# SERUM IL-33 LEVEL IN PREGNANT WOMEN EXPERIENCED MISCARRIAGE WITH / WITHOUT TOXOPLASMOSIS.

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**Abstract**

**Introduction:** Successful pregnancy is related tosafe immunotransitionfrom Th1 to Th2 immune response.Toxoplasmosis is a major cause of miscarriage. IL-33 is related to miscarriage and parasitic infection.

**Aim:** this study aims to investigate seropositivity status of IL-33 inpregnant women with/without toxoplasmosis, with miscarriage cases in first trimester of pregnancy.

**Materials and Methods:** this study implies a case- control design. Forty four blood samples were collected from pregnant women experienced miscarriage and 36 samples from another group with healthy pregnancy as control group. Toxoplasma gonidii was qualitatively and IL-33 was quantitatively detected by Enzyme linked Immunosorbent assay (ELISA), clinical symptoms were collected by the physicians.

**Results:** T. gonidiiwas detected in (15.9%) of pregnant women with miscarriage, and in in (13.8%) in control group, with no significant difference between the two groups.IL-33 concentration showed significant difference among healthy pregnant women positive for anti- T. gonidii IgG antibodies and those negative anti- T. gonidii IgG antibodies, P-value0.002. also ,IL-33 concentration showed significant difference between healthy pregnant women negative for anti- T. gonidii IgG antibodies and those with miscarriage and negative for anti- T. gonidii IgG antibodies, P-value0.008.

**Conclusion:** Disruption in serum IL-33 is contributed with miscarriage and with toxoplasmosis. Such finding may pave the way to imply IL-33 based treatment to control pregnancy failure.

**Keywords -** Toxoplasmosis, IL-33, Miscarriage

## I. INTRODUCTION

Toxoplasma gonidii is the sole species of Apicomplexa, it is an obligative intracellular parasite. Toxoplasmosis shows low specificity, as it infects many mammals and birds. Infection during pregnancy may result with severe consequences, intrauterine mental and physical retardation, fetal anomalies, and stillbirth. [1].

Toxoplasmosis in immunecompetent people goes asymptomatic and mild, comparing to severe clinical scours in immunocompromised people may ends with death. In patients who survives severe acute infection, reactivation may occur on immune suppression.[2].

Toxoplasmosis ransplacental infection estimated risk increase from 15% in 1st trimester, 44% in 2ed trimester and 71% in 3ed trimester of pregnancy. Severity of congenital toxoplasmosis is contributed to gestational phase. Highest disease severity is recorded in first and second trimester of pregnancy which results in miscarriage[3].

Although association of congenital toxoplasmosis with miscarriage has already been documented, immune -mediated mechanism is predicted but exact signaling pathway still questionable and requires investigation.[4].

IL-33 belongs to IL-1 family, play critical role in immune homeostasis.IL-33 and its receptor ST2 are abundantly expressed at the maternal –fetal interface which in turn provides fetus with nutrition ,ensures the adaptation to this semi allogeneic structure in addition to maintain defense against different pathogens [5]. Clinical studies recorded abnormal IL-33/ST2 signaling pathway is contributed with recurrent pregnancy loss.[6].

We propose the hypothesis that toxoplasmosis may induce IL-33 production that in turn will induce miscarriage due to its role in attenuate the conversion of Th1 to Th1 immune response.

This work aims to investigate the possible role of IL-33 in miscarriage in pregnant women infected with toxoplasma. That may pave the way explore immune-based therapy to attenuate the adverse effect of toxoplasmosis.

## II. MATERIALS AND METHODS:

This is a case control study. Samples were collected from the period of January- May /2024. From Alemamain Alkadhemain medical city. All patients were asked to assign a formed consent. This article has achieved ethical approval from (Research Ethics Committee/ Institute Review Board)/ NO. 20240541.

This study involved44 pregnant women, with miscarriage, in their 1st trimester of pregnancy),Inclusion criteria, pregnant with miscarriage negative for pathogens examined by Rubella, CMV, Herpes simples and HIV.And 36 healthy pregnant women. Exclusion criteria, any participant with infection, autoimmune disease or receiving anti-inflammatory drug.

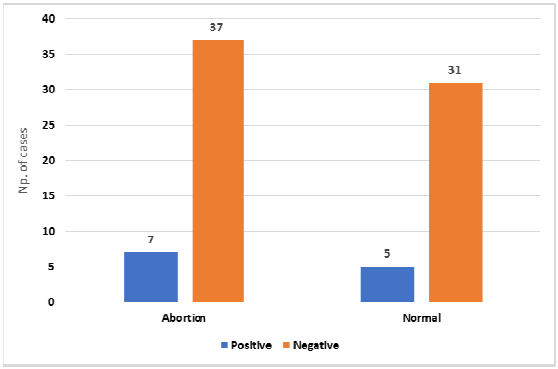
Clinical symptoms; fever, headache, sore throat abdominal painand cough; were collected by physicians in hospital. 5 ml of blood was collected from each participant, in a gel tube, after separation, serum was collected in eppendorf tube and stored in -20 $C^{o}$ till being tested. Anti- IgG Toxoplasma was examined by qualitative Enzyme Linked Immuno Sorbent assay (ELISA) kit , Sunlong Medical, China. IL-33 was quantitatively detected by ELISA kit, Sunlong Medical, China.

