Thyroid Autoimmune Antibodies and Major Depressive Disorder in Women

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Abstract

Introduction: Anti-thyroid antibodies are associated with extra-thyroid diseases such as Graves’ ophthalmopathy and Hashimoto’s encephalopathy. Some evidence suggests that anti-thyroid antibodies are also associated with depression. Interleukin (IL)-17 appears to play an important role in autoimmune thyroid disease. This study investigated whether specific thyroid autoantibodies and IL-17 distinguished persons with depression from non-depressed controls. Materials and Methods: Forty-seven adult females with non-psychotic, current major depressive disorder and 80 healthy female controls participated in this study. Thyroid peroxidase antibodies, thyroglobulin antibodies, thyroid-stimulating hormone (TSH) receptor antibodies, free T3 and T4, TSH and IL-17 were measured from the serum. Measurements were repeated to assess test-retest reliability. Receiver operating characteristic (ROC) curves were used to estimate discriminatory values of the measurements. Differences between groups and associations between the clinical and biochemical assessments were analysed. Results: Median TSH receptor antibody concentration was significantly higher in the depressed than control group (P <0.001). Area under the ROC curve was 0.80 (95% CI, 0.73 to 0.88). Higher TSH receptor antibody titres were associated with greater depression severity scores (r = 0.33, P <0.05). IL-17 levels were not associated with TSH receptor antibody levels or depression severity scores. Thyroid function and other thyroid autoantibodies were not associated with depression severity. Conclusion: TSH receptor antibodies might be a biomarker of immune dysfunction in depression.

Key words: Cytokines, Depression, Immunology

Introduction

Autoimmune thyroid diseases such as Graves’ disease and Hashimoto’s thyroiditis are defined by elevated serum levels of anti-thyroid antibodies and thyroid hormone dysfunction. Anti-thyroid antibodies could also affect extra-thyroidal tissues and organs.

In Graves’ ophthalmopathy, antibodies target thyroid stimulating hormone (TSH) receptor or thyroid peroxidase (TPO) antigens in orbital tissues to activate cytokine-mediated inflammatory changes. These cytokines stimulate glycosaminoglycan and metalloproteinase inhibitor production and fibroblast proliferation to cause soft tissue oedema and proptosis. Patients with Hashimoto’s encephalopathy may present with focal neurological symptoms, cognitive deficits, or psychiatric symptoms. Elevated levels of serum anti-thyroid antibodies, particularly antibodies to TPO (TPOAb) and thyroglobulin (TgAb), are hallmarks of this disease. TPO is a follicular cell enzyme involved in thyroid hormone synthesis, while Tg is a precursor protein used to synthesise thyroid hormones. Although serum levels of anti-thyroid antibodies are elevated in Hashimoto’s encephalopathy, thyroid hormone levels are...
Subjects aged 21 to 60 years were eligible to participate and all participants provided written informed consent. The study was approved by the Institutional Review Board. Volunteers were recruited via printed and online media. The adult psychiatry service at a general hospital. Healthy individuals (RR 1.4; 95% CI, 1.0 to 2.1). Another study reported an association between TPOAb positivity and lifetime diagnosis for major depressive disorder (OR 2.7; 95% CI, 1.1 to 6.7) based on the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV). These studies did not examine patients with current episode major depression. Few studies have investigated other autoantibodies in depressed patients. Two studies found no association between TgAb and depression, while 1 study reported higher levels of thyroid-binding inhibitory immunoglobulin in depressed patients compared to healthy controls. It is not clear whether neutral TSH receptor antibodies (TRAbs) are associated with depression. Thyroid autoantibody’s pathophysiological effect in depression may be cytokine mediated. Th17 lymphocytes and its primary cytokine, interleukin (IL)-17, have important roles in autoimmune disease. IL-17 is a critical signaling molecule that induces the release of proinflammatory cytokines and chemokines. Higher proportions of Th17 cells are present in patients with Hashimoto’s thyroiditis and Graves’ disease. Depression has been frequently associated with elevated levels of proinflammatory cytokines, especially IL-6 and tumour necrosis factor (TNF)-α. However, little is known about whether IL-17 is associated with depression.

The aim of this study was to determine whether depressed patients and healthy volunteers differed in terms of TRAb, TPOAb, TgAb and IL-17 levels. We hypothesised that thyroid autoimmune markers would be greater in patients with current major depressive episode than normal controls and that IL-17 levels would be associated with these thyroid antibody levels. We further explored the relationship between thyroid autoantibodies, IL-17 and depressive symptom severity.

