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Latent *Toxoplasma gondii* infection is associated with decreased serum triglyceride to high-density lipoprotein cholesterol ratio in male patients with schizophrenia

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ABSTRACT

Background: Previous studies suggested a complex association between *Toxoplasma gondii* (TG) infection and host lipid metabolism. Both TG infection and metabolic disturbances are very common in patients with schizophrenia, but this relationship is not clear.
Methods: In this cross-sectional study, we evaluated the association between TG seropositivity, serum lipid levels, body mass index (BMI) and metabolic syndrome (MetS) in 210 male inpatients with schizophrenia.
Results: In our sample of schizophrenia patients, with the mean age of 43.90 ± 12.70 years, the rate of TG seropositivity was 52.38% and the prevalence of MetS was 17%. Patients with the TG antibodies had lower serum triglyceride levels and body weight compared to TG seronegative patients, despite having more frequently received antipsychotics (clozapine, olanzapine risperidone and quetiapine), which are well known to induce weight gain and metabolic abnormalities. However, the only significant change in metabolic parameters, observed in TG seropositive patients with schizophrenia, was decreased serum triglyceride to high-density lipoprotein cholesterol (HDL-C) ratio. No associations were observed between TG seropositivity and serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and glucose levels, waist circumference, BMI and the rate of MetS.
Conclusion: This is the first report of the association between TG infection and decreased serum triglyceride to HDL-C ratio in a sample of carefully selected men with chronic schizophrenia.

1. Introduction

Latent infection with *Toxoplasma gondii* (TG) has recently raised significant interest in psychiatry, particularly in patients with schizophrenia [1,2]. Schizophrenia is a devastating disorder, which was consistently associated with increased rates of TG infection [2], but also with dyslipidemia [3,4]. Preclinical studies have reported a complex association between TG infection and host lipid metabolism. The parasite accumulates lipids from the host low-density lipoprotein cholesterol (LDL-C) [5]. Given that, TG is unable to synthesize cholesterol; its growth is dependent on the external cholesterol sources. Consequently, infection with TG decreased serum total cholesterol (TC) concentration in apolipoprotein E-deficient mice [6], LDL-C receptor knockout mice fed with hypercholesterolemic diet [5] and in wild-type mice [7]. In general, TG infection reduced serum TC levels in animal models due to its increased uptake [6].

In spite of such profound effects of TG on lipids in animal models, only two studies have investigated lipid levels in human subjects with evidence of TG infection. One study found no difference in serum TC levels from cord blood samples between TG seropositive and seronegative pregnant women [8]. Another study reported a correlation between TG seropositivity and serum TC and LDL-C levels in men with schizophrenia

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[9]. However, the latter study provided no data on other factors, which affect serum lipid levels in patients, like antipsychotics [4,10], lipid-lowering-agents and severity of symptoms.

Therefore, the link between the TG seropositivity, serum lipid levels and weight is poorly understood in patients with schizophrenia. In addition, there is no data on the relationship between TG infection and metabolic syndrome (MetS), neither in healthy individuals nor in psychiatric patients.

The aim of this study was to investigate the association between TG seropositivity, serum lipid indices, body mass index (BMI) and MetS in men with schizophrenia.

2. Materials and methods

2.1. Subjects and clinical evaluation

This cross-sectional study was conducted at University Hospital Centre Zagreb, Department of Psychiatry, and Psychiatric Hospital Popovaca. Inclusion criteria were: male inpatients aged 18 to 65 years, diagnosed with schizophrenia for at least 5 years. Diagnosis of schizophrenia was confirmed using the Structured Clinical Interview (SCID) [11]. Exclusion criteria were: intellectual disability, patients with first-episode psychosis and/or no previous treatment with antipsychotics, substance abuse and dependence in the previous year, any comorbid severe somatic or neurological disorder, including inherited dyslipidemia and current infection, treatment with lipid-lowering, antidiabetic and/or antihypertensive agents and the use of antidepressants in previous three months.

Only male patients were enrolled in the study in order to exclude the influence of gender on the results. Notably, gender differences were reported in metabolic issues, such as obesity [12], lipid levels [13], the prevalence of MetS [14], and treatment-outcome [15] in patients with schizophrenia. Patients were evaluated using structured interview for the Positive and Negative Symptom Scale (PANSS) [16], including the PANSS positive, negative, general psychopathology and cognitive (PANSS-COGN) subscale (consisting of P2, N5, N7, G7, G10, G11, G12, G14 and G15 PANSS items), as well as with Calgary Depression Scale for Schizophrenia (CDSS) [17] and International Suicide Prevention Trial (InterSePT) Scale [18].

At the time of the assessment, psychiatrists were not aware of the laboratory test results. The study was approved by the local Ethics Committees and was carried out in accordance with the Helsinki declaration (1975). All patients have signed approved informed consent prior to study procedures.

