

# Spleen size in patients with metabolic syndrome and its relation to metabolic and inflammatory parameters

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## Introduction

Spleen may be enlarged in patients with metabolic syndrome (MS). Many factors that are encountered in MS were incriminated as etiology of the splenomegaly. The aim of this study was to assess spleen longitudinal diameter (SLD) in patients with MS and to investigate the possible factors affecting spleen size.

## Patients and methods

The study included 60 patients with MS and 30 healthy controls. Assessment of full medical history, anthropometric measurements, and abdominal ultrasound to identify SLD and hepatic steatosis was carried out. Liver enzymes, lipid profile, and interleukin-10 (IL-10) were measured. Fatty liver index, which is considered a marker of nonalcoholic fatty liver disease, was calculated from serum triglyceride, BMI, waist circumference, and  $\gamma$ -glutamyl transferase.

## Results

SLD was significantly higher in patients with MS than controls ( $123.57 \pm 13.88$  vs.  $101.20 \pm 5.44$  mm,  $P < 0.001$ ), but IL-10 level was significantly lower ( $65.24 \pm 23.47$  vs.  $129.41 \pm 27.65$  pg/ml,  $P < 0.001$ ). Spearman correlation in patients with MS showed significant positive correlation between SLD and waist circumference ( $r = 0.398$ ,  $P = 0.002$ ), alanine aminotransferase ( $r = 0.295$ ,  $P = 0.027$ ), aspartate aminotransferase ( $r = 0.442$ ,  $P = 0.001$ ), alkaline phosphatase ( $r = 0.444$ ,  $P = 0.001$ ), but not with IL-10 ( $r = -0.047$ ,  $P = 0.730$ ). Linear regression analysis revealed that waist circumference ( $\beta = 0.265$ ,  $P = 0.044$ ) and alkaline phosphatase ( $\beta = 0.340$ ,  $P = 0.011$ ) were the only determinants of SLD.

## Conclusion

In this study, patients with MS had larger spleen size than healthy controls. SLD significantly correlated with waist circumference but not IL-10 in patients with MS.

## Keywords:

interleukin-10, metabolic syndrome, nonalcoholic fatty liver disease, spleen

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## Introduction

Metabolic syndrome (MS) becomes a universal problem that is related to obesity, diabetes, and dyslipidemia. Lipid accumulation in obesity triggers a low-grade inflammation resulting from an imbalance between proinflammatory and anti-inflammatory components of the immune system [1].

Spleen is the largest lymphoid organ in the body and has a critical role in the modulation of the immune system and differentiation and activation of inflammatory cells. Moreover, it is anatomically linked to the liver. Nonalcoholic fatty liver disease (NAFLD) is one of the most prominent causes of liver disease worldwide. The increasing incidence of NAFLD is linked to the obesity epidemic and MS [2]. Some previous studies reported the association between increased spleen size and obesity and NAFLD [3–5].

Interleukin-10 (IL-10) is a cytokine that has variable anti-inflammatory effects and regulates insulin

sensitivity and cholesterol uptake and efflux in macrophages [6,7]. IL-10 is synthesized within multiple organs, including spleen. It acts via the IL-10 receptor to activate the JAK/STAT pathway and exerts immunosuppressive effects by blocking IkK activity or by inducing tyrosine phosphorylation of STAT-3 [8,9]. Large amounts of IL-10 are produced from activated B cells that present in the marginal zone of spleen, and recent studies suggest that spleen-derived IL-10 has important suppressing effect on destructive immune responses induced by obesity [10,11].

Spleen-derived IL-10 had a protective effect against pathological inflammation in liver and IL-10 improves liver fibrosis [12,13]. However, obesity is associated with low IL-10 production by the spleen [10].

