Original Article

Chemokines profile as early predictor for complicated pregnancy among primigravida at high-risk of toxoplasmosis

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ABSTRACT

Background: Serum chemokines have variable functions related to angiogenesis that might be implicated in adverse pregnancy outcomes. Their assessment in toxoplasmosis during antenatal care will reduce fetal and neonatal morbidity.
Objective: To evaluate the distinguishing ability of chemokines in predicting the outcome of toxoplasmosis and its-related pregnancy complications in vulnerable primigravida.
Patients and Methods: The study included three groups of pregnant women; 60 high risk primigravida (A), 60 low risk primigravida (B) for criteria for toxoplasmosis, and 60 multigravida (C) who had a history of toxoplasmosis-related pregnancy complications. Blood samples were obtained to assess seropositivity for anti-*T. gondii* Igs, M and G and for ELISA estimation of interleukin-6 (IL-6), C-X-C chemokine-ligand-9 (CXCL9), chemotactic-competent motif ligand-2, and -5 (CCL2 and CCL5). Ability of the estimated parameters to predict the reported pregnancy complications was evaluated.
Results: In group A, the number of positive samples was higher and showed significantly higher CXCL9 levels than group B samples. In seropositive patients, CCL2 and 5 levels were significantly higher in group A, while in group B samples, only serum CCL2 levels were significantly higher. In addition, CCL2 levels were higher in IgG or IgG/IgM of group B than in group A subjects. Serum IL-6 levels were significantly higher in seropositive than seronegative women and in IgM than in IgG or IgG/IgM samples of both A and B groups. In group C with pregnancy complications, especially spontaneous abortion (SA), early pregnancy loss (EPL) and preterm labor were the most frequently encountered complications. Pregnancy complications were significantly frequent among group A women. While high CXCL9 levels alone could predict pregnancy complications, high IL-6 levels could predict seropositivity, and seropositivity might predict SA or EPL.
Conclusion: Serum CXCL9 and CCL2 levels could distinguish women vulnerable to developing pregnancy complications. High CXCL9 levels could predict SA or EPL, while coupled with high IL-6 levels it could distinguish seropositivity in primigravida.

Keywords: chemokines; early predictors; early pregnancy loss; high risk; spontaneous abortion; toxoplasmosis.

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INTRODUCTION

Toxoplasmosis affects various populations dealing with sources of transmission; contaminated water or food staffs[1-3]. Acute infection during pregnancy deleteriously affects both mother and fetus secondary to mother-to-fetus transmission[4]. Toxoplasmosis induces a disturbed immune milieu with shift of pro-inflammatory/anti-inflammatory equilibrium in direction of pro-inflammatory mediators[6]. This disturbed inflammatory milieu also stimulates the production of various chemokines that subsequently stimulate the production and or differentiation of various cellular components of immune system[6,7].

Chemokines are chemotactic-competent (C-C) molecules composed of a family of small cytokines[8]. Chemokine-receptor (CCR) system plays a pivotal role in leukocyte trafficking, inflammation, and immune cell differentiation[9]. Monocytes and dendritic cells (DCs) transport and present antigens to nodal T cells in a CCR5- and CCR7-dependent manner, respectively[10]. The C-C motif ligand 2 (CCL2) is an integral chemotactic factor which recruits macrophages for the immune response, and on coupling with its receptor (CCR2), it influences various diseases of different systems[11]. The C-X-C chemokine-9 (CXCL9) is crucial for recruiting immune T cells and inducing their accumulation into the infected areas to prevent chronic infection[7].

On the other hand, the interplay between chemokines recruiting leucocytes with subsequent release of inflammatory cytokines and chemokines

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recruiting immune T cells might be responsible for development of diseases and in case of infections it may be responsible for chronicity or infection-induced complications[12]. Therefore, the present study evaluates the predictive ability of estimated serum chemokines to distinguish primigravida liable to develop toxoplasmosis-related pregnancy complications.

PATIENTS AND METHODS

This prospective comparative observational study was performed at Benha University Hospital during the period from Jan 2022 to June 2023.

Study design: The study recruited newly pregnant women during their antenatal care. Anti-*Toxoplasma* IgG and IgM as well as determination of cytokines (IL-6, CXCL9, CCL5 and CCL2) levels were performed. The study assessed the relations between the frequency of pregnancy complications and outcomes, and *Toxoplasma* seropositivity and cytokines levels.

