

## How can toxoplasmosis effect in systemic lupus erythematosus women? An immunological insight

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### Abstract

The opportunistic intercellular blood parasite *Toxoplasma gondii* can occur a dangerous coinfection for pregnant women have general chronic systemic lupus erythematosus (GCSLE) may causes abortions or life threatening for both mother and fetus. 153 patients women divided into: G $\alpha$  (seropositive toxoplasmosis)/56, G $\beta$  (GCSLE seropositive toxoplasmosis)/43, and G $\gamma$  (GCSLE seronegative toxoplasmosis)/44, also 45 healthy women as control G $\delta$ , the average ages  $\pm$ S.d was  $31.34 \pm 3.25$  (22-45) years. The abortion status for all samples had studied. Both Anti-Toxo antibodies (IgM and IgG) detected via ELISA, and Anti $\beta$ 2-Glycoprotein I (anti $\beta$ 2GpI) IgM and IgG antibodies measured by full automated Alegria® device, all titer values compared with standard cut off for each indicator. Obviously the results of this treatise manifested there was a significant elevation in means values of the blood indicators (ESR and C-RP) and serum immunological antibodies anti-Toxo (IgM and IgG), and anti-GPI (IgM and IgG) were (35.5mm/hr. and 19.2mg/dl) and (1.86 and 9.93) Index, and (10.57 and 14.75) U/ml respectively in the group of GCSLE women infected with toxoplasmosis (G $\beta$ ) compared to all means values in the rest subjected groups statistically both  $p < 0.01$  and  $\alpha$  (Scheffe) = 0.01, in addition the highest percentage in number of recurrent abortion was in G $\beta$  21/44 (47.72%) at  $p < 0.05$ . The present study concluded that toxoplasmosis infection in women with GCSLE is a risk factor one through the progression of syndrome severity with accompany mischievous health effects, the other is the increasing in recurrent abortions occurrence probability, due to the activation of the parasite's dormant phases causes the elevation of antibodies and autoantibodies, because the strong synergistic correlation between them during pregnant stages.

**Keywords:** Systemic lupus erythematosus, Toxoplasmosis, ANA and anti-dsDNA, *Toxoplasma gondii*, anti- $\beta$ 2GPI

### Introduction

The opportunistic obligate intracellular from Apicomplexa phylum parasite causes toxoplasmosis is *Toxoplasma gondii* has infecting one third globally human beings ranked with different rang of threatening starting from the asymptomatic mild contagion to prolapse grave devastating for congenitally infected newborns and immunosuppressed individuals [1,2]. General Chronic Systemic Lupus Erythematosus (GCSLE) is inhomogeneity malady with numerous clinical semblances disorder depicted by over activity of both the main immune cells B and T, that creation of assorted self-antibodies containing immune complex in hole blood vessels and organs through human body, with scope incidence of 0.3-31.5 in 100.000 annually with continuous increasing prevalent that causes costing healthy management around \$(21.000-53.000) and €(2600-4800) in both USA and Europe respectively [3]. Many proinflammatory and inflammatory cytokines able enhance the function of numerous immune cells to proliferate many antibodies, these antibodies are suddenly shifting against immune tolerance to be

intolerant autoantibodies (Aub) that started attacked and damaged different parts of tissues and organs hole body which create the autoimmune disorders like GCSLE [4,5], some of the critical cytokines play pivotal role during the acute period of toxoplasmosis infection in autoimmune illnesses are  $\alpha$ -TNF, 12-IL,  $\gamma$ -INF, and CD8+T cell [6]. Large amount of autoantibodies are secreting from immune cells specific to own-antigens mainly of nuclear origin, The Antinuclear Antibody (ANA) and Anti-double strands DNA (Anti-dsDNA) are considered significant markers in diagnosis and prognosis of GCSLE [7] (Dema and Charles, 2016). Also the antiphospholipid syndrome (APLS) is another autoimmune infirmity may associate with GCSLE in co-occur status specially with lupus anticoagulant (Lac), the anti- $\beta$ 2 Glycoprotein I (anti $\beta$ 2GPI) an autoantibody associated with APLS rapport pregnant miscarriage and thrombosis [8,23]. This study is case-control and objectives to explore what is the range affective of toxoplasmosis co-infection on GCSLE women and the abortion situation they experience, through the determination of level titer values of some serum immunological indicators.

