

Cytokines Activating ILCs2 in Patients With Obesity Before and After Bariatric Surgery Shortened Title: ILCs2 in Patients With Obesity

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Abstract

Abstract/Purpose: Group 2 innate lymphoid cells (ILC2) are integral regulators of adipose tissue type 2 immunity and respond to epithelial signals such as IL-25, IL-33, thymic stromal lymphopoeitin (TSLP). Purpose was to assess cytokines activating ILCs2 in serum of patients with obesity and effect of bariatric surgery on these parameters

Material and Methods: In a single-center prospective study serum IL-25, IL-33, TSLP and ST2L levels were assayed at baseline and at 6 months after bariatric surgery and correlated with anthropometric changes and metabolism parameters.

Results Mean age and median of BMI of study participants were 41.9 years \pm 11 and 45.6 kg/m² (range 36.3-56.3), respectively. Six months after surgery %EWL was 43.1 \pm 10.2%. Serum TSLP was significantly lower in patients with obesity both before and after surgery than in healthy controls. TSLP values before operation were significantly correlated to HbA1c%, glucose plasma level and female sex. Serum IL-25, IL-33 and ST2L levels were comparable to controls both before and after operation.

Conclusions: Decreased serum levels of TSLP may be a characteristic trait for obesity, however non-modifiable by body mass surgical reduction in short time observation. Low serum levels of TSLP are related to disturbances in glucose metabolism and female sex.

Key Bullet

1. TSLP serum levels are significantly lower in patients with obesity than in healthy controls.
2. TSLP serum levels are related to glucose fasting and HbA1c in patients with obesity .
3. Short term body mass reduction resulting from bariatric surgery does not make TSLP serum levels higher .

Introduction/purpose

Obesity predisposes to several severe diseases such as hyperlipidemia, diabetes mellitus, cancer or cardiovascular diseases. Moreover it has been proved that with expanding adipose tissue chronic, low-grade inflammatory process increased [1-3], orchestrated by many various cytokines. Ones of them are cytokines belonging to a group 2 innate lymphoid cells (ILCs2) [4]. ILC2 are integral regulators of adipose tissue type 2 immunity, cooperate with regulatory T cells, support adipose tissue browning, control AT tissue remodeling and counterpart the progression of type 1 inflammation [5, 6]. Group 2 innate lymphoid cells (ILCs2) respond to epithelial signals such as IL-25, IL-33, thymic stromal lymphopoeitin (TSLP) [6-8]. The IL-33/ST2 axis is protective against obesity, insulin resistance, and type 2 diabetes (T2D) in animal models [9-10]. Interleukin-33 (IL-33), the ligand for the receptor ST2, is abundant in adipose tissue, including preadipocytes, adipocytes, and endothelial cells [11]. Recently it has been found that population of ILC2 are decreased in white adipose tissue (WAT) in mice and in humans and may be a conserved

feature of obesity [4]. Some data suggested the impaired function of TSLP gene in patients with obesity [12].

Thus taking together all the data suggesting that ILCs2 and TSLP may prevent obesity and atherosclerosis we decided to assess cytokines activating ILCs2 in serum of patients with obesity and effect of bariatric surgery on the these parameters.

Materials And Methods

Study design, settings, and participants

This single-center prospective observational cohort study included patients from the Department of General and Bariatric Surgery and Emergency Medicine. Patients with extreme obesity with a BMI > 35 kg/m², > 18 years of age, and indicated for bariatric surgery were included. Controls with BMI between 18.5 and 25 kg/m² were recruited at the above mentioned Department and at the department of Internal Medicine, Allergology and Clinical Immunology. Exclusion criteria both for patients and controls were as follows: allergic diseases such as allergic rhinitis, bronchial asthma and atopic dermatitis, any acute inflammation and chronic inflammatory conditions, or coexisting malignancies. The research was approved by the Bioethics Committee, Resolution No. PCN/0022/KB1/141/I/19/20. All subjects gave informed written consent.

Patient variables

Patients with obesity were examined directly before surgery and 6 months after surgery during routine follow-up in the outpatient clinic. Baseline body weight, BMI, excess body weight (EBW), waist-hip ratio, serum IL-25, IL-33, TSLP and ST2L were assayed in the cases and in controls. Moreover lipid metabolism parameters such as total cholesterol (mg/dl), HDL-cholesterol (mg/dl), LDL-cholesterol (mg/dl), triglycerides (mg/dl), glucose fasting and glycated hemoglobin (HbA1c, %) were assayed in the cases. The same variables were assessed 6 months after surgery.