Subjects: The study recruited all women who attended the Antenatal Care Unit, Benha University Hospital. Primigravida women who had at least one of the criteria of being high-risk for toxoplasmosis, according to the CDC diagnostic guidelines[13] were included in group (A). A similar number of primigravida free of the criteria of being high-risk for toxoplasmosis infection were enrolled as group (B). A third group of multigravida pregnant women, who had previously complicated pregnancies secondary to toxoplasmosis, were considered as a positive control group (C). Exclusion criteria included women with inflammatory disorders, maintenance on steroids, immunosuppressive, or non-steroidal anti-inflammatory drugs.

The collected data included participants' age, weight, and height. Body mass index (BMI) was calculated as the weight (kg) divided by height (m^2). Residence, educational levels, type of work, and socioeconomic status were recorded. Interrogation was particularly concerned about previous contact with pets, farm animals or their excreta, eating raw meats, vegetables or fruits, previous pregnancies for multigravida, positive serological tests for toxoplasmosis, and the presence of medical disorders. Baseline blood pressure measures and fasting blood glucose were determined, and clinical examination for exclusion of medical disorders was performed. Data concerning clinical evaluation, women's categorization, and pregnancy outcomes was withheld from the serological testing personnel. Table (1) shows the demographic and medical data for the enrolled women.

Blood sampling: Blood samples (5 ml) were withdrawn, and sera were frozen at -80°C for estimation of anti-*T. gondii* IgM and IgG, and serum chemokines.

Seropositivity: Anti-*T. gondii* IgM and IgG ELISA kits (Cat No. ab108778 and ab108776, respectively) were used (Abcam Inc., San Francisco, USA) according to the manufacturer's instructions[14]. The results were read using a 96 well microplate ELISA reader (Dynatech, MR 7000).

Serum Chemokines: We performed ELISA assay of human CXCL9 [15], IL-6 [16], CCL5[17] and CCL2[18] (Cat No. ab219047, ab46027, ab174446, ab179886, respectively).

Table 1. Demographic and medical data of women in the studied groups (A, B, C).

Variate		Group A (n=60)	Group B (n=60)	Group C (n=60)
Age (years)		28.7±3.2	30.2±4.2	29.8±4.0
Body mass index (kg/m^2)		30.5±2.2	30.6±2.0	30.7±2.7
Residence	**Rural**	22 (36.7%)	31 (51.7%)	27 (45%)
	Urban	38 (63.3%)	29 (48.3%)	33 (55%)
Educational level	**Illiterate**	17 (28.3%)	13 (21.7%)	21 (35%)
	1ry school	19 (31.7%)	20 (33.3%)	12 (20%)
	High school	11 (18.3%)	16 (26.7%)	18 (30%)
	University	13 (21.7%)	11 (18.3%)	9 (15%)
Work	**Housewife**	10 (16.6%)	22 (36.6%)	16 (26.6%)
	Farmer	13 (21.7%)	16 (26.7%)	19 (31.7%)
	Worker	19 (31.7%)	13 (21.7%)	15 (25%)
	Officer	18 (30%)	9 (15%)	10 (16.7%)
Contact with pets	**Yes**	17 (28.3%)	0	11 (18.3%)
	No	43 (71.7%)	0	49 (81.7%)
Contact with large animals	**Yes**	23 (38.3%)	0	19 (31.7%)
	No	37 (61.7%)	0	41 (68.3%)
Eating raw foods	**Yes**	16 (26.7%)	0	21 (35%)
	No	44 (73.3%)	0	39 (65%)
Presence of medical problems	**Yes**	5 (8.3%)	4 (6.7%)	7 (11.7%)
	No	55 (91.7%)	56 (93.3%)	53 (88.3%)
Fasting blood glucose (mg/ml)		79.9±6.6	79.3±7.3	78.1±6.3
Systolic blood pressure (mmHg)		109.6±4.8	108±5.2	108.1±5.6
Diastolic blood pressure (mmHg)		71.2±3.7	72.3±5	72±5.3

Follow-up: All enrolled women were asked to attend the outpatient clinic for clinical diagnosis of pregnancy as judged by the presence of a viable fetus and to attend every 2-w to assess the progress of pregnancy and development of complications. Besides, the enrolled women were advised to attend the clinic if any manifestations suggestive of disturbed pregnancy develop. The incidence of pregnancy complications was determined, and these complications included the development of SA, EPL, premature rupture of membranes (PRM), or systemic complications such as the development of gestational diabetes mellitus (GDM) or gestational hypertension (GH). Notably, the obstetrician was not informed of the serological examinations results till the end of the follow-up period. At the end of the study duration, all data were interpreted and analyzed and results were tabulated.

