Original Article

More Than an Association: Latent Toxoplasmosis Might Provoke a Local Oxidative Stress That Triggers the Development of Bipolar Disorder

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Abstract

Introduction: *Toxoplasma gondii*, a common parasitic infection, has a special affinity to the brain. It has a lifelong existence without an apparent clinical disease. While the etiology of bipolar disorder (BD) remains unclear, epidemiological studies suggest a role for infections. Central nervous system is particularly susceptible to oxidative stress (OS) because of its high metabolic rate and its low levels of antioxidant defenses. OS is a contributor to the initiation and progression of many neurological illnesses. OS injury is a constantly and compelling finding associated with BD and toxoplasmosis. **Aim:** This cross-sectional study has investigated a possible role of toxoplasma-induced OS in the development of BD. **Methods:** Healthy controls and BD patients were examined for anti-*Toxoplasma* immunoglobulin-G (IgG) and two protein (3-nitrotyrosine) and DNA (8-hydroxy-2' deoxyguanosine [8-OHdG]) OS markers. **Results:** *Toxoplasma* positivity was higher (40%) among BD patients compared to controls (12%). Significantly higher levels of anti-*Toxoplasma* IgG were detected in BD patients compared to controls. Nitrotyrosine (796.7 ± 106.28) and especially 8-OHdG (20.31 ± 8.38) were significantly higher among toxo-positive BD compared to toxo-negative BD (675.97 ± 144.19 and 7.44 ± 2.86) and healthy controls (464.02 ± 134.6 and 4.17 ± 1.43). **Conclusion:** These findings might indicate a role for *Toxoplasma* infection in the development of BD, possibly through creating a highly oxidative brain environment.

Keywords: 3-nitrotyrosine, 8-hydroxy-2'-deoxyguanosine, bipolar disorder, oxidative stress, *Toxoplasma*

Introduction

Bipolar disorder (BD) is a chronic mood disorder and a main cause of disability among young patients. Individuals with BD experience disruptive episodes of mania or hypomania and depression.[1] It has a worldwide prevalence of approximately 1%–2% among adults and leads to cognitive and functional impairment. The course of BD often has remissions, but recurrence usually happens.[2]

Toxoplasma gondii is a zoonotic intracellular protozoan parasite that infects over 30% of the human population. The parasite has a special predilection to the brain and survives mainly in neuronal tissue for a unique lifelong chronic phase of the disease.[3]

Like other psychiatric disorders, there is no diagnostic laboratory biomarker for BD. Diagnosis is solely made on clinical basis and directed interview with the patient and his/her relatives. Integration of clinical data with laboratory biomarkers and neuro-imaging findings is crucial to properly diagnose and monitor this disorder.[4,5]

Very little is known about the specific biological mechanisms underlying BD; however, research concerning the pathophysiology of BD has gained momentum over the past few decades.[6] Although this disorder exhibits a strong evidence of genetic influence, a multifactorial hypothesis is thought to better explain BD etiology where an interaction

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between environmental factors and genetic background is suggested.[7] Better understanding of the pathophysiological processes underlying BD, hopefully, might lead to objective biomarkers that could aid prognostic accuracy as well as early diagnosis.[8]

Many studies have demonstrated a correlation between *T. gondii* seropositivity and personality changes,[9,10] as well as various psychiatric disorders including schizophrenia[11] and depression.[12] Such a relationship has also been reported for other neuropsychiatric diseases such as epilepsy, Alzheimer's disease, and Parkinson's disease.[9,13] However, the pathophysiologic mechanisms underlying such changes are still not fully understood.[14]

