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Interleukin-8, CXCL10, CXCL11 and their role in insulin resistance in adult females with subclinical hypothyroidism and prediabetes

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ABSTRACT

In obesity, the hormonal secretion of the thyroid gland switches from homeostasis to type 2 allostasis in order to adapt to persistent modifications of adipose tissue and inflammation. Previous meta-analyses have linked obesity with an increased risk of developing thyroid diseases, prediabetes, and type 2 diabetes mellitus. We designed an observational cross-sectional study including all female patients presenting consecutively in an ambulatory clinic for 16 months. This study aimed to describe the level of serum cytokines and chemokines in relation to TSH, fT4 and insulin resistance (IR) indexes in patients with subclinical hypothyroidism (SCH). The study included 72 women with a median age of 59 ± 17.75 years, and a mean BMI (Body Mass Index) of 31.48 ± 6.75 kg/m^2. Modelling homeostasis model assessment of IR indices (HOMA-IR) based on chemokines (IL-8, CXCL10, CXCL11, leptin), C-reactive protein, the presence or absence of SCH, taking into account age, BMI, abdominal circumference, glycated haemoglobin (HbA1c), and anti-thyroid peroxidase antibodies (ATPO) as covariates, identified a single chemokine that was significantly associated with the dependent variable (IL-8). IR indices are negatively associated with IL-8 in female patients with subclinical hypothyroidism, but the effect of the cytokine is minimal. BMI rather than TSH influences the level of CXCL11 in our population. CXCL10 has a tendency to increase in patients with SCH, obesity and prediabetes, with no association with TSH.

Introduction

In obesity, the hormonal secretion of the thyroid gland switches from homeostasis to type 2 allostasis [1] in order to adapt to persistent modifications of adipose tissue and inflammation. This new predictive plasticity could be mediated by cytokines and their subclasses, namely interleukins and chemokines. These could establish a dynamic setpoint [2] for thyroid stimulating hormone (TSH), triiodothyronine (T3), thyroxine (T4), and their free plasmatic levels (fT3 and fT4).

The chemokine system is formed by approximately 40 chemokines and their receptors that belong to the seven-transmembrane G protein coupled receptor family [3]. Extensive efforts have been invested in describing their roles. Still, the task was hampered by the fact that one chemokine can bind to several receptors, and one receptor can be activated by multiple chemokines [4]. In general terms, chemokines mediate cell migration and are probably involved in complex crosstalk like the one between adipocyte and the thyroid cell [5].

The T3 level for peripheral and central tissues use is maintained through deiodinases activity, that regulates its level according to body composition and nutrition [1]. In obesity, the peripheral enzymatic activity of deiodinases is augmented, thus elevating the level of fT3 and T3 [6]. The distribution and affinity of deiodinases and thyroid hormone receptors (TR) are different among tissues [1]. The hypothalamus tanycytes (cells that line-up the cerebrospinal fluid to the portal

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capillaries) sense the fT3 and fT4 levels and regulate the secretion of TSH [7]; also, leptin that reaches these central cells stimulates the secretion of TSH. On the other side, at the peripheral level, TSH is able to stimulate the secretion of leptin from adipocytes [8].

Besides leptin, inflammation and insulin resistance (IR) [9], have been described as having a role in the allostatis mentioned above. In previous observational studies, monitoring the interleukin-8 (IL-8) level, but not interferon- alpha (IFN-α) in the sera of patients with thyroid diseases was associated with an advanced stage [10]. Other authors explored the T helper 1 lymphocytes (LTH1) pathogenical pathway which involves the production of interferon- gamma (IFN-V), and tumour necrosis factor alpha (TNF- α) [11]. These cytokines further stimulate the production of CXCL10 which perpetuated the autoimmune process [12], consequently altering the equilibrium between TSH and thyroid hormones which might overlap with the disturbance created by overweight.

Considering the metabolic syndrome, some authors hypothesised that IR is the promoter of inflammation in adipose tissue [13]. In addition, in individuals with prediabetes, hence insulin resistant, a low thyroid function was a risk factor for the development of diabetes [14,15]. Accordingly, if these conditions – insulin resistance, inflammation and hypothyroidism are met, regardless of the order of appearance, screening for diabetes mellitus should be performed.

