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

Azhar Hatif Al-Kuraishi,1 Hatham Ibraheem Khalil,2 Huda Hameed Hassan,3 Hayder Mutter Al-Kuraishy4

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, IL-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±1.92 pg/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.

Introduction

Toxoplasma gondii (T. gondii) is an intracellular parasite with the ability to infect various types of mammals. 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 naive 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

1-3Department of Microbiology, College of Medicine, 4Department of Clinical Pharmacology, Medicine and Therapeutic, College of Medicine, Al-Mustansiriya University, Baghdad, Iraq.

Correspondence: Hayder Mutter Al-Kuraishy. Email: hayderm36@yahoo.com
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.\textsuperscript{9,10}

Moreover, melatonin has immunomodulatory effects through regulating the release of pro-inflammatory cytokines and lymphocyte proliferation as well as decidual macrophages.\textsuperscript{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.\textsuperscript{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 Toxoplasma 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.

<table>
<thead>
<tr>
<th>Demographic characteristics</th>
<th>Mean ±SD, n, %</th>
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<tbody>
<tr>
<td>n</td>
<td>85(100)</td>
</tr>
<tr>
<td>Pregnant women with recurrent abortion</td>
<td>60(70.58)</td>
</tr>
<tr>
<td>With acute toxoplasmosis</td>
<td>28(46.66)</td>
</tr>
<tr>
<td>Without acute toxoplasmosis</td>
<td>32(53.33)</td>
</tr>
<tr>
<td>Healthy pregnant women</td>
<td>25(29.41)</td>
</tr>
<tr>
<td>Gestational age</td>
<td>11.23±4.21</td>
</tr>
<tr>
<td>Gravidity</td>
<td>3.14±2.27</td>
</tr>
<tr>
<td>Parity</td>
<td>1.5±0.94</td>
</tr>
<tr>
<td>Previous abortion</td>
<td>0.75±0.45</td>
</tr>
</tbody>
</table>

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.

**Figure-1:** Serum melatonin 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), 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 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).
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.\(^1\)

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 syncytiotrophoblast formation, suggesting an essential role of this hormone in the placental function and pregnancy well-being.\(^2\) Pregnant women with placental insufficiency have low level of melatonin with high level of pro-inflammatory cytokines.\(^3\) 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.\(^4\) 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.\(^5\) 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 \textit{T. gondii} during pregnancy disrupts the anti-inflammatory effects of melatonin leading to hyperproduction of inflammatory IL-12 cytokine and suppresses the production of anti-inflammatory cytokine IL-10.\(^6\) Indeed, melatonin enhances the production of steroid hormones which are present in higher concentrations at maternal-fetal interface to ensure successful pregnancies.\(^7\) 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.\(^8\) However, steroid hormone serum levels were not evaluated in the present study.

Thus, infection with \textit{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-\(\alpha\)), 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**