Reactive oxygen species (ROS) are constantly generated in cells as part of the physiological and metabolic processes. Under physiological conditions, a balance, involving reactive oxidants and various antioxidant defenses, is sustained.[15] Due to their reactive nature, these endogenously produced ROS induce changes to cell membrane lipids, nucleic acids, and proteins, particularly in mitochondria, impairing mitochondrial energy production.[16] Impaired energy and high ROS combined or individually can cause cell dysfunction and result in cell death, either by necrosis or by apoptosis. Symptoms of organ dysfunction appear once enough cells became either dysfunctional or dead.[17] Damage to macromolecules, membrane lipid-polyunsaturated fatty acids, DNA/RNA as well as proteins was reported to occur in BD by ROS and nitrogen species.[18-20] This oxidative overexpression could explain the hastened aging, premature mortality, and cognitive impairment encountered in BD.[21]

Toxoplasma-mediated oxidative stress (OS) is suggested to take part in the mechanism of neuropathology and neurodegeneration.[22] A pro-inflammatory immune reaction, dominated by Th1 and Th17 cytokines, particularly interferon (IFN-γ) and TNF-α, is triggered by *Toxoplasma* infection.[23] IFN-γ is the main and crucial cytokine for defense against *T. gondii*, particularly in central nervous system (CNS). However, a pathological arm of this defense mechanism does exist. IFN-γ-activated microglia produce toxic reactants, mainly nitric oxide (NO), that induce brain tissue injury and neuronal inflammatory pathologies. *Toxoplasma*-induced IFN-γ also triggers high levels of NO as a mechanism for arginine degradation.[24]

8-hydroxy-2-deoxyguanosine is a dominant form of free oxidative lesions of nuclear and mitochondrial DNA. It is a critical biomarker for measuring the effect of endogenous oxidative DNA damage and as a risk factor for many diseases including cancer.[25] Nitrotyrosine is generated through nitration of tyrosine residues in different proteins. This oxidative modifications of proteins can affect some of their functions such as enzymatic activity, DNA binding, and susceptibility to degradation.[26] 3-nitrotyrosine (3-NT) has been utilized as a pivotal biomarker for *in vivo* oxidative insult caused by ONOO−.[27] In the current study, both markers (8-hydroxy-2'-deoxyguanosine [8-OHdG] and 3-NT) were used to assess a possible role for *Toxoplasma*-induced OS in the pathogenesis of BD.

It is unlikely that a sole mechanism, utilized by *T. gondii* during infection, would justify all neuropsychiatric changes induced by toxoplasmosis and known to exist in BD. However, we suggest that oxidative and nitrosative stress, induced by *Toxoplasma* infection, has a major role in the development of BD, either directly or through mediating other pathways. If this suggestion stands, markers of OS those dominate, but at different levels, throughout the different phases of *Toxoplasma* infection could be utilized as diagnostic and/or prognostic indicators of BD. This study was conducted to investigate a possible role for *Toxoplasma*-induced OS in the development of BD.

Materials and Methods

Subjects

The study participants (n = 40) were recruited from inpatient wards and outpatient clinics of psychiatric departments of some general hospitals and psychiatric diseases/mental health hospitals in Makkah Region, Saudi Arabia. Patients were diagnosed as having BD according to the diagnostic criteria of the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders.[28] Patients had no other substantial medical conditions or taking medications other than those related to BD. Some rejection criteria were applied, mainly the absence of the complete data set of the patient as designed in the psychological evaluation report and the presence of current or past history of substance abuse or mental deficiencies or neurological disorders that could have an influence on cognitive performance such as epilepsy, mental retardation, head trauma, or history of encephalitis. Demographically matched healthy volunteers (n = 25) were selected from the same communities of the participating BD patients. For a volunteer to be included in the control group, he/she should be an apparently healthy person with no documented or suggestive present, past, or family history of psychiatric disorders and without current or past history of substance abuse. Controls were nonsmokers and were not taking medication. All participants were informed orally and in writing. All participants gave written informed consent.

The BD group (forty patients) was subdivided, according to anti-*Toxoplasma* immunoglobulin-G (IgG) positivity, into two subgroups: *Toxoplasm*a-positive BD group (16 patients) and *Toxoplasma*-negative BD group (24 patients).

