Insights into the interplay of latent toxoplasmosis, testosterone, cortisol and oxidative stress in screened schizophrenic patients in Egypt

Original Article

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ABSTRACT

Background: *Toxoplasma gondii* is a wide-spread infection. Most cases are asymptomatic latent toxoplasmosis and its link with schizophrenia is of wide interest. Toxoplasmosis induces immunopathological and neuroendocrine alterations that induce activation of hypothalamic pituitary-adrenal axis resulting in higher glucocorticoid and testosterone levels. Oxidative stress also has a role since antioxidants as glutathione (GSH) were found to be reduced.
Objective: To assess seroprevalence/serointensity of toxoplasmosis in schizophrenic patients in relation to the levels of testosterone, cortisol and GSH activity in order to evaluate these factors in the etiopathogenesis of schizophrenic patients harboring latent toxoplasmosis.
Subjects and Methods: In this cross sectional case control study, 120 schizophrenic inpatients were compared with 120 individuals attending the outpatients' clinics in the same age range, and after exclusion of schizophrenia. Schizophrenia was confirmed or excluded using positive and negative syndrome scale (PANSS). Levels of *Toxoplasma* IgG, free testosterone, cortisol and GSH were determined in both groups.
Results: Statistical analysis revealed no differences between cases and controls as regard age, gender, contact with pets, and *Toxoplasma* IgG seroprevalence. *Toxoplasma* IgG seroprevalence was higher in males compared to females and in rural areas than in urban areas. In *Toxoplasma*-positive patients, testosterone was higher in both genders and glutathione was lower, while no significant difference was documented in relation to PANSS, treatment with electroconvulsive-therapy (ECT) or cortisol level. Schizophrenic patients showed higher *Toxoplasma* antibody titer, cortisol, free testosterone levels in both genders and lower GSH than control. Male schizophrenic patients particularly showed lower GSH level and higher *Toxoplasma* antibody titer. Free testosterone level was significantly higher in male patients who had received ECT.
Conclusion: Latent toxoplasmosis may be involved in the etiology of schizophrenia. Screening and treatment of toxoplasmosis is advisable to prevent schizophrenia. Also, screening of schizophrenic patients for chronic toxoplasmosis and the fore mentioned parameters is recommended for prediction of complications and presents a promising approach for prevention and management.

Keywords: cortisol, glutathione, latent toxoplasmosis, schizophrenia, testosterone.

INTRODUCTION

Toxoplasma gondii, an obligate intracellular and opportunistic parasite, is one of the most widely spread infections all over the globe, affecting about 30% of the world population[1]. Unlike many other parasitic disorders, its distribution is not merely restricted to developing countries, but it also afflicts developed nations such as France, which is known for having a high incidence of toxoplasmosis[2]. The wide range of this infection is attributed to the multiplicity of its modes of infection. Besides, being a zoonosis, *T. gondii* oocysts shed by the feline definitive host can be acquired by the mammalian intermediate host through the ingestion of contaminated food and water. Also, improperly cooked meat containing tissue cysts constitute a potential source of infection. Additional modes of infection include organ transplantation, blood transfusion and congenital transmission from mother to fetus[3].

The clinical range includes acute toxoplasmosis presenting with a variety of nonspecific symptoms such as sore throat and lymphadenitis, opportunistic

toxoplasmosis in the immunocompromised and congenital toxoplasmosis especially known for its ocular and neurological manifestations[4]. Even though infection is common, clinical disease is manifested to a much lesser extent, where most cases are asymptomatic, a condition called latent toxoplasmosis[5]. Although patients that are harboring latent toxoplasmosis are asymptomatic, it was found to be incriminated in the development of mental retardation in children[6]. In addition to the mentioned presentations of toxoplasmosis, recent suggestions implicate *T. gondii* in other disorders of undefined etiology. Of special interest is its reported link to a group of neuropsychiatric disorders such as autism, bipolar disorder, schizophrenia and degenerative diseases such as Alzheimer's and Parkinson's diseases[7]. Association with these clinical conditions was motivated by the multiple effects of *T. gondii* on host physiology. Besides, the resulting immunopathological changes, *T. gondii* is also known to induce neuroendocrine alterations[8,9].