We designed an observational cross-sectional study to evaluate this high risk population including all female patients presenting consecutively in an ambulatory clinic for 16 months (May 2019–August 2020). The aim of this study was to describe the level of serum cytokines and chemokines in relation with TSH, fT4 and insulin resistance (IR) indexes in patients with subclinical hypothyroidism (SCH).

Materials and methods

After signing the informed consent to participate in the cross-sectional study at the initial visit, weight measurement and evaluation of the body composition was performed using the professional device Tanita SC-240®, and the software Tanita Pro version 3.4.0. We measured waist and hip circumference with a standard measuring tape. Patients were scheduled for a fasting blood sample (after 8–10 h of fasting) during the same week at the same laboratory. The following analyses were performed on fresh serum: blood glucose (mg / dL), glycated haemoglobin (HbA1c) (%), fasting insulinemia (μUI / mL), fasting C-peptide (ng / mL), total cholesterol / CT (mg / dL), HDL cholesterol (mg / dL), LDL cholesterol (mg / dL), triglycerides (TG) (mg / dL), TSH (μUI / mL), fT4 (ng / dL), ATPO (IU / mL), parathormone-PTH (ng / dL), cortisol (ug / dL), total calcium (mg / dL), magnesium (Mg) (mg / dL), 25-hydroxyvitamin D (25OHD) (ng / mL), C-reactive protein (PCR) (mg / L) and ferritin (ng / mL). The BA400® biochemistry analyzer (Biosystems SA, Barcelona, Spain, 2019), and Immulite® 1000 (Siemens GmbH, Erlangen, Germany) were used for these determinations. Part of the serum was kept at minus 70 degrees for further analysis of chemokines. These were determined at the "Victor Babeș" National Institute using the Luminex 200 Suspension Array Analyzer (Luminex Corporation) [16].

For chemokine determination we used immunofluorescence, the principle of which is resembling that of enzyme-linked immunosorbent assay (ELISA) sandwich. The difference is that reactions take place at the level of magnetic microspheres. In a meta-analysis of observational studies, the use of multiplex ELISA method had a higher sensitivity and specificity compared with ELISA in patients with T2DM [17]. We determined the following chemokines using a custom assay kit: TNF-α (Tumour Necrosis Factor α), MCP-1 (Monocyte Chemoattractant Protein-1 or CCL2), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-1β (IL-1β), I-TAC (Interferon-inducible T Cell Alpha Chemoattractant or CXCL11), IP-10 (interferon-inducible protein-10 or CXCL10), fractalkine (CX3CL1). Another kit was used for adiponectin, leptin, and resistin (Bio-Rad Laboratories, Inc.).

We used several formulas to estimate insulin resistance: QUICKI, TG / HDL cholesterol ratio, HOMA-IR1 (insulin) = fasting glycaemia (mg/dL) * fasting insulin (μUI/mL)/405, HOMA-IR1 (C-peptide) = 1.5 + fasting glycaemia (mmol/L)) × (fasting C-peptide (nmol/L)/2800, and HOMA-IR2 (insulin), HOMA-IR2 (C-peptide) calculated with a specific software version 2.2.3 available online (https://www.dtu.ox.ac.uk/homacalculator/) like previously described [18,19]. The inclusion criteria were: female patients age between 18 and 75 years presenting consecutively in the clinic. Exclusion criteria were: the presence of acute pathologies, the association of other autoimmune diseases besides thyroiditis (rheumatoid arthritis, coeliac disease, vitiligo, multiple sclerosis, the prior diagnosis of type 1 diabetes mellitus, type 2 diabetes mellitus, or other diseases that may alter insulin resistance: polycystic ovary syndrome or hypercortisolism. The diagnosis of diabetes and prediabetes was established according to the American Diabetes Association guidelines [20]. The diagnosis of subclinical hypothyroidism was based on American Association of Clinical Endocrinologists recommendations [21].

