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ORIGINAL ARTICLE

Difference of neuro- and immunomodulatory steroids and selected hormone and lipid concentrations between *Toxoplasma*-free and *Toxoplasma*-infected but not CMV-free and CMV-infected schizophrenia patients

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Abstract

OBJECTIVES: *Toxoplasma gondii*, the protozoan parasite infecting about 30% population worldwide, is suspected to be the etiological agent of certain form of schizophrenia disease. *Toxoplasma* is known to change levels of certain neurotransmitters, cytokines and several hormones in both infected animals and humans. A common feature of toxoplasmosis and schizophrenia is a disorder of immune system.

METHODS: Here we studied the levels of five neuro- and immunomodulatory steroids, selected hormones and lipids in sera of 173 schizophrenia patients.

RESULTS: *Toxoplasma* infected schizophrenia patients expressed only insignificantly lower concentration of neuro- and immunomodulatory DHEA metabolites. Infected women had higher concentration of glucose while infected men had higher concentration of cholesterol and LDL cholesterol. No significant effect of human cytomegalovirus infection on the concentration of the above parameters was observed. The difference in the concentration of DHEA metabolites faded with the decrease of the concentration of anti-*Toxoplasma* IgG antibodies (i.e. with the duration of *Toxoplasma* infection) while the difference in the concentration of cholesterol and LDL-cholesterol increased with the decrease of the concentration of anti-*Toxoplasma* IgG antibodies. The prevalence of toxoplasmosis in male (53.2%) but not female (29.8%) schizophrenia patients was unusually high in comparison with prevalence of toxoplasmosis in a general population.

CONCLUSION: Our results provided an explanation for seemingly decreasing prevalence of toxoplasmosis in schizophrenia patients observed in current studies

(increased concerns about the rights of patients resulting in absence of non-cooperative *Toxoplasma*-positive patients in the study population) and suggest possible explanation for reported positive correlation between prevalence of toxoplasmosis and incidence of cardiovascular diseases (accelerated atherosclerotic development due to increased level of cholesterol and LDL in *Toxoplasma* infected humans).

INTRODUCTION

Intracellular parasite *Toxoplasma gondii* is believed to infect about 30% of population worldwide (Tenter *et al.* 2000). Congenital toxoplasmosis acquired in the first trimester of gestation, the result of transmission of parasites from mother with acute infection to fetus, has very serious impacts on health of children and could even result in spontaneous abortion. Also, ocular forms of toxoplasmosis, the frequent result of the congenital toxoplasmosis or of postnatal infection with virulent strains of *Toxoplasma*, has a very serious impact on public health (Jones *et al.* 2007; Scallan *et al.* 2011). For a long time, the most common form of *Toxoplasma* infection, latent toxoplasmosis, was considered more or less harmless in immunocompetent subjects. However, results of many case-control, cohort as well as correlation studies suggest that latent toxoplasmosis is associated with various disorders and probably even plays an etiological role in certain diseases (Flegr 2013a). For example, a strong statistical association exists between toxoplasmosis and schizophrenia (Torrey *et al.* 2007), obsessive compulsory disease (Miman *et al.* 2010), epilepsy (Yazar *et al.* 2003) or risk of suicide (Pedersen *et al.* 2012). From nonpsychiatric diseases, the toxoplasmosis is suspected to play a role in etiology of some tumor diseases (Thomas *et al.* 2012; Vittecoq *et al.* 2012; Yazar *et al.* 2004) and possibly even in certain cardiovascular diseases (Flegr 2013a; Yazar *et al.* 2006). Subjects with latent toxoplasmosis differ in concentration of free testosterone (Flegr *et al.* 2008a; Flegr *et al.* 2008b), dopamine (Skallová *et al.* 2005), total leukocyte, monocytes, NK-cells and B-cells counts (Flegr *et al.* 2011). Most of these effects have been observed also in experimentally infected rodents suggesting that the *Toxoplasma* infection is the cause rather than the effect of observed changes in physiology of the infected hosts – for recent reviews see (Flegr 2013b; McConkey *et al.* 2013, Vyas 2013).