Most patients with schizophrenia suffer from a lifetime of disability and about 10% commit suicide[10]. The etiology of schizophrenia is thought to be due to several factors. In family studies, genetic factors were found to play a crucial role[11], whereas epidemiological researches indicated that environmental exposures, such as winter-spring births, peri- and postnatal infections and urban births are risk factors for the development of schizophrenia later in life[12]. Infectious agents, including *T. gondii*, were considered relevant in triggering schizophrenia[13]. Toxoplasmosis has been linked to schizophrenia through epidemiological data, serological evidence, neuropathological and molecular studies[14]. The causal relationship was speculated to involve susceptibility genes targeting, pro-inflammatory cytokines, neurotransmitters and hormones[15]. It was explained that *T. gondii* encysted in the host tissues constitutes a source of chronic infection, which leads to the activation of hypothalamic pituitary adrenal axis and subsequently, an increase in glucocorticoid concentration. This increase, in turn, is linked to neurosynaptic degeneration[9].

Another hormonal change that may explain the link between toxoplasmosis and schizophrenia is the elevation of serum testosterone, which was reported in several studies in *Toxoplasma* seropositive patients[5,16,17]. Alterations of serum testosterone levels have also been found to affect the severity of clinical disease in schizophrenic patients[18]. In addition to neuroendocrine alterations, oxidative stress is one of the mechanisms of pathology that is shared by both schizophrenia and toxoplasmosis. Oxidative stress can be assessed by several markers including malonedialdehyde (MDA), which reflects the degree of lipid peroxidation, and antioxidants such as GSH. MDA was found to be increased in *Toxoplasma* seropositive patients, while GSH was found to be reduced[19]. In parallel, psychotic patients suffering from schizophrenia

and bipolar disorder were shown to have a reduced serum level of glutathione[20].

To our knowledge, implication of the previously mentioned factors in the pathogenesis of toxoplasmosis and schizophrenia have been studied separately. The causative relation between endocrine changes and oxidative status and these two diseases remain under-investigated. The current study aims at assessing the seroprevalence/serointensity of toxoplasmosis in schizophrenic patients in relation to the levels of testosterone, cortisol and GSH activity in order to evaluate these factors in schizophrenic patients harboring latent toxoplasmosis.

SUBJECTS AND METHODS

This is a cross sectional case control study performed during the period from January 2018 to November 20 at Kasr-Al-Ainy Psychiatry and Addiction Hospital and Medical Parasitology Department, Faculty of Medicine, Cairo University, Cairo.

Subjects: A total number of 240 individuals divided into two equal groups were included in the study. Group 1 included inpatients (case group) ranging in age from 16 to 55 years; while group 2 included individuals attending the outpatients' clinics in the same age range, and after exclusion of schizophrenia. A data collection sheet covering sociodemographic and clinical information was fulfilled before serological testing. Non-cooperative patients, patients with other severe co-morbid disease or history of treatment of toxoplasmosis before sampling were excluded.

Study design: For psychiatric assessment of case group, diagnosis was confirmed by a structured clinical interview using the statistical manual of mental disorders (DSM) IV axis I disorder (SCID-I)[21]. Psychotic symptom severity was assessed according to PANSS. A general psychopathology scale was also performed. The validity and reliability of the total syndrome (TS) scale (the sum of positive, negative and general scales) was established[22]. ECT was included as a severity marker since it is only indicated for severe cases. Blood samples were collected from both groups for evaluation of the seroprevalence of anti-*Toxoplasma* IgG, and determination of levels of free testosterone, cortisol and GSH.

Specimen collection and preparation: Three ml venous blood samples obtained by venipuncture were centrifuged at 3000 rpm for 5 min[23] at the Department of Parasitology, Kasr-Al-Ainy School of Medicine and separated sera were frozen until use. Repeated freezing and thawing or heat inactivation were avoided. Insufficient samples or those with incomplete information in the data collection sheet, with hemolysis, turbid or contaminated serum samples, were excluded.

