

## SCIENTIFIC REPORT

## A correlation of pregnancy term, disease activity, serum female hormones, and cytokines in uveitis

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**Background/aims:** Pregnancy and the postpartum period are associated with the activity of autoimmune diseases including uveitis. Although the exact mechanism is unknown, hormones are reported to alter inflammatory cytokines and influence disease activity. The authors studied ocular inflammation, female hormones, and serum cytokine levels during and after pregnancy.

**Methods:** A prospective, observational case study was conducted. Four pregnant women in their first trimester with chronic non-infectious uveitis were followed monthly until 6 months after delivery. Serum female hormones (oestrogen, progesterone, prolactin) and various cytokines (IL-2, IL-4, IL-5, IL-6, IL-10, IFN- $\gamma$ , and TGF- $\beta$ ) were measured by ELISA.

**Results:** The four patients had five full term pregnancies. Uveitis activity decreased after the first trimester but flared in the early postpartum period. Serum female hormones, highly elevated during pregnancy, drastically dropped post partum. Cytokine levels except TGF- $\beta$  were mostly undetectable.

**Conclusion:** Female hormones and TGF- $\beta$  may contribute to the activity of uveitis during pregnancy and the postpartum period.

Many autoimmune diseases in females are known to improve during pregnancy but worsen in the postpartum period, because pregnancy induces in immune deviation promoting anti-inflammatory cytokines that prevent immunological rejection of the allogenic fetus.<sup>1,2</sup> This immunological balance imposed by pregnancy seems to be an antithesis of the immunological imbalance that promotes cell mediated autoimmunity.<sup>3</sup> Recent evidence indicates that there is a Th1, Th2, and transforming growth factor beta (TGF- $\beta$ ) shift during normal pregnancy and in the postpartum period resulting in a change in autoimmune disease activity.<sup>4–6</sup> The relation between female hormones, cytokines, and disease activity during and after pregnancy has not been reported in human uveitis. The aim of this study was to assess serum female hormones, cytokines, and ocular inflammation during and after pregnancy.

## PATIENTS AND METHODS

Four women (five full term pregnancies) in their first trimester of pregnancy with non-infectious uveitis for more than 2 years were enrolled in this National Eye Institute institutional review board approved clinical study after providing written informed consent. Patients had monthly ophthalmic examinations until 6 months post partum. At each assessment serum levels of the female hormones (oestrogen, progesterone, and prolactin) and Th1 (IL-2 and IFN $\gamma$ ); Th2 (IL-4, IL-5, IL-6, and IL-10); and Th3 (TGF- $\beta$ ) cytokine levels were measured with ELISA. The anti-human antibodies used in the ELISA assay were from the Quantikine

kit (R & D Systems, Inc, Minneapolis, MN, USA). TGF- $\beta$  assay measured only the active form.

For examination and presentation of the data, hormone and TGF- $\beta$  values were  $\log_{10}$  transformed and averaged by month of gestation and after delivery over the total pregnancies. To quantify the severity of uveitis each eye received one point for the presence of each of the following conditions: keratic precipitates, anterior chamber cells, vitreous cells, vitreous haze, active uveal lesions, active retinal lesions, cystoid macular oedema (CMO), active retinal vasculitis, other inflammatory findings (for example, synechiae), disease flare, or treatment since last study visit. The totals for the patient's eyes were then averaged to obtain the patient's uveitis severity score, which could range from 0 (no conditions present) to a maximum of 11 (all conditions).

To compare mean levels among the early phase of pregnancy (2–4 months), the late phase (5–9 months), and the postpartum phase (delivery to 6 months), a Monte Carlo of estimation of the p value from the permutation test of the hypothesis of no difference between the phases was conducted. Under this hypothesis the monthly sequence of values for each pregnancy is random and as likely to occur as any other. Within each of the five pregnancies the monthly values were randomly permuted. After permutation, mean values (hormone and TGF- $\beta$  levels were  $\log_{10}$  transformed before analysis) were calculated by phase for each pregnancy, and these means were then averaged over the five pregnancies by phase. This process was repeated for 100 000 iterations, and the fraction of times that the permutation induced difference exceeded the observed difference in absolute value was taken as the point estimate of the two sided p value. For 95% confidence against underestimating the p value, the upper 95% confidence limit was computed and reported as the p value. The testing level was two sided and set at 0.05. Since for each outcome variable there were three comparisons made, the Bonferroni criterion—that is,  $\alpha = 0.05/3$ , was applied to maintain the control of type I error for multiple comparisons.