During follow up, our primary study objective was the ability of the estimated chemokines to predict any oncoming pregnancy complications. The secondary objectives included:

- The relation between the estimated chemokines' levels and IgG seropositivity.
- The ability of the estimated chemokines to distinguish women who had acute toxoplasmosis as judged by IgM seropositivity only.
- The impact of acute-on-top of chronic toxoplasmosis as judged by IgM and IgG seropositivity.

Statistical analysis: Data were recorded using the SPSS (Version 22, 2015; Armonk, USA). One-way ANOVA test and Chi-square test (X2 test) were used to assess the significance of the results. Evaluation of predictability was conducted using the regression analysis and the receiver characteristic curve. The significance of the area under ROC curve (AUC) was assessed concerning AU of the reference curve. The significance of the results was determined using a *P* value at a cutoff point of 0.05.

Ethical considerations: The study was conducted after obtaining the preliminary approval of the study protocol by the Local Ethical Committee, Faculty of Medicine, Benha University, Egypt with code RC: 15-9-2023. Before acceptance to be enrolled, the study protocol was discussed with patients.

RESULTS

Demographic and clinical data: Throughout the case collection period, 213 women were evaluated, 23 women were excluded for not fulfilling the inclusion criteria and 10 women were missed during follow-up, while the remaining 180 women were categorized into the three groups (Fig. 1).

Seropositivity of anti-*Toxoplasma* antibodies: Serological diagnosis of toxoplasmosis in groups A and B women (72/120) proved seronegative; 25/60 (41.7%) and 47/60 (78.3%) respectively. Significant (*P*=0.0004) higher seropositive frequency (35/60; 58.3%) was recorded among those of group A. All 28/120 (23.3%) seropositive women, were anti-*T. gondii* IgM positive: 22/60 (36.7%) women of group A and 6/60 (10%) women of group B with significantly (*P*=0.0006) higher frequency among women of group A. Fifteen women had positive anti-*T. gondii* IgG, 8/60 among group A (13.3%) and 7/60 among group B (11.7%) with non-significance of higher frequency among women of group A. Only 5/60 women (8.3%) of group A had recurrence of chronic infections; positive anti-*T. gondii* IgM and IgG (Table 2). Among women of group C, 24/60 women (40%) had positive anti-T gondii IgM and 36/60 women were anti-T gondii IgM/IgG seropositive (Table 2, Fig. 2).

Chemokines levels: Serum levels of CXCL9 were significantly higher among group A seronegative (*P*<0.001) and seropositive (*P*<0.001) women compared

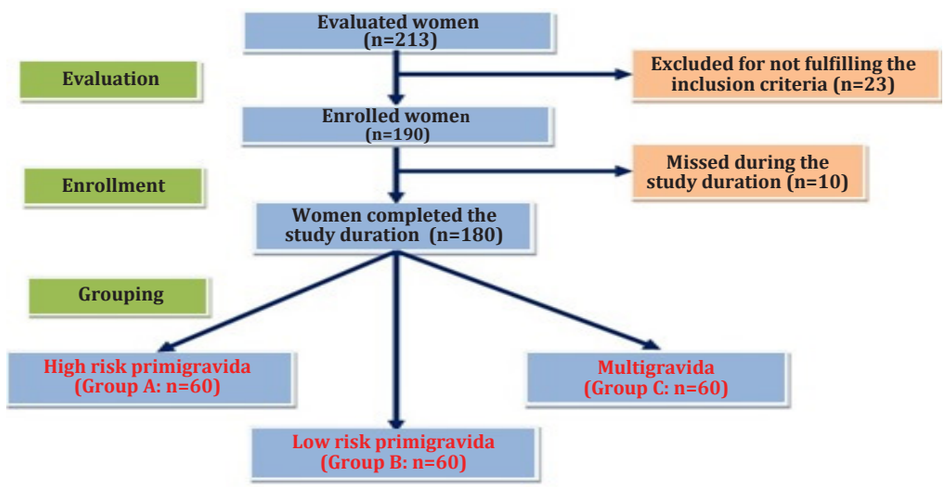

Fig. 1. Study flow chart.

Table 2. Distribution of groups A (high risk) and B (low risk) primigravida according to IgM and IgG seropositivity.

		Group A (n=60)	Group B (n=60)	*P* value
Serological diagnosis	**Negative**	25 (41.7%)	47 (78.3%)	0.0004*
	Positive	35 (58.3%)	13 (21.7%)	
Seropositive	**IgM**	22 (36.7%)	6 (10%)	0.0006*
	IgG	8 (13.3%)	7 (11.7%)	0.058*
	IgM/IgG	5 (8.3%)	0	

NB: All patients of group C were seropositive, and were included as positive control group. Data are analyzed using X^2 test; ***:** Significant (P<0.05).