Very close relation exists between latent toxoplasmosis and schizophrenia disease, probably the most important psychiatric disorder with incidence about 1% (McGrath *et al.* 2004). Prevalence of toxoplasmosis is usually higher in schizophrenia patients than in controls (Torrey *et al.* 2007; 2012; Zhou *et al.* 2011). Longitudinal cohort study performed on US soldiers showed that anti-*Toxoplasma* antibodies appear in serum of individuals between 6–18 months before onset of schizophrenia (Niebuhr *et al.* 2007). *Toxoplasma*-infected patients express more severe positive symptoms of schizophrenia (hallucinations, delusions) than *Toxoplasma*-free schizophrenics (Holub *et al.* 2013; Wang *et al.* 2006). This is probably related to increased concentration of dopamine that is synthetized with help of unique *Toxoplasma*-coded enzymes (Gaskell *et al.* 2009) and which is released from *Toxoplasma* cysts in the host brain (Prandovszky *et al.* 2011). Certain endophenotypes that were originally attributed to schizophrenia are probably typical only for *Toxoplasma*-infected subpopulation of schizophrenia patients. For example, the decreased density of gray matter in certain parts of brain (reduction of GM volume bilaterally in the caudate, median cingulate, thalamus, and occipital cortex and in the left cerebellar hemisphere) probably occurs only in *Toxoplasma*-positive schizophrenia patients, not in *Toxoplasma*-free patients or in *Toxoplasma*-infected controls (Horacek *et al.* 2012). Similarly, the reported earlier onset of schizophrenia in male than in female patients (Hafner 2003; Howard *et al.* 2000) occurs only in *Toxoplasma*-infected subjects (Holub *et al.* 2013).

A common feature of toxoplasmosis and schizophrenia is a disorder of immune system. Indeed, immunity disorders may be one of the connecting links between schizophrenia and toxoplasmosis infection (Bhadra *et al.* 2013; Hinze-Selch 2002; Muller *et al.* 2006). Recent reports demonstrated that some metabolites of dehydroepiandrosterone, namely its 7-hydroxylated derivatives, abundant in brain tissues, possess neuroprotective and neuromodulatory properties (Morfin *et al.* 2000; Morfin *et al.* 2001).

Therefore, in the present study we searched for differences in concentration of these DHEA metabolites, in *Toxoplasma*-free and *Toxoplasma*-infected schizophrenia patients in samples of sera collected by clinics for various reasons in the same psychiatric clinic during the period of 5 years.

METHODS

Subjects

The patient group consisted of 94 (54%) female patients, and 79 (46%) male patients with diagnosed schizophrenia made according to the Structured Clinical Interview for DSM-IV. Out of them there were 8 females and 13 males with first episode (drug naive) schizophrenia, the other patients were treated at least for six months with olanzapine or 'non-olanzapine' type of antipsychotic drugs. Antipsychotic treatment was prescribed in a flexible dosing schedule, adjusted according to the treating physician's discretion. The whole male and female groups were considered for statistical evaluation irrespective of the treatment, in order to avoid atomization of the data. Blood was collected at 08:00h after overnight fasting. The rests of sera from other study were used for analyses with approval of the physicians and the study was approved by the Local Ethical Committee of the Institute of Endocrinology, Prague.