Samples and assays

Sera extracted from the blood samples (3–5 ml) collected from all patients and controls, after a written informed consent, were subjected to analyses for anti-*Toxoplasma* IgG, 3-NT, and 8-OHdG.

Immunoglobulin-G enzyme-linked immunosorbent assay

Sera of all participants (patients and controls) were analyzed for specific anti-*Toxoplasma* IgG antibodies with a commercially available enzyme-linked immunosorbent assay (ELISA)

kit ("Toxoplasma IgG" NovaTec Immundiagnostics GmbH, Dietzenbach, Germany). The procedure was done following the manufacturer's instructions. Absorbance values of all control and test samples were converted to IgG concentration units (IU/ml) according to an absorbance versus concentration (standard calibration) curve. Positivity was considered for values >35 IU/ml.

Human 3-nitrotyrosine and 8-hydroxy-2'-deoxyguanosine by enzyme-linked immunosorbent assay

To assess protein oxidation, levels of 3-NT were determined, in sera of the study participants (patients and controls), with a commercially available ELISA kit (Uscan Life Science Inc., Wuhan, USA), for human nitrotyrosine, following the manufacturer's instructions. Participants' serum concentrations of 3-NT were expressed as pg/ml. Serum values of 8-OHdG were determined, as a biomarker for DNA oxidation products, using a commercially available 8-OHdG ELISA kit" (JaICA, NIKKEN SEIL co., Japan) conforming to the manufacturer's instructions. Serum concentrations of 8-OHdG for all participants were expressed in ng/ml.

Statistical analysis

The analyses for this study were performed using IBM SPSS Statistics for Windows, Version 23.0. (Armonk, NY: IBM Corp., USA). Descriptive analyses for demographic characteristics and laboratory values were expressed as means and standard deviations. Demographic and clinical characteristics were assessed using Chi-square test and one-way analysis of variance, with a significance level of $P < 0.05$. As laboratory results (anti-toxo IgG, 3-NT, and 8-OHdG) showed a nonparametric distribution, among different groups, they were analyzed using Student's *t*-test and Kruskal–Wallis test as indicated.

Results

The participants' characteristics, related to age and sex, are summarized in Table 1. A slightly greater, yet insignificant, prevalence of female over male patients was found in BD patients, especially those who are *Toxoplasma* positive. There was no significant difference on the mean age of the different groups.

The prevalence of significant anti-*Toxoplasma* IgG, indicative of *Toxoplasma* positivity, was higher (40%) among BD patients compared to controls (12%). Numerical values of IgG were significantly higher ($P < 0.001$) in patients with BD compared to normal controls [Table 1 and Figure 1].

Protein oxidative nitrosylation, as measured by 3-NT, was increased significantly [Table 1] in the bipolar group compared with controls, especially in *Toxoplasma*-positive patients. While 3-NT levels were still significantly higher ($P < 0.01$) in toxo-positive BD patients compared to toxo-negative ones [Figure 2], however, it was not as striking as in the case of 8-OHdG ($P < 0.005$) [Figure 3].

Levels of 8-OHdG, as a measure for DNA oxidation, were significantly higher [Table 1] in patients with BD, especially those positive for toxoplasmosis ($P < 0.01$ and $P < 0.0001$, respectively) compared to controls. We observed also an almost 3-fold increase in the levels of 8-OHdG in BD cases that are toxo positive compared to toxo negative ones ($P < 0.005$) [Figure 3].

Discussion

As far as we know, this study is the first to explore, not only the association between *Toxoplasma* infection and BD, but also to establish a causal link for the parasite in the pathogenesis of such illness.

In contrast to other psychiatric disorders, like schizophrenia, the relationship between *Toxoplasma* infection and BD is less recorded and more ambiguous. While some studies[29-31] positively correlate toxoplasmosis and BD, a considerable number of reports[32,33] deny such relationship.