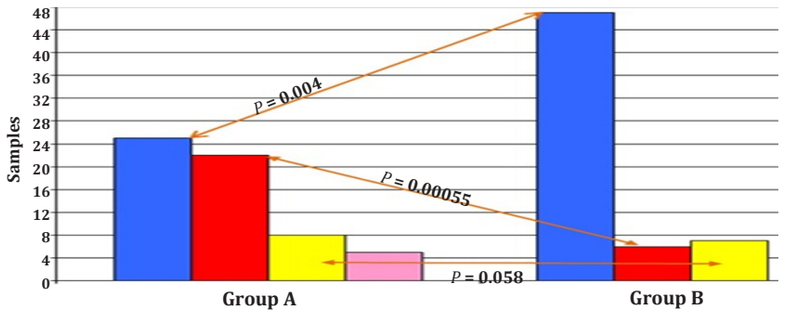

Fig. 2. Distribution of groups A (high risk) and B (low risk) primigravida samples according to *Toxoplasma* seropositivity. **Blue:** Seronegative; **Red:** Seropositive-IgM; **Yellow:** Seropositive-IgG; **Pink:** Seropositive-IgM/IgG

to the corresponding group B women. Further, serum CXCL9 levels in samples of group A and group B seropositive women were significantly (P=0.022) and non-significantly higher than values estimated in sera of seronegative women. Among seropositive women, serum CXCL9 levels were significantly (P<0.001) higher in those who had IgM than in samples of other women from both groups A and B with significant differences (P<0.001) between both groups. Serum CXCL9 levels of all women in groups A (P<0.001) and B (P=0.0074) were significantly higher than group C women with significantly (P=0.0012) higher levels in group A than in group B samples (Table 3).

Serum CCL5 levels were significantly (P=0.038) higher estimated in samples of group A seropositive than in samples of group A seronegative women and samples of IgG and IgG/IgM women than in samples of IgM women of group A. On the contrary, the differences between CCL5 estimate in samples of group B women was insignificant (P=0.159). Serum CCL5 estimated in samples of all patients in group B were significantly (P=0.0004) lower, but insignificantly lower than levels estimated in samples of women of groups C and A, respectively, with insignificant lower levels in samples of group A than group C (Table 3).

Serum CCL2 levels were significantly higher in group A seropositive than group A seronegative (P=0.0001) and group B seropositive (P=0.016) samples, and in samples of IgG or IgG/IgM women of group B than in corresponding samples of group A (P=0.032). Further, serum CCL2 levels in all samples of group B were significantly lower than levels estimated in samples of groups A (P=0.006) and C (P=0.0006) with insignificant differences between groups B and C (Table 3).

Serum IL-6 levels were significantly higher in seropositive than seronegative and in IgM than IgG or IgG/IgM women of groups A (P<0.001) and B (P =0.0005). In the total number of patients, serum IL-6 levels were significantly (P<0.001) lower in group C samples than in samples of other groups with significantly (P =0.046) lower levels in group B than in group A samples (Table 3).

Pregnancy complications: During the course of pregnancy, 98 women (54.4%) developed pregnancy complications. The frequency of complicated pregnancy was significantly higher among women of group C compared to women of groups A (P=0.036) and B (P=0.00003), with significantly (P=0.028) higher frequency of complicated pregnancy among women of group A than women of group B (Fig. 3).

Among women who had complicated pregnancies, early pregnancy disturbances were the commonest complications and accounted for 45.9% of encountered complications; and the next in frequency was the early pregnancy loss that occurred in 22.4% of women who had complicated pregnancies. Systemic complications were reported in 16.3%, and PRM occurred in another 16.3%. The frequency of complications according to their type showed non-significant differences between women of the three groups (Table 4).