Immunological tests for toxoplasmosis and human cytomegalovirus infection

The serological diagnosis of toxoplasmosis was performed in the National Reference Laboratory for Toxoplasmosis of the Czech Republic by two methods: ELISA (Enzyme-Linked Immunosorbent Assay; IgG: SEVAC, Prague, IgM: TestLine, Brno) and the Complement Fixation Test (CFT; SEVAC, Prague). The decrease in CFT titres compared with ELISA method detects more reliably the 'old' *Toxoplasma* infection (Kodym *et al.* 2007). CFT titres of antibodies to *Toxoplasma* were measured at dilutions between 1 : 4 and 1 : 1 024. All subjects testing IgG positive by IgG ELISA (positivity index >0.9) and those with CFT titres equal or higher than 1:8 were considered toxoplasmosis positive. Whenever more than one sample from the same patients were available, we repeated the test to detect possible seroconversion of patients. Only the patients with clear result of diagnosis were included into our study. The diagnosis of the cytomegalovirus CMV infection was performed in the National Reference Laboratory for herpes viruses of the National Institute of Public Health, Prague. Specific anti-CMV IgG antibodies were measured by quantitative ELISA (ETI-CYTOK-G plus, DiaSorin). Antibody concentration was expressed in arbitrary units (AU). Individuals with AU <40 were considered seronegative for CMV.

Steroid determination

Cortisol, dehydroepaiandrosterone and its sulfate (DHEA/S), and prolactin were determined by commercial kits from Beckman Coulter (previously Immunotech,Marseille,France).7-α-Hydroxydehydroepiandrosterone (7-α-OH-DHEA) and its 7-β-hydroxyisomer (7-β-OH-DHEA) were determined using an in-house radioimmunoassay as previously described (Lapcik *et al.* 1998, Lapcik *et al.* 1999).

Other biochemical tests

Thyrotropin (TSH) was measured by ECLIA (obtained from Roche Diagnostics GmBH, Mannheim, Germany) using a commercial Elecsys System 2010. Fasting blood glucose levels were measured with a Glucose analyser (Beckman, Fullerton, CA) using the glucoso-oxidase method. Lipid parameters, namely total serum cholesterol, high- and low density lipoproteins, and triacylglycerides, were measured using commercially available kits CHOL2 HiCo T 400, HDL-C III 200, LDL-D Gen 2 200, and TRIGL 250, respectively (Roche Diagnostics GmbH) with a Cobas 6000 module C analyser.

Statistics

The statistical analyses (statistical methods assumption tests, t-test, Logistic regression and General Linear Model (GLM) tests) were performed using the programe Statistica v. 9. (Stat Soft Inc.). The partial Kendall correlation test suggested by Siegel and Castellan (Siegel *et al.* 1988) based on Taus computed with standard Kendall correlations was used for nonparametric analyses (Kaňková *et al.* 2011); the Excel sheet for this analysis is available at http://web.natur.cuni.cz/flegr/programy.php.

RESULTS

We obtained both biochemical and serological data of 94 (54%) female patients (mean age: 33.9, S.D.: 6.92) and 79 (46%) male patients (mean age: 35.4, S.D.: 8.93), t=–1.33, p=0.186. Logistic regression of the biochemical and serological data, with sex and age as independent variables showed that the prevalence of toxoplasmosis in female patients was significantly lower (29.8%) than in male patients (53.2%), Wald's Chi^2=8.57, p=0.003, OR=2.57 (C.I.95=1.36–4.85). Prevalence of CMV was approximately the same in female patients (85.6%) and in male patients (83.3%), Wald's Chi^2=0.20, p=0.654, OR=0.82 (C.I.95=0.35–1.92). No association between toxoplasmosis and CMV was detected, Wald's Chi^2=2.27, p=0.132, OR=2.05 (C.I.95=0.80–5.27).

The GLM analyses suggested the existence of several significant or borderline effects of toxoplasmosis or toxoplasmosis-sex interaction on biochemical parameters, as demonstrated in the Table 1 and Figure 1. Separate nonparametric analyses for men and women showed that the effects of toxoplasmosis were mostly stronger in male than in female patients, see the Table 2. As may be seen in the Table 1, no significant effect of CMV or CMV-sex interaction on biochemical parameters was observed.

