Research

Soluble CD40 ligand levels in children with newly diagnosed Graves' disease

Metwalley KA et al. Soluble CD40 ligand levels in children with newly diagnosed Graves' disease

Kotb Abbass Metwalley1, Hekma Saad Farghaly1, Duaa Mohammed Raafat1, Asmaa Mohammed Ismail2, Ghada Mohamed Saied3
1Department of Pediatrics, Faculty of Medicine, Assiut University, Assiut, Egypt.
2Department of Pediatrics, Faculty of Medicine, Aswan University, Aswan, Egypt.
3Department of Clinical Pathology, Faculty of Medicine, Assiut University, Assiut, Egypt

What is already known on this topic?
Graves’ disease (GD) is believed to result from a complex interaction between genetic background, environmental factors, and the immune system. sCD40L might be involved in the evolution of many autoimmune diseases and may have diagnostic and therapeutic implications.

What this study adds?
To our knowledge, this is the first study to assess serum sCD40L concentrations in children with newly diagnosed GD. The study reported high concentrations of sCD40L in children with newly diagnosed GD and its correlations with TRAbs and thyroid volume which may suggest its biological active role in GD.

Abstract

Objectives: Soluble CD40 ligand (sCD40L) is elevated in various autoimmune disorders which may have diagnostic and therapeutic implications. The aims of the current study were to evaluate serum sCD40L concentrations in children with newly diagnosed Graves’ disease (GD) and to correlate its levels with patients’ clinical and laboratory parameters.

Methods: This study included 48 children with newly diagnosed GD and 48 healthy children. Serum thyroid hormones (TSH, FT4 and FT3), thyrotropin receptor antibodies (TRAbs), high sensitivity C reactive protein (hsCRP) and sCD40L levels and thyroid volume were measured.

Results: Compared to control subjects, children with GD had higher thyroid volume SDS (P = 0.001), hsCRP (P = 0.001), TRAbs (P = 0.001) and sCD40L (P = 0.001). Significant correlations were reported between sCD40L with age (P = 0.01), thyroid volume SDS (P = 0.001), hs-CRP (P = 0.01) and TRAbs (P = 0.001). In multivariate analysis, sCD40L concentrations were correlated with TRAbs (OR: 3.1, CI: 2.2–2.7, P = 0.001) and thyroid volume SDS (OR: 2.1, CI: 1.2–2.7, P = 0.001).

Conclusions: This preliminary study has evidence of high concentrations of sCD40L in children with newly diagnosed GD and its correlations with TRAbs and thyroid volume which may suggest its biological active role in GD.

Keywords: Graves’ disease; Soluble CD40 ligand (sCD40L); thyroid hormone; thyroid volume

Introduction
Graves’ disease (GD), the most common cause of spontaneous thyrotoxicosis, is believed to result from a complex interaction between genetics, environmental factors, and the immune system [1]. It is mediated by autoantibodies against thyroid stimulating hormone receptor (TRAbs) that activate TSH receptors, stimulate thyroid hormone synthesis, secretion and thyroid cell growth [2]. CD40L (L stands for ligand) is a trimeric transmembrane protein of the tumor necrosis family, was originally identified on the cells of the immune system [3]. It binds to CD40 which is mainly expressed on antigen-presenting cells and B cells as well as on other types of cells as thyroid follicular cells [4]. After cellular binding, the surface-expressed CD40L is then cleaved and/or released over a period of
minutes to hours generating a soluble fragment (sCD40L). sCD40L has full biological activity. It has number of
immune functions which include cell-to-cell interactions and antigen presentation and pathogen capture [5]. CD40-
sCD40L interaction has an emerging role in evolution of some autoimmune diseases (e.g. systemic lupus
erythematous, rheumatoid arthritis and mixed connective tissue diseases) [6]. Little is known about sCD40L role in
GD [7]. This study was conducted as a preliminary evaluation to estimate the serum concentrations of sCD40L in a
group of children with newly diagnosed GD and its relationship to patients’ clinical and laboratory variables.