Evaluation of laboratory findings as early predictors for the development of pregnancy complications using the ROC curve analysis showed the highest predictability for high serum levels of CCL2 and IL-6 followed by high serum levels of CXCL9 and seropositivity for toxoplasmosis (Fig. 4). Univariate regression analysis defined high serum CXCL9 and CCL2 levels and seropositivity for toxoplasmosis as

Table 3. Median values of serum levels of studied variate,

Parameters	Grouping	Group A (n=60)	Group B (n=60)	*P*1 value
Serum CXCL9 (pg/ml)	Seronegative	79.5 [23.3-133.5]	15 [10-77]	0.001*
	Seropositive	198 [26-298]	19 [10-402]	<0.001*
	P value	0.022*	0.215	
	IgM	278 [202.5-381.25]	402 [299.75-438.75]	0.021*
	IgG or IgM/IgG	22 [14.5-30]	11 [7-14]	0.048*
	P value	<0.001*	<0.001*	
	Total	112 [26-231.5]	16 [10.25-91.25]	0.0012*
	Group C (n=60) Level	9.5 [6.25-15.5]		
	Group C (n=60) *P*2 value	<0.001*	0.0074*	
Serum CCL5 (ng/ml)	Seronegative	14 [9.5-17]	13 [7-17]	0.429
	Seropositive	18 [10-33]	18 [6.5-40.5]	0.873
	P value	0.038*	0.177	
	IgM	13 [8-18]	16 [3-39]	0.865
	IgG or IgM/IgG	24 [16.5-39.5]	22.5 [10.75-47.25]	0.079
	P value	0.005*	0.285	
	Total	16 [10-23]	13 [7.25-18.75]	0.061
	Group C (n=60) Level	19 [14-30.5]		
	Group C (n=60) *P*2 value	0.078	0.0004*	
Serum CCL2 (pg/ml)	Seronegative	19 [15.5-29]	22 [16-29]	0.689
	Seropositive	36 [22.5-68.5]	18 [6.5-40.5]	0.016*
	P value	0.0001*	0.711	
	IgM	38 [22.5-68.5]	29 [19.75-34.75]	0.093
	IgG or IgM/IgG	34 [21-39.5]	15 [8-26]	0.032*
	P value	0.111	0.073	
	Total	27 [19-37]	22 [15.25-29.75]	0.006*
	Group C (n=60) Level	28.5 [20.5-40.5]		
	Group C (n=60) *P*2 value	0.741	0.0006	
Serum IL-6 (ng/ml)	Seronegative	6 [4.5-9]	6 [4-8]	0.689
	Seropositive	13 [10-17]	9 [7-19.5]	0.429
	P value	<0.001*	0.0005*	
	IgM	15.5 [10-19.75]	19.5 [13.75-22.75]	0.231
	IgG or IgM/IgG	10 [9.5-13.5]	8 [6-9]	0.046*
	P value	0.049*	0.038*	
	Total	10 [5.25-14]	7 [5-8.75]	0.0008*
	Group C (n=60) Level	4 [2-4]		
	Group C (n=60) *P*2 value	<0.001*	<0.001*	

Data are presented as a median and interquartile range in parenthesis; levels were compared using Mann-Whitney U test; **CXCL9:** C-X-C motif chemokine ligand 9; **CCL:** CC chemokine ligand; **IL-6:** Interleukin 6; ***:** Significant (*P*<0.05).

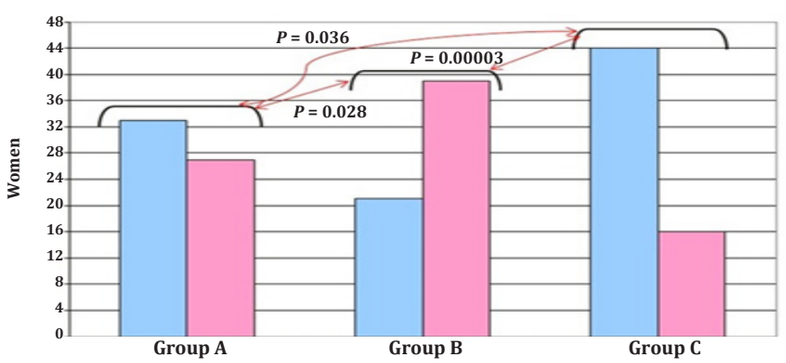

Fig. 3. The frequency of complicated pregnancy among women in studied groups A, B, C; **Blue:** Complicated pregnancy; **Red:** Uncomplicated pregnancy

the significant predictors for oncoming pregnancy complications, while multivariate regression analysis defined high serum level of CXCL9 as the persistently significant predictor for oncoming complications. The ROC curve analysis showed that all the estimated lab variates could predict the possibility of SA or EPL with the varied significance of AUC (Fig. 5), while regression analysis defined high serum CXCL9 and seropositivity as the significant predictors on univariate analysis and high serum CXCL9 on multivariate analysis (Table 5).