Statistically, the duration of the infection negatively correlates with concentration of anti-*Toxoplasma* IgG antibodies. Therefore, we can estimate whether observed effects are more probably the vanishing aftereffects of acute infection or the cumulative effects of latent toxoplasmosis. The partial Kendall analyses with concentration of anti-*Toxoplasma* IgG showed that the decreased level of DHEA metabolites in the *Toxoplasma*-infected patients were more probably only the aftereffect of acute toxoplasmosis, while increased level of cholesterol and LDL-cholesterol in sera of infected men more probably represented the cumulative effect of latent toxoplasmosis; see the Table 2 and Figures 2–3. No significant effect of CMV or CMV-sex interaction on biochemical parameters was observed, see the Table 1.

DISCUSSION

The aim of the present study was to find out whether schizophrenia patients with toxoplasmosis differ from those without serologically diagnosed toxoplasmosis in hormonal parameters, reflecting function of immune system. The subjects with toxoplasmosis had lower serum levels of DHEA and its sulfate, as well as its 7-hydroxylated metabolites, believed now to act as immune- and neuroprotective agents (Bicikova *et al.*

Tab. 1. Effects of toxoplasmosis, human cytomegalovirus infection, and sex on hormones and lipids in serum in schizophrenia patients.

	Mean				toxo		toxo-sex		CMV		CMV-sex	
	women Toxo-	women Toxo+	men Toxo-	men Toxo+	*p*-value	eta^2	*p*-value	eta^2	*p*-value	eta^2	*p*-value	eta^2
7-α-OH-DHEA	0.751	0.629	0.577	0.531	0.371	0.005	0.833	0.000	0.724	0.001	0.136	0.014
7-β-OH-DHEA	1.073	0.812	0.841	0.783	**0.056**	0.022	0.529	0.002	0.861	0.000	**0.031**	0.053
DHEA	25.723	21.570	25.729	22.276	0.315	0.006	0.745	0.001	0.861	0.000	0.357	0.005
DHEAS	5.450	4.462	5.917	5.036	0.346	0.005	0.897	0.000	**0.056**	0.022	0.904	0.000
cortisol	462.880	514.176	480.454	500.303	0.205	0.011	0.614	0.002	0.983	0.000	0.984	0.000
TSH	1.885	1.938	1.408	1.840	0.163	0.012	0.371	0.005	0.322	0.006	0.868	0.000
PRL	759.120	838.725	404.406	472.673	0.370	0.005	0.596	0.002	0.501	0.003	0.124	0.014
Glucose	4.131	4.977	4.338	4.294	**0.034**	0.027	**0.026**	0.029	0.754	0.001	0.419	0.004
TAG	1.364	1.489	1.462	1.843	0.129	0.014	0.370	0.005	0.622	0.001	0.460	0.003
Cholesterol	5.170	5.431	4.619	5.167	**0.048**	0.023	0.385	0.004	0.273	0.007	0.557	0.002
HDL	1.477	1.462	1.248	1.313	0.690	0.001	0.428	0.004	0.567	0.002	0.203	0.010
LDL	3.122	3.275	2.779	3.258	**0.089**	0.018	0.341	0.006	0.317	0.006	0.478	0.003

The table shows mean for *Toxoplasma*-infected and *Toxoplasma*-free male and female patients and results of GLM analyses, effect sizes (Eta^2), and significances (p-values) with toxoplasmosis (or CMV infection), sex, and toxoplasmosis-sex (or CMV infection-sex) interaction as independent variables and age of a patients as covariate. The trends (p<0.1) are printed in bold. No formal correction for multiple tests has been performed.

