ORIGINAL ARTICLE

Are alterations in melatonin and inflammatory cytokine serum levels linked with recurrent abortion in pregnant women with acute toxoplasmosis: The interacted nexus

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Abstract

Objectives: To investigate melatonin serum level and inflammatory biomarkers in acute toxoplasmosis in pregnant women with recurrent abortions.

Methods: The case-control study was conducted at the Department of Parasitology, together with the Department of Obstetrics, Al-Yarmouk teaching hospital, Baghdad Iraq, from September 2018 until February 2019. Included were 60 pregnant women in first trimester with acute toxoplasmosis (n=28) or without toxoplasmosis (n=32) and with the history of recurrent abortions with a control group of healthy pregnant women (n=25). Melatonin and interleukins (IL-10, 1L-12) serum levels were evaluated in all groups. The data analysis was done by using SPSS 20.

Results: The mean serum melatonin level was lower in the pregnant women with acute toxoplasmosis (69.57±17.37 pg/ml) as compared to the pregnant women without acute toxoplasmosis (77.25±24.35 pg/ml), but the difference was not significant (p=0.43). Healthy pregnant women had a higher serum melatonin level (117.48±34.88 pg/ml) compared with the pregnant women with/without acute toxoplasmosis and a history of recurrent abortions. IL-10 serum level was higher in the healthy controls (12.73±2.58 pg/ml) as compared with the pregnant women with acute toxoplasmosis (5.50±1.92pg/ml) or without acute toxoplasmosis (8.50±2.53 pg/ml), (p<0.0001). Serum melatonin level was positively correlated with serum IL-10 level (P<0.001, r=0.94), and negatively correlated with serum IL-12 level in pregnant women with recurrent abortions with or without acute toxoplasmosis.

Conclusion: Acute toxoplasmosis during pregnancy increases the risk of abortion through reduction of maternal serum melatonin level and dysregulation of pro-inflammatory/inflammatory cytokines axis.

Keywords: Melatonin, Inflammatory biomarkers, Acute toxoplasmosis, Recurrent abortion.

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Introduction

Toxoplasma gondii (*T. gondii*) is an intracellular parasite with the ability to infect various types of mammalians. *T. gondii* can cross the placental barrier causing congenital infection with foeto-maternal complications.[1] Infection with *T. gondii* during pregnancy can lead to various complications including recurrent abortion, preterm delivery, and congenital anomalies.[2,3]

The transmission of *T. gondii* can be through consuming food or water contaminated with the cyst or oocyst or it can be vertical from mother to foetus. A primary infection of the immunologically naïve mother can result in abortion. In general, toxoplasmosis is asymptomatic in the immune competent individual but serious disease can develop in immuno-compromised patients.[4]

Pregnancy is a state of immunological tolerance in which the inflammatory response with production of pro-inflammatory cytokines is necessary for initial implantation with invasion of trophoblasts and induction of angiogenesis. However, later the potential detrimental effects of the inflammatory response must be counteracted by anti-inflammatory cytokines involving Th1 to Th2 immune response.[5] During pregnancy, maternal hormones like progesterone, estradiol, and melatonin modify the maternal immune response during pregnancy in the presence of foetal antigens through shifting of Th1 to Th2 cytokines activity.[6]

Melatonin is a cyclical hormone released from pineal gland mainly at night involved in the regulation of body circadian rhythm. Extra-pineal melatonin has been identified to be released from placenta, retina, lymphocytes, and bone marrow cells that act in a paracrine manner in regulation of normal physiological functions.[7]

Indeed, interruption of the circadian rhythm of melatonin may undesirably disturb the embedding and pregnancy achievement and can be associated with complications as preeclampsia, preterm delivery, and abortion.[8]

During pregnancy, serum melatonin concentrations are

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altered, in particular, the first peak of serum melatonin occurs in the first 24 weeks of gestation, which is elevated all over again after 32 weeks and returns to the physiological level following delivery.[9,10]

Moreover, melatonin has immunomodulatory effects through regulating the release of pro-inflammatory cytokines and lymphocyte proliferation as well as decidual macrophages.[11] Melatonin also enhances the host immune response against the parasite by regulating the secretion of inflammatory mediators. In addition, the inhibition of the activation of inflammatory cells by melatonin has been demonstrated in various conditions of inflammation.[12]

Therefore, the objective of the current study was to explore the serum melatonin level and inflammatory biomarkers in acute toxoplasmosis in pregnant women with recurrent abortions.

