instance, in critically ill patients, adiponectin levels appear to be

transiently suppressed at the initial phase and then gradually elevated at the recovery phase [31,32]. The plasma concentrations in patients with active TB were further increased after starting treatment and then decreased close to the reference range by the end of treatment. Elevated adiponectin levels in chronic inflammatory diseases may be explained by compensatory response to the underlying disease as well as concomitant low body fat mass, which is postulated by others [33,34]. A study designed to measure alteration of adiponectin and BMI simultaneously throughout the treatment period would be able to characterize it further.

In most recent reports, leptin levels are low in TB [29,35–38], though other earlier or smaller studies have shown conflicting results [39–42]. In the present study, using a commercial ELISA, significantly lower levels of leptin were demonstrated in patients with active TB, which could be mostly explained by marked undernutrition in our disease population. Within the active-disease group, however, correlation between leptin and BMI was less clear. BMI-independent regulation of plasma leptin concentrations should also be taken into consideration in TB at least in part [13,37]. This idea is also supported by an *ex vivo* study by others demonstrating that continuous exposure of IL-1 or TNF-α provides a signal to downregulate leptin in human adipose tissue [43], though acute inflammation such as sepsis may rather upregulate circulating leptin levels transiently [44–46]. In addition to relatively high levels of adiponectin, low levels of leptin were observed in patients with large infiltrates, even after adjustment for BMI. This is concordant with a recent study showing that leptin levels were low in severe TB disease [29]. We have further demonstrated that low leptin/adiponectin ratio, or high adiponectin/leptin ratio is characteristic to severe TB disease in this study. This ratio was originally proposed as an atherogenic index indicating a balance between the two markers bearing apparently opposite functions in inflammation [47]. Our findings support the idea that suppressed production of leptin may be detrimental to host defense against TB by virtue of impairment of Th1 cell-mediated immunity [13,29,48]. After starting treatment, leptin levels were slightly elevated, but remained low during the treatment period. This is also compatible with reports made by others [37,38], although the mechanism remains unknown. Long-lasting low levels of leptin may be attributed to individual predisposition to TB or delayed recovery from wasting disease.

In our study, fetuin-A levels were considerably low in TB even after adjustment for BMI. Soon after starting treatment, the levels were increased in inverse proportion to the decrease in CRP. In TB, fetuin-A may be downregulated by at least dual mechanisms, strongly mediated by underlying inflammation [21] and partly controlled by depleted liver fat due to wasting or malnutrition [18]. Low fetuin-A levels may also result in impairment of macrophage function to kill the pathogen and ectopic calcification possibly in TB lesions [49,50].

RBP4 levels were also low in TB even after adjustment for BMI. Throughout the treatment course, the levels were gradually elevated close to the reference range inversely with the decrease in CRP. These findings are supported by a recent report demonstrating that RBP4 rapidly decreases during acute inflammation, possibly acting as a negative acute phase reactant, similar to fetuin-A, albumin and prealbumin [21,51,52]. This may partly explain a close positive correlation with fetuin-A demonstrated in the active-disease group. In addition to dual regulation of RBP4 by underlying inflammation and low body fat mass, reduced renal function is also known to cause retention of the circulating levels, such that further caution is needed to interpret RBP4 measurement in disease state [53].

Our study has several limitations. Firstly, many types of nutrients including micronutrients are essential to the human body but the potential interplay between each component of nutrients was not within our scope at that time. Secondly, since change of BMI was not measured during treatment, direct comparison of improved BMI with the corresponding marker levels was not possible. Thirdly, blood was collected during the daytime without enforced fasting. Although, of course, this increases the variance of measurements, it can be inferred that daytime variations on circulating adipokines and leptin [54] are not as large as to seriously affect conclusive results of comparisons within and between groups in this study. Finally, computer tomography, which has advantages over chest radiography as an imaging tool, was not available in our setting.

Overall, our data and recent literature would suggest that all of the four markers tested are controlled partly by low fat store and partly by inflammation in TB but their regulatory mechanisms are more or less different and interactions with other relevant factors including insulin sensitivity and cellular immunity are worth further investigation. In particular, leptin, adiponectin and their ratio may be promising markers for severity of the wasting disease. Since nutritional intervention has a potential to improve prognosis of intractable TB such as HIV co-infection and MDR-TB, large-scale prospective studies using selected biomarkers to investigate metabolic contributors to disease phenotype are desired. The more fully we understand the mechanisms linking diet, health, and disease, the more effective will be our ability to design optimal interventions.

