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Latent Toxoplasmosis is Not a Risk Factor for Pregnancy-induced Hypertension

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Authors' contributions

This work was carried out in collaboration between all authors. The study idea was conceived by author AAEH who also designed the study protocol. Author MMA recruited followed up patients and designed data collection form. Both authors AAEH and HAEN performed the laboratory work and run literature review. The manuscript was written by author AAEH in complete agreement with all authors who further reviewed the manuscript and approved the final draft.

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ABSTRACT

Aims: To compare between *Toxoplasma* IgG antibody seroprevalence in pregnancy-induced hypertensive females (cases) versus normotensive pregnant females (control), and to identify potential risk factors in *Toxoplasma* infected patients.
Study Design: A prospective case-control study.
Place and Duration of Study: Outpatient clinics of *Obstetrics & Gynecology* Department and Department of Medical Parasitology, faculty of medicine, Mansoura University in the period from January 2013 to February 2014.
Methodology: We included 78 pregnant females (39 hypertensive, and 39 normotensive; age range 18-39 years). Data concerning demographic and reproductive histories were recorded including previous pregnancies outcome and foetal complications. Comprehensive investigations of the current pregnancy including clinical examination and abdominal ultrasound scan were

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performed. Five ml venous blood was withdrawn from each female, processed and investigated for the presence of anti-*Toxoplasma* IgG antibodies by ELISA.

Results: The overall *Toxoplasma* seroprevalence was 40/78 (51.3%) among total participants. Anti- *T. gondii* IgG antibodies were found in 23/39 (57.5%) of pregnancy-induced hypertension patients and in 17/39 (42.5%) normotensive controls (OR=1.85; 95% CI: 0.7-4.6; *P*=.17). Of the anti- *T. gondii* IgG positive patients, 19 (82.6%) had high IgG levels. In comparison only 1 (6.2%) of the anti- *T. gondii* IgG positive controls showed high IgG levels (*P*<.0001). Regarding the specific characteristics of *Toxoplasma* positive pregnancy-induced hypertension patients, none of those characters displayed a significant correlation with hypertensive tendency except history of abortion (*P*=.004).

Conclusion: Chronic toxoplasmosis is not a likely risk factor for pregnancy-induced hypertension although significantly higher titre among hypertensive females necessitates further research.

Keywords: Toxoplasma; preeclampsia; pregnancy; ELISA; hypertension.

1. INTRODUCTION

Toxoplasmosis through its latent form had been involved in various clinical conditions as schizophrenia, Parkinson disease, auto-immune disease and liver cirrhosis where the immune system is likely to be a major player [1].

Pregnancy-induced hypertension affects 12–22% of all pregnancies and remains a major cause of complications [2]. Adverse foetal outcome is evident with intrauterine foetal growth restriction, prematurity [3], and increased risk of perinatal death [4]. Adverse maternal outcome manifests in the form of eclampsia, abruptio placenta, and HELLP (Hemolysis Elevated Liver enzymes, Low Platelet count) syndrome [5] as well as the long term sequelae of cardiac, cerebrovascular, peripheral arterial disease, and cardiovascular mortality [6]. It may be accompanied by proteinuria and/or oedema; pre-eclampsia (PE) or it may be isolated gestational hypertension [7].

Toxoplasmosis and preeclampsia share common characters immunologically and pathologically; both are associated with an increase in tumour necrosis factor (TNF) cytokine, toll-like receptor activation, skewness of immune system towards TH1 phenotype [8] and immunosuppression similar to that induced physiologically during pregnancy [9]. As in clinical or subclinical infections, modulation of immune response by toxoplasmosis may be involved in the pathogenesis of pre- eclampsia [10] e.g. toxoplasmosis may be a trigger for the cascade of events leading to pregnancy-induced hypertension [11]. As acute toxoplasmosis during pregnancy is just 1-2% while 84% of pregnant women may have latent toxoplasmosis [12], more attention should be paid to study the effects of latent toxoplasmosis on pregnant women. There are currently scarce data regarding the role played by Toxoplasma in pathogenesis of pregnancy-induced hypertension. Lack of studies as well as controversies in results necessitate further exploration for the role of Toxoplasma in pregnancy-induced hypertension. So, we aimed to compare between latent Toxoplasma sero-status among pregnancy associated hypertensive females and pregnant normotensive females. Besides, we intended to explore the role of Toxoplasma in pre-eclampsia (if any); whether it is associated or not with adverse foetal or maternal outcome, as well as to identify potential risk factors in infected patients.