Materials and Methods

Subjects

Psychiatric inpatients and outpatients were recruited from the adult psychiatry service at a general hospital. Healthy volunteers were recruited via printed and online media. The study was approved by the Institutional Review Board and all participants provided written informed consent. Subjects aged 21 to 60 years were eligible to participate. Major depressive disorder was diagnosed by psychiatrists and trained raters confirmed the diagnosis using the DSM-IV-based Mini International Neuropsychiatric Interview (MINI). Depressed subjects were included if they scored at least 16 on the Montgomery-Åsberg Depression Rating Scale (MADRS). The Quick Inventory of Depressive Symptomatology-Self Report (QIDS-SR) was also used to measure depression severity. Controls were included if they did not have current depression after screening with the MINI and MADRS. Only women were recruited to control for the known gender difference in autoimmune expression. Both control and depressed participants were excluded if they met the diagnostic criteria for bipolar or psychotic disorder (lifetime)—eating, obsessive-compulsive, alcohol or substance use-related disorders (past 1 year). Additional exclusion criteria for all participants included acute infections within the past 1 month; the presence of any allergic, autoimmune, neoplastic, endocrine and other general medical/surgical disorders within the past 3 months; and pregnancy. None of the participants took prescribed or over-the-counter drugs that had known effects on immunological or inflammatory responses.

Serum Measurements

Thyroid autoantibodies (TPOAb, TgAb and TRAb), thyroid hormones (fT3, fT4 and TSH) and cytokine IL-17 were measured from the serum. The blood sample was drawn once from each participant in the day. Samples were then aliquoted into separate sample tubes, stored at -20°C, and assayed in batches. Each blood sample was measured twice in the laboratory to determine test-retest assay reliability.

fT3, fT4 and TSH were measured using the ADVIA Centaur immunoassay system (Siemen Healthcare Diagnostics, Germany). For the fT3 assay, the minimum detectable concentration (analytical sensitivity) was 0.2 pg/mL, reference range was 5.0 pmol/L to 8.9 pmol/L, and manufacturer defined (md) intra-assay CV was <4%. For the fT4 assay, the analytical sensitivity was 0.1 ng/dL, reference range was 9.6 pmol/L to 19.1 pmol/L, and md intra-assay CV was <4%. For the TSH assay, the analytical sensitivity was 0.010 μIU/mL, reference range was 0.65 mol/L to 3.7 mol/L, and md intra-assay CV was <6%.

Thyroid autoantibodies were measured using radioimmunoassays (BRAHMS Diagnostica, Germany). Human receptor assays for the antibodies were used. The analytical sensitivities of TPOAb and TgAb were both 5.5 U/mL, and their measured intra-assay CVs were <5%. The analytical sensitivity of TRAb was 0.3 U/mL and the measured intra-assay CV was <2%.

IL-17 was measured using the Merck-Millipore Milliplex Human Cytokine Panel (Merck KGaA, Darmstadt,

often normal. Both TPOAb and TgAb have been detected in the cerebrospinal fluid of patients with Hashimoto’s encephalopathy, which suggests that anti-thyroid antibodies may have direct effects on the central nervous system.

Could depression be a model of extra-thyroidal autoimmune disease? A higher lifetime prevalence of depression, based on self-report, was found in TPOAb positive versus negative individuals (RR 1.4; 95% CI, 1.0 to 2.1). Another study reported a higher levels of thyroid-binding inhibitory immunoglobulin in depressed patients compared to healthy controls. It is not clear whether neutral TSH receptor antibodies (TRAbs) are associated with depression. Thyroid autoantibody’s pathophysiological effect in depression may be cytokine mediated. Th17 lymphocytes and its primary cytokine, interleukin (IL)-17, have important roles in autoimmune disease. IL-17 is a critical signaling molecule that induces the release of proinflammatory cytokines and chemokines. Higher proportions of Th17 cells are present in patients with Hashimoto’s thyroiditis and Graves’ disease. Depression has been frequently associated with elevated levels of proinflammatory cytokines, especially IL-6 and tumour necrosis factor (TNF)-α. However, little is known about whether IL-17 is associated with depression.

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Germany). The assay was performed using a self-antigen specific capture antibody technique in a multiplex immunoassay quantification system. The intra-assay CV was <10%.

**Statistical Analysis**

All serum specimens were measured twice in the laboratory to assess test-retest reliability of the test procedures. Reliability of the measurements was evaluated by agreement testing (Wilcoxon signed-rank test) and intraclass correlation (Spearman’s correlation). Differences between groups and associations between the clinical and biochemical variables were analysed with the appropriate t-test, Mann-Whitney U test, Pearson’s or Spearman’s correlation after normality checks. Chi-square test was used for categorical data. Generalised linear regression was used to evaluate potential confounding. Receiver operating characteristic (ROC) curves were used to estimate potential discriminatory values of the laboratory measures.