Trends in the scatter plots were traced by non-parametric local regression (loess) with the LOESS procedure of the Statistical Analysis System (SAS Institute, Inc, Cary, NC, USA). A local second degree least squares fitting at every point were made with the smoothing parameter (that is, fraction of data in the neighbourhood of a point) automatically selected according the Akaike information criterion.

## RESULTS

The four patients were diagnosed with panuveitis secondary to sarcoidosis, idiopathic granulomatous panuveitis with retinal vasculitis, idiopathic anterior uveitis, and idiopathic posterior uveitis (table 1). The patient with sarcoidosis

**Abbreviations:** CMO, cystoid macular oedema; EAU, experimental autoimmune uveitis; EU, experimental induced uveitis; TGF- $\beta$ , transforming growth factor beta

**Table 1** Clinical data of five pregnancies among four women

Case	Age	Diagnosis	Last flare before pregnancy	Change of medication in early pregnancy, late pregnancy, post partum
1	40	Sarcoidosis	4 years	decrease, no change, no change (*Score: 7.45, 5.7, 4.3, 5.3)
	41		5 years	no change, decrease, increase (Score: 5.3, 5.3, 4.3, 6.4)
2	41	Panuveitis	2 months	increase, decrease, increase (Score: NA, 10.5, 8.9, 11.0)
3	20	Iridocyclitis	5 month	increase, decrease, increase (Score: 7.0, 7.5, 5.6, 3.8)
4	33	Intermediate uveitis	>2 years	no change, no change, no change (Score: NA, 5.3, 4.3, 6.4)

\*Uveitis score (before pregnancy, early pregnancy ( $\leq 5$  months), late pregnancy (6, 9 months), postpartum period).

1 Patients were recruited under an institutional review board approved clinical study after providing written informed consent.

2 No patients experienced ocular, systemic, or teratogenic complications during the five pregnancies.

**Table 2** Mean of uveitis scores and geometric means of hormone levels

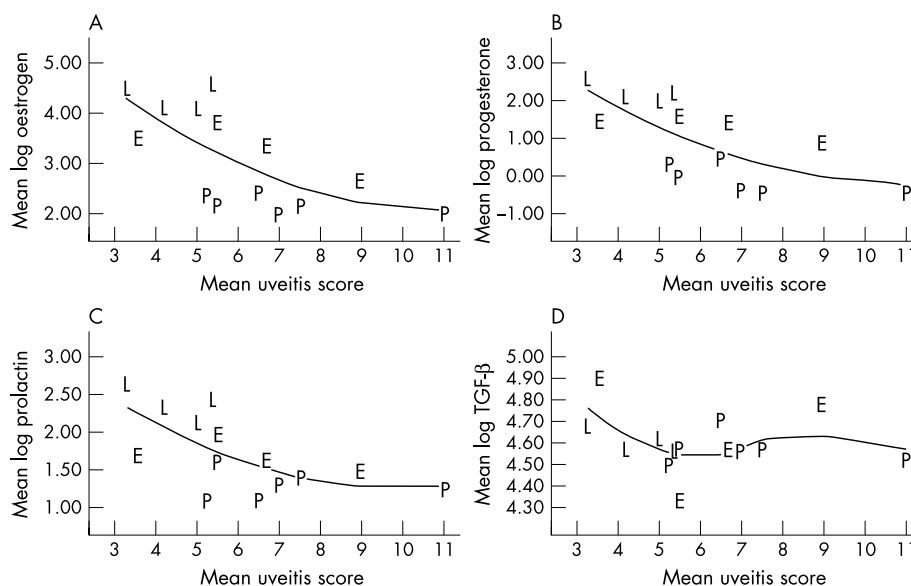
Phase	Uveitis score	Oestrogen*	Progesterone*	Prolactin*	TGF- $\beta$
Early (2–5 months)	7.4	1469	15	31	44 838
Mid (5–9 months)	5.1	10 911	79	148	32 225
Post partum (to 6 months)	6.2	244	1	28	38 134

\*The unit for the hormone and TGF- $\beta$  is pg/ml.

enrolled for two consecutive pregnancies. No patient developed any systemic medical or obstetric complications during pregnancy or in the postpartum period.