2. MATERIALS AND METHODS

2.1 Study Design

This is a prospective case-control study.

2.2 Participants

Thirty nine pregnant women with hypertension matched with 39 normotensive pregnant females recruited from the obstetrics and gynaecology department at Mansoura University Hospitals, Egypt over the period from January 2013 to February 2014.

2.2.1 Eligibility criteria

Pregnant females with gestational age >20 weeks. Blood pressure >=140/90 mmHg on two or more occasions with or without proteinuria. Proteinuria was considered positive if 24-h urine collection showed a total protein excretion of at least 300 mg. Gestational hypertension was

defined as blood pressure >=140/90 mmHg without proteinuria while preeclampsia (PE) was defined as hypertension associated with proteinuria. Cases were matched regarding age, residence, stage of pregnancy and parity with pregnant normotensive controls with uneventful pregnancy attending department of obstetrics for routine follow up.

2.2.2 Exclusion criteria

Pre-existing endocrine disorders (diabetes mellitus or thyrotoxicosis), renal disease, collagen vascular disease (lupus), cardiovascular disease as valvular lesions and autoimmune disease. Patients with multiple pregnancy as well as patients with pre-gestational chronic hypertension (based on physician diagnosis and/or proven medical record) were also excluded.

2.3 Demographic and Clinical Data Collection

At the time of enrolment in the study, data concerning demographic and reproductive histories were recorded including previous pregnancies outcome and foetal complications. Comprehensive investigation of the current pregnancy including abdominal ultrasound scan was performed. Data: maternal age: <25, 25-34, >=35 years, parity: primipara and multipara, gestational age: 2nd trimester 15-28 weeks, 3rd >=29 weeks, systolic/diastolic ratio: moderate >140/90), and severe >160/110. Presence of either HELLP (Hemolysis Elevated Liver enzymes Low Platelet count) syndrome or eclampsia classify cases as severe even with moderate hypertension.

2.4 Laboratory Tests

A 5 ml of venous blood were withdrawn from patients and control participants. Blood samples were processed immediately by centrifugation at 4,000 rpm for 5 minutes after which they were kept at -20℃ until analysis. Samples were analyzed for anti-*Toxoplasma* IgG antibody, using commercial *Toxoplama* IgG detection kit NovaLisa® (Novatec, Germany) following manufacturer instructions. The absorbance of all wells was read at 450 nm by ELISA Microwell Plate Reader. Anti-*T. gondii* IgG antibody levels were expressed as IU/ml and a test is considered positive if greater than 35 IU/ml. Based on the standards provided with the kit, we further grouped positive results into low positive (>35-50), medium positive (51-100), and high positive (101-200) IU/ml.

2.5 Statistical Analysis

Qualitative variables were described as numbers and percentages. Quantitative variables were described as mean + standard deviation (SD). Chi-square tests or Fisher's exact tests were applied to analyze categorical variables, as appropriate. For comparison of continuous variables, independent-sample t-test was used. Odds ratios (OR) and 95% CI were calculated by univariate logistic regression. All reported values were two-sided and were considered statistically significant at P<.05. Data analyses were performed using SPSS Statistics software (version 20.0, SPSS Inc., Chicago, IL, USA).