Parameters: ELISA was performed according to the manufacturer's instructions as described for *Toxoplasma* IgG ELISA (Calbiotech Inc, Beta Lab, Cairo, Egypt). For free testosterone and cortisol, ELISA was performed as described by Diametra Inc., and Bioactivia Diagnostic, respectively; both supplied by El-Mottahedeh Inc, Cairo, Egypt. Competitive immunoenzymatic colorimetric method was used for quantitative determination of free testosterone concentration. The cortisol kit used is a solid phase competitive ELISA, in which the intensity of color was considered inversely proportional to the cortisol concentration. Colorimetric method was performed as described for GSH (Bioactivia Diagnostic, El-Mottahedeh Inc, Cairo, Egypt). The method is based on the reduction of 5,5' Dithiobis (2-nitrobenzoic acid) by GSH producing a yellow compound. According to the instruction manual, the reduced chromogen was considered directly proportional to GSH concentration and its absorbance was measured at 405 nm.

Statistical analysis: Microsoft excel 2013 was used for data entry and the statistical package for social science (SPSS version 24) was used for data analysis. Simple descriptive statistics (arithmetic mean and standard deviation) were used for analysis of normal quantitative data using paired t test, one-way ANOVA and post-hoc tests. Frequencies were used for qualitative data and statistically analyzed by Chi-square and Fisher's exact tests where appropriate. The level of significance was set at probability (P) value <0.05[24].

Ethical considerations: Both schizophrenic and non-schizophrenic patients were properly informed of the purpose of the study and were provided with consent forms. The study was performed in accordance to the ethical guidelines of the Faculty of Medicine, Cairo University; in addition to the approval of the Inpatient Psychiatry Hospital ethical committee.

RESULTS

All 120 schizophrenic patients and 120 non-schizophrenic control samples were subjected to measurement of *Toxoplasma* IgG, cortisol, free testosterone and GSH levels. Table (1) describes data of patients as regards duration of illness, different scales done to assess their schizophrenic illness and history of ECT to assess severity; besides past and family history of psychotic and mood disorders.

No significant difference was recorded between cases group and control group as regard age, gender, occupation, contact with pets, and *Toxoplasma* IgG seroprevalence (P<0.05). There were significant higher *Toxoplasma* titers (P=0.049), free testosterone levels in both genders (P<0.001 and 0.005 in males and females, respectively) and cortisol levels (P<0.001) in both groups; while GSH level was significantly lower in cases group than control group (P<0.001) (Table 2).

In tables (3 and 4), *Toxoplasma* prevalence in schizophrenic cases showed significantly higher rate in male patients than in females (P=0.03), and in rural than in urban areas (P=0.002); while it was significantly lower in patients with history of drug abuse (P=0.005) and patients with positive family history of psychotic disorders (P=0.011). Prevalence was not higher in patients with history of contact with cats. There was no significant difference in patients with positive family history of mood disorders; and there were no significant differences related to positive syndrome scale (PS), negative syndrome scale (NS), general syndrome scale (GS), TS, treatment with ECT or cortisol level. In *Toxoplasma* positive patients; free testosterone levels in both genders were significantly higher (P<0.001) while GSH was significantly lower (P=0.045). Gender specification showed no significant difference in PS, GS and cortisol level (Table 4).

Table 1. Mean ± SD of duration of illness, PANSS and history of schizophrenic cases.

Relevant data		Mean ± SD
Duration of illness (Years)		5 ± 4
PS (7-49)		22 ± 8
NS (7-49)		19 ± 10
GS (16-112)		26 ± 6
TS		67 ± 15
Relevant data		**No. (%)**
Electro convulsive therapy	**Negative**	48 (40.0)
	Positive	72 (60.0)
Past history of psychosis	**Negative**	120 (100.0)
	Positive	0 (0.0)
Past history of medical disorders	**Negative**	120 (100.0)
	Positive	0 (0.0)
Past history of drug abuse	**Negative**	114 (95.0)
	Positive	6 (5.0)
Family history of psychosis	**Negative**	87 (72.5)
	Positive	33 (27.5)
Family history of mood disorders	**Negative**	99 (82.5)
	Positive	21 (17.5)

Table 2. Comparison of sociodemographic data, *Toxoplasma* prevalence, mean ± SD of *Toxoplasma* titers, cortisol, free testosterone and GSH levels among cases and control.