Measurement of serum levels of IL-25, ST2, IL-33, and TSLP

Commercial enzyme-linked immunosorbent assays were used to measure serum level of IL-25 (Wuhan), IL-33, TSLP and ST2L/IL-1 R4 (R&D Systems). The assays were performed using the protocols recommended by the manufacturers. Sensitivity and range of IL-25 determinations are 10 pg/ml and 62.5 pg/ml - 4000 pg/ml, respectively, of IL-33 1.51 pg/ml and 3.1 - 200 pg/ml, respectively, of TSLP 9.87 pg/ml and 31.2 - 2,000 pg/ml, respectively and of ST2L//IL-1 R4 – 13.5 pg/ml and 31.3 - 2,000 pg/ml, respectively. [13-16]

Statistical analysis

Results are expressed as mean and standard deviation or median values with interquartile ranges. Normality was checked using the Shapiro-Wilk test. Categorical variables were presented by count and

percentage. Nonparametric tests were used (U-Mann-Whitney test, Wilcoxon rank sum test). Multiple regression analysis was performed with age, sex, BMI, serum glucose, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, and HbA1c to determine the association with serum cytokine concentration before and after surgery. All analyses were performed with a software package (The STATISTICA PI 13.5). P values less than 0.05 were considered significant.

Results

Patient characteristics

30 patients with severe obesity, 25 female (83.3%), mean age 41.9 ± 11 years, BMI - 45.6 (36.3-56.3) kg/m^2 were included into the study and qualified to bariatric surgery. All patients were re-evaluated 6 months after surgery. Their body weight, BMI, and WHR were significantly reduced compared with baseline. Percentage of excess weight loss (EBL) at six months after surgery was $43.1 \pm 10.2\%$. HbA1c, HDL-cholesterol and triglycerides levels were significantly lower than baseline, HDL-cholesterol levels significantly increased. The patients characteristics at baseline and at 6 months after surgery are shown in Table 1. The control group included of 15 non-obese, age-matched participants, six women (40%) with a mean age of 39.7 ± 8.3 years and BMI of $23.6 \pm 2.6 \text{ kg/m}^2$.

Serum IL-25, IL-33, TSLP and ST2L were assayed at baseline and at 6 months after surgery (Table. 2). Baseline serum TSLP was significantly lower in patients with obesity both before ($p = 0.0008$) and at six months after surgery ($p = 0.005$) than in healthy controls (Mann-Whitney U rank sum test, figure 1). Serum levels of all four cytokines in the 6 months after surgery did not change significantly as compared to values before operation ($p > 0.05$, Wilcoxon rank sum test). Multiple regression analysis revealed that TSLP values before operation were significantly correlated to HbA1c%, glucose plasma level and female sex ($p = 0.003$). At six months after operation TSLP was not correlated to any of estimated parameters.

Discussion

In this study we assessed the serum levels of cytokines IL-25, IL-33 and thymic stromal lymphopoietin that activate ILCs2 and a soluble form of receptor for IL-33 called ST2L in patients with severe obesity who underwent bariatric surgery. We found that both at baseline and at six months after bariatric surgery TSLP serum levels were significantly lower in patients with obesity than in controls. TSLP was related to fasting glucose and HbA1c and female sex before surgery. IL-33, IL-25 and ST2L serum levels in patients with obesity were comparable to results in healthy non-obese controls both before and at six months after bariatric surgery. Moreover bariatric surgery did not significantly change serum levels of any of the studied cytokines.

TSLP is known as crucial inflammatory cytokine in immune homeostasis [17]. This molecule is related to promotion of autoimmunity and cancer [18,19]. It was found that the serum levels of TSLP may be suitable as a biomarker for prediction of prognosis in a subgroup of patients with sepsis who are

exhibiting hyperleukocytosis and a high neutrophil ratio [20]. Moreover its role was confirmed in atopic diseases [21], pediatric allergic asthma [22] and atopic dermatitis [23] where may be even a potential therapeutic agent. Much less is known about its role in obesity. TSLP is expressed in human adipose tissue and is produced by human differentiated adipocytes derived from subcutaneous or omental depots in response to a variety of agonists such as TSH, IL-1 β , and TNF- α [24]. A previous expression profiling of visceral adipose tissue revealed lower expression of TSLP gene in severely obese men with metabolic syndrome than in obese without metabolic syndrome [16].

