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Original Research Article

Overt hypothyroidism is associated with blood inflammatory biomarkers dependent of lipid profile

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ABSTRACT

To investigate the association between inflammatory biomarkers and overt hypothyroidism (OH). We measured inflammatory cytokines, interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) as well as cell-free DNA (cf-DNA) levels in 40 OH patients and 40 healthy controls. Total cholesterol, high and low density lipoprotein subfractions, triglyceride, fibrinogen, and D-dimer were recorded. Increased inflammatory profile was evidenced through significant elevations in the concentrations of all cytokines and cf-DNA levels in the OH group. Lipids and prothrombotic markers were also increased in hypothyroid subjects. A significant association between the inflammatory cytokines and lipid profile was observed. A multivariate analysis showed that this result was independent of the sex, age and BMI status of the subjects. Hypothyroidism is associated with proinflammatory state. Lipid abnormalities have a stronger influence on inflammation, increasing cardiovascular risk and atherosclerosis development in hypothyroidism.

Introduction

Overt hypothyroidism (OH) is characterized by high thyroid-stimulating hormone (TSH) blood concentration, low triiodothyronine (T3) and thyroxine levels (T4), and alterations of plasma lipid concentration, which could also be involved in the progress of atherosclerosis (Donnini et al., 2003). Hypercholesterolemia is very common in OH patients, mainly due to higher low-density lipoprotein cholesterol

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(LDL-C) levels. In addition, OH presents association with other atherosclerosis risk factors such as diastolic hypertension, coagulopathy and impaired endothelial function. As described in the review performed by Ichiki (2010) who summarized the basic and clinical studies on the role of thyroid hormone in atherogenesis, emerging risk factors have been associated with atherosclerosis and OH as a high C-reactive protein levels (CRP).

Atherosclerosis is currently regarded as a low-grade chronic inflammation and maintained by the chronic activation of autoimmune reactions against self-proteins modified by oxidative stress that sustains endothelial dysfunction and plaque development (Profumo et al., 2012). In these terms, a potential OH association with inflammatory cytokines could be expected.

Thyroid hormones influence specific immune responsiveness as well as several aspects of innate and adaptive immunity. However, the relationship between thyroid hormones and immune cells is complex and needs to be clarified with additional investigations. T3 and T4 are able to modulate immune responses through both genomic and nongenomic mechanisms and at physiological concentrations (De Vito et al., 2011).

The acute phase response to inflammation is characterized by the combination of hepatocyte-derived plasma proteins induced by the inflammatory cytokines tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) as well as those induced by interleukin-6 (IL-6) (Salvi et al., 2000). A key regulator of the inflammatory response is IL-6, which stimulates the synthesis of acute phase proteins including CRP and fibrinogen.

Moreover, IL-1 has been identified as a chemical mediator released from monocytes/macrophages and exhibits important biologic activity in inflammatory and immunologic responses (Hamaguchi et al., 1991). TNF-α is another important cytokine mediating the induction of adhesion molecules and other cytokines (Salvi et al., 2000) and modulating of the immunologic reactions produced by interferon-γ (IFN-γ) of HLA class II molecules in human thyroid follicular cells (Miyakoshi et al., 1992). Despite the relevance of these molecules in metabolic dysfunctions associated to atherogenesis studies of alternating cytokine levels and OH are still incipient.

Another emerging inflammatory biomarker is the cell-free DNA (cf-DNA) that has been associated with outcome in several conditions as cancer (Schwarzenbach et al., 2011), stroke (Boyko et al., 2011), coronary heart disease (Liu et al., 2015) and reports concerning the outcomes after cardiac arrest that found association of circulating DNA quantities at admission with mortality (Gornik et al., 2014). The possible sources of cell-free DNA in plasma are passive release through cell death (necrosis or apoptosis) and active release by cell secretion (González-Masiá et al., 2013).

Despite the potential influence of OH on blood inflammatory biomarkers there are few studies investigating this potential association. Therefore, we performed here a case-control study that evaluated the association between inflammatory biomarkers and OH. Lipid, prothrombotic and other biochemical markers related with body functions were also evaluated in the sample studied here.

Methods

Study design

A case-control study was performed with eighty subjects enrolled prospectively from clinical laboratory LABIMED, located in Santa Maria-RS, Brazil. Subjects were divided into two groups as follows: control group, 40 healthy subjects (male = 18; female = 22) and newly diagnosed OH group, 40 patients (male = 19; female = 21) without previous pharmacological treatment. OH was defined as TSH higher than 10 mIU/L and low T3 and free thyroxine (fT4) levels (Nanda et al., 2008).

At enrollment, all the participants were tested for serum TSH. When serum TSH levels were higher than 10 mIU/L, we tested fT4, T3, anti-thyroperoxidase antibodies (Anti-TPO Abs) and anti-thyroglobulin antibodies (Anti-TgAbs) levels to evaluate the presence of overt hypothyroidism and if your etiology was autoimmune.

Subjects with previous diseases and dysfunctions that could influence the results were excluded. The exclusion criteria were as follows: subjects taking lipid-lowering drugs, antioxidant vitamin supplements, acetylsalicylic acid, antihistamines, antihypertensive, and exposure to high iodine condition, smokers, alcoholics, pregnant women, women on hormone replacement therapy, diabetics and subjects with acute, chronic or malignant diseases. The protocol was approved by the Human Ethics Committee of the Federal University of Santa Maria (number 2010-87). All subjects gave written informed consent to participate in the study.

Biochemical determinations

Blood samples were collected after 12 h overnight fasting by venous puncture into blue, gray and red top Vacutainers® (BD Diagnostics, Plymouth, UK) tubes. The samples were centrifuged for 15 min at 2500 × *g*, and aliquots of serum were kept at −20 °C for maximum of 4 weeks. Serum TSH, T3, fT4, Anti-TPOAbs and Anti-TgAbs concentrations were measured by chemiluminescent immunometric assay on IMMULITE 2000® (Siemens Healthcare Diagnostics, Los Angeles, USA). Detection limits for TSH was 0.004–14.000 mIU/L, fT4 was 3.9–77.2 pmol/L and T3 was 0.29 nmol/L.

The biochemical markers were spectrophotometry determined using Hitachi U-2800A® equipment (Hitachi High-Technologies Corporation, Japan). High-density lipoprotein cholesterol was measured in the supernatant plasma after the precipitation of apolipoprotein B-containing lipoproteins with dextran sulfate and magnesium chloride as previously described (Bachorik and Albers, 1986). LDL-C was estimated with the Friedewald equation (Friedewald et al., 1972).

The cytokines IL-1, IL-6, TNF-α and IFN-γ were analyzed using ELISA capture, according to the manufacturer's instructions (Biomyx Technology, San Diego, CA). The D-dimer levels were measured by immunoturbidimetric method on Cobas INTEGRA 400® (Roche Diagnostics, Basel, Switzerland). Fibrinogen levels were measured using coagulation analyzer Sysmex® CA-1500 (Siemens Healthcare Diagnostics, Los Angeles, USA).