There is a considerable overlap between neurobiological and biochemical characteristics of BD and the changes inflicted by *Toxoplasma* infection in the local brain environment. *Toxoplasma* infection might represent a main environmental

Table 1: Demographic and laboratory parameters in the different study groups

	Controls ($n=25$)	BD ($n=40$)	*Toxoplasma*-negative BD ($n=24$)	*Toxoplasma*-positive BD ($n=16$)	*P*
Gender (%)					
Male	14 (56)	19 (47.5)	12 (50)	7 (43.75)	0.65*
Age (years)	24.4±4.6	32.6±8.2	26.8±9.4	41.3±7.2	0.72**
Anti-*toxoplasma* IgG (IU/ml)	7.95±3.76	85.96±74.96	12.95±3.28	195.49±86.77	[a] versus [b] (>0.05) [a] versus [c] (<0.0001) [b] versus [c] (<0.0001)
NT (pg/ml)	464.02±134.6	724.26±143.2	675.97±144.19	796.7±106.28	[a] versus [b] (<0.01) [a] versus [c] (<0.0001) [b] versus [c] (<0.01)
8-OHdG levels (ng/ml)	4.17±1.43	12.21±8.79	7.44±2.86	20.31±8.38	[a] versus [b] (<0.01) [a] versus [c] (<0.0001) [b] versus [c] (<0.005)

*Chi-square test, **Student's *t*-test. BD: Bipolar disorder, IgG: Immunoglobulin-G, NT: Nitrotyrosine, 8-OHdG: 8-hydroxy-2'-deoxyguanosine