Supporting Information

Table S1 Pairwise correlations between four tested markers.
(DOC)

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Author Contributions

Conceived and designed the experiments: N. Keicho IM TT N. Kobayashi SS. Performed the experiments: IM. Analyzed the data: N. Keicho IM NTLH TS. Contributed reagents/materials/analysis tools: IM TT NTLH SS MH PHT LTL. Wrote the paper: N. Keicho.

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INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Can We Determine Depressive Conditions on the Basis of Somatic Symptoms? A Cross-sectional Study of Depressive Conditions among Japanese Patients at a University Hospital General Medicine Clinic

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Abstract

Objective We evaluated the relationship between somatic symptoms and depressive conditions among patients visiting the general medicine clinic of a university hospital.

Methods We distributed interview forms to 332 consecutive patients who visited our clinic for the first time between March and July 2011. Somatic symptoms were rated using a symptom checklist, and depressive conditions were evaluated using the Zung Self-Rating Depression Scale (SDS). We categorized and compared 2 groups of patients: patients with an SDS score of more than 48 (depressive group) and patients with an SDS score of less than 48 (non-depressive group).

Results A total of 284 (85.5%) patients returned the forms. The SDS scores were obtained from the forms of 182 patients (64.1%). The average age of these 182 patients was 46.5±18.04 years. The mean number of checked symptoms was 4.3±3.03, and the most common symptom was general fatigue (n=106; 58.2%). The number of checked symptoms in the survey was higher in the depressive group patients than in the non-depressive group patients. Multiple logistic regression analysis indicated that general fatigue, headache, and sleeping problems were significant dependent variables which were related to depressive conditions. We defined these 3 symptoms as depression-related somatic symptoms (DRSS). On a receiver-operating characteristic curve, the optimal cutoff scores were 2 of 3 DRSS and 4 of 20 somatic symptoms.

Conclusion General physicians should consider possible depressive conditions when patients have 2 or more DRSS or 4 or more somatic symptoms.

Key words: depression, SDS, somatic symptoms, screening, general medicine clinic

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Introduction

Depression is a common mental disorder with a reported prevalence of 2.7-9.3% (1). In some outpatient settings, such as a menopausal clinic (2), smoking cessation clinic (3), or emergency department (4), the prevalence of depressive conditions has been reported to be high, similar to that previously described in a general medicine clinic (5).

Depressed patients often present with somatic symptoms such as general fatigue, sleeping problems, headache, and palpitations (6, 7), and therefore, their mental health condition may be overlooked (8). A systematic review of reports in the literature indicated that the accuracy of depression recognition by non-psychiatrist physicians was low (9, 10). In this study, we evaluated the relationship between somatic symptoms and depressive conditions using the Zung Self-Rating Depression Scale (SDS) (11) among patients visiting

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Table 1. Items Used for the Self-Rating Depression Scale (SDS)

	Rarely	Sometimes	Often	Always
1 I feel down-hearted and blue	1	2	3	4
2 Morning is when I feel the best	4	3	2	1
3 I have crying spells or feel like it	1	2	3	4
4 I have trouble sleeping at night	1	2	3	4
5 I eat as much as I used to	4	3	2	1
6 I still enjoy sex	4	3	2	1
7 I notice that I am losing weight	1	2	3	4
8 I have trouble with constipation	1	2	3	4
9 My heart beats faster than usual	1	2	3	4
10 I get tired for no reason	1	2	3	4
11 My mind is as clear as it used to be	4	3	2	1
12 I find it is easy to do the things I used to	4	3	2	1
13 I am restless and can't keep still	1	2	3	4
14 I feel hopeful about the future	4	3	2	1
15 I am more irritable than ususal	1	2	3	4
16 I find it easy to make decisions	4	3	2	1
17 I feel that I am useful and needed	4	3	2	1
18 My life is quite full	4	3	2	1
19 I feel that others would be better off if I were dead	1	2	3	4
20 I still enjoy the things I used to do	4	3	2	1

Table 2. Checklist of 20 Symptoms and Number (%) of Patients who Checked Each Symptom

	n	%		n	%
1 General fatigue	106	58.2%	11 Shortness of breath	22	12.1%
2 Body weight change	51	28.0%	12 Swelling (hands, feet, face etc.)	32	17.6%
3 Fever	35	19.2%	13 Palpitations	31	17.0%
4 Skin symptoms	28	15.4%	14 Chest pain, pressure	39	21.4%
5 Headache	51	28.0%	15 Abdominal pain	28	15.4%
6 Sleeping problems	53	29.1%	16 Back pain, lumbago	39	21.4%
7 Eye symptoms	38	20.9%	17 Appetite loss	32	17.6%
8 Nose symptoms	40	22.0%	18 Diarrhea or constipation	44	24.2%
9 Oral symptoms	49	26.9%	19 Urinary symptoms	24	13.2%
10 Difficulty swallowing	15	8.2%	20 Muscle or joint pain	42	23.1%

the general medicine clinic of our hospital.

