Research Article

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Serum interleukin 1 receptor antagonist as an independent marker of non-alcoholic steatohepatitis in humans

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Background & Aims: Mechanisms leading to non-alcoholic steatohepatitis (NASH) have remained unclear, and non-invasive diagnosis of NASH is challenging. In this study, we investigated the benefits of measuring serum interleukin 1 receptor antagonist (IL-1RA) levels.

Methods: Liver biopsies from 119 morbidly obese individuals (47.5 ± 9.0 years, BMI 44.9 ± 5.9 kg/m^2) were used for histological and gene expression assessment. In a cross-sectional population-based cohort of 6447 men (58 ± 7 years, BMI 27.0 ± 3.9 kg/m^2) the association of serum IL1-RA with serum alanine aminotransferase (ALT) levels was investigated.

Results: Serum levels of IL-1RA, and liver mRNA expression of *IL1RN* are associated with NASH and the degree of lobular inflammation in liver (p <0.05). The decrease in serum IL-1RA level and expression of *IL1RN* after obesity surgery correlated with the improvement of lobular inflammation (p <0.05). We developed a novel NAFLD Liver Inflammation Score, including serum Il-1RA concentration, which performed better to diagnose NASH than did previously published scores. Results from the population study confirmed the potential of measuring serum IL-1RA level. The strongest determinants of the ALT concentration at the population level were Matsuda insulin sensitivity index (r^2 = 0.130, $p = 7 \times 10^{-197}$) and serum IL-1RA concentration (r^2 = 0.074, $p = 1 \times 10^{-110}$). IL-1RA concentrations associated significantly with ALT levels even after adjusting for BMI, alcohol consumption and insulin sensitivity ($p = 2 \times 10^{-21}$).

Conclusions: IL-1RA serum levels associate with liver inflammation and serum ALT independently of obesity, alcohol consumption and insulin resistance, suggesting a potential use of IL-1RA as a non-invasive inflammatory marker for NASH.

Keywords: Nonalcoholic steatohepatitis; Interleukin 1 receptor antagonist; IL-1RA; Cytokines; Steatohepatitis; Population study; Obesity; Insulin resistance.
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Abbreviations: BMI, body mass index; DM2, type 2 diabetes; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; IL-1β, interleukin 1β; IL-1RA, interleukin 1 receptor antagonist; OGTT, oral glucose tolerance test; Matsuda ISI, Matsuda insulin sensitivity index; NAFLD, non-alcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; TNFα, tumour necrosis factor α.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is rapidly becoming the most common cause of liver disease [1], estimated to affect 10–50% of humans in different studies [2]. NAFLD has two important consequences. First, hepatic lipid accumulation contributes to metabolic alterations, such as insulin resistance, hyperglycemia and hyperlipidemia [3–5]. In fact, liver fat accumulation is likely to be the major determinant of metabolic abnormalities related to the metabolic syndrome [5,6]. Second, NAFLD can lead to non-alcoholic steatohepatitis (NASH) and liver cirrhosis, and ultimately to liver failure [7].

The challenge in the diagnosis of NASH is that there are no reliable non-invasive methods to determine the degree of steatohepatitis [8]. Several mechanisms could explain why obesity is related with NAFLD. Liver may serve as an alternative depot for free fatty acids (FFA) when adipose tissue capacity is exceeded [9] or mitochondrial oxidative capacity is decreased [10]. Adipose-derived inflammatory signals, increased production of reactive oxygen species [11,12], increased cholesterol synthesis and accumulation of free cholesterol [13] have been suggested to contribute to the development of NASH. All these mechanisms have been linked to steatosis but their role in liver inflammation is less clear.

Levels of interleukin 1 receptor antagonist (IL-1RA) are elevated in conditions related to liver fat accumulation, such as obesity and type 2 diabetes (T2DM) [14]. In this study, we tested the hypothesis that serum levels of interleukin 1 receptor antagonist (IL-1RA) [14], could also help in the detection of inflammation and activity of NASH. Additionally, an association of serum IL-1RA level with serum alanine aminotransferase (ALT) level, a marker of liver disease, in a large population study of 6447 men [15], was investigated. Based on the findings that serum IL-1RA level associated with NASH and serum ALT level independently of other known risk factors, we suggest that that

Table 1. Clinical characteristics and selected liver histology (details in Supplementary Table 1) in the obesity surgery study.