3. RESULTS AND DISCUSSION

Thirty-nine pregnant females with mean age±SD= 26.23±5.12 years were enrolled in the study matched with 39 normotensive females; their mean age±SD was 25.28±5.72 years which analogues age of cases (P=.443). The overall *Toxoplasma* seroprevalence was 40/78 (51.3%) among total participants. No statistically significant difference between seroprevalence of anti- *T. gondii* IgG in pregnancy-induced hypertension compared to control group as anti-*T. gondii* IgG antibodies were found in 23/39 (57.5%) of pregnancy-induced hypertension patients and in 17/39 (42.5%) normotensive controls (OR=1.85; 95% CI: 0.7-4.6; P=.17). Of the anti- *T. gondii* IgG positive patients, 19 (82.6%) had high IgG levels. In comparison only 1 (6.2%) of the anti- *T. gondii* IgG positive controls showed high IgG levels; P<.0001; Table 2. With respect to clinical data, a history of abortion imposes a greater possibility for Toxoplasmosis in pregnancy-induced hypertension patients (P<.0001) and a history of still birth (SB) was significantly associated with Toxoplasma positive cases (P=.01; Table 2).

Regarding the specific characteristics of *Toxoplasma* positive pregnancy-induced hypertension patients, neither clinical nor demographic data displayed a significant correlation with hypertensive tendency except history of abortion (P=.004) as Table 4 clarified. After stratification of data by *Toxoplasma* antibody titre, a history of abortion in pregnancy-induced hypertension patients still seemed to be significantly associated with *Toxoplasma* infection (P=.03) as shown in Table 4.

Table 1. Demographic and obstetric data of study population[a]

Variable	Cases (Pregnancy-induced hypertension) (n=39, %)	Control (n=39, %)	P[b]
Age	26.23±5.12	25.28±5.72	.443
Age groups			
<25 years	4 (10.3)	8 (20.5)	.2
>25 years	35 (89.7)	31 (79.5)	
Parity	1.67±1.06	1.54±0.97	.58
Parity groups			
Primi	23 (59)	27 (69.2)	.35
Multi	16 (41)	12 (30.8)	
Pregnancy week	33.49±3.3	35.03±6.4	.19
Residence			
Rural	24 (61.5)	18 (46.2)	.17
Urban	15 (38.5)	21 (53.8)	

[a]Data are presented as mean±standard deviation or n _%, [b]P-value based on x2-test for categorical variables and Student's t-test for continuous ones

Table 2. Demographic and clinical data of *Toxoplasma* positive pregnancy- induced hypertensive patients versus positive controls

Characteristic	Cases (Pregnancy-induced hypertension) *Toxoplasma* positive n=23 (%)	Control *Toxoplasma* positive n=17 (%)	P
***Toxoplasma* titre**			
Low >35-50 IU/ml	3 (13.1)	13 (76.5)	†<.0001
Medium 51-100 IU/ml	1 (4.3)	3 (17.6)	
High 101-200 IU/ml	19 (82.6)	1 (5.9)	
Age group			
<25 years	3 (13)	5 (29.4)	.2
>=25 years	20 (87)	12 (70.6)	
Residence			
Rural	15 (65.2)	10 (58.8)	.68
Urban	8 (34.8)	7 (41.2)	
Parity group			
Primi	12 (52.2)	11 (64.7)	.4
Multi	11 (47.8)	6 (35.3)	
Pregnancy trimester			
2nd (15-28 w)	3 (13)	2 (11.8)	
3rd (>=29 w)	20 (87)	15 (88.2)	.90
Foetal presentation			
Cephalic	21 (91.3)	14 (82.35)	
Malpresentation‡	2 (8.7)	3 (17.65)	.40
History of abortion			
No	3 (13)	14 (82.35)	†<.0001
Yes	20 (87)	3 (17.65)	
***IUFD**			
No	20 (87)	17 (100)	.12
Yes	3 (13)	0 (0)	
***IUGR**			
No	21 (91.3)	17 (100)	.21
Yes	2 (8.7)	0 (0)	
Still birth			
No	18 (78.3)	17 (100)	†.01
Yes	5 (21.7)	0 (0)	
Oligohydraminos			
No	21 (91.3)	17 (100)	.21
Yes	2 (8.7)	0 (0)	

†P is significant at <.05, ‡ any other presentation, IUFD: intrauterine foetal death * IUGR: intrauterine growth retardation*

Table 3. Data of hypertensive cases according to *Toxoplasma* serostatus[a]