Materials and Methods

The subjects were 332 Japanese patients who visited the general medicine clinic of our hospital for the first time between March and July 2011. After obtaining written informed consent, each patient was requested to complete an interview form consisting of SDS questions and a symptom checklist, excluding those who were severely ill or required emergency treatment. Participants were then requested to submit the form to an attending doctor before the initial medical examination.

We used the Japanese version of the SDS developed by Fukuda and Kobayashi in 1983 (12). Its usefulness has since been reported in various clinical settings in Japan (2, 3, 13, 14). The SDS scale consists of 20 items, as shown in Table 1. Patients chose their answer to each item from 4 categories: always, often, sometimes, or rarely. The total score was the sum of the 20 items. If 1 or 2 items were not answered, total scores were calculated by multiplying the raw scores of 19 or 18 items by 1.05 or 1.11, respectively. The SDS scores ranged from 20 to 80, and were interpreted as follows: less than 40, no evidence of depressive condition; 40-47, mild; 48-55, moderate; greater than 56, severe (14).

We developed a symptom checklist which rated 20 common symptoms based on a previous report (15) (Table 2). Patients could check as many symptoms as they had recently experienced. We then counted the number of checked symptoms.

We collected patient data regarding age, sex, chief complaints, history of mental disorders, and clinical diagnosis from the medical records 1 to 3 months after the initial visit. We categorized patients with moderate or severe depressive conditions into a depressed group. Patients with an SDS

Table 3. Patient Characteristics

	n = 182
Age; mean (SD)	46.5 (18.04)
range	15-84
Women (%)	109 (59.9%)
SDS score; mean (SD)	39.9 (10.66)
range	23-68
Severity of depression	
non-SDS ≤ 40	97 (53.3%)
mild 40-47	47 (25.8%)
moderate 48-55	20 (11.0%)
severe SDS ≥ 56	18 (9.9%)
History of mental disorders; n (%)	9 (4.9%)
Patients with medical referral letters; n (%)	9 (4.9%)
Number of checked symptoms; mean (SD)	4.3 (3.03)
range	0-14

SD: standard deviation

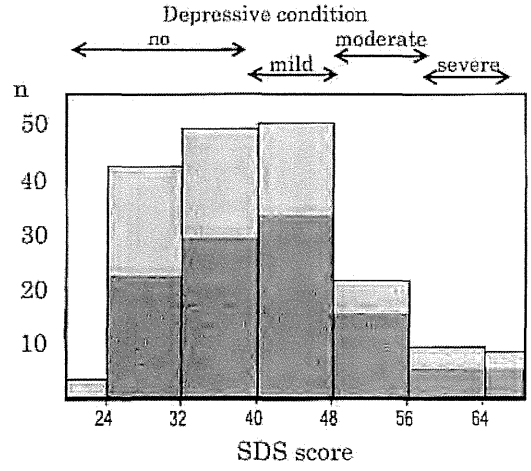


Figure 1. Distribution of SDS scores (dark grey bars indicate numbers of women).

score of less than 48 who showed no evidence of depressive conditions or mild depressive conditions were categorized into a non-depressed group. The chi-square test was used for identifying categorical variables and the Mann-Whitney U-test was used for comparisons of continuous variables between the 2 groups on bivariate analysis. We performed multivariate analysis using non-conditional logistic regression to determine which symptoms were independently associated with depressive conditions. We determined significant dependent variables and defined them as depression-related somatic symptoms (DRSS).

In addition, we calculated a receiver-operating characteristic (ROC) curve for indexes to predict depressive conditions, and analyzed the relationship between the numbers of somatic symptoms and depressive conditions, and between the numbers of DRSS and depressive conditions. Analysis was performed using JMP statistical software for Windows, Version 9 (SAS Institute, Cary, NC, USA). A P-value of less than 0.05 was considered to indicate a statistically significant difference.