		No. of cases (%)	No. of control (%)	*P* value
Gender	Male	81 (67.5)	88 (73.3)	
	Female	39 (32.5)	32 (26.7)	0.322
Occupation	Negative	84 (70.0)	80 (66.7)	
	Positive	36 (30.0)	40 (33.3)	0.578
Contact with pets	Negative	99 (82.5)	97 (80.8)	
	Positive	21 (17.5)	23 (19.2)	0.739
Toxoplasma titers	Negative	66 (55.0)	58 (48.3)	
	Positive	54 (45.0)	59 (49.2)	0.154
	Borderline	0 (0.0)	3 (2.5)	
		No. (Mean ± SD)	No. (Mean ± SD)	*P* value
Age		120 (30.23 ± 8.252)	120 (31.5 ± 8.750)	0.248
Toxoplasma titers		120 (1.43 ± 1.217)	120 (1.129 ± 1.129)	**0.049***
Cortisol		120 (176.972 ±130.522)	120 (98.39 ± 46.255)	**< 0.001***
Free testosterone	Male	81 (17.968 ±10.481)	88 (9.014 ± 6.64)	**< 0.005***
	Female	39 (6.5 ± 8.52)	32 (2.37 ± 3.53)	**< 0.001***
GSH		120 (2.49 ± 1.002)	120 (4.026 ± 1.479)	**< 0.001***

*Significant *P* value < 0.05

Table 3. Prevalence of *Toxoplasma* in schizophrenic patients in relation to gender, residence, pets, ECT, history of drug abuse, family history of psychotic and mood disorders.

Relative data		No. of negative (%)	No. of positive (%)	*P* value
Gender	Male	39 (59.1)	42 (77.2)	
	Female	27 (40.9)	12 (22.8)	**0.03***
Residence	Urban	45 (68.2)	24 (44.4)	
	Rural	21 (31.8)	30 (55.6)	**0.002***
Contact with pets	Negative	54 (81.8)	45 (83.3)	
	Positive	12 (18.2)	9 (16.7)	0.828
ECT	Negative	23 (34.8)	25 (46.3)	
	Positive	43 (65.2)	29 (53.7)	0.202
History of drug abuse	Negative	66 (100.0)	48 (88.9)	
	Positive	0 (0.0)	6 (11.1)	**0.005***
Family history of psychosis	Negative	54 (81.8)	33 (61.1)	
	Positive	12 (18.2)	21 (38.9)	**0.011***
Family history of mood disorders	Negative	54 (81.8)	45 (83.3)	
	Positive	12 (18.2)	9 (16.7)	0.828

*Significant *P* value < 0.05

Table 4. PS, NS, GS, TS, *Toxoplasma* antibody titer, cortisol, free testosterone and GSH levels in relation to *Toxoplasma* prevalence and gender in schizophrenic patients.

		Toxplasma			Gender		
	Neg./Pos.	No. (Mean ± SD)	*P* value	Male/Female	No. (Mean ± SD)	*P* value
PS (7-49)	Negtive	66 (21.5 ± 8.6)		Male	81 (22.5 ± 9.0)	
	Positive	54 (22.3 ± 7.9)	0.607	Female	39 (20.6 ± 6.53)	0.241
NS (7-49)	Negtive	66 (18.3 ± 9.13)		Male	81 (21.11 ± 9.71)	
	Positive	54 (19.8 ± 10.76)	0.403	Female	39 (14.7 ± 8.93)	**0.001***
GS (16-112)	Negtive	66 26.7 ± 7.09)		Male	81 26.1 ± 5.53)	
	Positive	54 (24.8 ± 5.04)	0.101	Female	39 (25.3 ± 7.69)	0.527
TS	Negtive	66 (66.6 ± 16.2)		Male	81 (69.8 ± 12.9)	
	Positive	54 (67.1 ± 12.43)	0.860	Female	39 (60.6 ± 15.9)	**0.001***
Toxoplasma titers	-	-		Male	81 (1.27 ± 1.13)	
	-	-	-	Female	39 (83.0 ± 1.06)	**0.043***
Cortisol	Negtive	66 (163.8 ± 138.6)		Male	81 (190.3 ± 135.2)	
	Positive	54 (193.0 ± 119.0)	0.223	Female	39 (149.1 ± 116.9)	0.105
Males free testosterone	Negtive	39 (7.0 ± 6.59)		Male	81 (17.9 ± 10.48)	
	Positive	42 (10.8 ± 6.23)	**0.01***			
Females free testosterone	Negtive	27 (2.2 ± 1.65)		Female	39 (6.5 ± 8.52)	**0.001***
	Positive	12 (8.5 ± 9.62)	**0.003***			
GSH	Negtive	66 (2.8 ± 1.0)		Male	81 (2.3 ± 1.01)	
	Positive	54 (2.0 ±0.82)	**<0.001***	Female	39 (2.7 ± 0.92)	**0.045***