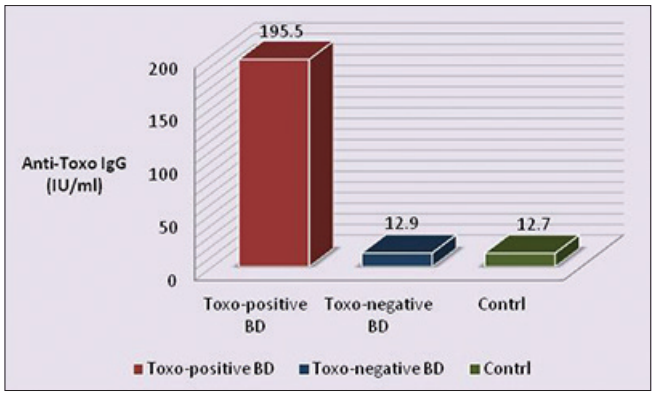

Figure 1: Anti-Toxo IgG Changes observed in relation to BD

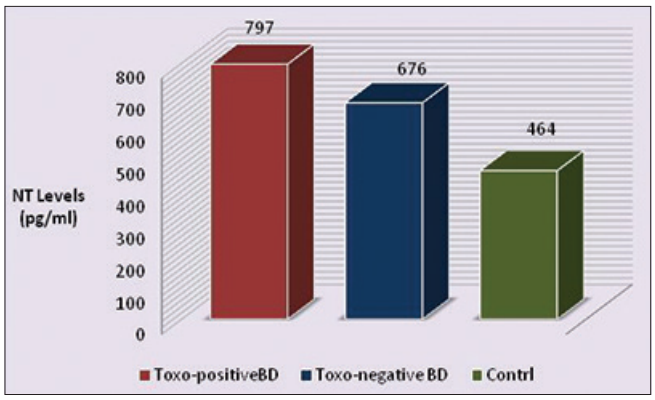

Figure 2: Changes observed in NT levels in relation to BD and toxoplasmosis

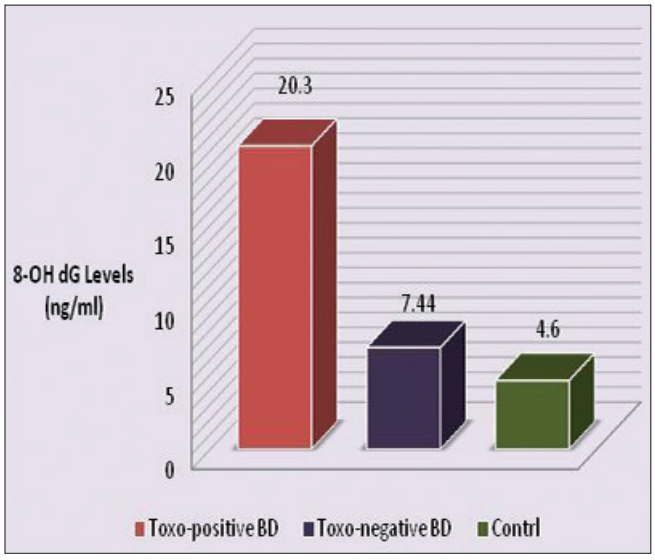

Figure 3: Changes observed in 8-OH dG levels in relation to BD and toxoplasmosis

insult that mediates a possible interaction between genetic vulnerability, neurotransmission, and modulation of the immune system in BD. *Toxoplasma* infection creates a host's immunological environment with escalating OS marked by overproduction of toxic free radicals such as ROS and NO.[34] Several studies have indicated a crucial share of lipid, protein, and DNA peroxidation in the pathophysiology of BD.[35,36] Therefore, the current study investigated the role of protein and DNA peroxidation biomarkers in patients with BD, especially those infected with *T. gondii*.

The results of this study showed that *Toxoplasma* infection has a significant link to BD which could be through the OS induced by the parasite, which was also demonstrated in our results. This interesting triology of interplaying *T. gondii*, OS, and BD, revealed in this study, could be explained on the basis of mitochondrial dysfunction, which is one of the most accepted hypotheses for the development of BD.[37] Dysfunctional mitochondria produce ATP less efficiently, but more competent in producing ROS. Oxidative overexpression and apoptosis due to mitochondrial dysfunction play a role in the pathophysiology of common neurodegenerative disorders such as Parkinsonism[38] and Alzheimer's disease.[39] Recently, a mitochondrial dysfunction-based model has been hypothesized[40] to interpret the phenomenal biphasic energy dysregulation in BD mania and depression phases. A significant decline in the activity of antioxidant defense system has been demonstrated in *Toxoplasma*-seropositive patients.[41] Some studies have shown that inhibitors of mitochondrial function and inducers of OS can induce *Toxoplasma* encystment *in vitro*.[42]

This study demonstrated a high percentage of BD cases that are seronegative for toxoplasmosis. This does not invalidate the hypothesis of the study, as it was not expected that *T. gondii* will be the sole infectious agent responsible for all BD cases. We believe that infectious agents, other than Toxoplasma, could precipitate BD if they have the same brain predilection, and can trigger the same OS pathways. Cytomegalovirus (CMV), for example, shares some of the *Toxoplasma* infectious patterns that could make it a possible candidate to trigger BD. CMV is a common infection and even more common than *T. gondii*;[43] has a lifelong existence within the host, with a special affinity to nervous tissues; and its congenital infection might lead to severe CNS consequences.[44] Similarly, most immunocompetent individuals who acquire CMV as children or adults show no signs of illness or have mild symptoms, while immunocompromised ones will experience the most severe forms of nervous system involvement.[45] Sero-negative pregnant women are at a high risk of *in utero* transmission of CMV infection if primarily infected with the virus during pregnancy,[46] a phenomenon that is well documented in toxoplasmosis.[3] Latent infection with CMV was reported to have a significant association with overexpressed OS.[47] Weis *et al*.[48] reported an upregulated OS caused by CMV in endothelial cells, playing a part in transplant arteriosclerosis. Therefore, *Toxoplasma*-negative BD patients could be latently infected with other infectious agents that have similar criteria of pathogenesis as *T. gondii*, an assumption that was not investigated in the current study.