Patients and Methods

This case-control study was done at the Department of Clinical Parasitology in alliance with the Department of Obstetrics, Al-Yarmouk Teaching Hospital, College of Medicine, Al-Mustansiriyah University, Baghdad Iraq, from September 2018 until February 2019. It was approved by the Scientific Committee of Department of Parasitology and certified by the Ethics Committee of Medical College, according to the Declaration of Helsinki.

Included in the study were sixty pregnant women with a gestational age < 20 weeks with a history of recurrent abortions with or without AT. Twenty-five healthy pregnant women were included as controls. Three groups were formed as follows: Group I: Pregnant women with recurrent abortions with acute toxoplasmosis (n=28), Group II: Pregnant women with recurrent abortions without acute toxoplasmosis (n=32), Group III: Healthy pregnant women (n=25). A detail medical history was noted and a physical and obstetric examination was performed on all included women.

The inclusion criteria were, pregnant women with gestational age <20 weeks with a history of recurrent abortions and with/without acute toxoplasmosis. Healthy pregnant women with gestational age <20 weeks were taken as controls.

The exclusion criteria were, pregnant women with chronic diseases like gestational hypertension, preeclampsia, gestational diabetes, and other associated disorders.

For biochemical studies, 5 ml venous blood was collected in a plain tube from each study participant. The serum was separated and stored under -20°C until it was tested. Anti Toxoplasama IgM Abs was tested for each women included in the study using ELISA kit method (Bioactiva Diagnostica/ Germany). The serum concentration of melatonin was estimated by using ELISA kit method (CUSABIO, Mabtech Company/ Germany and Biosoures/ Belgium). Interleukins 10 and 12 (IL-10, IL-12) were estimated by ELISA kit methods (Bioactiva Diagnostica/ Germany).

Data of the present study was analyzed by using SPSS version 20 and presented as means and standard deviations. Un-paired student t test was applied to detect the significance of differences between two groups. Besides, one way analysis of variance (ANOVA), post-hoc test, and correlation coefficient were applied to detect significance of differences among different treated groups and correlation respectively. P value less than 0.05 was considered significant.

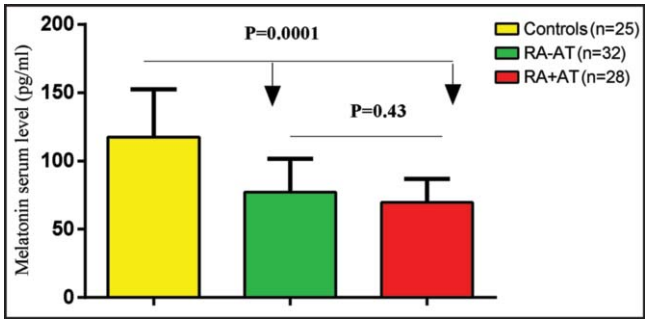
Results

Of the 85 women included in the study, 60 (70.58%) gave a history of recurrent abortions.

Table: Demographic characteristics of the present study.