The ROC curve analysis defined high levels of CCL5 and CCL2 as significant (*P*<0.001) predictors for seropositivity, while high serum CXCL9 showed significance (*P*=0.018) and excluded IL-6 levels (Fig. 6). Moreover, univariate regression analysis excluded

IL-6, and multivariate regression analysis excluded CCL5 as predictors for seropositivity among the three groups of pregnant women (A, B, C) and assured the predictability of high levels of CXCL9 as the most significant (P<0.001) predictor followed by high levels of CCL2 (P=0.001). Among primigravida, ROC curve

Table 4. Frequency of complicated pregnancies in studied groups A, B, C.

		Group A (n=60)	Group B (n=60)	Group C (n=60)
Frequency of complicated pregnancy	**Yes**	27 (45%)	21 (35%)	44 (73.3%)
	No	33 (55%)	39 (65%)	16 (26.7%)
	***P*1 value**		0.028*	0.036*
	***P*2 value**			0.00003*
Pregnancy complications	**Spontaneous abortion**	8 (24.2%)	6 (28.6%)	9 (20.5%)
	Early pregnancy loss	7 (21.2%)	7 (33.3%)	8 (18.2%)
	Preterm labor	7 (21.2%)	4 (19%)	10 (22.7%)
	Premature rupture of membrane	5 (15.2%)	3 (14.3%)	8 (18.2%)
	Gestational diabetes mellitus	4 (12.1%)	1 (4.8%)	6 (13.6%)
	Gestational hypertension	2 (6.1%)	0	3 (6.8%)
	Total	27 (100%)	21 (100%)	44 (100%)
	***P*1 value**		0.719	0.905
	***P*2 value**			0.478

Data are analyzed using X^2 test; ***:** Significant (P<0.05).

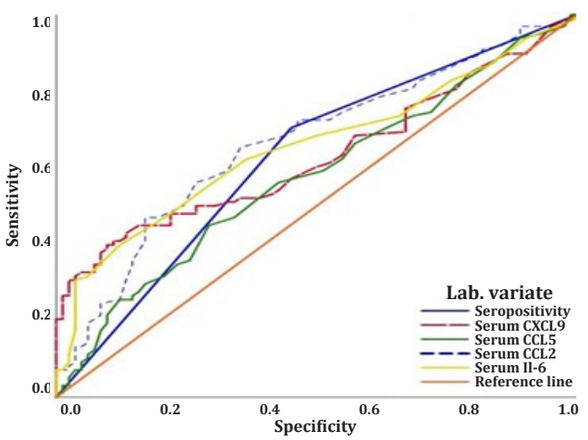

Fig. 4. ROC curve for the predictability of the estimated lab variate for pregnancy complications.

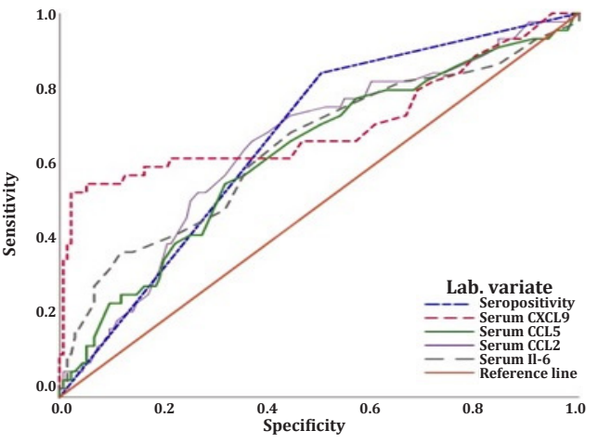

Fig. 5. ROC curve for the predictability of the estimated lab variate for the possibility of development of SA or EPL.

Table 5. Statistical analyses for Lab variate as early predictors for oncoming pregnancy complications.

Variate complications	Receiver operating characteristic curve				Univariate		Multivariate	
	AUC	SE	*P* vlue	95% CL	β	*P* vlue	β	*P* vlue
Prediction of pregnancy complications								
Seropositivity	0.626	0.042	0.004	0.544-0.709	0.168	0.020*	0.221	0.002*
CXCL9	0.630	0.041	0.003	0.549-0.711	0.300	<0.001*	0.321	<0.001*
CCL2	0.666	0.040	<0.001	0.586-0.745	0.166	0.023*	Excluded	Excluded
CCL5	0.577	0.043	0.074	0.494-0.661	Excluded	Excluded	Excluded	Excluded
IL-6	0.655	0.041	<0.001	0.575-0.734	Excluded	Excluded	Excluded	Excluded
Prediction of spontaneous abortion or early pregnancy loss								
Seropositivity	0.670	0.044	0.001	0.585-0.756	0.245	<0.001*	Excluded	Excluded
CXCL9	0.711	0.054	<0.001	0.606-0.819	0.496	<0.001*	0.523	<0.001*
CCL2	0.653	0.047	0.002	0.561-0.746	Excluded	Excluded	Excluded	Excluded
CCL5	0.632	0.049	0.008	0.537-0.728	Excluded	Excluded	Excluded	Excluded
IL-6	0.651	0.050	0.002	0.552-0.750	Excluded	Excluded	Excluded	Excluded