**Results**

Forty-seven women with non-psychotic, current episode major depressive disorder and 80 female controls participated in this study. Baseline characteristics are shown in Table 1. Depressed subjects had moderate to severe depression, with a mean MADRS score of 31.3 (±

<table>
<thead>
<tr>
<th>Table 1. Baseline Characteristics and Serum Measurements</th>
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<tbody>
<tr>
<td><strong>Depressed (n = 47)</strong></td>
</tr>
<tr>
<td>Mean age, years (SD)</td>
</tr>
<tr>
<td>Gender (%)</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Ethnicity (%)</td>
</tr>
<tr>
<td>Chinese</td>
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<tr>
<td>Education level (%)</td>
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<tr>
<td>Primary or below</td>
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<td>Secondary</td>
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<td>Tertiary and above</td>
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<tr>
<td>Mean MADRS score (SD)</td>
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<td>Mean QIDS-SR score (SD)</td>
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<tr>
<td>Mean age of onset for first major depressive episode, years (SD)</td>
</tr>
<tr>
<td>Number of major depressive episodes including current episode (%)</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
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<tr>
<td>≥3</td>
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<tr>
<td>Number of adequate antidepressant trials (%)</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>1</td>
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<tr>
<td>≥2</td>
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<tr>
<td>Median anti-thyroid antibody titres (range)</td>
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<tr>
<td>TRAb (U/mL)</td>
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<tr>
<td>TPOAb (U/mL)</td>
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<tr>
<td>TgAb (U/mL)</td>
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<tr>
<td>Median thyroid hormone levels (range)</td>
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<tr>
<td>fT3 (pmol/L)</td>
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<td>fT4 (pmol/L)</td>
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<tr>
<td>TSH (μU/mL)</td>
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<tr>
<td>Median cytokine level (range)</td>
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<tr>
<td>IL-17 (pg/mL)</td>
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</tbody>
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IL-17: Interleukin-17; MADRS: Montgomery-Åsberg Depression Rating Scale; QIDS-SR: Quick Inventory of Depressive Symptomatology-Self Report; TgAb: Anti-thyroglobulin antibody; TPOAb: Anti-thyroid peroxidase antibody; TRAb: Anti-thyroid stimulating hormone receptor antibody; TSH: Thyroid stimulating hormone

*P<0.001 (Mann-Whitney U test)
11.9 SD) and QIDS-SR score of 14.8 (± 4.6 SD). Seventy-nine percent of those depressed were on antidepressant medication (either a selective serotonin reuptake inhibitor, serotonin-norepinephrine reuptake inhibitor or tricyclic antidepressant). Eight depressed and 1 control were smokers. None of the participants had a past history of thyroid disease. Median measures of fT3, fT4 and TSH did not differ between the groups.

All antibody and cytokine measurements in Table 1 had acceptable agreement, as indicated by non-significant differences between repeat laboratory measurements. Correlations between repeat measurements were good. The correlation coefficients for TPOAb, TgAb, TRAb and IL-17 were 1.00, 0.99, 0.78 and 0.94 respectively for cases, and 1.00, 1.00, 0.95 and 0.97 respectively for controls, with all P values less than 0.001.

**Anti-thyroid Antibodies**

The median TRAb level was significantly higher (P <0.001) in those depressed compared with controls. There was 1 TRAb outlier but the difference remained significant (P <0.001) even after exclusion of the outlier. Higher TRAb titres correlated with greater depression scores on the QIDS-SR (r = 0.33, P <0.05). The association between TRAb levels and depression remained significant (P = 0.033) after adjusting for age and smoking. No difference was found between the depressed and control groups for TPOAb and TgAb.

TRAb levels differentiated the depressed from controls, based on the ROC curve for TRAb. The area under the curve (AUC) was 0.80 (95% CI, 0.73 to 0.88) (Fig. 1), using a cut-off of 0.30 U/mL. At this threshold, TRAb had 87% sensitivity and 73% specificity in discriminating depressed from controls.

Clinically, upper reference limits of >60 U/mL for TPOAb and TgAb, and >10 U/mL for TRAb are used to define positive thyroid autoimmunity in relation to autoimmune thyroid disease. TPOAb positivity was found in 8.5% of depressives and 5% of controls, while TgAb positivity was found in 6.4% of depressives and 7.5% of controls. Depressed individuals did not have significantly greater TPOAb or TgAb positivity compared to controls. None of the subjects were TRAb positive.