Variable	*Toxoplasma* positive n=23	*Toxoplasma* negative n=16	*P*[b]
Age	26.2±5	26.3±5.4	.94
Parity	1.70±0.97	1.63±1.2	.84
Pregnancy week	32.65±3.43	34.69±2.8	.06
Systolic (mmHg)	165.43±13.89	170.63±10.94	.22
Diastolic (mmHg)	107.52±9.4	108.13±7.93	.84

[a]Data are presented as mean±standard deviation, [b]P-value based on Student's t-test

Table 4. Demographic and clinical data of *Toxoplasma* seropositive hypertensive patients

Variable	*Toxoplasma* positive n=23, %	*P*	Low n=3, %	Med n=1, %	High n=19, %	*P*
Age group						
< 25 years n=4	3 (13)	.54	0 (0)	0 (0)	3 (15.8)	.7
>=25 years n=35	20 (87)		3 (100)	1 (100)	16 (84.2)	
Parity group						
Primi n=23	12 (52.2)	.4	2 (66.7)	0 (0)	10 (52.6)	.5
Multi n=16	11 (47.8)		1 (33.3)	1	9 (47.4)	
Residence						
Rural n=24	15 (65.2)	.57	2 (66.7)	0 (0)	13 (68.4)	.38
Urban n=15	8 (34.8)		1 (33.3)	1 (100)	6 (31.6)	
Pregnancy trimester						
2nd (15-28 w) n=3	3 (87)	.13	0 (0)	0 (0)	3 (15.8)	.7
3rd (>=29 w) n=36	20 (13)		3 (100)	1 (100)	16 (84.2)	
Pre-eclampsia degree						
Moderate n=14	9 (39.1)	.61	1 (33.3)	0 (0)	8 (42.1)	.69
Severe n=25	14 (60.9)		2 (66.7)	1 (100)	11 (57.9)	
Abortion history						
Yes n=27	20 (87)	†.00	3 (100)	0 (0)	17 (89.5)	†.03
No n=12	3 (13)	4	0 (0)	1 (100)	2 (10.5)	
Eclampsia						
Yes n=3	1 (4.3)	.35	0 (0)	0 (0)	1 (5.3)	.9
No n=36	22 (95.7)		3 (100)	1 (100)	18 (94.7)	
***HELLP**						
Yes n=2	2 (8.7)	.23	0 (0)	0 (0)	2 (10.5)	.79
No n=37	21 (91.3)		3 (100)	1 (100)	17 (89.5)	
***IUGR**						
Yes n=3	2 (8.7)	.78	0 (0)	0 (0)	2 (10.5)	.79
No n=36	21 (91.3)		3 (100)	1 (100)	17 (89.5)	
Oligohydraminos						
Yes n=3	2 (8.7)	.78	0 (0)	0 (0)	2 (10.5)	.79
No n=36	21 (91.3)		3 (100)	1 (100)	17 (89.5)	
Still birth						
Yes n=6	5 (21.7)	.19	0 (0)	0 (0)	5 (26.3)	.51
No n=33	18 (78.3)		3 (100)	1 (100)	14 (73.7)	

*†P is significant at <.05, *HELLP: Hemolysis Elevated Liver enzymes Low Platelet count syndrome *IUGR: intrauterine growth retardation*

Latent toxoplasmosis is an apparently asymptomatic infection characterized by the presence of *Toxoplasma* bradyzoite cysts and lifelong protective immunity where immunomodulation elicited by the parasite allows ultimate coping with the host [13].

Although a wealth of data covers nearly all aspects of immunity to acute Toxoplasma infection, little is known about the long term sequelae of its more prevalent chronic (latent) form. Latent Toxoplasma infection is no longer considered asymptomatic in the view of various clinical conditions linked to its presence e.g. psychiatric effect, liver disease, auto-immune diseases and even on the haematological parameters of infected patients [1].

There had been few attempts to identify the prevalence of latent toxoplasmosis in patients with pre-eclampsia, a medical important point particularly in our region due to the commonness of both *Toxoplasma* infection and preeclampsia.