Tab. 2. Correlation of toxoplasmosis with hormones and lipids in serum in schizophrenia patients.

	toxoplasmosis						anti-Toxo IgG					
	All		women		men		All		women		men	
	Tau	*p*-value	Tau	*p*-value	Tau	*p*-value	Tau	*p*-value	Tau	*p*-value	Tau	*p*-value
7-α-OH-DHEA	–0.108	**0.026**	–0.083	0.234	–0.046	0.547	–0.112	0.160	–0.055	0.680	–0.216	**0.044**
7-β-OH-DHEA	–0.155	**0.001**	–0.167	**0.017**	–0.060	0.434	–0.018	0.826	0.127	0.343	–0.229	**0.032**
DHEA	–0.097	**0.047**	–0.129	**0.066**	–0.102	0.185	–0.031	0.694	–0.093	0.486	–0.025	0.817
DHEAS	–0.082	**0.092**	–0.135	**0.054**	–0.123	0.109	0.002	0.977	0.043	0.747	–0.065	0.543
cortisol	0.027	0.597	0.105	0.153	0.065	0.422	–0.039	0.653	–0.052	0.713	–0.027	0.818
TSH	–0.020	0.674	–0.053	0.452	0.134	**0.081**	–0.110	0.168	–0.175	0.191	–0.075	0.485
PRL	–0.023	0.631	0.075	0.285	0.001	0.986	–0.062	0.440	–0.213	0.111	0.117	0.275
Glucose	0.046	0.341	0.172	**0.014**	–0.022	0.771	–0.116	0.146	–0.117	0.383	–0.078	0.467
TAG	0.073	0.134	0.031	0.659	0.131	**0.087**	–0.045	0.576	0.139	0.298	–0.070	0.512
Cholesterol	0.071	0.143	0.074	0.293	0.187	**0.015**	–0.083	0.301	0.077	0.567	–0.102	0.342
HDL	–0.029	0.552	–0.050	0.478	0.105	0.170	–0.026	0.741	–0.071	0.594	0.026	0.805
LDL	0.053	0.282	0.047	0.511	0.198	**0.012**	–0.072	0.383	0.126	0.356	–0.158	0.157

The left part of the table shows results of partial Kendall correlation (age controlled) between concentration of particular molecules in serum of a patient and binary variable toxoplasmosis for all patients, the right part of the table shows results of partial Kendall correlation (age controlled) between concentration of particular molecules in serum of a patient and concentration of anti-*Toxoplasma* IgG antibodies for a subpopulation of *Toxoplasma*-infected patients. *Toxoplasma*-infected patients were coded as 1 and *Toxoplasma*-free patients as 0, therefore, positive partial Tau corresponds to positive correlation between the *Toxoplasma* infection and the concentration of particular molecules. The positive Tau in the right part of the table reflects positive correlation between concentration of particular molecules and concentration of specific antibodies in serum, i.e. the negative correlation between concentration of particular molecules and duration of the *Toxoplasma* infection. No formal correction for multiple tests has been performed.

2013; Morfin *et al.* 2000; 2001; Muller *et al.* 2006). This shift was observed in both sexes, however it was much stronger in women than in men. The generally worsened spectrum of biochemical parameters in schizophrenic patients with toxoplasmosis reflects also their higher glucose levels in female patients and less favor-

able lipid spectrum in male patients. The decreased levels of DHEA and its 7-hydroxylates metabolites return to norm with the decrease of concentration of anti-*Toxoplasma* antibodies and therefore, most probably, with duration of the *Toxoplasma* infection. That means that these decreases could by possibly just transient after-effect of acute *Toxoplasma* infection. On the other hand, no correlation with the anti-*Toxoplasma* antibodies concentration was observed for lipids or glucose, suggesting that these shifts are stable during the infection.

Toxoplasma infected patients, especially the men, had increased level of cholesterol and LDL. No such data have been published for humans, however, a similar effect, namely the increased level of LDL was observed in outbred mice infected in laboratory. *Toxoplasma* imports LDL for synthesis of its membrane lipids from the host organism. It was observed that *T. gondii* diverts cholesterol from low-density lipoproteins for cholesteryl ester synthesis and storage in lipid bodies (Nishikawa *et al.* 2005). Acute phase of infection is characterized by decreased levels of cholesterol and LDL in normal (Milovanovic *et al.* 2009) and ApoE-deficient (Portugal *et al.* 2004) mice and postacute phase (42 days after the infection) by an increased concentration of LDL (Milovanovic *et al.* 2009). The increase of LDL correlated with cyst counts in 44% of mice with more than 300 cysts per brain. The authors suggested that *Toxoplasma* induces serum lipoprotein changes by influencing on lipid receptors and apolipoproteins (Milovanovic *et al.* 2009).