2.2. Determination of TG IgG antibodies

TG immunoglobulin G (IgG) antibodies in serum were determined using VIDAS Toxo IgG test produced by Biomerieux, France. Vidas Toxo IgG is an automated quantitative test for use on the VIDAS family instruments for the quantitative measurement of anti-TG IgG in human serum or plasma using ELFA (Enzyme-Linked-Fluorescent Assay) technique. The assay combines a two-step enzyme immunoassay sandwich method with a final fluorescent detection at 450 nm. Once the assay is completed, the results are analysed automatically by the instrument using calibration curves, which are stored and expressed in international units (IU)/ml. The cut off values are determined by the test kit manufacturer. It has been considered that results up to 4 IU/ml were negative, results from 4 IU/ml to 8 IU/ml were considered equivocal, and results above 8 IU/ml were considered positive.

2.3. Determination of biochemical and anthropometric parameters

Waist and hip circumference (cm), height (cm), and body mass (kg) were measured by hospital nursing stuff. The height and weight were recorded in standing position, barefoot, and in light clothes. Body mass index (BMI) was calculated as body weight in kilograms divided with squared height value in metres. Waist circumference was measured at minimal respiration at the high point of the iliac crest and at the level of the umbilicus.

Venepuncture was performed between 7 and 9 a.m., after 12 h overnight fast. Immediately after collecting, blood samples were transferred to the Department for Laboratory Medicine, University Hospital Centre Zagreb. Serum levels of triglycerides, TC, LDL-C, high-density lipoprotein cholesterol (HDL-C) and glucose were determined on Roche Cobas C501 Chemistry Analyzer. Serum glucose level was determined by enzymatic UV test with hexokinase. LDL-C was calculated using the Friedewald equation. If serum triglyceride concentration exceeded 4.52 mmol/l, LDL-C was estimated by homogeneous enzymatic colorimetric test with cholesterol oxidase. LDL-C/HDL-C, TC/HDL-C and triglycerides/HDL-C ratios have been also calculated.

MetS definition was used according to NCEP ATP III criteria [19], which requires the presence of three or more of the following criteria: 1) abdominal obesity (waist circumference > 102 cm in men); 2) hypertriglyceridemia (≥1.7 mmol/l); 3) low HDL-cholesterol (<1.04 mmol/l in men); 4) high blood pressure (≥135/85 mm Hg); 5) high fasting glucose (≥6.1 mmol/l).

2.4. Statistical analysis

Statistical analyses were performed with GraphPad Prism version 4.00 (GraphPad Software, San Diego, CA, USA) and MedCalc Statistical Software version 14.8.1 (MedCalc Software bvba, Ostend, Belgium).

Normality of data distribution was assessed with the D'Agostino-Pearson omnibus normality test. The differences in the age, the scores on the PANSS total, positive, negative, cognitive and general psychopathology subscales, CDSS and InterSePT total scores, dose of antipsychotics expressed as chlorpromazine equivalent, glucose, TC, HDL-C, LDL-C and triglyceride levels in the blood, LDL-C/HDL-C, TC/HDL-C and triglycerides/HDL-C ratios, as well as the differences in the waist and hip circumference, body weight, and BMI (all expressed as mean ± SD), between TG seropositive and TG seronegative group were analysed by Student *t*-test (when data showed normal distribution) or by Mann-Whitney test (when normality of the data failed).

The frequencies of smoking, current treatment with clozapine or olanzapine or risperidone or quetiapine, as well as MetS, between different groups of patients with schizophrenia were evaluated by a $\chi 2$-test of independence.

G*Power 3 Software was used for conducting power analyses, i.e. to determine a priori sample size and power. For Mann-Whitney test (with $\alpha = 0.05$; power $(1 - \beta) = 0.80$; median effect size = 0.35), total desired sample size was 210, and the actual sample size was 210. For Student *t*-test (with $\alpha = 0.05$; power $(1 - \beta) = 0.80$; median effect size = 0.35), total desired sample size was 204, and the actual sample size was 210. For analyses with a $\chi 2$-test (with $\alpha = 0.05$; power $(1 - \beta) = 0.80$ and small effect size ($\omega = 0.20$; df = 1)), total desired sample size was 197, while the actual total sample size was 210. Therefore, power analysis revealed that the study included appropriate sample size and statistical power to detect significant differences.

The results obtained by investigating various demographic and clinical data in TG seropositive and TG seronegative schizophrenia patients (Table 1) were corrected for multiple testing (11 tests) using Bonferroni correction, and the *p*-value was set to 0.0045. Bonferroni correction for multiple testing was also applied to the results obtained by comparing different metabolic parameters (13 tests) between TG seropositive and TG seronegative group of patients with schizophrenia (Table 2) and the p-value for these data was set to 0.00385.

Finally, multiple regression analysis (Table 3, Supplementary Table) was performed in order to assess the influence of various demographic and clinical data (independent variables), listed in the Table 1, on the 13 different metabolic parameters (dependent variables), listed in the