Results

Patient profiles

Among the 332 patients who received the interview forms, 284 patients (85.5%) returned them to the doctors. A total of 152 patients answered all 20 items of the SDS, 22 patients answered 19 items, and 8 patients answered 18 items. Thus, the scores were calculated on the basis of a total of 182 forms (64.1%). The demographic data of the 182 patients are summarized in Table 3.

SDS scores

The mean SDS score was 39.9±10.66. Fig. 1 shows the distribution of SDS scores among the 182 patients. The SDS scores of 38 (20.9%) patients were greater than 48, indicating moderate or severe depressive conditions.

Somatic symptoms

The mean number of checked symptoms was 4.3±3.03, and the most common symptom was general fatigue (106; 58.2%), followed by sleeping problems (53; 29.1%) (Table 2).

Comparison between depressed group and non-depressed group

The characteristics of the patients in the 2 groups are shown in Table 4. There were no statistically significant differences in sex or age between the 2 groups. Patients in the depressed group had a significantly greater history of mental disorders and consultations with a psychiatrist than those in the non-depressed group. The number of checked symptoms was significantly larger in the depressed group than in the non-depressed group (6.7 vs. 3.7, $p<0.01$). Overall, 9 of the 20 checklist symptoms were more prevalent in the depressed group than in the non-depressed group.

Multiple logistic regression analysis to identify variables related to depressive condition

Multiple logistic regression analysis indicated that the significant dependent variables which were related to depressive conditions were general fatigue (checklist item 1), headache (item 5), and sleeping problems (item 6) (Table 5). We defined these 3 symptoms as DRSS.

Factors predicting depressive conditions

The areas under the ROC curves of the number of checklist symptoms and the number of DRSS were 0.751 and 0.752, respectively (Fig. 2). The sensitivities and specificities are also shown in Fig. 2.

Table 4. Patient Characteristics by Depressive Conditions

	Non-depressive group SDS < 48 n = 144	Depressive group SDS ≥ 48 n = 38	p value
Mean age (SD)	46.7 (17.49)	45.6 (20.22)	0.748
Women (%)	84 (58.3%)	25 (65.8%)	0.46
SDS score (SD)	35.6 (6.86)	55.9 (6.37)	< 0.0001*
History of mental disorders; n (%)	4 (2.8%)	5 (13.2%)	0.022*
Consultations with psychiatrists; n (%)	2 (1.4%)	4 (10.5%)	0.031*
Symptom checklist ; number of positive patients; n (%)			
1 General fatigue	72 (50.0%)	34 (89.5%)	< 0.01*
2 Body weight change	36 (25.0%)	15 (39.5%)	0.08
3 Fever	28 (19.4%)	7 (18.4%)	1.00
4 Skin symptoms	19 (13.2%)	9 (23.7%)	0.13
5 Headache	33 (22.9%)	18 (47.4%)	< 0.01*
6 Sleeping problems	28 (19.4%)	25 (65.8%)	< 0.01*
7 Eye symptoms	26 (18.1%)	12 (31.6%)	0.08
8 Nose symptoms	33 (22.9%)	7 (18.4%)	0.66
9 Oral symptoms	33 (22.9%)	16 (42.1%)	0.02*
10 Difficulty swallowing	10 (6.9%)	5 (13.2%)	0.32
11 Shortness of breath	11 (7.6%)	11 (28.9%)	< 0.01*
12 Swelling (hands, feet, face etc.)	24 (16.7%)	8 (21.1%)	0.63
13 Palpitations	16 (11.1%)	15 (39.5%)	< 0.01*
14 Chest pain, pressure	26 (18.1%)	13 (34.2%)	0.03*
15 Abdominal pain	20 (13.9%)	8 (21.1%)	0.31
16 Back pain, lumbago	28 (19.4%)	11 (28.9%)	0.20
17 Appetite loss	20 (13.9%)	12 (31.6%)	0.02*
18 Diarrhea or constipation	27 (18.8%)	17 (44.7%)	< 0.01*
19 Urinary symptoms	19 (13.2%)	5 (13.2%)	1.000
20 Muscle or joint pain	29 (20.1%)	13 (34.2%)	0.08
Number of DRSS; mean (SD)	0.98 (0.869)	1.82 (0.834)	< 0.01*
Number of checked symptoms; mean (SD)	3.7 (2.51)	6.7 (3.59)	< 0.01*

DRSS, depression-related somatic symptoms: general fatigue,headache, and sleeping problems