## Materials and Methods

### The study design

The current scientific research was carried out over a period extended from 5th of November/2024 to 6th of May/2025 taken place within Al-Nassiriyah City of Thi-Qar province, Iraq. Three patient's categories; (G $\alpha$ ) were 56 seropositive toxoplasmosis (IgG or IgM) detected in Bint-Alhuda Teaching Hospital, Al-Nassiriyah City, Iraq. The CCESL patients obtained from Rheumatology Consult Unit in Artificial Kidney Dialysis Center of AlHussien Teaching Hospital, Thi\_Qar, Iraq, subjected to anti-(IgG and IgM) Ab of toxoplasmosis detection to separate between CCESL seropositive(coinfection) (G $\beta$ ) and CCESL seronegative (G $\gamma$ ) were 44 and 53 respectively. The study also included 45 women apparently in strong healthy as control (G $\delta$ ). Regarding the all CCSLE patients included in the study subtended the revised European League against Rheumatism (EULAR)/American College of Rheumatology (ACR) classification criteria and according to diagnostic by specialist physicians [9,10], for married patients in all groups the recurrent abortion times recorded. Each patient if had any chronic illness, another overlapping inflammatory or connective tissue disease, was not included in this study. The different information of patient's clinical history had been recorded, also the study was completed approving to the Helsinki Declaration of ethical rules for medicinal investigations involving human subjects[11].

### Methodology of the experiments

Commonly pattern, 5ml of venous blood with aseptic situations from patients and control group collected that divided into; 3ml in gel-activator (left for 8-10min.) and aliquot of 2ml potassium EDTA tubes for serum and ESR tests respectively, for serum obtaining from 3ml blood was centrifuged at 3500rpm/min. for 20min., the serum products were distributed into many aliquots of 125 $\mu$ l as a replicates that stored at -400 $^{\circ}$ C for requirement assessed understanding indicators later. ESR and CRP content tested via Westergren-Katz tube and laboratorial routine measurement respectively [12]. Toxoplasmosis detected according procedure[13]by screening

anti(IgM and IgG) antibodies kits(CAMP medica-Romania), the kits accomplished through the Human Reader and wisher microplate spectrophotometer(Human HS-Germany) in Department of Pathological Analysis, College of science, University of Thi-Qar-Iraq. The anti $\beta$ 2GPI (IgM and IgG) autoantibodies detected via full automated Alegria® instrument by ready test strip package reagents kit (ORGENTEC Diagnostika GmbH-Germany) according manufacturer's instructions in private laboratory. All results of immunological indicators compared with cut off values of manufacturer's instructions each kit.

### The study ethicality

The project study got approval ethics from the Scientific Committee of the Faculty of Science, University of Thi-Qar, Iraq, No.3/11/1330 in 10-6-2024 and ethical approval according scientific partnership contract with the Research Ethics Committee of the General Health Directory, Thi-Qar, Iraq. No.240/2024 in 10-23-2024. All subjected women signed admittance precursory of data gathering, were obtained after being informed of the study's goal. Every subjected woman had the full, unconditional right to withdraw at any time. The population studied were given assertiveness that the data would just be exercised for project targets during the research, hide personal information, and ensured would be governing.

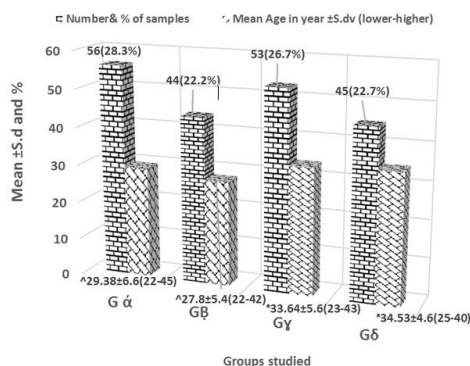
### Statistical analysis

The statistical interpretation for outputs of this study through the ready software IBM-SPSS statistic program (version26). All titer values and percentages expressed as mean of triplicate  $\pm$  standard deviation (S.dv ) or error (S.E), the homogeneity variances of samples in groups investigated by Levene statistic test, also the differences between means and the highest significant mean detected by post-hoc test of Scheffe at  $\alpha < 0.01$  of one-way ANOVA analysis, also the liner correlation between immunological markers and severity of CCESL progression scrutinized with Pearson coefficient bivariate, the p value  $< 0.01$  was considered high significant(\*\*)[14].

## Results

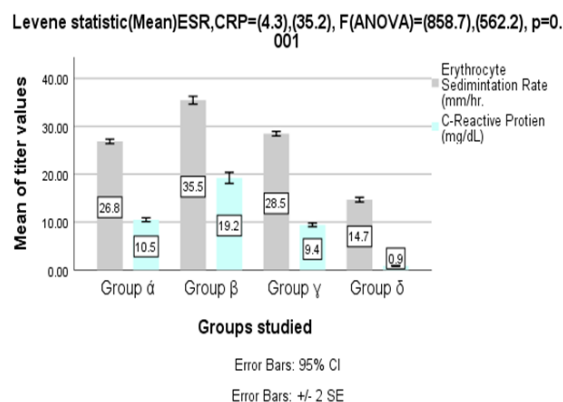
### Data of samples and blood indicators

The general data of all subjected women in this study had been distributed into subgroups which classified based on percentage of immunological markers, clinical manifestation and medicinal history which illustrated in (Figure 1).