*Significant *P* value < 0.05

While free testosterone levels were found to be significantly higher in male patients receiving ECT (*P*=0.026), no significant difference was found between ECT and PANSS, cortisol and anti-*Toxoplasma* IgG. Moreover, no significant difference between drug abuse and the previous mentioned study parameters was detected (Table 5). As regards the family history for mood and psychotic disorders, statistical significance was only detected between the family history for mood disorders and the GSH (*P*=0.021) (Table 6).

Table 5. PS, NS, GS, TS, *Toxoplasma* antibody titer, cortisol, free testosterone and GSH levels in relation to ECT and history of drug abuse in schizophrenic patients.

		ECT			History of drug abuse	
	Neg./Pos.	No. (Mean ± SD)	*P* value	Neg./Pos.	No. (Mean ± SD)	*P* value
PS (7-49)	Negtive	48 (22.0 ± 5.93)		Negtive	114 (21.71 ± 8.46)	
	Positive	72 (21.8 ± 9.6)	0.915	Positive	6 (25.5 ± 2.739)	0.278
NS (7-49)	Negtive	48 (21.1 ± 10.45)		Negtive	114 (19.24 ± 9.92)	
	Positive	72 (17.6 ± 9.3)	0.060	Positive	6 (15.5 ± 9.311)	0.369
GS (16-112)	Negtive	48 (26.5 ± 5.58)		Negtive	114 (25.89 ± 6.397)	
	Positive	72 (25.4 ± 6.73)	0.349	Positive	6 (26 ± 4.382)	0.968
TS	Negtive	48 (69.6 ± 12.8)		Negtive	114 (66.84 ± 14.545)	
	Positive	72 (64.9 ± 15.43)	0.081	Positive	6 (67.0 ± 16.432)	0.979
Toxoplasma titers	Negtive	48 (1.2 ± 1.0)		Negtive	114 (1.7371 ± 1.08)	
	Positive	72 (1.03 ± 1.14)	0.251	Positive	6 (2.385 ± 0.312)	0.146
Cortisol	Negtive	48 (161.9 ± 112.8)		Negtive	114 (174.0921 ± 128.294)	
	Positive	72 (187.0 ± 140.9)	0.284	Positive	6 (231.7 ± 172.313)	0.294
Males free testosterone	Negtive	27 (7.8 ± 6.69)		-	-	
	Positive	54 (11.3 ± 6.0)	**0.026***	-	-	-
Females free testosterone	Negtive	21 (4.0 ± 5.33)		-	-	
	Positive	18 (9.5 ± 10.55)	0.055	-	-	-
GSH	Negtive	48 (2.3 ± 0.96)		Negtive	114 (2.5053 ± 1.009)	
	Positive	72 (2.5 ± 1.0)	0.307	Positive	6 (2.2 ± .876)	0.469

*Significant *P* value < 0.05

Table 6. PS, NS, GS, TS, *Toxoplasma* antibody titers, cortisol, free testosterone and GSH levels in relation to family history of psychosis and family history of mood disorders in schizophrenic patients.