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The aim of this study was to evaluate spleen longitudinal diameter (SLD) in patient with MS and to look for its possible association with different

Fatty liver index (FLI) was calculated from TG, GGT, BMI, and WC, by using the following formula [15]:

$$FLI = \left[ \frac{e^{0.953 \times \ln(TG) + 0.139 \times BMI + 0.718 \times \ln(GGT) + 0.053 \times WC - 15.745}}{1 + e^{0.953 \times \ln(TG) + 0.139 \times BMI + 0.718 \times \ln(GGT) + 0.053 \times WC - 15.745}} \right] \times 100.$$

variables of MS including anthropometric, metabolic parameters, IL-10 as anti-inflammatory marker, and fatty liver.

### Patients and methods

This case-control study included 60 patients who attended the internal medicine outpatient clinic and diabetes outpatient clinic between August 2016 and April 2017. A group of 30 healthy nonobese patients taken as a control group were recruited from the relatives of the patients who were well matched for age and sex. All study patients were recruited after approval of the institutional ethical committee.

MS was diagnosed if three or more of the following five criteria are met: waist circumference over 102 (men) or 88 cm (women), blood pressure over 130/85 mmHg, fasting triglyceride (TG) level over 150 mg/dl, fasting high-density lipoprotein cholesterol level less than 40 (men) or 50 mg/dl (women), and fasting blood sugar over 100 mg/dl [14].

Patients who had positive anti-hepatitis C virus antibody or hepatitis B surface antigen, history of alcoholism, bilharzial infestations, autoimmune hepatitis, drug intake, metabolic liver disease, or hematological disorders were excluded from the study. All patients provided informed consent to participate in this study.

All participants were subjected to thorough medical evaluation including presence of diabetes or hypertension and physical examination including blood pressure measurement and anthropometric measures such as weight, height, and BMI. The waist circumference (WC) was assessed midway between the lowest rib margin and the iliac crest in a standing position by the same examiner.

Biochemical tests included fasting blood glucose, total cholesterol, high-density lipoprotein cholesterol, Low-density lipoprotein cholesterol, TG, alanine aminotransferase, aspartate aminotransferase,  $\gamma$ -glutamyl transferase (GGT), alkaline phosphatase (ALP), and serum IL-10.

Patients with FLI of at least 60 were defined as having NAFLD [15].

All patients underwent ultrasonography (US) after fasting overnight using a Toshiba Aplio XV scanner (Toshiba, Tokyo, Japan) with a convex 2.5–5 MHz probe. The spleen was viewed along its longitudinal diameter in the midaxillary line; the margin between the lung and the spleen was considered as the upper limit of the spleen [16]. The presence of liver steatosis was defined as follows: (i) diffuse hyperechoic echo texture, (ii) increased liver echogenicity in contrast to the kidney, (iii) vascular blurring, and (iv) deep attenuation [17].

### Interleukin-10 measurement

Samples were assembled in serum separator tubes and were allowed to clot for 30 min. Centrifugation was done for 15 min at  $\sim 1000 \times g$ , and grossly hemolyzed samples were excluded. Serum was separated and stored at  $-80^\circ\text{C}$ . Serum IL-10 level was estimated by Human IL-10 ELISA kit (Quantikine; R&D Systems, Shanghai, China) that employs the quantitative sandwich enzyme immunoassay technique using monoclonal antibody specific for the human IL-10. The test was performed according to manufacturer's instructions.

### Statistical analysis

Data were coded and entered using the statistical package SPSS, version 24 (SPSS Inc., Chicago, Illinois, USA). Data were summarized using mean and SD for quantitative variables and frequencies (number of cases) and relative frequencies (percentages) for categorical variables. Comparisons between groups were done using unpaired *t*-test when comparing two groups. For comparing categorical data,  $\chi^2$ -test was performed. Exact test was used instead when the expected frequency is less than 5. Correlations between quantitative variables were done using Pearson correlation coefficient. Linear regression analysis was done to detect predictors of spleen diameter. *P* values less than 0.05 were considered as statistically significant.



