

# Serum levels of interleukin-1 beta associate better with severity of simple steatosis than liver function tests in morbidly obese patients

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**Background:** In high-fat diet-fed mice, interleukin-1 beta (IL-1 beta) has been shown to play a key role in hepatic steatosis. However, it remains unknown whether IL-1 beta could be associated with different grades of steatosis in obese humans. **Materials and Methods:** Morbidly obese patients ( $n = 124$ ) aged 18–65 years were divided into four groups: no steatosis (controls), mild steatosis, moderate steatosis, and severe steatosis using abdominal ultrasound. IL-1 beta serum levels and liver function tests were measured and significant differences were estimated by one-way ANOVA followed by Tukey test. **Results:** IL-1 beta serum levels significantly increased in morbidly obese patients with mild ( $11.38 \pm 2.40$  pg/ml), moderate ( $16.72 \pm 2.47$  pg/ml), and severe steatosis ( $23.29 \pm 5.2$  pg/ml) as compared to controls ( $7.78 \pm 2.26$  pg/ml). Liver function tests did not significantly change among different grades of steatosis. **Conclusion:** IL-1 beta serum levels associate better with steatosis degree than liver function tests in morbidly obese population.

**Key words:** Fatty liver, interleukin-1 beta, liver functions tests, morbid, obesity

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## INTRODUCTION

Simple steatosis is characterized by progressive fat accumulation in the liver and is considered to be the initial step in the development of nonalcoholic steatohepatitis (NASH), cirrhosis, and liver failure.<sup>[1,2]</sup> Liver function tests are typical biochemical markers to identify patients with NASH and cirrhosis, without showing utility in steatosis until now.<sup>[3]</sup>

Cytokines have been shown to play a key role in the pathogenesis and progression of steatosis in high-fat diet (HFD)-fed mice especially interleukin (IL)-1 beta (IL-1 beta).<sup>[4]</sup> However, as far as we know, there are no studies evaluating whether IL-1 beta could be modified depending on the simple steatosis degree in obese patients.<sup>[5]</sup>

Our main goal was to determine the serum levels of IL-1 beta in morbidly obese patients with different degrees of simple steatosis, while also comparing the association level of IL-1 beta and liver function tests with severity of steatosis.

## MATERIALS AND METHODS

### Study population

Morbidly obese women and men ( $n = 124$ ) aged 18–65 years, attending to the Department of Internal Medicine and the Clinic for Obesity of the General Hospital of Mexico from October 2014 to December 2017, were included in the study by convenience sampling and divided into four groups: controls (no steatosis), mild steatosis, moderate steatosis, and severe steatosis. Steatosis degree was scored by two independent senior hepatologists using

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abdominal ultrasound imaging, as previously described.<sup>[6]</sup> All participants provided written informed consent, and the study was conducted in strict adherence to the Helsinki Declaration. Exclusion criteria included previous diagnosis of acute or chronic hepatic disease, inflammatory or autoimmune disorders, acute or chronic infectious diseases, and neoplastic disorders, as well as pregnancy, lactation, and any kind of anti-inflammatory medical treatment. Blood samples were collected from each participant after 10–12 h fasting to avoid procedural variations.

#### Liver function tests and interleukin-1 beta serum levels

Liver function tests, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), and total bilirubin, were measured from serum samples by enzymatic assay (Megazyme International, Ireland). Blood glucose, insulin, HbA1c, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglycerides were also measured according to the manufacturer's instructions (Megazyme International, Ireland). IL-1 beta serum levels were measured by enzyme-linked immunosorbent assay (Peprotech, Mexico).

#### Statistical analysis

Data are expressed as mean  $\pm$  standard deviation and were analyzed by one-way ANOVA followed by a *post hoc* Tukey test. ANOVA results were corrected using the Bonferroni's multiple comparison test. Women/men proportion in each group was evaluated using the Chi-square test. All statistical analyses were performed using the GraphPad Prism 6.01 software, GraphPad Software, La Jolla, CA 92037 USA. Differences were considered statistically significant when  $P < 0.05$ .

## RESULTS

No significant differences were found in women/men proportion, age, body mass index, blood pressure, HDL, and triglycerides between control subjects ( $n = 27$ ) and patients with mild ( $n = 35$ ), moderate ( $n = 31$ ), or severe steatosis ( $n = 31$ ) [Table 1]. Severe steatosis was accompanied by significantly increased levels of blood glucose, insulin resistance, insulin, glycated hemoglobin, cholesterol, and LDL. Moderate steatosis was characterized by hyperinsulinemia resulting in low glucose values, even though insulin resistance was also increased in these patients. Mild steatosis was hallmarked by having similar levels of blood glucose, insulin, insulin resistance, cholesterol, and LDL than those found in morbidly obese patients without steatosis [Table 1].