		ECT			History of drug abuse	
	Neg./Pos.	No. (Mean ± SD)	*P* value	Neg./Pos.	No. (Mean ± SD)	*P* value
PS (7-49)	Negtive	99 (21.33 ± 7.77)		Negtive	87 (22.17 ± 8.93)	
	Positive	21 (24.57 ± 10.24)	0.105	Positive	33 (21.18 ± 6.43)	0.562
NS (7-49)	Negtive	99 (19.30 ± 9.55)		Negtive	87 (19.38 ± 10.21)	
	Positive	21 (17.86 ± 11.52)	0.545	Positive	33 (18.18 ± 9.05)	0.556
GS (16-112)	Negtive	99 (25.09 ± 5.55)		Negtive	87 (26.14 ± 6.43)	
	Positive	72 (25.4 ± 6.73)	**0.021***	Positive	33 (25.27 ± 5.98)	0.504
TS	Negtive	21 (29.71 ± 8.15)		Negtive	87 (67.69 ± 15.4)	
	Positive	99 (65.73 ± 14.81)	0.067	Positive	33 (64.64 ± 12.04)	0.307
Toxoplasma titers	Negtive	21 (72.14 ± 12.31)		Negtive	87 (1.02 ± 1.11)	
	Positive	99 (1.08 ± 1.06)	0.414	Positive	33 (1.42 ± 1.15)	0.077
Cortisol	Negtive	21 (1.35 ± 1.42)		Negtive	87 (178.25 ± 136)	
	Positive	99 (192.28 ± 104.24)	0.077	Positive	33 (173.59 ± 116.74)	0.862
Males free testosterone	Negtive	21 (243.35 ± 175.72)		Negative	60 (9.22 ± 6.47)	
	Positive	69 (9.18 ± 6.69)	0.589	Positive	21 (8.41 ± 7.24)	0.634
Females free testosterone	Negtive	12 (8.05 ± 6.57)		Negative	27 (5.54 ± 5.26)	
	Positive	30 (6.57 ± 8.95)	0.985	Positive	12 (8.92 ± 13.29)	0.258
GSH	Negtive	99 (2.33 ± 0.94)		Negtive	87 (2.55 ± 1.01)	
	Positive	21 (1.98 ± 0.97)	0.124	Positive	33 (2.31 ± 0.98)	0.225

*Significant *P* value < 0.05

DISCUSSION

The current study aims to assess the seroprevalence/serointensity of toxoplasmosis in schizophrenic patients in relation to the levels of free testosterone, cortisol and GSH activity in order to evaluate these factors in schizophrenic patients harboring latent toxoplasmosis. Our results revealing significant higher titers of *Toxoplasma* in cases group than control group, are assured by other studies[25,26] that reported the risk of schizophrenia in offspring of mothers with maternal antibodies to toxoplasmosis. Accordingly, this appears to implicate the mothers of our cases group in transplacental transmission of toxoplasmosis. Our result also conforms with a meta-analysis that involved forty-two studies carried out in 17 countries over five decades[27]. Analysis of meta data that included 3873 individuals with schizophrenia and 7046 controls, revealed that individuals with schizophrenia have an increased prevalence of antibodies to *T. gondii*. Concepts for this statement include that schizophrenia is known to have a genetic component, and that genes are also known to influence the susceptibility of animals to *T. gondii*. Schizophrenia is believed to be a disease of neurodevelopment, which is consistent with the known ability of *T. gondii* to cause prenatal infections and remain latent for many years before reactivation. Also while, schizophrenia is known to include abnormalities of neurotransmitters; animal studies have demonstrated an effect of *T. gondii* on dopamine and serotonin. It is also noticed that there are persistent behavioral changes in *Toxoplasma*-infected animals[27].

Leweke *et al.*[28] reported that the IgG antibody levels in untreated schizophrenic patients were the highest followed by that in treated patients then controls. This was confirmed in other reports[29,30] in which the prevalence of *T. gondii* IgG antibodies among patients with schizophrenia was significantly higher than in the controls but not the IgM antibody levels indicating the latency of the infection. Furthermore, other studies[31,32] observed no significant rise in IgG titer and attributed this to the possible effect of antipsychotic medications in causing a reduction of *T. gondii* IgG level by inhibiting the replication of *T. gondii*. Controversially, the study by Khademvatan *et al.*[33] indicated differences in *Toxoplasma* antibody seroprevalence between males and females schizophrenic patients, but not between patients with schizophrenia and healthy individuals nor among different subtypes of schizophrenia, various age groups, residential area, or clinical course of treatment.