SDS, Self-Rating Depression Scale

*p<0.05; chi-square test or Mann-Whitney U-test

SD = standard deviation

Table 5. Results of Multiple Logistic Regression Analysis in Relation to Moderate or Severe Depressive Condition

	Odds ratio	95% CI	p value
General fatigue	5.09	1.641-19.78	< 0.01*
Headache	2.57	1.013-6.672	0.047*
Sleeping problems	5.67	2.237-15.228	< 0.01*
Oral symptoms	0.78	0.268-2.119	0.63
Shortness of breath	2.92	0.744-11.567	0.12
Palpitations	1.77	0.553-5.419	0.33
Chest pain, pressure	1.69	0.584-4.746	0.33
Appetite loss	2.03	0.668-6.105	0.21
Diarrhea or constipation	1.90	0.739-4.831	0.18

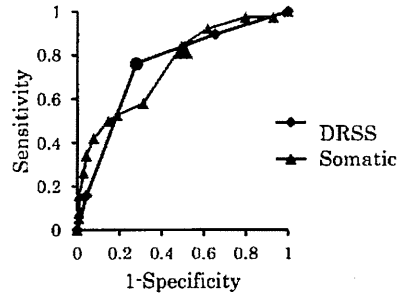
* p< 0.05

Discussion

The prevalence of depression among outpatients in primary care or a general medicine clinic is reported to be between approximately 5% and 50%, depending on the population studied and the methods used (8, 10). In the present study, 20% of patients in the general medicine clinic were suspected to have moderate or severe depressive conditions. This ratio is not significantly different from those reported by Zung et al. (5) (13%: general medicine clinic), Yoshihara et al. (13.1%: university hospital general medicine clinic) (16), and Coulehan et al. (16%: academic general medicine clinic) (17). Yoshihara et al. reported that the mean SDS score was significantly higher in patients in a university hospital general medicine clinic than in patients in rural area clinics (38.3±9.6 vs. 34.2±7.5, p<0.05).

Garcia-Campayo et al. reported positive associations between the characteristics of somatic symptoms (number, frequency, and associated disabilities) and depressive severity in patients with major depressive disorder (18). In the current study, we did not investigate the frequency or associated disabilities of somatic symptoms. However, we identified 3 somatic symptoms which were related to depressive conditions and confirmed that the number of symptoms was associated with depressive conditions.

From the ROC curves, the sensitivities and specificities for detecting depressive conditions were observed to be similar for the total number of somatic symptoms and the 3



			Sensitivity	Specificity
AUC of DRSS	0.752	● 2	76.3%	72.2%
		(95% CI	60.8-87.0	64.4-78.9)
AUC of somatic symptoms	0.751	▲4	84.2%	50.7%
		(95% CI	69.6-92.6	42.6-58.7)

AUC, area under the curve
DRSS, depression-related somatic symptoms

Figure 2. Receiver operating characteristic curve for DRSS to predict the depressive condition (SDS ≥ 48).

DRSS. In a time-limited environment such as a primary care setting or a general medicine clinic, the number of DRSS might be a simple and useful predictor of depressive conditions.

The early detection and treatment of depression are important to prevent suicide. In a recent study regarding the Japanese health service, the authors reported that patients with depression often present with physical symptoms, and therefore medical doctors should properly coordinate with psychiatrists (19).

Study limitations

There were some limitations in the present study. First, this was a single-center study, and therefore the clinical characteristics of the present patients may differ from those of other primary care clinics in Japan. Second, about one-third of the patients did not answer all 20 questions. The patients with severe depression and/or elderly patients might be unable to fill out the questionnaires. In this study, patients who answered 17 or less items of SDS were significantly older than those who answered more than 18 items (57.8±19.34 vs. 46.5±18.04, $p<0.01$). The exclusion of these patients would likely lead to an underestimation of the prevalence of depression. Further studies, with improved study design to facilitate a higher completion rate, are necessary.

Williams and Macdonald (20) determined non-response bias in the results of a two-stage screening survey of psychiatric disorder. They reported two types of non-response bias. The first one is bias due to illness, which resulted in prevalence estimates being some 5% lower than the true prevalence. The second one is bias due to defensiveness, which results in the sensitivity being overestimated by about 6%. Physicians should recognize the limitation of the tools. Despite these limitations, SDS has been a useful screening tool for depression in primary care settings and has shown reasonable and consistent accuracy.

The authors state that they have no Conflict of Interest (COI).

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