Patients and Methods

Patients
This is a cross-sectional case-control study. It included 48 children (age range: 11-18 years; mean age = 14.4± 3.6)
carried the diagnosis of GD (girls = 34; boys = 14). All were newly diagnosed before the start of medical treatment.
They were consecutively recruited over a period of 2 years (2015-2017) from the Pediatric Endocrinology Clinic of
Children’s Hospital, Assiut University, Assiut, Egypt. Diagnosis of GD was based on the presence of clinical
manifestations of hyperthyroidism, low serum level of thyroid-stimulating hormone (TSH), high serum levels of free
thyroxine (FT4), free triiodothyronine (FT3), and high titers of thyrotropin receptor antibodies (TRAbs) [8].
Excluded from the study were those with: systemic or other immune-mediated diseases, subclinical
hyperthyroidism, previous relapse, Graves's ophthalmopathy toxic adenoma, toxic multinodular goiter and cases
coming from iodine deficient areas. Forty eight healthy children recruited from the general population and matched
for age, gender pubertal status, and socioeconomic status were also included as control subjects for statistical
comparisons. The inclusion criteria for the control group were demonstration of normal serum TSH and FT4,
negative antithyroid antibodies, and no past or family history of thyroid disease.

Methodology

All participants underwent detailed medical histories and clinical examinations with special emphasis on age at
onset of GD and its duration. Anthropometric measurements (height and weight) and vital signs were recorded.
Body mass index (BMI) was calculated using the following formula: BMI = weight (kg)/height (m) 2. BMI was
expressed as standard deviation scores (SDs) to normalize for age and sex [9] using national growth reference data
[10]. Blood pressure was recorded and expressed as standard deviation scores (SDs) to normalize for age and sex
[11]. Pubertal development was assessed by determining Tanner stage [12]. Thyroid volume was estimated using
ultrasonography (7.5-MHz linear array transducer) (GE Healthcare Bio-Systems, Milwaukee, WI, USA). Thyroid
volume values were obtained by calculating the volumes of both lobes as follows: Lobe (ml) = length x width x
depth (mm) x 0.479. Thyroid volume was expressed as SDS on the basis of the references values for age and gender
[13, 14]. Imaging data were reviewed by the same pediatric radiologist who was blinded to the biological data.

Laboratory investigations

Blood samples were obtained at 8.00 a.m. after an overnight fast for estimation of serum levels of TSH, FT4, and
FT3 (Immulite™ 2000 Third Generation, Diagnostic Products Corporation, Los Angeles, CA). The reference ranges
for thyroid hormones were as follows: TSH = 0.4-4.0 mU/L, FT4 = 10.0-26.0 pmol/L, and FT3 = 3.5–5.5 pmol/L.
The coefficients of variations (CV) for thyroid hormones were as follows: TSH = 5.0 and 5.1% at concentrations of
4.0 and 10.0 mU/L, respectively; FT4 = 6.5% at concentrations of 10.0 pmol/L; and FT3 = 8.9% at concentrations of
3.5 pmol/L. The serum TRAbs was measured with the 3rd generation TBII assay (TRAb3rd) using the automated
Cobas electrochemiluminescence (Elecsys, Roche Diagnostics GmbH, Penzberg, Germany). The cut-off value for
positive concentration of TRAbs was 1.75 IU/L. The serum level of hsCRP was measured using the high sensitivity
C reactive protein (hsCRP) enzyme immunoassay test (ELISA) kit (catalog no. E29-056; Immunospec Corp.,
Canoga Park, CA, USA). Measurement of serum sCD40L levels was done using a specific ELISA (Biosource Int.,
CA, USA) according to the manufacturer’s instructions. The reference range of sCD40L level is 0.16-10 ng/ml [15].

Ethical consideration

The protocol of the study was carried out in accordance with the Declaration of Helsinki ethical principles for
medical research involving human subjects. it has been approved by the ethical committee of Assiut University and
informed consent and assent were obtained from all participant before inclusion in the study.