Multivariate statistical analyses were performed with the R programming environment, v.4.1.1. [22], under RStudio v.1.4.1717. To assess the normality of residual values we used graphical methods (quantile-quantile graphs - QQ plots and histograms of student residual values - R package "MASS" [23], taking into account the limitations inherent in inferential methods. We also explored several inferential tests (implemented in the "nortest" package) for this purpose [24]. Homoskedasticity was assessed graphically and by the Breusch-Pagan test, implemented in the R package "lmtest" [25]. Because we identified slight deviations from normality and homoskedasticity, we used robust regression models as sensitivity analyses in the implementation of the "robustbase" R package [26], based on an MM-type estimator [27,28]. We also explored the use of a linear model with mixed effects, treating the categorical variable by identifying the subgroup of patients both having a fixed and a random component. For this purpose, we applied the R "lme4" packages (for model construction) [29], and lmerTest [30] for estimating coefficients.

Because several outliers were identified in the analytical determinations of the chemokines, for the purpose of sensitivity analyses we replaced them with the median value, using the "imputate_outlier" function from the R dlookr package [31]. In the case of leptin determinations, three values were not available and their imputation was performed by applying the function "imputate_na" in the same package. The imputation was performed based on the algorithm of the nearest neighbors (k near neighbors) [31]. The R package "ggstatsplot" was used to estimate the standardized regression coefficients (beta) and their graphical representation [32].

Results

The study included 72 women with a median age of 59 ± 17.75 years and a mean BMI (Body Mass Index) of 31.48 ± 6.75 kg / m^2. The general characteristics of the population are provided in Table 1. After analysing the values of TSH, fT4, blood glucose and HbA1c, the population was divided into 6 groups: group 1 - subclinical hypothyroidism (SCH), prediabetes and obesity (n = 11); group 2 - SCH and obesity (n = 11); group 3 - prediabetes and obesity (n = 13); group 4 - obesity (n = 14); group 5 - newly diagnosed type 2 diabetes –T2DM (n = 13); and group 6 - SCH and normal weight (n = 10). We also divided the population into a group with IR (n = 44) and one without IR (n = 28), respectively with SCH (n = 32) and without SCH (n = 27), excluding those with T2DM. Thirty-eight women (52.77%) had metabolic syndrome.

After analysing the HOMA-IR (insulin and C-peptide) and QUICKI values, we noticed that 61.11% of the population had IR, defined as at least one of the 5 indices above the cut-off. Approximately 30% of the population had SCH, and 13.88% were newly diagnosed with T2DM.

Modelling HOMA-IR1 (insulin) based on chemokines (IL-8, CXCL10, CXCL11, leptin), inflammation- CRP, the presence or absence of SCH,

Table 1
General characteristics of the population.