We found the relations between TSLP levels and glucose fasting and HbA1c in patients with obesity before operation. This relation disappeared after bariatric operation resulting in improvement of glucose metabolism parameters. Data on the role of TSLP in glucose metabolism are scarce. TSLP was suggested to have therapeutic potential for the treatment of type 1 diabetes [25]. Moreover in another study TSLP improved insulin sensitivity and caused a robust increase in Tregs and decrease in CD8 T cells and NK cells in the adipose of MC903 and TSLP-treated mice, suggesting that activation of TSLP signaling may be a therapeutic immunotarget for improving insulin sensitivity and preventing type 2 diabetes. Thus our finding of the relationship between lower levels of serum TSLP and glucose fasting and HbA1c in patients with obesity may be in some aspects in line with the data of possible protective role of TSLP against both type 1 and type 2 diabetes at least in murine models. Further studies on the role of TSLP in humans with obesity and diabetes are warranted.

In our study we found that serum levels of mesenchymal-derived cytokines such as IL-33, IL-25 were comparable in patients with severe obesity and healthy controls with normal weight and did not change after bariatric procedure and significant body mass reduction. The role of IL-33 in obesity has been proved. IL-33 is related to the beigning of adipocytes and to the maintenance of adipose tissue homeostasis. However the differences of action between exogenous IL-33 and adipose tissue-driven IL-33 was observed [26]. Our results may suggest that the known interactions of IL-33 and IL-25 may a play a role on tissue levels and are not reflected in the systemic circulation.

There are some limitations of our study. One of them is a relatively low size of control group. Another limitation is the fact that all female and majority of male patients with obesity had central type of obesity so we could not compare results of TSLP measurements between patients with various types of obesity. Moreover it would be interesting to investigate other cytokines that stimulate ILC2s such as IL-2 and IL-7 and cytokines produced by ILC2s such as IL-5, IL-9 and IL-13 and amphiregulin and epidermal growth factor receptor [5, 27]. It may be a suggestion for further research in this field.

Conclusion

Summarizing, we found that lower serum levels of TSLP may be a characteristic trait for obesity and the reduction of body mass as a result of bariatric surgery did not change it. Moreover low serum levels of TSLP are related to disturbances in glucose metabolism. These data may suggest that deficiency in TSLP may play a role in obesity development together with diabetes, especially in women.

Declarations

Conflict of Interest: The authors declare that they have no conflict of interest.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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Tables

Table 1 Characteristics of the study obese population at baseline and at 6 months after surgery

Characteristics	Median and IQR or mean \pm SD	Range	<i>p</i> value
Age (years)	41.9 \pm 11	21-61	
Weight (kg)			
Baseline	129.2 \pm 17.7	103-160	p=0.000003
6 months post-operative	99.2 \pm 12.3	79-123	
BMI (kg/m ²)			
Baseline	45.6 (36.3-56.3)	41.8-50.5	P=0.000002
At 6 months	34.9 (32.3-38.1)	28.7-43.1	
WHR			
Baseline	0.9 \pm 0.05	0.8-1.0	p=0.002
At 6 months	0.9 \pm 0.1	0.8-1.1	
%EWL			
At 6 months	43.1 \pm 10.2	20.9-68.7	
HbA1c (%)			
Baseline	5.7 (5.2-6.2)	4.9-9.5	p=0.01
At 6 months	5.4 (5.2-5.6)	5.2-5.6	
Glucose fasting (mmol/l)			
Baseline	5.2 (4.9-6.)	4.3-9.6	p=ns
At 6 months	5.6 (5.2-5.9)	4.5-10.3	
Total cholesterol (mg/dl)			
Baseline	194 (183-230)	129-268	p=ns
At 6 months	215 (184-234)	152-303	
HDL cholesterol			
Baseline	49 (42-55)	35-81	p=0.002
At 6 months	52 (48-59)	36-123	
LDL cholesterol			
Baseline	112.1 \pm 36.7	35-81	p=0.04
At 6 months	131.5 \pm 42.3	11-207	
Triglycerides (mg/dl)			

Baseline	146 (113-175)	58-575	p=0.002
At 6 months	101 (81-152)	61-224	

BMI indicates body mass index, WHR – waist-hip ratio, EWL – excess weight loss, HbA_{1c} – glycated hemoglobin, HDL – high-density lipoprotein, LDL – low-density lipoprotein

Table 2 Comparison of serum levels of IL-25, IL-33, TSLP and ST2L before and after operation in obese patients and in controls

	Obese before operation	Obese at 6 months after operation	Controls
IL-25 (pg/ml)	9.5 (4-23; 1-86)	18 (9-41; 0.9-143)	20 (6-223; 1-400)
IL-33 (pg/ml)	7 (3,5-11; 1,6-29)	7 (3-12; 1,4-26)	11 (7-12; 3-32)
TSLP (pg/ml)	7.1* (5.4-15; 1.4-56.8)	8.7† (4.7-17.3; 2.3-64)	16.3 (11.9-68.6; 8.1-85.4)
ST2L (pg/ml)	672.5 (539-761; 359-1644)	615 (475-792; 370-1252)	583 (511-903; 230-1324)

Values are shown as median, interquartile range and total range.