**Figure 1.** Percentage of samples population subgroups, and average age. ^ No significant (between G $\alpha$  and G $\beta$ ), \* no significant (between G $\gamma$  and G $\delta$ ) at  $p < 0.01$ . The average of total ages =  $31.34 \pm 3.25$  (22-45)

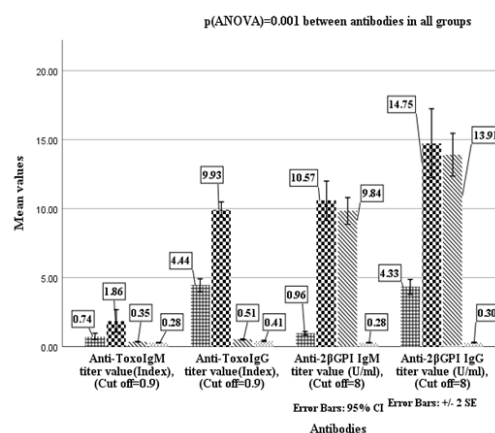
About hematological criteria, whether blood Erythrocyte Sedimentation Rate (ESR) and serum C-Reactive Protein (CRP) the results shown there was high significant elevation both values in women of G $\beta$  than rest subjected groups at statistical scheffe test  $\alpha = 0.01$ , (Figure 2).



**Figure 2.** Simple error bar histogram for both means of ESR (mm/hr.) and of CRP (mg/dL) between groups studied.

### The estimation of immune auto and antibodies

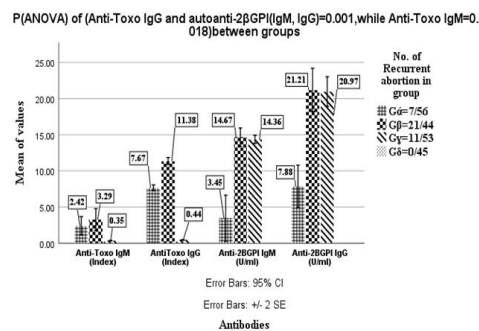
According the consequence gained from subjected antibodies measurement there was high significant difference between four groups ( $p = 0.001$ ), also was obvious elevation of anti-Toxo IgG, anti- $\beta$ 2GPI IgM and IgG in serum G $\beta$  at  $\alpha = 0.001$  (Scheffe) than other groups, (Figure 3), although the anti-Toxo IgM antibody shown weak mean values but G $\beta$  stilled highest statistically than rest groups.



**Figure 3.** Clustered bar mean histogram of Anti-Toxo (IgM and IgG) and Anti- $\beta$ 2GPI (IgM and IgG) antibodies titre values by INDEX of subjected groups.

### The relationship between antibodies and recurrent abortion (ReA)

Regarding the rapport effect between diversity of antibodies (anti-Toxo (IgM and IgG)) and autoantibodies (anti- $\beta$ 2GPI (IgM and IgG)) to number abortion in subjected women the results shown in (Figure 4) and (Table 1).



**Figure 4.** Clustered Bar Mean of anti-Toxo (IgM, IgG) and autoanti- $\beta$ 2GPI (IgM, IgG) antibodies titer values by index of women with recurrent abortions in groups studied.



**Table 1.** Statistical correlations between antibodies effect and number of women with recurrent abortions in subjected groups

Antibodies and autoantibodies				
Number of recurrent abortion	Anti-Toxo IgM	Anti-Toxo IgG	Anti-2BGPI IgM	Anti-2BGPI IgG
Pearson Correlation	-0.301-	-0.635- <sup>**</sup>	0.631 <sup>**</sup>	0.517 <sup>**</sup>
Sig. (2-tailed)	0.063	0	0.002	0.001
N	39	39	39	39

<sup>\*\*</sup>. Correlation is significant at the 0.01 level (2-tailed)