One of the limitations of the current study is that individual groups have a small sample size, restricting the adjustment for confounding variables other than age, gender, and *Toxoplasma*

infection. This consequently hindered the use of such markers for staging BD. The cross-sectional design of the study disabled us from concluding meaningful information about the course of the illness. While the results of the current study should be deduced within the context of such limitations, they create the basis for new prospective large-scale studies in the same direction.

Conclusion

These results provide an evidence for the involvement of oxidative DNA and proteins damage in BD. They also indicate a role for *Toxoplasma*-induced OS in triggering such illness. We speculate that other infectious agents that have the same predilection to brain tissue like *T. gondii* and could utilize its OS-triggering pathways, could have a role in the development of BD. These findings, if further consolidated by large-scale longitudinal studies, will validate OS biomarkers as reliable diagnostic and/or prognostic indicators that have significant clinical utility.

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Conflicts of interest

There are no conflicts of interest.

References

1. Anderson IM, Haddad PM, Scott J. Bipolar disorder. BMJ 2012;345:e8508.
2. Grande I, Berk M, Birmaher B, Vieta E. Bipolar disorder. Lancet 2016;387:1561-72.
3. Halonen SK, Weiss LM. Toxoplasmosis. In: Garcia HH, Tanowitz HB, Del Brutto OH, editors. Handbook of Clinical Neurology. Neuroparasitology and Tropical Neurology. 3rd ser., Vol. 114. Cambridge, MA, USA: Elsevier BV; 2013.
4. Keener MT, Phillips ML. Neuroimaging in bipolar disorder: A critical review of current findings. Curr Psychiatry Rep 2007;9:512-20.
5. Canales-Rodríguez EJ, Pomarol-Clotet E, Radua J, Sarró S, Alonso-Lana S, Del Mar Bonnín C, *et al.* Structural abnormalities in bipolar euthymia: A multicontrast molecular diffusion imaging study. Biol Psychiatry 2014;76:239-48.
6. Fears SC, Reus VI. Bipolar disorder. In: Rosenberg R, Pascual J, editors. Rosenberg's Molecular and Genetic Basis of Neurologic and Psychiatric Disease. 5th ed, Academic Press, Cambridge, Massachusetts, USA. 2015.
7. Craddock N, Sklar P. Genetics of bipolar disorder. Lancet 2013;381:1654-62.
8. Frey BN, Andreazza AC, Houenou J, Jamain S, Goldstein BI, Frye MA, *et al.* Biomarkers in bipolar disorder: A positional paper from the International Society for Bipolar Disorders Biomarkers Task Force. Aust N Z J Psychiatry 2013;47:321-32.
9. Fekadu A, Shibre T, Cleare AJ. Toxoplasmosis as a cause for behaviour disorders – overview of evidence and mechanisms. Folia Parasitol (Praha) 2010;57:105-13.
10. Hinze-Selch D, Däubener W, Erdag S, Wilms S. The diagnosis of a personality disorder increases the likelihood for seropositivity to *Toxoplasma gondii* in psychiatric patients. Folia Parasitol (Praha) 2010;57:129-35.
11. Brown AS, Schaefer CA, Quesenberry CP Jr., Liu L, Babulas VP, Susser ES, *et al.* Maternal exposure to toxoplasmosis and risk of schizophrenia in adult offspring. Am J Psychiatry 2005;162:767-73.
12. Kar N, Misra B. Toxoplasma seropositivity and depression: A case report. BMC Psychiatry 2004;4:1.
13. Hurley RA, Taber KH. Latent toxoplasmosis gondii: Emerging evidence for influences on neuropsychiatric disorders. J Neuropsychiatry Clin Neurosci 2012;24:376-83.
14. Parlog A, Schlüter D, Dunay IR. *Toxoplasma gondii*-induced neuronal alterations. Parasite Immunol 2015;37:159-70.
15. Halliwell B, Gutteridge JM. Free Radical in Biology and Medicine. 3rd ed. Oxford: Oxford University Press; 1999.