AUC: Area under the ROC curve; **SE:** Standard error; **CI:** Confidence interval; β: Standardized coefficient; *: Significant (P<0.05).

analysis defined high serum levels of CXCL9 and IL-6 as high predictors for seropositivity and followed by high serum levels of CCR2 and CCR5 (Fig. 7). However, regression analyses excluded CCR2 and CCR5 as predictors and assured CXCL9 and IL-6 (Table 6).

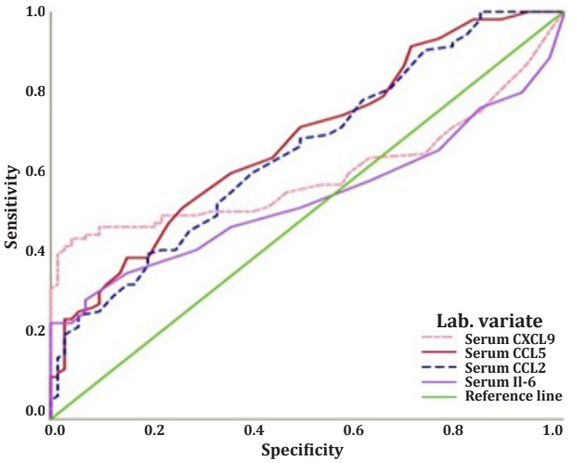

Fig. 6. ROC curve for the predictability of the estimated lab variate for seropositivity among low risk primigravida (group B).

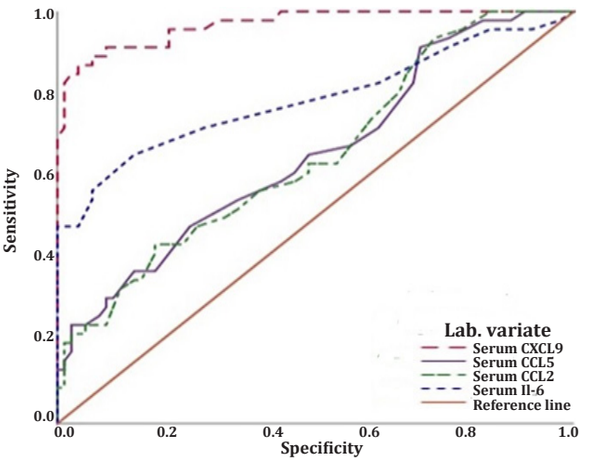

Fig. 7. ROC curve for the predictability of the estimated lab variate for seropositivity among high risk primigravida (group A).

Table 6. Statistical analyses for Lab variate as predictors for seropositivity among primigravida groups (B and A).

Variate complications	Receiver operating characteristic curve				Univariate		Multivariate	
	AUC	SE	*P* vlue	95% CL	β	*P* vlue	β	*P* vlue
Prediction of seropositivity among group B primigravida								
CXCL9	0.604	0.042	0.018	0.521-0.687	0.279	<0.001	0.332	<0.001*
CCL2	0.659	0.041	<0.001	0.579-0.738	0.206	0.003	Excluded	Excluded
CCL5	0.683	0.039	<0.001	0.606-0.761	0.182	0.011	0.231	0.001
IL-6	0.543	0.043	0.329	0.459-0.626	Excluded	Excluded	Excluded	Excluded
Prediction of seropositivity among group A primigravida								
CXCL9	0.968	0.014	<0.001	0.941-0.996	0.673	<0.001	0.779	<0.001*
CCL2	0.652	0.051	0.005	0.552-0.752	Excluded	Excluded	Excluded	Excluded
CCL5	0.654	0.051	0.005	0.553-0.755	Excluded	Excluded	Excluded	Excluded
IL-6	0.786	0.048	<0.001	0.693-0.879	0.213	0.009	0.275	0.001

AUC: Area under the ROC curve; **SE:** Standard error; **CI:** Confidence interval; β: Standardized coefficient; *: Significant (P<0.05).