**Cytokine IL-17**

IL-17 levels did not differ between controls and those depressed. IL-17 levels were neither associated with TRAb levels nor depression severity scores. IL-17 was also not associated with age, thyroid hormone levels and other thyroid autoantibody levels.

**Discussion**

Patients with major depressive disorder in a current major depressive episode did not differ from healthy controls in terms of TPOAb and TgAb levels. This is consistent with previous studies that found no difference in TPOAb and TgAb positivity between currently depressed patients and controls. However, we found significantly higher TRAb levels in depressed patients than controls.

Approximately 69% of those with systemic autoimmune disease are depressed and autoimmune disease is associated with a 45% increased risk of subsequent mood disorder. Higher titres of autoantibodies are reported in patients with autoimmune disease suffering from depression compared to those without depression and healthy controls. In systemic lupus erythematosus (SLE), elevated levels of autoantibodies that are associated with depression include anti-endothelial cell, anti-ganglioside, anti-nuclear, anti-N-methyl-d-aspartate receptor, anti-phospholipid, and anti-P ribosomal autoantibodies. To test whether such autoantibodies had a direct pathogenic role in depression, Shoenfeld and colleagues injected purified human anti-P ribosomal autoantibodies into mice intracerebroventricularly. This induced depression-like behaviour in the animal model. The experiment further showed that this depressive behaviour could be blocked by a 4-week treatment with fluoxetine. Immunohistologic staining of anti-P ribosomal autoantibodies revealed stain patterns in the limbic system (cingulate cortex, hippocampus and piriform cortex), a neural network implicated in affect regulation. Although autoimmune antibodies may be involved in depression pathophysiology, studies have yet to show that depression could be induced by thyroid autoantibodies in specific.
The TRAb assayed in our study is not known to be stimulatory or inhibitory, and is thought to be functionally neutral in terms of thyroid hormone production. However, TRAbs may have direct effects on extra-thyroidal tissue and organs, such as the brain. TRAbs were recently shown to bind to human cortical neurons, with localisation to cell bodies and axons.24 Neutral TRAbs activate unique cellular signal cascades that include protein kinase A II, phosphatidylinositol 3K/Akt, MAPK-ERK1/2/p38α, mammalian target of rapamycin/p70 S6K and nuclear factor-κB.25 Chronic exposure to these antibodies leads to oxidative stress-induced apoptosis and excess oxidative stress, and generation of reactive oxygen species have been described in depression.26,27 Another mechanism through which TRAbs can act is in the stimulation of proinflammatory cytokines. Antibodies that bind to TSH receptors on bone marrow cells have been shown to stimulate IL-6 secretion in the absence of TSH.28

Proinflammatory cytokines are known to cause emotional and cognitive disturbances. Interferon is commonly associated with depression when administered exogenously. Twenty-five percent of patients who started on interferon therapy for hepatitis C go on to develop a major depressive episode.29 Endogenously released cytokines can also cause depressed mood. Healthy volunteers, when given endotoxins to induce proinflammatory cytokine release, experienced both depressed mood and memory impairment. These endotoxins had no effect on physical illness symptoms but caused an increase in cytokines levels that correlated positively with depressive symptoms.30 We measured IL-17 in this study based on previous studies that associated it with autoimmune thyroid disease. IL-17 levels were not significantly raised in our depressed patients. This may be because thyroid autoantibody titres in depression are not as elevated as that in autoimmune thyroid disease, hence there is minimal IL-17 stimulation.

While it is not conclusive that TRAbs are directly involved in the pathogenesis of depression, our findings suggest that neutral TRAbs may be a biomarker of low-grade immunological dysfunction in depression. The AUC for TRAb was 0.8 in this study, which is fairly good for a diagnostic test, considering that there is no reliable laboratory test for depression at present. Other stimulatory and inhibitory TRAbs may be examined in future. Besides the aforementioned autoantibodies that are associated with depression, other cellular markers of altered immune function have been reported in depression, such as leukocytosis, decreased natural killer cell cytotoxicity and reduced lymphocyte proliferation in response to mitogens.31 Future studies, with a larger sample size, could look into using the most consistent immunological markers together to characterise depression diagnosis and treatment response.

Conclusion

Our study showed that serum TRAb titres were able to discriminate between depressed cases and controls. IL-17 levels were not associated with TRAb titres or depression severity. TRAbs may be a useful indicator of immune dysfunction in depression.

REFERENCES