## Discussion

The data of all women underwent in the current research shown that the average age was in youthfulness and fertility period, also according statistical test of Levene based mean was =4.36 ( $p=0.005$ ) which indicates to homogeneity of variance in population studied, there were significant difference(S.D) between groups but No Significant Difference(NS.D) between  $G\alpha$  and  $G\beta$  as same as with  $G\gamma$  and  $G\delta$ , the study in line with[15] indicated to the elevation level of seroprevalence of toxoplasmosis infection in pregnant women globally, this be contingent on the person's age, manner of life, immune activity plane, and disease awareness regarding *T. gondii* in expectant women. GCSLE is multifactorial autoimmune disorder with heterogeneity semblances and can influenced any organ in body, regarding to the women of  $G\beta$  and  $G\gamma$  in this study had been diagnosed through the detection of ANA, antidsDNA, ant Nucleosome(ANuA), and ant Ribosomal P protein (ARPP) autoantibodies according the protocol of [16], that obtained from data of history patients, in addition according to numerous manifestation features within criteria system of both EULAR/ACR[17] by the consultants of autoimmune diseases and rheumatologist. The ESR and C-RP utilized in this study as premonitory clinically indicators to prove the healthiness of control women ( $G\zeta$ ), also to investigate the comparison of range inflammation and elevation in serum between four subjected

groups, the output showed that  $G\beta$  was the highest titer values than  $G\gamma$  and  $G\alpha$  they were equal where  $G\zeta$

the lowest one.

The general results of measurement for level titer values of anti-Toxo(IgM and IgG) and autoanti-2 $\beta$ GPI (IgM and IgG)antibodies in serum of subjected four groups showed in (Figure 3) that the highest values significantly in  $G\beta$  at  $\alpha=0.01$ (Scheffe), synoptically the results in (Table 2)manifest that there were a strong direct rapport between both autoanti-2 $\beta$ GPI (IgM and IgG)antibodies and the number of abortion according Pearson correlation Sig. (2-tailed) while there were inverse relationship and non-relation with anti-Toxo IgG and IgM respectively, it's obvious the autoantibodies 2 $\beta$ GPI (IgM and IgG)antibodies have greatest influence on the occurrence of ReA in subjected women specially in  $G\beta$  that mean the GCSLE women with toxoplasmosis have continuous situation with high number recurrent abortions, where in  $G\gamma$  with  $G\alpha$  the number of ReA decreased based on kind disorder or infection individually compare with control group( $G\zeta$ ) which hadn't cases (0/45) of ReA. Regarding anti-Toxo IgG and IgM antibodies were having synergic role with autoantibodies 2 $\beta$ GPI (IgM and IgG) to increase number of RA especially in  $G\beta$ . Although [18] clarified that was no considerable correlation between acute or chronic *T. gondii* infection with ReA in women with toxoplasmosis but with once or twice abortions there was rapport, while the recent study found that both Anti-Toxo(IgM and IgG) had a magnificent influence



in GCSLE women (G $\beta$ ) which had 47.7%(21/44) RA individuals. The upshots in this research conformity with the results of numerous studies found that the toxoplasmosis infection have a strong an influential correlation and the progression of the severity and complications of immune disorders and various symptoms of GCSLE, including it's effects on ReA in women carry this syndrome, Iraqi study by Abdulhussein et al.,2017 indicated to toxoplasmosis as provoke factor disarray for immune functions in heart maladies patients[19], with Egyptian investigation by Aboukamar et al.,(2023) which proved that both anti-Toxo(IgM and IgG)antibodies associated with graveness advancement[20], Li et al., (2024) suggested that anti-toxoplasmosis antibodies may be a significant risk for SLE prevalence and severity in Chinese community[21].

Although the recent study restricted to investigate its aim around anti-Toxo antibodies and anti-2 $\beta$ GPI autoantibodies, but there may be a belief by this study that there is another immunological factors can affected on the increasing and continuous ReA in GCSLE women like various complements(Cs)[14], autoantibodies(anticardiolipin and Lupus anticoagulant)in phospholipid syndrome[22], and cytokines[5]. On other hand, the current study notarizes that there is an urgent requirement to conduct more diverse comprehensive investigations with precise aspects on the modulations in immunological factors and components that occur during the infection of GCSLE patients with toxoplasmosis, and to determine the levels of the severity of the rapport between them, also the damage that may result. there for further investigation required to study the relationship between toxoplasmosis effectiveness on SLE women through other immunological markers and their impact on disease severity this study recommends.

## Conclusion

The current investigation was able to reach numerous illations that illustrates there were elevated the level titers of serum anti-Toxo(IgM and IgG) antibodies and anti-2 $\beta$ GPI(IgM and IgG) autoantibodies in GCSLE women infected with toxoplasmosis, also there was a synergistic correlation between these immunological antibodies which are causing the increasing rate of the recurrent abortions in GCSLE women, that can be considered

one of the serious complications that accompany patients with this syndrome.

## Conflict of interest

Both authors advertised that there are no conflicts of interest.

## Authors collaborations

Nuha Jabbar Alrikaby designed, statistical analyzed data and supervised the project. Zahraa A.Rzaij recruited patients and provided clinical information, performed the experiments. Both authors wrote the manuscript, revised the manuscript, and approved the final manuscript.

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