Statistical analysis:Data analysis was performed using "package for the social science software" SPSS-23. Data normal distribution was first studied.Non- normally distributed data were presented as median, mean ± standard deviation (±SD) comparison using Mann- Whitney U- test. Categorical data were described as count and percentage. 95% confidence interval was applied, P-value less or equal than 0.05 considered as significant.

## III. RESULTS

This study involved 44 pregnant women with miscarriage, in their 1st trimester of pregnancy with age mean (28.18 ± SD 6.74),and 36 healthy individuals with age mean (26.44±6.36), no significant difference was detected between the two groups, P-value 0.243.

Among women experienced miscarriage 7of 44 were positive for T.gonidii (15.9%) , while in control group 5 out of 36 (13.8%)were positive for T.gonidii.Fisher exact test showed no significant difference between the two groups. P-value, 1.(Figure-1).




**(Figure-1): Number of toxoplasmosis in pregnant women with miscarriage and in healthy pregnant women.**

Serum IL-33 was investigated in both groups.In women experienced miscarriage the median concentration was 51.05 pg/ml comparing with 12.3 pg/ml in healthy controls. Significant difference was recorded between the two groups, P-value 0.018.

Among the 44 women with miscarriage, most miscarriage casesoccurred in 3ed month, 2ed month and 1stmonth, 20, 18 and 6, respectively. Further, among this group, IL-33 was highest among women in second month of 1st trimester, 3ed month and 1st month, 75.5pg/ml (4.6-113.5),52.72pg/ml (2.2-178) and 16.1pg/ml (4-187.3), respectively. (Table -1).

**Table-1: Concentration of IL-33 in pregnant women experienced miscarriage during 1st trimester of pregnancy.**

| Miscarriage | 1st month N=6 Median (Range) | 2nd month N=18 Median (Range) | 3rd month N=20 Median (Range) | P value |
|---|---|---|---|---|
| S. IL-33 (pg/ml) | 16.1 (4-187.3) | 75.5 (4.6-113.5) | 52.72 (2.2-178) | 0.643 |

IL-33 concentration showed significant difference among healthy pregnant women positive for anti- T. gonidiiIgG antibodies and those negativeanti- T. gonidii IgG antibodies, median (range) 104(11.6-109) and 10.1(1.2-101) respectively, P-value0.002.

While no significant difference was recorded among pregnant women with miscarriage positive for anti- T. gonidii IgG antibodies and those negative anti- T. gonidii IgG antibodies, median (range) 81.8 (13.2-106.5) and 31(2.2-187.3) respectively, P-value0.450. Further,IL-33 concentration showed significant difference between healthy pregnant women negative for anti- T. gonidii IgG antibodies and those with miscarriage and negative for anti- T. gonidii IgG antibodies, median (range) 10.1 (1.2-101) and 31 (2.2-187.3) respectively, P-value0.008. in contrast, no significant difference in serum IL-33 between

healthy pregnant women positive for anti- T. gonidii IgG antibodies 104(11.6-109) and those with miscarriage and positive for anti- T. gonidii IgG antibodies 81.1 (13.2-106.5), P-value0.343.(Table-2).

**(Table-2): Serum concentration of IL-33 in women with miscarriage and in healthy pregnant women.**

| Parameter | Group | Positive Median (Range) | Negative Median (Range) | P value |
|---|---|---|---|---|
| S. IL-33 (pg/ml) | Miscarriage | 81.8 (13.2-106.5) | 31 (2.2-187.3) | 0.450 |
| | Healthy pregnancy | 104 (11.6-109) | 10.1 (1.2-101) | 0.002 |
| P -value | | 0.343 | 0.008 | |

Regarding recorded clinical symptoms, IL-33 showed higher concentration in women with miscarriage suffer sore throat comparing with healthy group, P-value 0.008. While did show any significant difference between the same two groups regarding other clinical symptoms, P-value ≥0.05. (Table-3)

**(Table-3):Comparison of serum concentration of IL-33 in women with miscarriage according to clinical symptoms.**

| Parameter | Present (N=25) Median (Range) | Absent (N=19) Median (Range) | P - value |
|---|---|---|---|
| Fever | 65 (2.2-187.3) | 37.1 (7.33-136) | 0.896 |
| Headache | 31 (2.2-178) | 87.4 (4.2-187.3) | 0.101 |
| Abdominal pain | 51.05 (2.2-187.3) | 47.5 (4.6-136) | 0.665 |
| Sore throat | 15.4 (2.2-136) | 88.3 (4.6-187.3) | 0.008 |
| Cough | 51.05 (2.2-187.3) | 49.67 (4.6-113.5) | 0.906 |