All patient groups significantly differed from each other regarding IL-1 beta serum levels. As a matter of fact, IL-1 beta progressively elevated as the severity of steatosis also increased [Table 2]. Morbidly obese participants without steatosis (controls) showed  $7.78 \pm 2.26$  pg/ml IL-1 beta in blood as compared to patients with mild, moderate, and severe steatosis, who exhibited  $11.38 \pm 2.40$ ,  $16.72 \pm 2.47$ , and  $23.29 \pm 5.2$  pg/ml IL-1 beta, respectively [Table 2]. Lower-upper 95% confidence intervals (95% CIs) for controls were 6.88–8.67 pg/ml IL-1 beta. On the contrary, lower-upper 95% CI for mild, moderate, and severe steatosis rose to 10.55–12.21, 15.81–17.63, and 21.34–25.23 pg/ml IL-1 beta, respectively. No significant differences were found in ALT, AST, GGT, ALP, and total bilirubin between control participants and patients with mild, moderate, or severe steatosis [Table 2].

**Table 1: Demographic and biochemical characteristics of the study subjects**

Parameters	Steatosis			
	Controls <sup>a</sup>	Mild <sup>b</sup>	Moderate <sup>c</sup>	Severe <sup>d</sup>
Sex (women/men)*	21/6	27/8	24/7	25/6
Age (years)	33.6 $\pm$ 9.1	34.8 $\pm$ 9.4	32.6 $\pm$ 8.2	37.7 $\pm$ 8.6
BMI (kg/m <sup>2</sup> )	41.1 $\pm$ 4.2	42.8 $\pm$ 6.2	42.2 $\pm$ 6.9	41.8 $\pm$ 6.5
Blood pressure (mmHg)	132.86 $\pm$ 13.10	131.86 $\pm$ 13.9	131.86 $\pm$ 12.10	131.87 $\pm$ 14.11
Blood glucose (mg/dl)	102.4 $\pm$ 11.6	103.5 $\pm$ 18.1	96.7 $\pm$ 12.7	132.9 $\pm$ 23.6 <sup>a,b,c</sup>
Serum insulin (mU/l)	14.4 $\pm$ 5.7	14.2 $\pm$ 5.3 <sup>c,d</sup>	20.9 $\pm$ 4.8 <sup>a,b</sup>	21.2 $\pm$ 5.2 <sup>a,b</sup>
HOMA-IR	3.7 $\pm$ 1.5	3.5 $\pm$ 1.7 <sup>c,d</sup>	5.1 $\pm$ 1.3 <sup>b,d</sup>	7.1 $\pm$ 2.6 <sup>a,b,c</sup>
HbA1C (%)	4.1 $\pm$ 0.9	5.2 $\pm$ 1.4 <sup>a,d</sup>	5.4 $\pm$ 0.8 <sup>a,d</sup>	7.6 $\pm$ 1.6 <sup>a,b,c</sup>
Total cholesterol (mg/dl)	187.2 $\pm$ 26.1	179.8 $\pm$ 31.1 <sup>c,d</sup>	208.5 $\pm$ 39.4 <sup>b</sup>	223.2 $\pm$ 44.6 <sup>a,b</sup>
LDL (mg/dl)	101.6 $\pm$ 18.6	103.8 $\pm$ 32.7 <sup>c,d</sup>	136.8 $\pm$ 24.3 <sup>a,b</sup>	138.8 $\pm$ 19.8 <sup>a,b</sup>
HDL (mg/dl)	32.6 $\pm$ 6.1	39.3 $\pm$ 9.2	37.3 $\pm$ 6.3	33.8 $\pm$ 6.4
Triglycerides (mg/dl)	195.1 $\pm$ 74.5	184.5 $\pm$ 91.7	212.7 $\pm$ 79.3	230.3 $\pm$ 76.9

Data are expressed as mean $\pm$ SD except for \*that is expressed as absolute values. Significant differences were estimated by means of one-way ANOVA followed by a *post hoc* Tukey test except for \*that was analyzed by means of the Chi-square test. ANOVA results were corrected using the Bonferroni's multiple comparisons test. Differences were considered significant when  $P < 0.05$ , as follows: <sup>a</sup>Significant difference versus controls; <sup>b</sup>Significant difference versus mild steatosis; <sup>c</sup>Significant difference versus moderate steatosis; <sup>d</sup>Significant difference versus severe steatosis. BMI=Body mass index; HOMA-IR=Homeostatic model assessment of insulin resistance that resulted of multiplying glucose by insulin then divided by 22.5; HbA1c=Glycated hemoglobin; LDL=Low-density lipoprotein; HDL=High-density lipoprotein; SD=Standard deviation