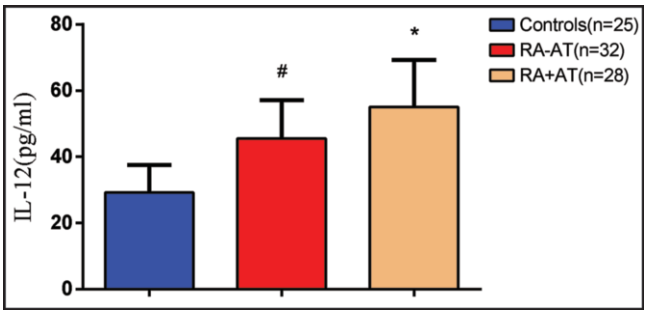
Demographic characteristics	Mean ±SD, n, %
n	85(100)
Pregnant women with recurrent abortion	60(70.58)
With acute toxoplasmosis	28(46.66)
Without acute toxoplasmosis	32(53.33)
Healthy pregnant women	25(29.41)
Gestational age	11.23±4.21
Gravidity	3.14±2.27
Parity	1.5±0.94
Previous abortion	0.75±0.45
Anti-Toxo (IgM)	
Pregnant women with acute toxoplasmosis	26.60±10.30
Pregnant women without acute toxoplasmosis	0

Data are presented as mean ±SD, Anti-Toxo (IgM): anti-toxoplasma antibody titer.



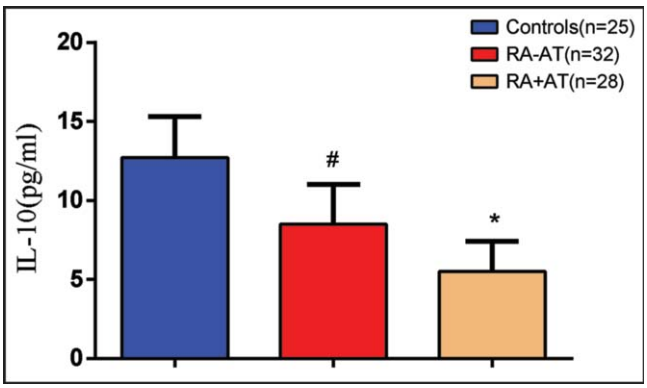
RA-AT: Recurrent abortion without acute toxoplasmosis.
RA+AT: Recurrent abortion with acute toxoplasmosis.

Figure-1: Serum melatonin level in the pregnant women with recurrent abortion with or without acute toxoplasmosis.



RA-AT: Recurrent abortion without acute toxoplasmosis.
RA+AT: Recurrent abortion with acute toxoplasmosis.
*** P<0.01 as compared with control and RA-AT.**
 # P<0.01 as compared with control and RA+AT.

Figure-2: IL-12 serum level in the pregnant women with recurrent abortion with or without acute toxoplasmosis.



RA-AT: Recurrent abortion without acute toxoplasmosis.
RA+AT: Recurrent abortion with acute toxoplasmosis.
*** P<0.01 as compared with control and RA-AT.**
 # P<0.01 as compared with control and RA+AT.

Figure-3: IL-10 serum level in the pregnant women with recurrent abortion with or without acute toxoplasmosis.

Of these 60 women, 28(46.66%) were associated with acute toxoplasmosis and 32 (53.33%) were not infected. The mean of gestational age was 11.23±4.21 weeks and the mean gravidity and parity was 3.14±2.27 and 1.5±0.94 respectively, (Table).

Serum Melatonin level was lower in the pregnant women with acute toxoplasmosis (69.57±17.37 pg/ml) as compared with the pregnant women without acute toxoplasmosis (77.25±24.35 pg/ml), but the difference was not significant (p=0.43). The serum melatonin level was higher in healthy pregnant women (117.48±34.88 pg/ml) as compared to the pregnant women with/without acute toxoplasmosis with history of recurrent abortions P<0.0001 (Figure-1).

Regarding the inflammatory biomarkers, IL-12 serum level was increased in the pregnant women with acute