## IV. DISCUSSION

Successful embryo implantation, pregnancy maintenance and safe parturition implies essential uterine immunological transformation. Continuous and excessive pro-inflammatory milieu had been contributed to poor outcome inpregnancy.[7]. Dysregulated IL-33/ ST2 signaling pathway was recorded to be implicated in recurrent pregnancy loss and preterm birth.[8].Congenital toxoplasmosis may result in miscarriage, growth retardation, and stillbirth. Transplacental transmission occurs in 1st trimester of gestation.[2]. Aim of this work is to investigate possible role of IL-33 in women experienced miscarriage with/withouttoxoplasmosis.

This study revealed that no significant difference in number of toxoplasmosis cases between women with miscarriage (15.9%) and healthy pregnant women (13.8%). This is thought to be due to the high endemecity of toxoplasmosis in Iraq.Several national studies recorded the high prevalence of toxoplasmosis in Iraq. A study conducted in Al-Najaf city, recorded that anti- T. gonodii IgG antibodies was found in 42.1% of healthy people using both ELISA and Immunochromotography tests, while anti- T. gonodii IgM antibodies had not been detected in any sample.[9].

In another study conducted in Al-Anbar city/Iraq,used nested-PCR and real time polymerase chain reaction (RT-PCR) techniques revealed that prevalence of toxoplasmosis was 30% and 38.5% in healthy women visiting veterinary clinics, respectively.[10].

Prevalence of anti-T. gonodiiIgG antibodies are also high in regional countries , a study conducted in Saudi Arabia, included studies during 18 years from 2000- 2018 revealed that 1/3 of population are positive for anti- T. gonodii IgG antibodies and 4.6% of population had IgM seropositivity. [11]. In Cairo, 46.6% toxoplasmosis prevalence was recorded.While , 22.4% prevalence anti-T. gonodii IgG antibodies was recorded in Sudan[9].

Present workalso revealed that IL-33 is higher in pregnant women (negative for toxoplasmosis) with miscarriage comparing with those have healthy pregnancy, P-value≤0.05.This result is in accordance with other published data.Previous experimental study conducted on mice showed that IL-33/ST2 signaling pathway play essential role in immune regulation and modification during pregnancy. Animals with knocked-out IL-33 genesuffer defects in embryo implantation, abnormal vascularization and decidualization during first trimester of pregnancy.[8].

IL-33 promotes Th-1 cell differentiation and is considered as an important inducer for the Th-1 cytokine, INF-y.[12].In turn INF-g is well documented cytokine that play crucial role in recurrent pregnancy loss.[2].

Further, IL-33 has the potential to activate and recruit NK- cells, which are potential producer of INF-y and TNF- a. Those cytokines are important mediators in Th-1 immunity and proper concentration of those cytokines are crucial in implantation, placentation pregnancy success. [13] and [2].

Long zhaoand his colleagues in 2020 conducted a research in Chian, found that IL-33 and its receptor, suppressor of tumorgenecity 2 (ST2)pathway isimplicated in attenuating the immunoconvergence from Th-1 to Th-2 during pregnancy, which

considered a potential step to secure pregnancy success, resulting in fetus rejection and subsequently miscarriage.Up- and down regulation of IL-33 would either directly or indirectly affects pregnancy maintenance and fetal development through affecting levels of other related cytokines like INF-g and TNF-α .[6].

Another main finding of this study is that IL-33 was significantly different between healthy women negative and positive for anti-T. gonodii IgG antibodies, P-value ≤0.05.

Interaction of T. gonidii with host immune system still questionable, further investigations are needed to exhibitinformation regarding this field. Toxoplasmosis was recorded to record significant increment in plasma proinflmmatory cytokines INF-y and TNF-α , while no significant change was recored in anti-inflammatory cytokines IL-10 and TGF-β [1]. It seem that toxoplasmosis enhance Th1 inflammatory cytokines,results in dangerous pregnancy outcome. However contribution of toxoplasmosis with IL-33 productionhas not been verified. To the far of our knowledge, this is the first study which contribute toxoplasmosis with IL-33 and miscarriage.

## V. CONCLUSION

Toxoplasmosis is endemic in Iraq with high prevalence in women with/without miscarriage. Mechanism of interaction between T. gonidiiand hostIL-33 is not fully specified and further studies are required to venture such crucial research area.Disruption in serum IL-33 is contributed with miscarriage and with toxoplasmosis. Thisstudy highlights IL-33 as critical biomarker for early prediction of pregnancy loss, providing novel target for idiopathic miscarriage treatment.

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