**Table 1 Comparison of clinical, laboratory, and spleen measurements between patient and control groups**

Variables	Patients		Control		P value
	Mean	SD	Mean	SD	
Age (years)	50.33	11.1	49.3	10.1	0.451
Weight (kg)	95.00	14.90	71.40	7.54	<0.001*
Height (cm)	164.77	4.33	166.32	6.41	0.205
BMI (kg/m <sup>2</sup> )	34.96	4.89	22.81	2.38	<0.001*
Waist (cm)	105.64	9.19	89.12	4.93	<0.001*
FBG (mg/dl)	118.45	19.8	89.23	10.07	0.01*
ALT (IU/l)	62.39	43.29	16.12	6.11	<0.001*
AST (IU/l)	64.14	41.70	16.52	6.55	<0.001*
ALP (IU/l)	122.02	47.27	74.36	21.78	<0.001*
GGT (IU/l)	93.52	87.38	22.48	5.19	<0.001*
Triglyceride (mg/dl)	168.80	80.02	72.36	16.44	<0.001*
HDL (mg/dl)	41.86	11.90	51.76	9.32	<0.001*
Cholesterol (mg/dl)	168.71	43.32	120.24	23.11	<0.001*
Spleen diameter (mm)	123.57	13.88	101.20	5.44	<0.001*
IL-10 (pg/ml)	65.24	23.47	129.41	27.65	<0.001*
FLI	88.67	10.86	25.71	11.39	<0.001*

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; FBG, fasting blood glucose; FLI, fatty liver index; GGT,  $\gamma$ -glutamyl transferase. \* $P < 0.05$  is considered significant.

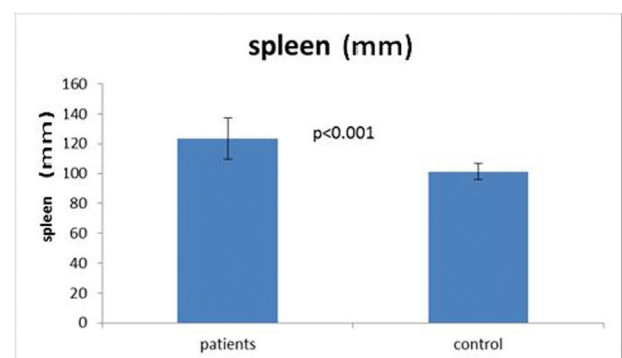
## Results

Sixty patients with MS (13 male and 47 female; mean age:  $50.33 \pm 11.1$  years) and 30 age-matched and sex-matched controls (six male and 24 female; mean age:  $49.3 \pm 10.1$  years) participated in the study. Forty-nine (87.5%) patients had diabetes and 39 (69.6%) patients were hypertensive. Almost all patients in the current study had NAFLD as detected by FLI of more than 60 and confirmed by US.

Clinical, laboratory, and ultrasonographic findings of patients and controls are illustrated in Table 1. Patients with MS had significantly larger SLD than healthy individuals ( $123.57 \pm 13.88$  vs.  $101.20 \pm 5.44$ ,  $P < 0.001$ ) (Fig. 1). IL-10 levels were significantly lower in patients with MS compared with controls ( $65.24 \pm 23.47$  vs.  $129.41 \pm 27.65$ ,  $P < 0.001$ ).

Correlations between SLD and study variables in patients with MS are reported in Table 2. SLD correlated positively with WC ( $r = 0.398$ ,  $P = 0.002$ ), alanine aminotransferase ( $r = 0.295$ ,  $P = 0.027$ ), aspartate aminotransferase ( $r = 0.442$ ,  $P = 0.001$ ), ALP ( $r = 0.444$ ,  $P = 0.001$ ), and FLI ( $r = 0.334$ ,  $P = 0.012$ ). However, there was no significant correlation between SLD and IL-10 level ( $r = -0.047$ ,  $P = 0.730$ ).

Linear regression analysis to detect independent predictors of spleen diameter revealed that waist circumference ( $\beta = 0.265$ ,  $P = 0.044$ ) and ALP ( $\beta = 0.340$ ,  $P = 0.011$ ) were the only determinant of the SLD.

**Figure 1**

Comparison of mean longitudinal diameter of the spleen between patients and control. Spleen longitudinal diameter was significantly larger in patients than control.

## Discussion

MS is a growing problem worldwide because of rapid increase in the prevalence of obesity. Spleen has been found to have a new role of metabolic and endocrine functions in obesity and MS [18].