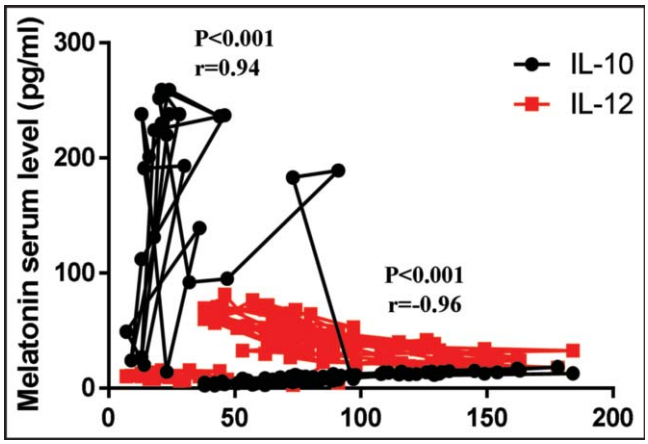


Figure-4: Correlation of serum melatonin level with IL-10 and IL-12 serum levels in the pregnant women with recurrent abortion with or without acute toxoplasmosis.

toxoplasmosis and recurrent abortions (55.009±14.31pg/ml) as compared to the pregnant women without acute toxoplasmosis with recurrent abortions (45.58±11.59 pg/ml), p=0.03. However, IL-12 serum level was lower in normal healthy pregnant women (29.24±8.25pg/ml), p<0.0001, (Figure-2). Besides, IL-10 serum level was higher in the healthy controls (12.73±2.58pg/ml) as compared to pregnant women with acute toxoplasmosis (5.50±1.92pg/ml) or without acute toxoplasmosis (8.50±2.53 pg/ml), P<0.0001, (Figure-3).

Serum melatonin level was positively correlated with IL-10 serum level (P<0.001, r=0.94), and negatively correlated with IL-12 serum level in the pregnant women with recurrent abortions with or without acute toxoplasmosis (Figure-4).

Discussion

Spontaneous abortion is a cessation of pregnancy prior to 20 weeks of gestation, which is assessed to happen in 40% of all pregnancies.[13] The precise mechanisms of abortion induced by *T. gondii* infection are still unclear. However, abnormal immune response induced by acute toxoplasmosis leads to dysregulation of immunological tolerance due to over-production of Th1 pro-inflammatory cytokines. Aberrant production of pro-inflammatory cytokines, have been suggested to be related to the frequency of abortion caused by *T. gondii* infection.[14] These findings support results of the present study which confirmed the association between recurrent abortions and acute toxoplasmosis as evident by high circulating Anti-IgM serum levels.

Besides, the present study illustrated that the serum melatonin level was markedly reduced in the pregnant women with acute toxoplasmosis.

Throughout pregnancy, changes in the serum melatonin level have a noticeable role in the action of the immune response. Notably, melatonin is assumed to govern a number of the T-cell subpopulation, which is involved in the development of abnormal gestation.[15]

Moreover, serum melatonin level is increased noticeably during pregnancy, up to 100 fold higher in third trimester compared with healthy non-pregnant women, to promote syncytium formation, suggesting an essential role of this hormone in the placental function and pregnancy well-being.[16] Pregnant women with placental insufficiency had low level of melatonin with high level of pro-inflammatory cytokines.[17] Herein, low serum melatonin in the pregnant women with acute toxoplasmosis might be due the placental dysfunction.

Besides, a compound like melatonin, which has a pro-inflammatory effect, can also efficiently suppress inflammatory responses during acute toxoplasmosis.[18] Melatonin could stimulate release of IL-2 from dendritic cells and lymphocytes, which inhibit release of melatonin. At an early stage of implantation melatonin stimulates the production of pro-inflammatory cytokines, which facilitate the implantation. Later melatonin acts as an immune suppressive molecule through release of anti-inflammatory cytokines to counteract the effect of pro-inflammatory cytokines.[19] These findings could explain the positive association between melatonin, pro-inflammatory cytokines, and negative association with inflammatory cytokines of the present study.

A search on different studies in literature, it was observed that *T. gondii* during pregnancy disrupts the anti-inflammatory effects of melatonin leading to hyper-production of inflammatory IL-12 cytokine and suppresses the production of anti-inflammatory cytokine IL-10.[20] Indeed, melatonin enhances the production of steroid hormones which are present in higher concentrations at maternal-foetal interface to ensure successful pregnancies.[21] Reduction of melatonin leads to decline in the level of steroid hormones, so low levels of steroid hormones and high levels of pro-inflammatory cytokines are attributable for increased risk of abortions.[22] However, steroid hormone serum levels were not evaluated in the present study.