Variable	Total (n = 79)	Group 1 (n = 11)	Group 2 (n = 11)	Group 3 (n = 13)	Group 4 (n = 14)	Group 5 (n = 13)	Group 6 (n = 10)
Weight (kg)	81.56 ± 18.89	88.45 ± 26.77	88.55 ± 14.95	79.85 ± 9.81	87.00 ± 18.8	85.00 ± 12.74	56.40 ± 5.29
BMI (kg/m^2)	31.48 ± 6.75	34.31 ± 9.23	33.75 ± 4.53	32.25 ± 3.86	31.91 ± 5.92	33.66 ± 4.57	21.4 ± 2.58
AC (cm)	100.36 ± 14.77	103.73 ± 20.84	101.82 ± 9.57	104.23 ± 7.55	103.79 ± 14.11	104.23 ± 9.05	80.2 ± 11.59
HC (cm)	112.44 ± 12.59	116.27 ± 17.38	117.41 ± 11.71	113.38 ± 8.26	116.86 ± 10.44	111.77 ± 9.74	96.25 ± 4.10
Fat mass (kg)	34.13 ± 14.11	38.94 ± 16.5	36.52 ± 11.06	33.20 ± 5.79	37.40 ± 12.96	40.12 ± 15.50	15.03 ± 6.29
Visceral fat (%)	10.33 ± 3.94	11.73 ± 4.73	11.55 ± 3.26	11.23 ± 2.38	10.29 ± 3.07	12.00 ± 2.51	4.20 ± 2.39
Glycaemia (mg/dl)	109.26 ± 30.67	114.98 ± 10.40	102.58 ± 12.53	121.27 ± 23.44	99.17 ± 12.41	150.15 ± 31.34	94.55 ± 10.08
HbA1c (%)	5.7 ± 0.57	5.90 ± 0.4	5.5 ± 0.5	5.8 ± 0.3	5.4 ± 0.13	6.7 ± 0.95	5.25 ± 0.32
Insulin (uUI/mL)	11.15 ± 13.48	20.2 ± 10.5	8.21 ± 5.63	11.7 ± 16.02	9.74 ± 8.17	21.00 ± 14.85	6.32 ± 4.28
C-Peptide (ng/mL)	2.65 ± 2.15	3.87 ± 1.78	2.64 ± 0.82	2.67 ± 4.83	2.08 ± 2.09	2.85 ± 2.73	1.46 ± 1.27
HOMA-IR1 (insulin)	3.09 ± 4.19	5.68 ± 3.24	2.33 ± 1.54	3.59 ± 4.35	2.46 ± 1.98	6.61 ± 5.63	1.41 ± 1.10
HOMA-IR2 (insulin)	1.06 ± 1.8	2.71 ± 1.63	1.18 ± 0.7	1.68 ± 2.86	1.35 ± 0.76	3.01 ± 2.14	0.85 ± 0.52
HOMA-IR1 (C-peptide)	1.6 ± 0.1	1.64 ± 0.07	1.59 ± 0.05	1.62 ± 0.16	1.57 ± 0.07	1.66 ± 0.17	1.54 ± 0.05
HOMA-IR2 (C-peptide)	1.99 ± 1.76	2.81 ± 1.32	1.67 ± 1.15	2.30 ± 2.91	1.31 ± 1.82	2.48 ± 2.49	0.98 ± 1.03
QUICKI	0.33 ± 0.06	0.29 ± 0.02	0.33 ± 0.03	0.31 ± 0.07	0.33 ± 0.05	0.29 ± 0.03	0.36 ± 0.06
TG/HDLC	1.85 ± 1.45	2.76 ± 4.01	1.65 ± 1.16	1.27 ± 1.16	1.61 ± 1.11	2.07 ± 1.31	1.23 ± 1.19
TSH (uUI/mL)	3.39 ± 3.95	5.56 ± 5.45	5.99 ± 3.71	1.17 ± 0.28	2.08 ± 1.32	1.43 ± 1.81	6.65 ± 5.92
fT4 (ng/dl)	1.12 ± 0.21	1.1 ± 0.19	1.14 ± 0.1	1.17 ± 0.28	1.13 ± 0.26	1.11 ± 0.23	1.03 ± 0.29
ATPO UI/mL)	10.00 ± 35.38	20.00 ± 217.57	18.6 ± 391.77	10.00 ± 13.35	10.00 ± 13.51	5.08 ± 20.31	110.24 ± 633.09
25OHD (ng/mL	30.8 ± 16.21	25.26 ± 11.75	25.13 ± 14.57	31.83 ± 14.8	37.99 ± 12.65	25.28 ± 16.26	33.81 ± 8.95
HDLc (mg/dl)	65.00 ± 25.00	61.00 ± 26.00	64.00 ± 20.00	85.00 ± 30.5	65.5 ± 17.5	57.00 ± 21.95	72.00 ± 46.85
TG (mg/dl)	119.9 ± 74.9	165.75 ± 158.89	121.24 ± 114.33	102.95 ± 50.79	102.78 ± 75.82	138.38 ± 68.79	97.16 ± 80.22
CRP (mg/L)	2.48 ± 4.24	3.29 ± 4.09	4.88 ± 7.45	2.45 ± 4.58	1.98 ± 7.57	2.58 ± 2.65	1.25 ± 1.82
Feritin (ng/mL)	82.19 ± 69.58	67.31 ± 86.25	65.7 ± 90.22	89.68 ± 34.85	90.94 ± 71.87	84.83 ± 162.86	50.88 ± 59.91
Fractalkine (pg/mL)	175.83 ± 119.14	190.16 ± 103.63	191.88 ± 103.37	221.93 ± 184.63	182.89 ± 191.5	145.89 ± 106.67	150.22 ± 67.14
CXCL11 (pg/mL)	10.4 ± 6.13	12.68 ± 10.52	9.72 ± 19.16	9.56 ± 8.42	10.86 ± 4.87	12.64 ± 8.62	7.32 ± 4.81
IL-1β (pg/mL)	0.53 ± 0.13	0.53 ± 2.05	0.49 ± 0.19	0.49 ± 0.04	0.53 ± 0.26	0.53 ± 2.52	0.53 ± 1.54
IL-6 (pg/mL)	4.63 ± 4.46	8.52 ± 41.62	5.62 ± 1.96	4.63 ± 3.99	4.63 ± 3.02	4.63 ± 3.96	6.09 ± 8.52
IL-8 (pg/mL)	21.28 ± 58.8	16.48 ± 227.2	34.08 ± 48.48	6.24 ± 11.92	8.96 ± 31.6	58.72 ± 143.52	32.96 ± 89.2
CXCL10 (pg/mL)	82.00 ± 59.88	102.00 ± 63.64	82.40 ± 49.84	124.32 ± 66.8	85.54 ± 54.27	59.72 ± 42.52	58.42 ± 53.57
MCP-1 (pg/mL)	2903.93 ± 4360.18	3305.47 ± 6066.26	2941.33 ± 1339.41	3150.22 ± 5838.21	1867. 66 ± 5840.24	2934.28 ± 3518.22	2567.31 ± 2950.24
TNF-α (pg/mL)	3.82 ± 5.07	3.82 ± 7.16	4.54 ± 3.95	3.06 ± 3.25	3.44 ± 4.15	3.82 ± 5.92	2.22 ± 5.07
Adiponectin (ng/mL)	109.17 ± 328.72	89.12 ± 185.00	106.64 ± 285.11	12.98 ± 156.89	271.64 ± 385.37	108.09 ± 513.67	128.62 ± 378.87
Leptin (pg/mL)	232.33 ± 442.88	326.85 ± 264.1	267.41 ± 466.24	155.68 ± 121.92	217.55 ± 375.69	816.74 ± 907.36	164.22 ± 737.38
Resistin (pg/mL)	55.10 ± 49.69	54.68 ± 55.22	67.21 ± 55.07	39.91 ± 27.49	56.73 ± 55.18	55.82 ± 53.14	31.99 ± 38.97