Statistical analysis:

All statistical analyses were carried out using Statistical Package for the Social Sciences (SPSS) version 18.0 (SPSS
Inc., Chicago, IL, USA). Quantitative variables were presented means as standard deviations (SDs), and qualitative
variables were presented as percentages. The Kolmogorov-Smirnov test was used for assessing normality of data
distribution. Comparisons between parametric and nonparametric values were done using a two-tailed Student t and
Mann-Whitney U tests, respectively. Categorical variables were compared using the Chi-square or Fisher’s exact
tests. Correlations between sCD40L and clinical, and laboratory variables were done using Pearson’s correlation
coefficient test. Multivariate analysis was used to determine the factors that were significantly associated with high
sCD40L concentrations. The odds ratios, 95% confidence intervals and significances were calculated. For all tests, values of $p<0.05$ were considered statistically significant.

**Results**

Compared to healthy children, patients had significantly lower BMI SDS ($P = 0.01$) and higher heart rate ($P = 0.01$). They had significantly higher hsCRP and sCD40L levels ($P = 0.001$ for each) (Table 1). Patients’ sCD40L levels had significant positive correlation with age ($r = 0.319, P = 0.01$), thyroid volume SDS ($r = 0.447, P = 0.001$), hsCRP ($r = 0.323, P = 0.01$) and TRAbs concentrations ($r = 0.632, P = 0.001$) but not with FT3, FT4, or TSH levels (Table 2). Multivariate analysis showed that sCD40L levels were significantly correlated with TRAbs (OR: 3.1, CI: 2.2–2.7, $P = 0.001$) and thyroid volume SDS (OR: 2.1, CI: 1.2–2.7, $P = 0.001$).

**Discussion**

The current study demonstrated that sCD40L levels were significantly higher in children with GD compared with control ($P = 0.001$). Moreover, sCD40L correlated positively with TRAbs concentrations that remained significant after regression analysis (OR: 3.1, CI: 2.2–2.7, $P = 0.001$). Janusz Mysliwiec et al. [7] reported that sCD40L levels were greater in adult patients with GD than in control subjects, although the difference did not reach statistical significance. Experimental studies found that the increased sCD40L concentrations were associated with in vitro adhesion molecules and monocyte chemoattractant protein-1 release, impaired migration of endothelial cells and O2 generation in monocytes [16], which reflect that sCD40L plays an important role in the regulation of autoimmune and inflammatory responses that might be involved in the pathogenesis of GD [7]. Blockade of the CD40–CD40L pathway with BI 655064 in MTX-IR patients with rheumatoid arthritis resulted in marked improvement in clinical and biological parameters [18], suggesting the attracted role of CD40–CD40L pathway as a target for novel therapeutic strategies of autoimmune diseases.

hsCRP is an acute-phase protein associated with systemic inflammation. In this study, the circulating levels of hsCRP were significantly higher in the children with GD than in the control children. Furthermore, the hsCRP levels were positively correlated with sCD40L levels ($r = 0.323, P = 0.01$). These findings are consistent with those of previous studies [19,20], that show increased systemic inflammation in adult patients with GD. In this study, age was significantly associated with sCD40L ($r = 0.319, P = 0.01$). This is in agreement with El-Asrar et al. [21] who reported significant positive correlations between sCD40L and age in a cohort of children with type 1 diabetes mellitus. On the other hand, Cholette et al. [22] reported that sCD40L levels are high at birth and remain significantly higher than those of adults throughout childhood. Future research may help to answer questions regarding the underlying reasons for developmental changes in sCD40L serum levels.

In the current study, thyroid volume SDS was significantly higher in children with GD compared with control ($P = 0.01$). Furthermore, we demonstrated a significant positive correlation between sCD40L levels and thyroid volume SDS that reminded significant after regression analysis (OR: 2.1, CI: 1.2–2.7, $P = 0.001$) suggesting a direct causal relationship between sCD40L and thyroid volume. Previous studies indicated that increased levels of sCD40L may reflect more massive T cells infiltration of thyroid gland in patients with GD as the degree of surface CD40 expression was shown to closely correlate with intensity of lymphocyte infiltration in addition to the thyroid growth-stimulating role of sCD40L that may result in diffuse goiter [7,23]. In this study, we reported a lack of correlation between sCD40L levels with either FT3 or FT4 concentration. This in agreement with Yamamoto et al. [24], who reported the same data among adult patients with GD. Despite the important role of sCD40L in the pathogenesis of GD [25], it seems that the high serum levels of this parameter are associated with the presence of goiter but not with thyroid hormones levels. However, further studies are needed to clarify the role of sCD40L in relation to the thyrotoxic activity of GD.