In our results *Toxoplasma* prevalence rate in schizophrenic cases was significantly higher in male patients than in females, and in rural than in urban areas. It was significantly lower, and therefore could not be incriminated, in patients with history of drug abuse and patients with positive family history of psychotic disorders. Prevalence was not higher in patients with history of contact with cats which apparently discounts that issue as a risk factor in these patients. Also, no significant difference was recorded in patients with positive family history of mood disorders, in relation to PS, NS, TS, GSH, treatment with ECT, or level of cortisol. Holub *et al.*[34] confirmed that higher PANSS scores of positive, negative and disorganized psychopathology were associated with lower titer of anti-*Toxoplasma* antibodies. In addition, it was postulated that due to the presence of the parasites within neurons, host-mediated neuroimmune and hormonal responses to infection are present[8].

Our results revealed significant higher free testosterone levels (in both genders) and significant higher cortisol levels in cases group than control group. These results conform with the report by Zouei *et al.*[35] who found that testosterone concentration in toxoplasmosis patients was higher than that in control group with statistically significant difference for men and women. Zghair *et al.*[36] had previously reported that both acute and chronic toxoplasmosis in males recorded significant higher mean concentration of total and free testosterone. Analysis of our data concerning patients on ECT revealed that free testosterone levels in male patients were significantly higher. However, patients on ECT did not show any other significant relationships with the other parameters. On the other hand, our results were different from those of Eslamirad *et al.*[17], and Oktenli *et al.*[37] who demonstrated significantly lower concentration of total and free testosterone in male patients during acute toxoplasmosis. The authors explained this controversy as due to involvement of different parasite strains with variable virulence and epidemiological occurrence; and concluded that the parasite genotype seems to be an important parameter influencing the clinical infection in humans.

GSH is a non-enzymatic antioxidant that eliminates free radicals and has been suggested to have a role in schizophrenia[19]. We found that the GSH level was significantly lower in cases group than control group, and in the *Toxoplasma* positive patients it was significantly lower. Our results conform with those of Karaman *et al.*[19] and Nucifora *et al.*[20] who found a statistically significant difference between *T. gondii*-infected patients and the control group as regard serum MDA, GSH, and nitric oxide levels. While a decrease in GSH activity was detected, MDA and nitric oxide levels increased significantly; and this decrease in GSH level correlated with PANSS total and positive scores for schizophrenia. In our study gender showed no significance concerning PS, GSH and cortisol levels. While NS, TS and *Toxoplasma* antibody titers were more significant in male patients than females, GSH level was significantly lower, and family history of mood disorders was found to be significantly related to GSH. Family history of psychotic disorders did not show any significant difference and drug abuse did not show any significant relationship.

It was reported that behavioral changes associated with latent *Toxoplasma* infection are attributed to a presumed increase in neurotransmitters signaling[38]. Therefore, *Toxoplasma* may play a role in the etiopathogenesis of psychiatric disorders by affecting neurotransmitters which are implicated in the emergence of psychosis and behavioral abnormalities; and by inducing brain inflammation by the direct stimulation of inflammatory cytokines in the central nervous system[39]. Accordingly, neurotransmitter-blocking agents have shown some efficacy in the treatment of this pathology. However, the possible association between toxoplasmosis and positive symptoms should be proven by future studies involving neurotransmitters investigations[40]. It can therefore be hypothesized that toxoplasmosis is involved in the etiology of schizophrenia at least partially through the elevation of testosterone and the generation of oxidative stress.

Accordingly, it is concluded that in schizophrenic patients, there were high *Toxoplasma* antibody levels as well as free testosterone and cortisol levels, while there was reduced GSH levels suggesting that latent toxoplasmosis may be involved in the etiology of schizophrenia via neuro-endocrine alterations. The implication of toxoplasmosis in schizophrenia paves the way to a promising approach to prevention and management of this disease and other psychiatric disorders. This approach adds to the conventional anti-psychotic management strategy by suggesting epidemiological and infectious disease dimension that could provide a background for adjunctive prevention and management plans for patients with schizophrenia.

Author contribution: MSA Abd-El-Tawab suggested research concept. AMR Hamed, NA Mahfouz, SA Hassan and RAE Ibrahim collected samples and data. AM Mahfoz performed ELISA, Elsebaei ES did the statistical analysis, while MK Rehan and NSM El-Gebaly analyzed data. NSM El-Gebaly, MSA Abd-El-Tawab, AMR Hamed and MK Rehan wrote and revised the manuscript.

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