* BMI- Body mass index; AC- Abdominal circumference; HC- Hip circumference; TG- Triglyceride; TC- Total cholesterol; ATPO- Anti-thyroid peroxidase antibodies; 25OHD- 25- hydroxyvitamin D; CRP- C reactive protein; IL- Interleukin; MCP-1 Macrophage chemoattractant protein 1; TNF-α Tumour necrosis factor α; HbA1c-glycated haemoglobin.

taking into account age, BMI, abdominal circumference, glycated haemoglobin (HbA1c), and ATPO as covariates, identified a single chemokine that was significantly associated with the dependent variable (IL-8). The coefficient of determination of this model was 0.493, and the adjusted coefficient of determination (corrected for the multiplicity of variables) was 0.395, which means that the respective variables explained almost half of the variability observed in the analysed sample. HOMA-IR1 (insulin) was positively and significantly associated with HbA1c ($p = 0.003$), and negatively associated with IL-8 ($p = 0.026$). The group with T2DM was significantly associated with HOMA-IR1 (insulin) ($p = 0.01$), but not the group with SCH, which did not show any difference from the control group ($p = 0.50$). The BMI approached the conventional threshold of statistical significance without reaching it. As the examination of standardised coefficients (beta) shows, the most pronounced effect corresponds to the group of patients with newly diagnosed diabetes and HbA1c; all other variables, even statistically significantly associated with HOMA-IR1 (insulin), have a much smaller effect, almost negligible in terms of effect size. By treating the variable group of patients as having a fixed and a random component, it did not significantly change the results: the same variables remained significant in the linear model with mixed effects, with the particularity that the difference for the subgroup of patients with T2DM was not significant ($p = 0.462$).