**Table 2: Serum levels of interleukin-1  $\beta$  and liver function tests in morbidly obese patients with hepatic steatosis**

Parameters	Steatosis			
	Controls <sup>a</sup>	Mild <sup>b</sup>	Moderate <sup>c</sup>	Severe <sup>d</sup>
IL-1 $\beta$ (pg/ml)	7.78 $\pm$ 2.26	11.38 $\pm$ 2.40 <sup>a,c,d</sup>	16.72 $\pm$ 2.47 <sup>a,b,d</sup>	23.29 $\pm$ 5.29 <sup>a,b,c</sup>
ALT (U/L)	33.21 $\pm$ 16.83	37.49 $\pm$ 15.59	41.03 $\pm$ 19.32	40.03 $\pm$ 20.17
AST (U/L)	27.35 $\pm$ 11.90	32.16 $\pm$ 16.83	37.26 $\pm$ 20.25	36.58 $\pm$ 15.86
GGT (U/L)	28.87 $\pm$ 16.41	32.51 $\pm$ 16.26	32.78 $\pm$ 18.69	37.89 $\pm$ 21.60
ALP (U/L)	70.00 $\pm$ 19.70	68.93 $\pm$ 26.57	73.63 $\pm$ 23.84	69.86 $\pm$ 22.28
Total bilirubin (mg/dL)	0.78 $\pm$ 0.26	0.73 $\pm$ 0.31	0.84 $\pm$ 0.39	0.93 $\pm$ 0.30

IL-1 $\beta$  serum levels progressively increase according to the steatosis degree. ALT, AST, GGT, ALP, and total bilirubin do not significantly change among different grades of hepatic steatosis in the study population. Steatosis degree was scored by two independent senior hepatologists using abdominal ultrasound imaging. Data are expressed as mean $\pm$ SD. Significant differences were estimated by means of one-way ANOVA followed by a *post hoc* Tukey test. ANOVA results were corrected using the Bonferroni's multiple comparisons test. Differences were considered significant when  $P < 0.05$ , as follows: <sup>a</sup>Significant difference versus controls; <sup>b</sup>Significant difference versus mild steatosis; <sup>c</sup>Significant difference versus moderate steatosis; <sup>d</sup>Significant difference versus severe steatosis. IL=Interleukin; ALT=Alanine aminotransferase; AST=Aspartate aminotransferase; GGT=Gamma-glutamyl transpeptidase; ALP=Alkaline phosphatase; SD=Standard deviation

## DISCUSSION

IL-1 beta is a member of the IL-1 cytokine family with prominent functions in the regulation of the inflammatory response as well cell proliferation, differentiation, and apoptosis.<sup>[7,8]</sup> However, IL-1 beta has now emerged as a major contributor in steatosis development by inducing hepatic lipogenesis and triglyceride overproduction in HFD-fed mice.<sup>[3,9]</sup> Consistent with previous information, our results demonstrate for the first time that IL-1 beta serum levels clearly increase in morbidly obese patients that show the most severe stages of steatosis, which in turn concurs with prior studies also reporting IL-1 beta elevation in portal and systemic blood of HFD-fed mice with hepatic steatosis.<sup>[10]</sup>

A growing body of studies have consistently reported increasing serum levels of tumor necrosis factor alpha, IL-6, IL-8, and IL-10 in NASH patients.<sup>[11,12]</sup> However, as far as we know, IL-1 beta had never been reported in morbidly obese patients with simple steatosis.<sup>[6,11,13]</sup> Interestingly, in morbidly obese participants without steatosis, serum IL-1 beta ranged from 2.02 to 11.63 pg/ml. On the contrary, in morbidly obese patients with mild, moderate, or severe steatosis, IL-1 beta levels were found between 4.87 and 19.07, 11.82 and 23.53, and 10.62 and 34.47 pg/ml, respectively, which suggest that serum values of this cytokine considerably increase only in severe grades of hepatic fat accumulation.

Finally, numerous studies have persistently shown that patients with simple steatosis exhibit normal liver function tests, except those who are progressing to NASH.<sup>[14,15]</sup> Our data expand on this body of work by revealing that liver function tests do not change in morbidly obese patients with different degrees of steatosis. In this sense, IL-1 beta appears to associate better with hepatic steatosis than liver function tests in patients with morbid obesity and should be further investigated in clinical prospective studies.

## CONCLUSION

The serum levels of IL-1 beta associate better with severity of simple steatosis than liver function tests in morbidly obese population.

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### Conflicts of interest

There are no conflicts of interest.

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