An average of nine (range 7–12) clinical visits were recorded for each pregnancy. Overall, uveitis activity was slightly worse in the first trimester but became either inactive or milder in the late pregnancy, which allowed for reduction of immunosuppressive medications in all patients. However, a slight flare of the uveitis was likely in the first 3 postpartum months.

Cases 1 and 4 had milder uveitis activity (uveitis scores in 0–7 range) while cases 2 and 3 had more severe uveitis (uveitis scores in 3–11 range). The ocular disease in patients with milder uveitis was characterised by trace to 2+ anterior chamber cells, trace to 1+ vitreal haze, CMO, inactive chorioretinal lesions, and vasculitis. In contrast, the ocular findings in the two patients with more severe uveitis included 3–4+ anterior chamber cells, 3–4+ vitreal haze, CMO, active chorioretinal inflammatory lesions, and retinal vasculitis.



**Figures 1** (A–D) Plots of monthly mean  $\log_{10}$  of hormones and of TGF- $\beta$  by monthly mean uveitis score. To quantify the severity of uveitis each eye received a point for the presence of each of the following conditions: keratic precipitates, anterior chamber cells, vitreous cell, vitreous haze, active uveal lesions, active retinal lesions, cystoid macular oedema, active retinal vasculitis, other inflammatory findings (for example, synechiae), disease flare, or treatment since last study visit. The totals for the patient’s eyes were then averaged to obtain the patient’s uveitis severity score, which could range from 0 (no conditions present) to a maximum of 11 (all conditions). The lines are loess fittings that non-parametrically track the relations between the variables. “E” designates early pregnancy, up to month 5 of gestation; “L,” late gestation, from month 6 to delivery; and “P,” postpartum period.

**Table 3** Estimated p values for comparisons between pregnancy phases

Comparison	Uveitis score	Oestrogen	Progesterone	Prolactin	TGF- $\beta$
Early v late	0.005*	0.025	0.126	0.002	0.069
Early v postpartum	0.093	0.026	0.016	0.818	0.322
Late v postpartum	0.111	<0.001*	<0.001*	<0.001*	0.302

\*Significantly different according to the Bonferroni criterion  $\alpha=0.05/3$ .

All four patients were on oral prednisone throughout their pregnancies. Two patients (cases 2 and 3) required an increased dose of prednisone in early pregnancy, one from 12.5 mg/day to 30 mg/day and the other from 30 mg/day to 40 mg/day. However, later in the pregnancies of these two patients their prednisone dose was decreased to 7.5 mg/day and 17.5 mg/day, respectively. During the late stage of pregnancy, the uveitis scores in these patients dropped into the milder range of 3–6. The two patients with mild uveitis were maintained on low dose prednisone throughout the pregnancy (case 1, 3 mg/day; case 4, 5 mg/day) (table 1). In the postpartum period following four of the five pregnancies, there was evidence of disease flare requiring increased immunosuppressive therapy.

As expected, all three female hormones—oestrogen, progesterone, and prolactin—steadily and markedly elevated during pregnancy and drastically dropped in the postpartum period (table 2). Interestingly, most Th1 and Th2 cytokines were below detectable levels. Among a total of 270 assays from all five pregnancies, Th1 and Th2 cytokines were detected in only four assays from three patients with posterior uveitis; the Th2 cytokines during pregnancy (IL-5, 42 pg/ml; IL-6, 291 pg/ml; and IL-10, 40.7 pg/ml; respectively), and the Th1 cytokine (IL-2, 42 pg/ml) in the first month postpartum. TGF- $\beta$ , a Th3 cytokine, was consistently detected in all patients throughout the study.

Table 3 provides estimated p values for the comparisons of these means between phases, generated by the Monte Carlo permutation test. At the 0.0166 level of testing, uveitis scores dropped significantly from the early to late phase. All hormones decreased significantly from late phase to postpartum. Progesterone in the postpartum phase was significantly lower compared to both early and late phases. Prolactin significantly increased from early to late while it significantly decreased from late to postpartum. There were no significant differences between phases for TGF- $\beta$ .