Because HbA1c and fasting blood glucose are autocorrelated variables, we preferred to include a single variable (HbA1c) in the model. Also, because BMI and visceral fat (%) are autocorrelated variables, we included only BMI; its replacement with visceral fat led to similar results. If we exclude from the independent variables HbA1c, then the variables with the most pronounced effect on HOMA-IR1 (insulin) are represented by the category of patients with onset of diabetes and visceral fat (Fig. 1). The use of a robust regression model (the "robustbase" package) led to very similar results (BMI reached the significance threshold, along with age), so we limited ourselves to presenting the results of the conventional model in Fig. 1.

Including only IL-8 in the model had very similar results, except that the BMI reached the conventional threshold of statistical significance ($p = 0.04$). Building separate models for each of the other chemokines did not identify a statistically significant association with HOMA-IR1 (insulin) ($p = 0.29$ for CXCL11, $p = 0.70$ for CXCL10 and $p = 0.99$ for leptin). We also investigated the other three indices of insulin resistance: HOMA-IR2 (insulin), HOMA-IR1 (C-peptide), HOMA-IR2 (C-peptide), and also QUICKI with similar results to the HOMA-IR1 (insulin)-based model.

The second hypothesis tested was whether the presence of subclinical hypothyroidism causes changes in the chemokine level. For this purpose, we first evaluated the influence of TSH on each of the cytokines, chemokines and CRP in simple linear regression models, without including covariates. In these models TSH did not significantly influence the levels of IL-8 ($p = 0.392$, negative adjusted r^2), CXCL11 ($p = 0.944$, negative adjusted r^2), CXCL10 ($p = 0.705$, negative adjusted r^2), leptin ($p = 0.982$, negative adjusted r^2) or CRP ($p = 0.729$, negative adjusted r^2).