DISCUSSION

Among the studied primigravida in groups A and B, 48/120 (40%) women were *T. gondii* seropositive; 35/60 (58.3%) women had high-risk (group A) and 13/60 (21.7%) women had a low risk (group B) of infection with a significantly higher possibility of having toxoplasmosis among those at high risk of infection. This significant difference between both groups signified the impact of exposure to the sources of infections, especially domestic cats. In support of this assumption, it was documented that members of the family Felidae can serve as both intermediate and definitive hosts for *T. gondii*[19] and shed unsporulated oocysts, that sporulate within 1-5 days and become infective[20]. The reported seropositivity rate (21.7%) among women who are at low risk of infection could be attributed to their residence in rural areas with a high possibility of contact with contaminated soil, or through ingestion of infected raw food stuffs[21].

Serum levels of estimated chemokines and IL-6 were significantly higher in high risk primigravida of group A than primigravida of low risk group B and multigravida group C, and in primigravida of group B than multigravida of group C. These data indicated increased levels of CXCL9 and CCL2 with high than low exposure and with acute than chronic infection. However, the increased levels of CCL5 showed non-significant differences between primigravida categorized according to the risk of exposure. These findings indicated deregulation of the expression of the studied chemokines with *T. gondii* infection, which is associated with overexpression levels of inflammatory cytokines. The obtained results coincided with Denis *et al*.[23] who studied the dynamic cytokine patterns through *T. gondii* infection phases and reported significantly elevated levels of 11 of the cytokines including IL-6, CXCL9 and CCL2 during acute infection, but their levels were significantly lower with chronic infection in comparison to uninfected patients. Dos

Santos *et al*.[24] also detected significantly higher levels of CCL2, CXCL16, and IL-33 in *T. gondii*-seropositive than in seronegative pregnant women, and in a systemic review of studies including 806 pregnant women. The investigators recorded significantly higher plasma levels of IL-5, 6, 8, 10, and 17 and CCL5 and concluded that the equilibrium between inflammatory and regulatory cytokines might alleviate the harmful placenta and fetus effects due to toxoplasmosis[24].

Unfortunately, 98/180 (54.4%) women developed pregnancy complications and its incidence was significantly higher among multigravida of group C, who had previous complicated pregnancies attributed to toxoplasmosis and in primigravida of high (A) than those of low (B) risk of toxoplasmosis. Similarly, Dos Santos *et al*.[25] observed that multiparous women are more likely to be infected with *T. gondii* with its subsequent complications than primiparous women.

The estimated serum chemokines' levels at pregnancy particularly levels of CXCL9 and CCL2 showed high predictability for the oncoming pregnancy complications especially SA and EPL. However, the high serum levels of CXCL9 were the most pronounced predictor for pregnancy complications in general, especially SA or EPL. In line with these data, Spathakis *et al*.[12] detected altered expression of CXCL7, 4, 9 and 11 in the placentae and overexpression of CXCL7, 8, 9, 11 and 20 in the decidua of women who had SA. It was concluded that dysregulation of angiogenesis in the form of angiostatic overexpression and diminished expression of angiogenic chemokines in the placenta and decidua, might contribute to the pathogenesis of miscarriage[12]. Mei *et al*.[26] also reported overexpression of CXCL9, 10, and 11 and Th1 cells specific transcription factors in human fetal membranes from PTL in comparison to full-term labor women. Further, Yang *et al*.[27] reported that altered expression levels of CXCL9, CXC receptor 3, and Integrin Subunit αX genes may be closely associated with the development of fetal growth restriction and ROC curve revealed that these three pivotal genes had a significant diagnostic ability for fetal growth restriction.

As regards the systemic complications, 11 women developed GDM and 5 developed GT. The frequency of GDM and GT was related to the estimated levels of chemokines and IL-6 that could predict its development in the affected women. Similarly, Wang *et al*.[28] found the deregulated expression levels of CXCL9 and CXCL10 are closely related to the development of GDM. Brien *et al*.[29] also observed higher levels of IL-6 and CXCL10 in women who developed preeclampsia, and CXCL9 in women who developed PTL. Thereafter, Liu *et al*.[30] reported that GDM is associated with disturbed levels of several chemokines and detected a potential role of chemokines as biomarkers concerning laboratory detection and clinical characteristics of GDM patients.

In conclusion, acute toxoplasmosis during pregnancy is associated with the activation of pro-inflammatory cytokines and chemokines pathways. Estimation of serum levels of chemokines CXCL9 and CCR2 at the time of pregnancy diagnosis could distinguish women vulnerable to developing complications during pregnancy, and high levels of CXCL9 could predict the oncoming SA or EPL. Further, high levels of CXCL9 and IL-6 could distinguish infected primigravida more precisely than other investigations.

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