Although uveitis scores did not vary freely, as they may have been controlled by immunosuppressive therapy, there was a discernible decrease in the female hormones with increasing uveitis score, but no change in TGF- $\beta$  (fig 1).

## DISCUSSION

Pregnancy is associated with remediation of many autoimmune diseases including rheumatoid arthritis and multiple sclerosis, yet with exacerbation of other autoimmune conditions such as systemic lupus erythematosus.<sup>7–11</sup> Few observational publications on non-infectious ocular inflammation have shown improvement during pregnancy and exacerbation after parturition.<sup>12–17</sup> In a recent large series of 76 pregnancies among 50 women with Vogt-Koyanagi-Harada syndrome, Behçet's disease, and idiopathic uveitis, Rabbiah and Vitale observed a flare up within the first 4 months of pregnancy, a relative inactivity in late pregnancy, and a rebound in activity within 6 months of delivery.<sup>12</sup> We have reported a significantly lower incidence and severity of experimental autoimmune uveitis (EAU) due to higher elevation of circulating TGF- $\beta$  and selective inhibition of Th1 responses in pregnant mice,<sup>18</sup> as well as the effect of sex hormones on cytokine balance in EAU.<sup>19</sup>

This study agrees with other observations demonstrating amelioration of human non-infectious ocular inflammation in mid and late pregnancies and flare up during the postpartum period. Furthermore, we showed the relations among female hormones, TGF- $\beta$ , a Th3 cytokine, and disease activity in a small heterogeneous group. These results are compatible with previous reports in EAU and experimental induced uveitis (EIU) models.<sup>18–20</sup> Although other serum cytokine levels were mostly below the detectable levels, the four measurable Th2 cytokines were found in the pregnancy and the one measurable Th1 cytokine was in the postpartum period. The data suggest that physiological changes of sex hormones before and after delivery may polarise the immune response towards a Th2/Th3 response,<sup>6</sup> which may counterbalance the augmented Th1 response observed in non-infectious uveitis.

Data concerning the fluctuation in uveitis status is certainly quite complex. However, one of the main factors is associated with cytokine changes during pregnancy. In general, Th2 cytokines are associated with the downregulation of Th1 cytokines and may confer protection from Th1-mediated autoimmune diseases. During pregnancy, there is a shift from Th1 to Th2 that occurs both locally,<sup>21</sup> at the fetal maternal interface, and systemically.<sup>22</sup> This immune shift is thought to be necessary to avoid fetal rejection, since failure to achieve a Th1 to Th2 immune deviation has been associated with increased risk of spontaneous abortion.<sup>5</sup> This naturally occurring, systemic shift in immune responses may underlie the improvement in Th1 mediated autoimmune diseases including most autoimmune mediated uveitis during pregnancy.

Female patients with uveitis may require close follow up and treatment in the early postpartum period. Pregnancy and pregnancy related hormones including female hormones and cortisol influence the signs and symptoms of autoimmune diseases.<sup>4–23</sup> The current study also suggests a correlation between uveitis severity and female hormone levels in the graphic display of the data, although the sample size was too small to achieve statistical significance. Recently oral oestriol therapy has been reported to benefit patients with relapsing remitting multiple sclerosis.<sup>24</sup> The influence of female hormones on inflammatory cytokines and the interplay between circulating and cellular mechanisms causing uveitis remain to be further investigated.