Preeclampsia originates pathologically from insufficient development of uteroplacental arteries by invading extravillous cytotrophoblast in the first and second trimesters of pregnancy [14]; the site preferentially invaded by *Toxoplasma*. *Toxoplasma* has an estimated prevalence of 20-80% worldwide in its latent form and a prevalence of 0.1-1% of all pregnancies [15] while pre- eclampsia complicates about 12-22 % of worldwide pregnancies [16], and is a leading cause of maternal mortality, especially in developing countries [17]. In Egypt, PE had a prevalence rate (10.7%) according to a community based study [18]. While results from other studies showed a range of 9.1% to 12.5% [19,20] of all deliveries.

In the present study, seroprevalence of anti- T. gondii IgG antibodies in pregnancy-induced hypertension was comparable to control group (P=.17). There is a substantial amount of research documenting lack of association between *Toxoplasma* and hypertension during pregnancy. Our results corroborate another study from Mexico [21] where a similar prevalence of *Toxoplasma* antibodies was found among cases and controls although an indirect evidence was clarified through the effect of spiramycin (Toxoplasmicidal) drug administration during pregnancy on the incidence of PE, where there is a reduction in incidence of PE in the treated group [2] Contradictory results from studies on other subclinical or chronic infections had been reported eg. Trogstad et al. [22] reported that seronegativity to viruses as herpes simplex, cytomegalovirus and Epstein-Barr virus antibodies among pregnant women was a possible risk factor for PE. Saetta et al. [23] found an association between CMV virus infections and risk of PE. Recently, a positive correlation between Chlamydia trachomatis and PE had shown-up in a longitudinal study by Haggerty et al., [24]. Various case-control studies confirmed this association [25-27]. Certain bacterial and viral infections were reported to be associated with a higher risk of preeclampsia in a comprehensive systematic review on the association between maternal infections and PE [28] and even considered as a risk marker for Pre-ecalmpsia according to Ponzetto et al. [27].

We found significant association between anti-*Toxoplasma* antibodies and PE cases as most of pregnancy-induced hypertension cases (82.6%) were within high titre group (P<.0001). In other studies apart from PE, Level of *Toxoplasma* antibodies was significantly associated with other clinical conditions e.g schizophrenia, traffic accidents. Besides, a correlation exists between level of IgG antibody titre and severe or reactivated infection. So, cases with high antibody are associated with a recent reactivated infection due to dormant infection than cases with mild or moderate titres [29]. So, level of anti-*Toxoplasma* antibodies could be a determining factor in relevance to PE that requires further investigations.

Among demographic and clinical data of positive participants, only history of abortion and still birth were significantly associated with *Toxoplasma* infection in pregnancy-induced hypertension group (P<.0001 and .01 respectively). In a previous study from Egypt, the seroprevalence of IgG antibodies to *T. gondii* in women with early spontaneous abortion was 46.1% [30] and in another study from Qualyobia governorate a seroprevalence of 44.7% had been reported [31]; both were comparable with our findings.

While stillbirths due to toxoplasmosis are sporadic [32], they still exist as in Zimbabwe; a 4-fold increase in stillbirths of mothers with serological evidence of *Toxoplasma* [33]. Based on the mentioned explanations which provided that history of stillbirth occurred exclusively in PE patients in a placenta already diseased with reduced blood flow to the foetus, any other aggravating factor as infection may be associated with more placental blood flow compromization resulting in stillbirth.

4. CONCLUSION

Results of the current study indicate irrelevance of pregnancy induced hypertension to latent *Toxoplasma* infection. However, level of anti-*Toxoplasma* IgG antibodies was significantly higher among hypertensive cases especially the severe form. Our study was a provisional cross-sectional where we restricted cases to 2nd and 3rd trimesters only as it is that period with first presentation of pregnancy-induced hypertension. So in the future, we will aim to include 1st trimester cases as a separate entity and follow them throughout pregnancy in longitudinal studies.

ETHICAL APPROVAL

All authors declare that written informed consent was obtained from each patient after explaining in details the aims and the procedure of the study and that all experiments have been examined and approved by Mansoura University ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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