Thus, infection with *T. gondii* leads to hyper-production of the inflammatory cytokine IL-12 and hypo-production of anti-inflammatory cytokine IL-10, which in turn may exert direct or indirect effects that may be lethal to the foetus. A state of immune-pathological effect may arise after immune response to the infection, which may be protective to the mother but it is harmful to the foetus.

The present study had several limitations as a small sample size. The pro-inflammatory cytokines like tumour necrosis factor alpha (TNF-α), which affects pregnancy outcomes and melatonin effects were not estimated. However, the present study gave a clue to the association between recurrent abortions and low melatonin serum levels. Large prospective clinical trials are warranted for more authentic results.

Conclusion

Acute toxoplasmosis during pregnancy increases the risk of abortion through reduction of maternal serum melatonin level and dysregulation of pro-inflammatory/inflammatory cytokines axis. Therefore, melatonin supplement may prevent risk of recurrent abortions in pregnant women with acute toxoplasmosis. Large-scale prospective studies are warranted in this regards.

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References

1. Al-Kuraishi AH, Al-Windy SD, Al-Kuraishy HM, Al-Gareeb AI. Endothelial dysfunction in acute acquired toxoplasmosis. Trop Parasitol 2020;10:29-33. doi: 10.4103/tp.TP_26_19.
2. Al-Kuraishy HM, Al-Kuraishi AH, Al-Windy S, Al-Gareeb AI. Toxoplasmosis and Risk of Endothelial Dysfunction: Role of Oxidative Stress and Pro-Inflammatory Mediators, Arch Clin Infect Dis 2019;14:e95563. doi: 10.5812/archcid.95563.
3. Olariu TR, Blackburn BG, Press C, Talucod J, Remington JS, Montoya JG. Role of Toxoplasma IgA as Part of a Reference Panel for the Diagnosis of Acute Toxoplasmosis during Pregnancy. J Clin Microbiol 2019;57:e01357-18. doi: 10.1128/JCM.01357-18.
4. Oyeyemi OT, Oyeyemi IT, Adesina IA, Tiamiyu AM, Oluwafemi YD, Nwuba RI, et al. Toxoplasmosis in pregnancy: a neglected bane but a serious threat in Nigeria. Parasitology 2020;147:127-34. doi: 10.1017/S0031182019001525.
5. Al-Maiahy TJ, Al-Gareeb AI, Al-kuraishy HM. Prolactin and risk of preeclampsia: A single institution, cross-sectional study. Asian Pac J Reprod 2019;8:112-7. DOI: 10.4103/2305-0500.259168
6. Al-Maiahy TJ, Al-Gareeb AI, Al-kuraishy HM. Testosterone is a surrogate and proxy biomarker for severity of late-onset preeclampsia: A cross-sectional study. Asian Pac J Reprod 2020;9:1-8. DOI: 10.4103/2305-0500.275522
7. Cecon E, Liu L, Jockers R. Melatonin receptor structures shed new light on melatonin research. J Pineal Res 2019;67:e12606. doi: 10.1111/jpi.12606.
8. McCarthy R, Jungheim ES, Fay JC, Bates K, Herzog ED, England SK. Riding the Rhythm of Melatonin Through Pregnancy to Deliver on Time. Front Endocrinol 2019;10:e616. doi: 10.3389/fendo.2019.00616.
9. Al-kuraishy HM, Al-Maiahy TJ, Al-Gareeb AI, Musa RA, Ali ZH. COVID-19 pneumonia in an Iraqi pregnant woman with preterm delivery. Asian Pac J Reprod 2020;9:156-8. DOI:

10.4103/2305-0500.282984
10. Al-Nami MS, Al-Kuraishy HM, Al-Gareeb AI, Al-Mamoori F. Metabolic profile and prolactin serum levels in men with type 2 diabetes mellitus: Old-new rubric. Int J Crit Illn Inj Sci 2019;9:120-6. doi: 10.4103/IJCIIS.IJCIIS_40_19.
11. Al-Kuraishy HM, Hamada MT, Al-Samerraie AY. Effects of metformin on omentin levels in a newly diagnosed type II diabetes mellitus: Randomized, placebo controlled study. Mustansiriya Med J 2016;15:49-55.
12. Al-Kuraishy HM, Al-Gareeb AI, Faidah H, Al-Maiahy TJ, Cruz-Martins N, Batiha GE. The Looming Effects of Estrogen in Covid-19: A Rocky Rollout. Front Nutr 2021;8:e649128. doi: 10.3389/fnut.2021.649128.
13. Al-Kuraishy HM, Al-Gareeb AI, Al-Maiahy TJ. Concept and connotation of oxidative stress in preeclampsia. J Lab Physicians 2018;10:276-82. doi: 10.4103/JLP.JLP_26_18.
14. Arefkhah N, Pourabbas B, Asgari Q, Moshfe A, Mikaeili F, Nikbakht G, et al. Molecular genotyping and serological evaluation of Toxoplasma gondii in mothers and their spontaneous aborted fetuses in Southwest of Iran. Comp Immunol Microbiol Infect Dis 2019;66:e101342. doi: 10.1016/j.cimid.2019.101342.
15. Bae H, Yang C, Lee JY, Park S, Bazer FW, Song G, et al. Melatonin improves uterine-conceptus interaction via regulation of SIRT1 during early pregnancy. J Pineal Res 2020;69:e12670. doi: 10.1111/jpi.12670.
16. Mokhtari F, Akbari Asbagh F, Azmoodeh O, Bakhtiyari M, Almasi-Hashiani A. Effects of Melatonin Administration on Chemical Pregnancy Rates of Polycystic Ovary Syndrome Patients Undergoing Intrauterine Insemination: A Randomized Clinical Trial. Int J Fertil Steril 2019;13:225-9. doi: 10.22074/ijfs.2019.5717.
17. Nehme PA, Amaral FG, Middleton B, Lowden A, Marqueze E, França-Junior I, et al. Melatonin profiles during the third trimester of pregnancy and health status in the offspring among day and night workers: A case series. Neurobiol Sleep Circadian Rhythms 2019;6:70-6. doi: 10.1016/j.nbscr.2019.04.001.
18. Berbets AM, Barbe AM, Yuzko OM. Constant light exposure terminates pregnancy in rats with pineal gland dysfunction, low melatonin level and pro-inflammatory response. Melatonin Res 2019;2:9-24. DOI: 10.32794/mr11250038.
19. Liu WX, Tan SJ, Wang YF, Li L, Sun XF, Liu J, et al. Melatonin ameliorates murine fetal oocyte meiotic dysfunction in F1 and F2 offspring caused by nicotine exposure during pregnancy. Environ Pollut 2020;263:e114519. doi.10.1016/j.envpol.2020.114519
20. Machado NI, Dos Santos TAT, de Souza W, DaMatta RA, Seabra SH. Treatment with melatonin induces a reduction of Toxoplasma gondii development in LLC-MK2 cells. Parasitol Res 2020;119:2703-11. doi: 10.1007/s00436-020-06766-5.
21. Soni N, Pandey AK, Kumar A, Verma A, Kumar S, Gunwant P, et al. Expression of MTNR1A, steroid (ER?, ER?, and PR) receptor gene transcripts, and the concentration of melatonin and steroid hormones in the ovarian follicles of buffalo. Domest Anim Endocrinol 2020;72:e106371. doi: 10.1016/j.domaniend.2019.06.003.
22. Kale K, Vishwekar P, Balsarkar G, Jassawalla MJ, Alkahtani S, Kishore U, et al. Serum Levels of Collectins Are Sustained During Pregnancy: Surfactant Protein D Levels Are Dysregulated Prior to Missed Abortion. Reprod Sci 2020;27:1894-1908. doi: 10.1007/s43032-020-00209-3.