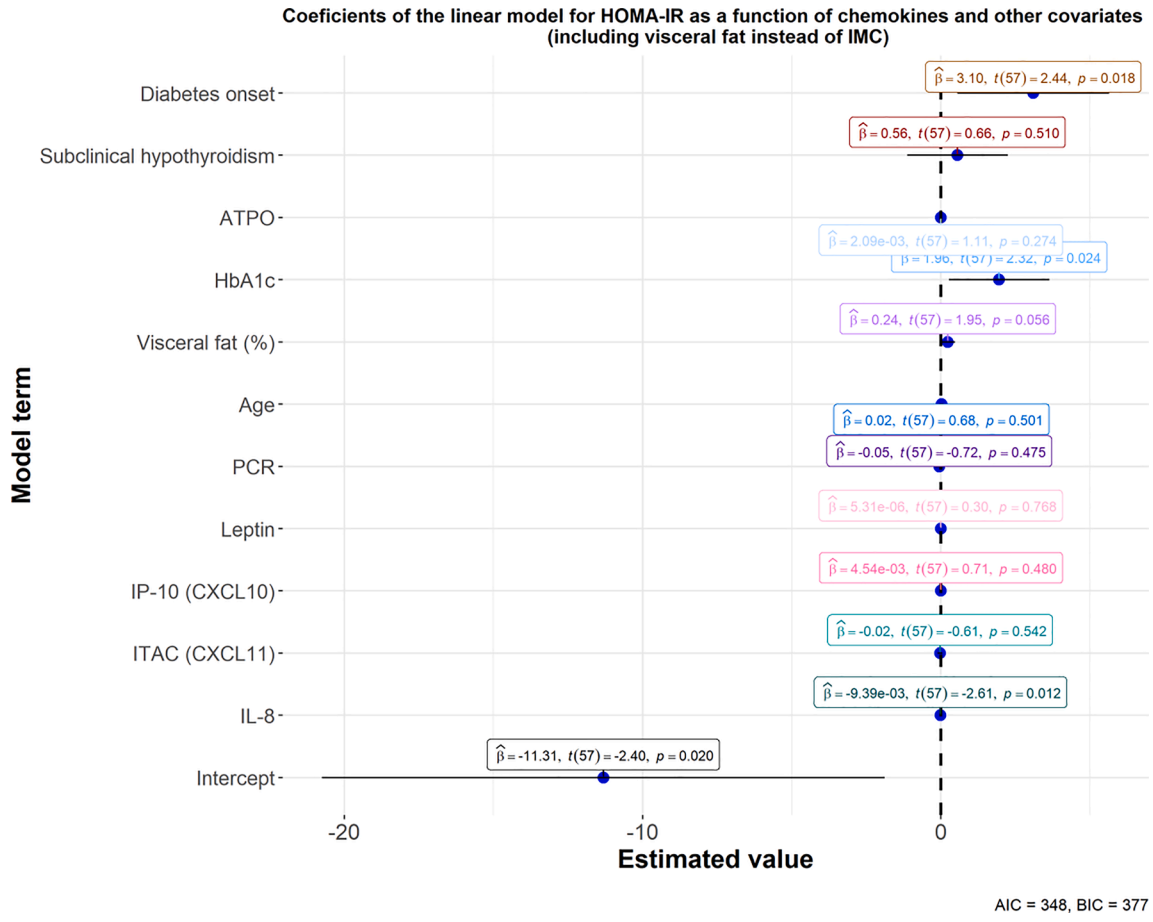

Fig. 1. Multivariate linear regression model using HOMA-IR (insulin) as a dependent variable.

In a second step, we built other models for each of the chemokines or CRP, adding the following covariates to TSH: age, BMI, and ATPO. Because BMI was strongly correlated with abdominal circumference (r = 0.870) and visceral fat (0.926), these two variables were not included, but we tested BMI substitution successively with the other two variables to identify if there is any noticeable difference. In the case of IL-8, the only variable that was significantly associated with this chemokine was BMI (p = 0.029), with TSH having no influence (p = 0.861); however, the effect of visceral fat and abdominal circumference was less strongly associated with IL-8 (p values of 0.16 and 0.13). For CXCL11 the situation is similar, the BMI association was lower for this cytokine (p = 0.097), but TSH had no influence (p = 0.892). In the case of CXCL10, only age had a tendency to associate with this chemokine (p = 0.07), TSH showing no association (p = 0.99).

Discussion

We designed a cross-sectional study evaluating IR in the female population with SCH, and the association between the TSH level and different cytokines values. Based on HOMA-IR (insulin) indices, the groups with new-onset T2DM, and those with prediabetes and SCH were associated with increased IR. The group with SCH without dysglycaemia was no different from the control group. The association between subclinical hypothyroidism and IR was previously observed [33], as well as the higher risk for metabolic syndrome [34]. This increases the risk of T2DM onset in this population, especially if low access to healthcare is a problem [35,36].

In the multivariate analysis, HOMA-IR1 and 2 insulin resistance indices for C-peptide and insulin were negatively associated with IL-8, but given the very low beta coefficients for the chemokine, we can consider the effect to be minimal. Patients with SCH, regardless of weight, had elevated median values of this chemokine (34.08 and 32.96 pg / ml vs. 8.96 pg / ml), similar to those with T2DM, but not affected by TSH or IR. On the other hand, IL-8 is significantly influenced by BMI when we created a statistical model that included only this chemokine, but is not influenced by visceral fat, fat mass, or ATPO titre. Therefore, IL-8 is elevated in patients with SCH, as demonstrated in previous studies [10], but we hypothesise that the source may be the vascular endothelium [37], circulatory monocytes, or immune system cells [38].