NAFLD has become the most common cause of liver disease because of the rapid increase in the prevalence of obesity [19]. The results of the current study underline that patients with MS and fatty liver had spleen enlargement. This confirms the results of one study that found an association between NAFLD and increased spleen volume [19]. Another study revealed that patients with NAFLD had a higher SLD and significantly higher inflammatory markers than healthy controls [5]. Furthermore, Savastano *et al.* [3] reported higher SLD in patients with more severe hepatic steatosis.



**Table 2 Correlation between spleen longitudinal diameter and different variables in patients with metabolic syndrome**

Variables	<i>r</i>	<i>P</i> value
Age (years)	-0.138	0.312
BMI (kg/m <sup>2</sup> )	0.195	0.150
Waist (cm)	0.398	0.002*
ALT (IU/l)	0.295	0.027*
AST (IU/l)	0.442	0.001*
ALP (IU/l)	0.444	0.001*
GGT (IU/l)	0.117	0.390
Triglyceride (mg/dl)	0.238	0.078
HDL (mg/dl)	-0.134	0.324
Cholesterol (mg/dl)	-0.024	0.861
IL-10 (pg/ml)	-0.047	0.730
FLI	0.334	0.012*

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; FBG, fasting blood glucose; FLI, fatty liver index; GGT,  $\gamma$ -glutamyl transferase; HDL, high-density lipoprotein; IL-10, interleukin-10. \* $P < 0.05$  is considered significant.

The mechanism of splenomegaly in NAFLD and MS remains unclear. One explanation of this link is that spleen as immune organ could increase in size as a result of chronic inflammatory status associated with insulin resistance and MS. This inflammatory condition results from overproduction of inflammatory mediators such as tumor necrosis factor- $\alpha$  and IL-6 and a decrease of the anti-inflammatory cytokine IL-10 production [5,20]. In support of this hypothesis, the current study showed that IL-10 level was significantly lower in obese patients than nonobese healthy individuals. However, no significant correlation was found between SLD and IL-10. The degree of obesity is the major determinant of IL-10 level [21]. First, obesity results in inhibition of IL-10 secretion by the spleen, and then proinflammatory cytokines arise from tissues other than the spleen and become the key player of the obesity-associated inflammatory state that leads to progression of NAFLD [10].

Another explanation of increased splenic diameter in patients with MS in the current study was abdominal obesity, presented with significant correlation of SLD with waist circumference after adjustment of other confounders. This relation could be explained by sinusoidal dilatation and intracellular deposits of lipid in the spleen as a result of obesity. In support of this explanation, Gallagher and colleagues recently reported reduction in spleen size after weight reduction [22,23].

Other factors related to splenomegaly in NAFLD are portal hypertension and splenic congestion induced by progressive liver damage, in addition splenic volume increases according to the severity of the compromised liver function [24]. In this study, spleen size was

significantly correlated with transaminases. Moreover, SLD has been reported as a good discriminator between simple fatty infiltration of the liver and the presence of mild fibrosis in patients with NAFLD [5,21]. This finding indicates that spleen diameter could be a result of progression of hepatic steatosis and development of liver fibrosis. In support of this hypothesis, we found that ALP, which is an indicator of liver fibrosis in nonalcoholic steatohepatitis, predicted the SLD after adjustment for other confounders [25].

Study limitations should be mentioned. First, this was cross-sectional study. Second, assessment of the presence of NAFLD was done noninvasively by US and chemical markers not by liver biopsy. However, FLI index, a valuable and sensitive noninvasive indicator of hepatic fibrosis in patients with NAFLD was used. Third, insulin resistance has not been evaluated in our study; however, IL-10 which is an anti-inflammatory cytokine secreted by the spleen and regulates insulin sensitivity has been measured. Future researches are needed to determine the precise mechanism of spleen enlargement in patients with MS.

## Conclusion

The present study showed a clear association of spleen enlargement and waist circumference in patients with fatty liver and MS. However, there was no significant correlation between IL-10 and spleen size in these patients.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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