16. Kehrer JP. Free radicals as mediators of tissue injury and disease. Crit Rev Toxicol 1993;23:21-48.
17. Myhill S, Booth NE, McLaren-Howard J. Chronic fatigue syndrome and mitochondrial dysfunction. Int J Clin Exp Med 2009;2:1-6.
18. Selek S, Savas HA, Gergerlioglu HS, Bulbul F, Uz E, Yumru M, *et al.* The course of nitric oxide and superoxide dismutase during treatment of bipolar depressive episode. J Affect Disord 2008;107:89-94.
19. Andreazza AC, Frey BN, Erdtmann B, Salvador M, Rombaldi F, Santin A, *et al.* DNA damage in bipolar disorder. Psychiatry Res 2007;153:27-32.
20. Wang JF, Shao L, Sun X, Young LT. Increased oxidative stress in the anterior cingulate cortex of subjects with bipolar disorder and schizophrenia. Bipolar Disord 2009;11:523-9.
21. Kapczinski F, Dal-Pizzol F, Teixeira AL, Magalhaes PV, Kauer-Sant'Anna M, Klamt F, *et al.* Peripheral biomarkers and illness activity in bipolar disorder. J Psychiatr Res 2011;45:156-61.
22. Dincel GC, Atmaca HT. Role of oxidative stress in the pathophysiology of *Toxoplasma gondii* infection. Int J Immunopathol Pharmacol 2016;29:226-40.
23. Mordue DG, Monroy F, La Regina M, Dinarello CA, Sibley LD. Acute toxoplasmosis leads to lethal overproduction of Th1 cytokines. J Immunol 2001;167:4574-84.
24. Nishikawa Y, Kawase O, Vielemeyer O, Suzuki H, Joiner KA, Xuan X, *et al. Toxoplasma gondii* infection induces apoptosis in noninfected macrophages: Role of nitric oxide and other soluble factors. Parasite Immunol 2007;29:375-85.
25. Valavanidis A, Vlachogianni T, Fiotakis C. 8-hydroxy-2' -deoxyguanosine (8-OHdG): A critical biomarker of oxidative stress and carcinogenesis. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 2009;27:120-39.
26. Andreazza AC, Shao L, Wang JF, Young LT. Mitochondrial complex I activity and oxidative damage to mitochondrial proteins in the prefrontal cortex of patients with bipolar disorder. Arch Gen Psychiatry 2010;67:360-8.
27. Naoi M, Maruyama W, Shamoto-Nagai M, Yi H, Akao Y, Tanaka M, *et al.* Oxidative stress in mitochondria: Decision to survival and death of neurons in neurodegenerative disorders. Mol Neurobiol 2005;31:81-93.
28. Angst J. Bipolar disorders in DSM-5: Strengths, problems and perspectives. Int J Bipolar Disord 2013;1:12.
29. Tedla Y, Shibre T, Ali O, Tadele G, Woldeamanuel Y, Asrat D, *et al.* Serum antibodies to *Toxoplasma gondii* and herpesvidae family viruses in individuals with schizophrenia and bipolar disorder: A case-control study. Ethiop Med J 2011;49:211-20.
30. Sutterland AL, Fond G, Kuin A, Koeter MW, Lutter R, van Gool T, *et al.* Beyond the association. *Toxoplasma gondii* in schizophrenia, bipolar disorder, and addiction: Systematic review and meta-analysis. Acta Psychiatr Scand 2015;132:161-79.
31. Hamdani N, Daban-Huard C, Lajnef M, Richard JR, Delavest M, Godin O, *et al.* Relationship between *Toxoplasma gondii* infection and bipolar disorder in a French sample. J Affect Disord 2013;148:444-8.
32. Mortensen PB, Pedersen CB, McGrath JJ, Hougaard DM, Nørgaard-Petersen B, Mors O, *et al.* Neonatal antibodies to infectious agents and risk of bipolar disorder: A population-based case-control study. Bipolar Disord 2011;13:624-9.
33. Mortensen PB, Nørgaard-Pedersen B, Waltoft BL, Sørensen TL, Hougaard D, Yolken RH, *et al.* Early infections of *Toxoplasma gondii* and the later development of schizophrenia. Schizophr Bull 2007;33:741-4.
34. Mun HS, Aosai F, Chen M, Piao LX, Norose K, Iwakura Y, *et al.* Pathogenicity of *Toxoplasma gondii* through B-2 cell-mediated downregulation of host defense responses. Microbiol Immunol 2003;47:533-42.
35. Andreazza AC, Gildengers A, Rajji TK, Zuzarte PM, Mulsant BH, Young LT, *et al.* Oxidative stress in older patients with bipolar disorder.