	Not NASH n = 72	Possible NASH n = 19	Definitely NASH n = 28	*p*
Clinical				
Sex (male/female)	22/50	9/10	11/17	0.143*
Age (yr)	47.9 ± 9.2	45.8 ± 7.3	46.7 ± 8.0	0.449
Weight (kg)	128.3 ± 17.0	134.1 ± 21.3	132.1 ± 24.5	0.559
BMI (kg/m^2)	6.0	44.5 ± 4.9	44.3 ± 6.9	0.658
Diabetes n (%)	27 (37)	10 (56)	11 (44)	0.076*
Fasting glucose (mmol/L)	6.4 ± 1.4	6.9 ± 1.6	6.5 ± 1.6	0.789
Fasting insulin (mU/L)	17.6 ± 9.0	21.5 ± 12.4	25.4 ± 17.1	0.046
Plasma adiponectin (μg/ml)	9.3 ± 4.9	8.0 ± 3.5	7.6 ± 2.7	0.310
ALT U/L	39 ± 21	51 ± 26	57 ± 34	0.021
AST U/L	29 ± 10	36 ± 13	47 ± 36	0.007
Histology				
NAFLD activity score (definition below)	0.75 ± 0.94	2.68 ± 1.11	4.00 ± 1.24	1 x 10^{-10}
Steatosis grade				1 x 10^{-6}
<5%	40	4	1	
5-33%	24	8	10	
33-66%	4	3	11	
>66%	4	4	6	
Lobular Inflammation				1 x 10^{-27}
None	71	0	0	
<2 foci per 200x field	1	18	17	
2-4 foci per 200x field	0	1	11	
Liver cell ballooning				
None	63	14	10	1 x 10^{-6}
Few balloon cells	9	5	13	
Many cells/prominent ballooning	0	0	5	
Fibrosis stage				4 x 10^{-7}
None	57	9	4	
Perisinusoidal or periportal	12	7	21	
Perisinusoidal and portal/periportal	2	3	1	
Bridging fibrosis	1	0	1	
Cirrhosis	0	0	1	

NAFLD activity score is unweighted sum of steatosis (0–3), lobular inflammation (0–3) and hepatocellular ballooning (0–2) scores.
*Kruskal–Wallis test for continuous variables and Chi square-test for categorical variables.

measurement of serum IL-1RA concentration, as an inflammatory marker, could improve non-invasive diagnosis of NASH.

Patients and methods

Liver biopsy study of morbidly obese subjects

One hundred nineteen subjects (42 men, 77 women, (47.5 ± 9.0 years, BMI 44.9 ± 5.9 kg/m^2 other characteristics see Table 1) were selected from an ongoing study including all subjects undergoing bariatric surgery at the Kuopio University Hospital [16]. Every participant had one-day visit including an interview on the history of previous diseases and current drug treatment, and an evaluation of glucose tolerance and cardiovascular risk factors. Fasting blood samples were drawn after 12 h of fasting followed by an oral glucose tolerance test (OGTT). Weight loss in this cohort during the 1 year follow-up was 32.6 ± 11.6 kg (-25%). All patients with alcohol consumption >2 doses per day were excluded from the study.

Population study

A total of 6447 men from the population-based cross-sectional METSIM Study (Metabolic Syndrome in Men Study) were included in the study, as previously described [15]. Subjects, aged from 45 to 70 years, were randomly selected from the population register of Kuopio town, Eastern Finland (population of 95 000). Their age was 58 ± 7 years, and BMI 27.0 ± 3.9 kg/m^2.

Informed consent was obtained from each participant and the study protocol was approved by the Ethics Committee of Northern Savo Hospital District and was in accordance with the Helsinki Declaration.

Clinical measurements

Body composition was determined by bioelectrical impedance (RJL Systems) in subjects in the supine position after a 12-h fast. Self-reported alcohol consumption was below 20 g/week in the lowest tertile and above 120 g/week in the highest tertile of alcohol consumption. A 2-h OGTT (75 g of glucose) was