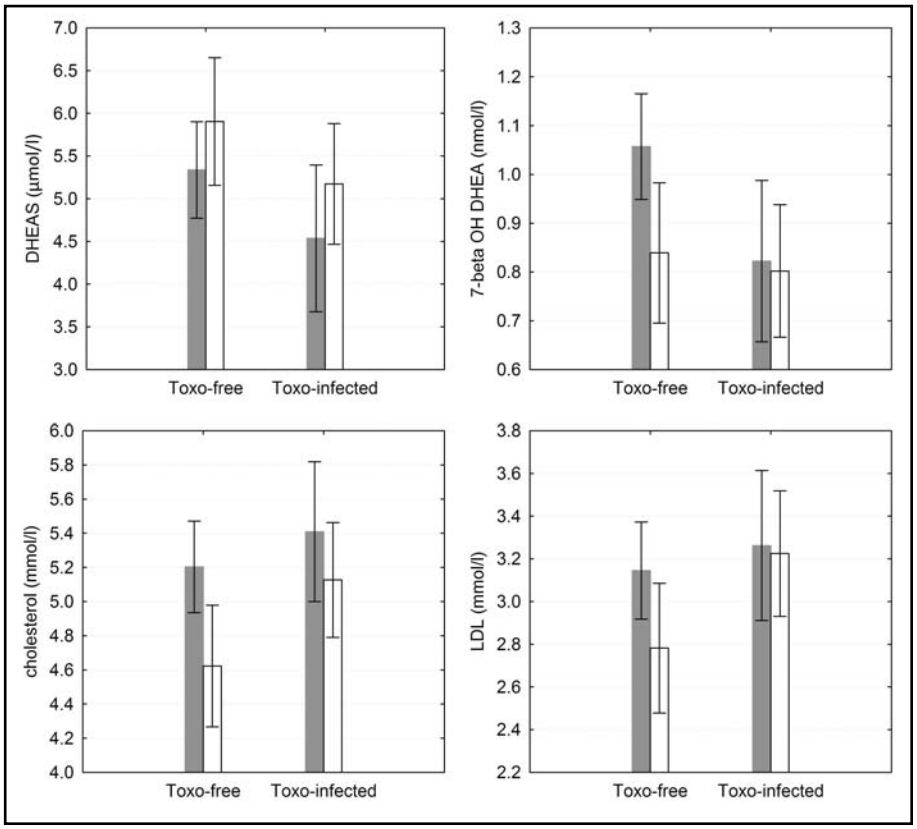

Fig. 1. Effect of *Toxoplasma* infection on DHEA metabolites, cholesterol and LDL-cholesterol in female (dark columns) and male (white columns) patients.