For patients with T2DM, HOMA-IR (insulin) has a minor effect on IL-8, regardless of BMI. In a cross-sectional study, on a different population (Mexican Americans), IL-8 was increased in previously diagnosed T2DM patients, with a lower level of IL-8 in the obese group. Consistent with our results, IL-8 did not correlate with HOMA-IR, and the authors assumed that the source of IL-8 is most likely other than the adipocyte [39,40]. Cimini et al. have shown that in patients with T2DM, IL-8 is associated with a more severe inflammatory and metabolic profile: elevated IL-6, TNF-α, HbA1c, and fasting glycaemia [41]. Regarding our results, in the group of patients with recent onset T2DM, IL-8 correlated positively with IL-1β, IL-6 and MCP-1; in SCH it was associated with IL-6, leptin and resistin. The level of IL-8 is increased in diabetes and SCH by the same mechanism, by an independent one, or by chance. Other prospective or interventional studies are needed to clarify this observation.

CXCL10 level is increased in autoimmune thyroiditis [11], and 26.38% of our population had a high antibody titre. In the multivariate linear regression model with ATPO, age and BMI as co-variates, we observed a tendency for BMI to associate with CXCL11, and age with CXCL10, although the values did not reach statistical significance. This is an argument in favour of the theory that obesity increases the risk of autoimmune thyroid pathology, possibly mediated by TH1 lymphocytes

and the production of CXCL10 or CXCL11 [11]. Compared with other studies, the median levels of CXCL10 and CXCL11 were lower in our population indicating a lower level of tissue inflammation [42,43]. The relative risk for SCH was 1.7 in a meta-analysis of studies including obese patients. There was a positive correlation between ATPO and obesity, hence the increased risk of Hashimoto's thyroiditis [44]. We argue that a precocious nutritional intervention might result in a prevention of thyroid disease, and T2DM.

A meta-analysis of chemokine system studies by Pan et al. revealed there was no difference in their concentration between patients with prediabetes vs. the control group [17]. Among the chemokines that have been elevated in patients with T2DM, and might play a role in the progression from prediabetes to actual disease are: CCL1, CCL2 (MCP-1), CCL4, CCL5, CCL11, CXCL8 (IL-8), CXCL10 (IP-10) and CX3CL1 (fractalkine) [17]. In order to expand the research to other cytokines, interleukins 6, 12 or 17 can be added [45]. In patients with SCH and prediabetes, progression to T2DM could be determined by IL-8, CXCL10, and CXCL11.

The small number of patients, exclusion of men due to low addressability, lack of a dynamic determination of serum cytokines and chemokines represent the limitations of our study. By its cross-sectional nature, this study can generate hypotheses without demonstrating them.

The strengths of the study come from the degree of novelty, being one of the rare studies that analysed a complex panel of chemokines in the Romanian population [46] with hypothyroidism and prediabetes, respectively newly diagnosed T2DM. Biochemical and hormonal analyses were performed by the same laboratory, and immunofluorescence determinations by the same experienced research assistant.

Conclusions

Insulin resistance indices are negatively associated with IL-8 in female patients with subclinical hypothyroidism, but the effect of the cytokine is minimal. BMI rather than TSH influences the level of CXCL11 in our population. CXCL10 has a tendency to increase in patients with subclinical hypothyroidism, obesity and prediabetes, with no association with TSH. Further studies are needed to investigate the association between the increase of chemokine levels in patients with obesity and the risk of thyroid dysfunction, prediabetes, and T2DM onset.

CRediT authorship contribution statement

Roxana Adriana Stoica: Investigation, Writing – original draft, Funding acquisition. **Nicoleta Drăgana:** Validation, Writing – original draft. **Robert Ancuceanu:** Software, Formal analysis, Data curation, Writing – original draft. **Ovidiu Ionuț Geicu:** Investigation, Writing – original draft. **Cristian Guja:** Conceptualization, Writing – review & editing. **Anca Pantea-Stoian:** Writing – review & editing. **Damaris-Cristina Gheorghe:** Writing – review & editing. **Raluca-Ioana Stefan-van Staden:** Methodology, Resources, Funding acquisition. **Cristian Serafinceanu:** Visualization, Supervision. **Adrian Costache:** Investigation. **Constantin Ionescu-Tîrgoviște:** Conceptualization, Validation, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Institutional review board statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of "NC Paulescu" National Institute of Diabetes, Nutrition, and Metabolic Diseases (protocol number 1 date of approval-9th April 2019).

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

Data availability statement

Data are available on request with the permission of R.A.S.

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