Am J Geriatr Psychiatry 2015;23:314-9.
36. Jacoby AS, Vinberg M, Poulsen HE, Kessing LV, Munkholm K. Increased DNA and RNA damage by oxidation in patients with bipolar I disorder. Transl Psychiatry 2016;6:e867.
37. Scaini G, Rezin GT, Carvalho AF, Streck EL, Berk M, Quevedo J, *et al.* Mitochondrial dysfunction in bipolar disorder: Evidence, pathophysiology and translational implications. Neurosci Biobehav Rev 2016;68:694-713.
38. Jenner P. Parkinson's disease, pesticides and mitochondrial dysfunction. Trends Neurosci 2001;24:245-7.
39. Aliev G, Seyidova D, Lamb BT, Obrenovich ME, Siedlak SL, Vinters HV, *et al.* Mitochondria and vascular lesions as a central target for the development of Alzheimer's disease and Alzheimer disease-like pathology in transgenic mice. Neurol Res 2003;25:665-74.
40. Morris G, Walder K, McGee SL, Dean OM, Tye SJ, Maes M, *et al.* A model of the mitochondrial basis of bipolar disorder. Neurosci Biobehav Rev 2017;74:1-20.
41. Karaman U, Celik T, Kiran TR, Colak C, Daldal NU. Malondialdehyde, glutathione, and nitric oxide levels in *Toxoplasma gondii* seropositive patients. Korean J Parasitol 2008;46:293-5.
42. Soête M, Camus D, Dubremetz JF. Experimental induction of bradyzoite-specific antigen expression and cyst formation by the RH strain of *Toxoplasma gondii in vitro*. Exp Parasitol 1994;78:361-70.
43. Staras SA, Dollard SC, Radford KW, Flanders WD, Pass RF, Cannon MJ, *et al.* Seroprevalence of cytomegalovirus infection in the United States, 1988-1994. Clin Infect Dis 2006;43:1143-51.
44. Kylat RI, Kelly EN, Ford-Jones EL. Clinical findings and adverse outcome in neonates with symptomatic congenital cytomegalovirus (SCCMV) infection. Eur J Pediatr 2006;165:773-8.
45. Nelson CT, Demmler GJ. Cytomegalovirus infection in the pregnant mother, fetus, and newborn infant. Clin Perinatol 1997;24:151-60.
46. Cheeran MC, Lokensgard JR, Schleiss MR. Neuropathogenesis of congenital cytomegalovirus infection: Disease mechanisms and prospects for intervention. Clin Microbiol Rev 2009;22:99-126.
47. Lee YL, Liu CE, Cho WL, Kuo CL, Cheng WL, Huang CS, *et al.* Presence of cytomegalovirus DNA in leucocytes is associated with increased oxidative stress and subclinical atherosclerosis in healthy adults. Biomarkers 2014;19:109-13.
48. Weis M, Kledal TN, Lin KY, Panchal SN, Gao SZ, Valantine HA, *et al.* Cytomegalovirus infection impairs the nitric oxide synthase pathway: Role of asymmetric dimethylarginine in transplant arteriosclerosis. Circulation 2004;109:500-5.