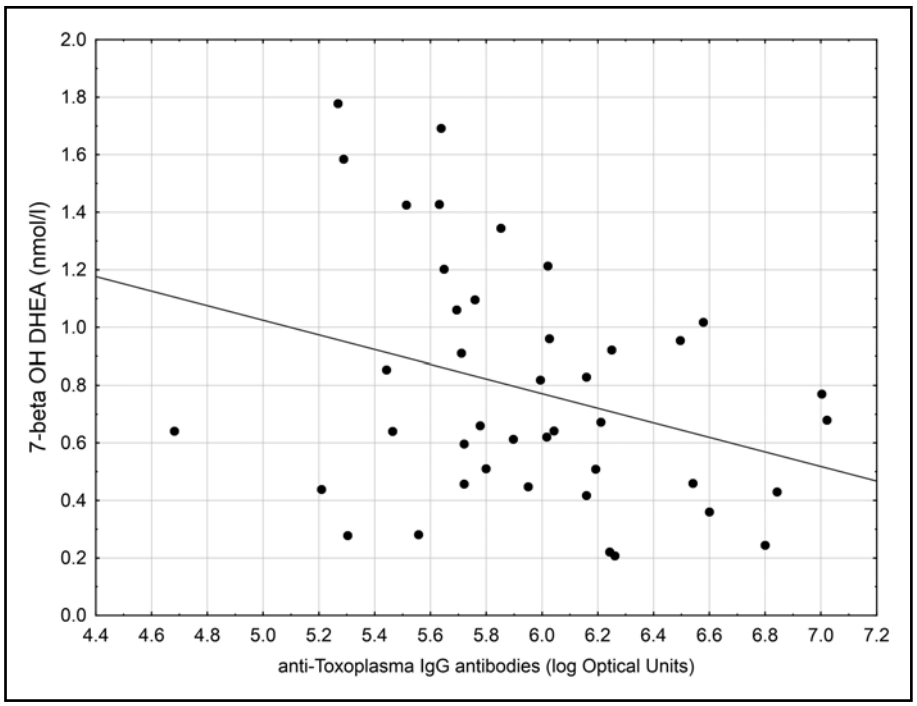

Fig. 2. Correlation between the concentration of 7-beta DHEAS and the level of anti-*Toxoplasma* antibodies in *Toxoplasma*-infected male patients.

Infection with *Toxoplasma gondii* increased atherosclerotic lesion in ApoE-deficient mice, which was considered to be a result of increased interferon production in chronic phase of *T. gondii* infection. Our results suggest that *Toxoplasma* infection could accelerate atherosclerotic development not only by continu-

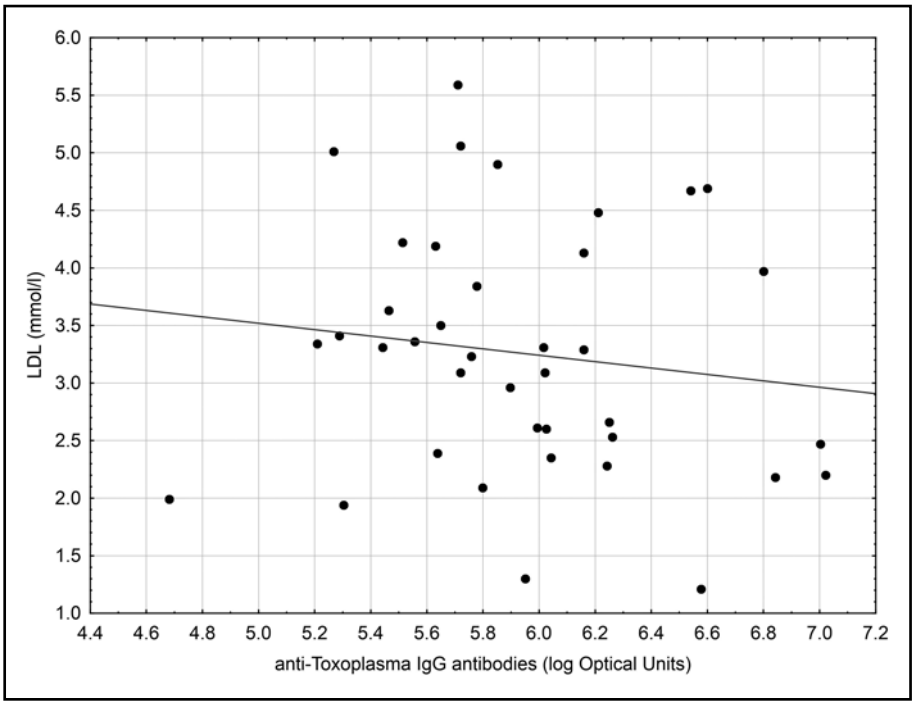

Fig. 3. Correlation between concentration of LDH and level of anti-*Toxoplasma* antibodies in *Toxoplasma*-infected male patients.

ous stimulation of synthesis of lymphokines (Flegr *et al.* 2011; Gazzinelli *et al.* 1991;1992; Milovanovic *et al.* 2009) but also by increasing levels of cholesterol and LDL in latently infected humans and in mice in postacute phase of the infection. This can explain the observed positive correlation between prevalence of toxoplasmosis and incidence of cardiovascular diseases in particular European countries (Flegr 2013a).