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## REFERENCES

- 1 **Raghupathy R**. Th1-type immunity is incompatible with successful pregnancy. *Immunol Today* 1997;**18**:478–82.
- 2 **Wilder RL**. Hormones, pregnancy, and autoimmune diseases. *Ann N Y Acad Sci* 1998;**840**:45–50.
- 3 **Makhseed M**, Raghupathy R, El-Shazly S, *et al*. Pro-inflammatory maternal cytokine profile in preterm delivery. *Am J Reprod Immunol* 2003;**49**:308–18.
- 4 **Elenkov IJ**, Chrousos GP. Stress hormones, proinflammatory and antiinflammatory cytokines, and autoimmunity. *Ann N Y Acad Sci* 2002;**966**:290–303.
- 5 **Marzi M**, Viganò A, Trabattani D, *et al*. Characterization of type 1 and type 2 cytokine production profile in physiologic and pathologic human pregnancy. *Clin Exp Immunol* 1996;**106**:127–33.
- 6 **Olivieri A**, De Angelis S, Vaccari V, *et al*. Postpartum thyroiditis is associated with fluctuations in transforming growth factor-beta1 serum levels. *J Clin Endocrinol Metab* 2003;**88**:1280–4.
- 7 **Khamashta MA**, Ruiz-Irastorza G, Hughes GR. Systemic lupus erythematosus flares during pregnancy. *Rheum Dis Clin North Am* 1997;**23**:15–30.
- 8 **Ostensen M**. Sex hormones and pregnancy in rheumatoid arthritis and systemic lupus erythematosus. *Ann N Y Acad Sci* 1999;**876**:131–43, discussion 144.
- 9 **Barrett JH**, Brennan P, Fiddler M, *et al*. Does rheumatoid arthritis remit during pregnancy and relapse postpartum? Results from a nationwide study in the United Kingdom performed prospectively from late pregnancy. *Arthritis Rheum* 1999;**42**:1219–27.
- 10 **Sicotte NL**, Liva SM, Klutch R, *et al*. Treatment of multiple sclerosis with the pregnancy hormone estradiol. *Ann Neurol* 2002;**52**:421–8.
- 11 **Whitaker JN**. Effects of pregnancy and delivery on disease activity in multiple sclerosis. *N Engl J Med* 1998;**339**:339–40.
- 12 **Rabiah PK**, Vitale AT. Noninfectious uveitis and pregnancy. *Am J Ophthalmol* 2003;**136**:91–8.
- 13 **Hyman BN**. Postpartum uveitis. *Ann Ophthalmol* 1976;**8**:677–80.
- 14 **Steahly LP**. Vogt-Koyanagi-Harada syndrome and pregnancy. *Ann Ophthalmol* 1990;**22**:59–62.
- 15 **O'Connor GR**. Factors related to the initiation and recurrence of uveitis. XL Edward Jackson memorial lecture. *Am J Ophthalmol* 1983;**96**:577–99.
- 16 **Nohara M**, Norose K, Segawa K. Vogt-Koyanagi-Harada disease during pregnancy. *Br J Ophthalmol* 1995;**79**:94–5.
- 17 **Chavis PS**, Tabbara KF. Effects of pregnancy on the course of uveitis. In: Dodds EM, Couto CA, eds. *Uveitis in the third millennium: Elsevier Science* 2000:167–70.
- 18 **Agarwal RK**, Chan CC, Wiggert B, *et al*. Pregnancy ameliorates induction and expression of experimental autoimmune uveitis. *J Immunol* 1999;**162**:2648–54.
- 19 **Buggage RR**, Matteson DM, Shen DF, *et al*. Effect of sex hormones on experimental autoimmune uveoretinitis (EAU). *Immunol Invest* 2003;**32**:259–73.
- 20 **Miyamoto N**, Mandai M, Suzuma I, *et al*. Estrogen protects against cellular infiltration by reducing the expressions of E-selectin and IL-6 in endotoxin-induced uveitis. *J Immunol* 1999;**163**:374–9.
- 21 **Wegmann TG**, Lin H, Guilbert L, *et al*. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon? *Immunol Today* 1993;**14**:353–6.
- 22 **Elenkov IJ**, Wilder RL, Bakalov VK, *et al*. IL-12, TNF-alpha, and hormonal changes during late pregnancy and early postpartum: implications for autoimmune disease activity during these times. *J Clin Endocrinol Metab* 2001;**86**:4933–8.
- 23 **Langer-Gould A**, Garren H, Slansky A, *et al*. Late pregnancy suppresses relapses in experimental autoimmune encephalomyelitis: evidence for a suppressive pregnancy-related serum factor. *J Immunol* 2002;**169**:1084–91.
- 24 **Soldan SS**, Retuerto AI, Sicotte NL, *et al*. Immune modulation in multiple sclerosis patients treated with the pregnancy hormone estradiol. *J Immunol* 171:6267–74.



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