CON – controls; *p=0.0008 vs. controls; † p=0.005 vs. controls; U Mann-Whitney test

Figures

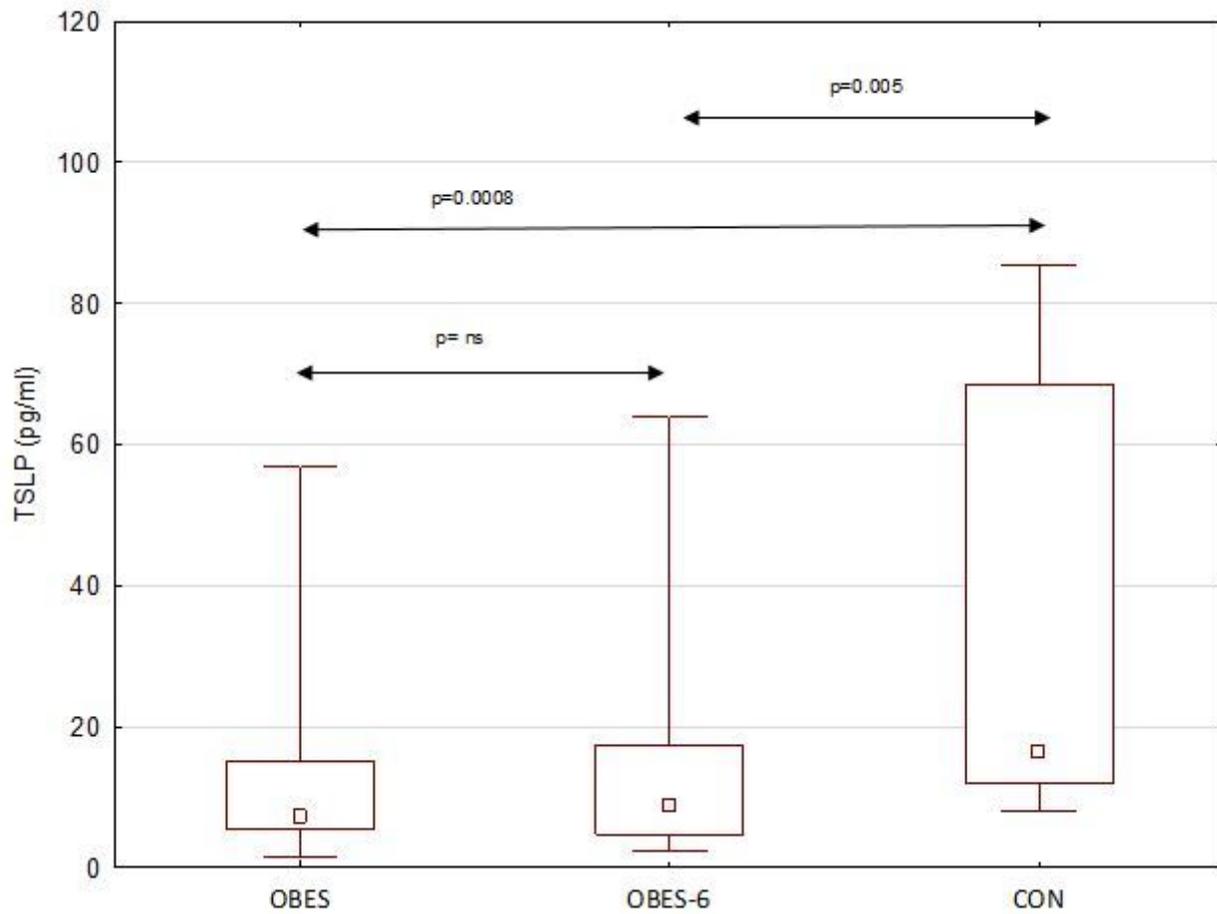


Figure 1

Serum levels of TSLP (pg/ml) in patients with obesity before (OBES) and at six months after operation (OBES-6) and in the control groups. Data are medians, interquartile, and total ranges.