Though insignificantly, *Toxoplasma*-infected subjects had higher cortisol levels, with cortisol being known to act as an immunosuppressive hormone. Concentration of cortisol is known to decrease after victory in a competition events (Salvador 2005). However, in some situations, an opposite shift in concentration of cortisol after the victory, or for example, after success in written university examination was observed (Flegr *et al.* 2010; Kirschbaum *et al.* 1995; Suay *et al.* 1999; Wirth *et al.* 2006). Generally, an increased level of cortisol is associated with various types of acute and especially chronic stress, including physical and mental stress (Fukuda *et al.* 2001a,b; Russell *et al.* 2012; Staufenbiel *et al.* 2013). Indirect evidence suggest that, in fact, so called "asymptomatic" latent toxoplasmosis represents a mild but long term stressor (Lindová *et al.* 2006; 2010). It can be speculated that the latent toxoplasmosis-associated chronic stress accompanied with increased level of an immunosuppressor cortisol could be the proximal cause of impaired immunoreactivity of *Toxoplasma*-infected subjects. Of course, it is also possible that observed toxoplasmosis-associated immunosuppression represents the result of adaptive manipulative activity of the parasite, aimed to increase its chance for survival in a host organism.

The most striking finding, in fact accidental, was the higher prevalence of toxoplasmosis in schizophrenic men than in women, irrespectively to age and even to presence of anti-*Toxoplasma* antibodies (IgG) in serum, in contrast to common population. This may be influenced by the fact that samples from all the patients were evaluated, irrespective to the severity of their schizophrenia disease and associated willingness to participate in basic research. It has been already suggested that absence of differences in prevalence of toxoplasmosis between schizophrenia patients and general population observed in certain recent studies performed in developed countries (Hinze-Selch *et al.* 2007; Horacek *et al.* 2012) is the result of increased concerns about the rights of patients (Flegr 2013b). In the past, all patients of a particular hospital were automatically included in the study. Currently, only the patients who are able and willing to sign the informed consent document participate in the studies. It is known that *Toxoplasma*-infected men are more suspecting (Flegr *et al.* 1994; 1996) and both men and women are less cooperative and conscientious than their *Toxoplasma*-free peers (Lindová *et al.* 2010; 2012). Also, *Toxoplasma*-infected schizophrenia patients express more severe symptoms of psychosis than *Toxoplasma*-free patients (Amminger *et al.* 2007; Holub *et al.* 2013; Wang *et al.* 2006; Yolken *et al.* 2009). Higher suspiciousness and lower cooperativeness and conscientiousness of *Toxoplasma*-infected subjects as well as their more severe positive symptoms of schizophrenia increase the probability of rejecting the participation in research study.

Limitation of the study. The major limitation of the presented study is the absence of control populations of *Toxoplasma*-free and *Toxoplasma*-infected subjects without any psychiatric diseases. Without such data we cannot decide whether observed differences between *Toxoplasma*-free and *Toxoplasma*-infected subjects can be observed only in schizophrenia patients or whether they can be detected also in a general population. It is therefore highly desirable to repeat this study also with non-psychiatric subjects matched for age and gender with our schizophrenia patients.

Our study (accidently) provided possible explanation for seemingly decreasing prevalence of toxoplasmosis in schizophrenia patients observed in current

studies. It also suggests that latent toxoplasmosis could play a role in the development of atherosclerosis, which offered a possible explanation for reported positive correlation between prevalence of toxoplasmosis and incidence of cardiovascular diseases.

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