**Limitations:** The cross-sectional survey and a small number of subjects represent the major limitations to conclude whether higher sCD40L levels are directly involved in the pathogenesis of GD or just a consequence of the immune-mediated process.

**Conclusions:** This preliminary study has evidence of higher concentrations of sCD40L in children with newly diagnosed GD and its correlations with TRAbs and thyroid volume which may suggest its biological active role in GD.

**References**


**Table (1)** Clinical and laboratory characteristics of the studied groups

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients (n = 48)</th>
<th>Controls (n = 48)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female / male</td>
<td>34/14</td>
<td>32/16</td>
<td>NS</td>
</tr>
<tr>
<td>Age(years)</td>
<td>14.4± 3.6</td>
<td>15.4 ± 3.6</td>
<td>NS</td>
</tr>
<tr>
<td>BMI SDS</td>
<td>-0.37 ± 1.06</td>
<td>0.30 ± 2.16</td>
<td>&lt; 0.01*</td>
</tr>
<tr>
<td>Heart rate (beat per minute)</td>
<td>113± 13</td>
<td>98 ± 8</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>Systolic BP SDS</td>
<td>0.72 ± 03</td>
<td>0.63 ± 0.2</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic BP SDS</td>
<td>0.37 ± 0.1</td>
<td>0.32 ± 0.05</td>
<td>NS</td>
</tr>
<tr>
<td>TSH(mIU/ml)</td>
<td>0.061 ± 0.02</td>
<td>1.95 ± 0.9</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>FT4(pmol/l)</td>
<td>35.8 ± 9.3</td>
<td>13.32 ± 2.55</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>FT3 (pmol/l)</td>
<td>13.4 ± 4.4</td>
<td>4.22 ± 2.1</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>TRAbs (IU/L)</td>
<td>16.32 ± 4.65</td>
<td>0.7 ± 0.7</td>
<td>&lt; 0.01*</td>
</tr>
<tr>
<td>Thyroid volume SDS</td>
<td>5.1 ± 1.1</td>
<td>2.1 ± 0.4</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>hs-CRP (mg/L)</td>
<td>329 ± 20.5</td>
<td>67.9 ± 12.8</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>sCD40L (ng/ml)</td>
<td>16.2±3.5</td>
<td>3.66±1.2</td>
<td>&lt; 0.001*</td>
</tr>
</tbody>
</table>

Data are means ± standard deviation (SD)

BMI-SDS, body mass index standard deviation score; TSH, thyroid stimulating hormone; FT4, free thyroxine; FT3, free triiodothyronine; TRAbs, thyrotropin stimulating hormone receptor antibodies; sCD40L, soluble CD40 ligand; hs-CRP, high-sensitivity C-reactive protein

NS: non-significant

* Significant difference.

**Table 2. Correlation between sCD40L and the other parameters in children with GD**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>r value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>0.319</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>BMI SDS</td>
<td>- 0.204</td>
<td>NS</td>
</tr>
<tr>
<td>Heart rate (beat per minute)</td>
<td>0.119</td>
<td>NS</td>
</tr>
<tr>
<td>Systolic BP SDS</td>
<td>0.123</td>
<td>NS</td>
</tr>
<tr>
<td>Diastolic BP SDS</td>
<td>0.125</td>
<td>NS</td>
</tr>
<tr>
<td>Thyroid volume SDS</td>
<td>0.447</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TSH(μIU/Ml)</td>
<td>- 0.212</td>
<td>NS</td>
</tr>
<tr>
<td>FT4 (pmol/l)</td>
<td>0.135</td>
<td>NS</td>
</tr>
<tr>
<td>FT3 (pmol/l)</td>
<td>0.199</td>
<td>NS</td>
</tr>
<tr>
<td>hs-CRP (mg/L)</td>
<td>0.323</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>TRAbs (IU/L)</td>
<td>0.632</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

BMI-SDS, body mass index standard deviation score; TSH, thyroid stimulating hormone; FT4, free thyroxine; FT3, free triiodothyronine; TRAbs, thyrotropin receptor antibodies;NS: non-significant

* Significant difference.