Brief report

CSF and plasma cytokines at delivery and postpartum mood disturbances

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Abstract

Background: Immune activation has been shown to be involved in the pathophysiology of anxiety states and major depression and pregnancy is associated with a characteristic immune activation to sustain the fetus. Despite the possibility of a relation between immune parameters and postpartum mood disturbance, few studies have explored this association. Further, no study to-date has examined CSF.

Methods: Fifty-six Greek parturients were recruited and a detailed medical and obstetric history was recorded. All of them completed the Postpartum Blues Questionnaire (on admission and on days 1–4 postpartum) and the Edinburgh Postnatal Depression Scale (at first and sixth week postpartum). At delivery, a blood sample and a CSF sample while puncturing for epidural analgesia were taken from 33 participants; blood samples only were obtained from the rest of the 23 parturients. TNF-a and IL-6 were quantified with an ELISA assay.

Results: A multiple regression analysis of psychometric scores depending on cytokine levels revealed that cytokine levels were positively associated with depressive mood during the first four days postpartum ($p=0.035$ for CSF IL-6, $p=0.025$ for CSF TNF-a, $p=0.023$ for serum TNF-a) and also at sixth week postpartum ($p=0.012$ for CSF IL-6, $p=0.072$ for CSF TNF-a). Pregnancy duration had an adverse association to psychometric scores.

Conclusions: It is suggested that immune mechanisms may play a role in the etiopathology of postpartum depressive mood shifts. The role of a “rebound” reaction of the maternal immune system postnatal should be further investigated.

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Keywords: Maternity blues; Postpartum depression; Cytokines; TNF-a; IL-6; Immune activation

1. Introduction

Maternity blues, appearing a few days after delivery (Pitt, 1973), could be a marker of affective vulnerability for depression (Henshaw, 2003) possibly leading to Postpartum Depression (PPD) or/and Postpartum Major Depression (PPMD).

The pathophysiology of postpartum mood disturbances remains elusive (Henshaw, 2003). Meanwhile, accumulating evidence supports the involvement of immune activation in the pathophysiology of anxiety states (Leonard and Song, 1999; Anisman and Merli,
2003) and major depression (Dantzer, 2006; Irwin and Miller, 2007). Additionally, the existence of a brain–cytokine system that reorganizes perception and action of the host in response to activation of the peripheral innate immune system (Dantzer, 2004) is an interesting model with possible important implications in pathophysiology and therapeutics (Müller et al., 2004).

Pregnancy is characterized by a certain immune status necessary for its maintenance (Hunt et al., 2006) and the fact that at the end of this immunological entity certain mood disorders occur (Moses-Kolko and Kraus Roth, 2004) appears as an intriguing aspect for psychoneuroimmunology. In the present study we investigated a possible relation between postpartum depressive mood shifts and immune activation by measuring in both serum and cerebrospinal fluid (CSF) two key proinflammatory cytokines, namely, interleukin 6 (IL-6) and tumor necrosis factor-alpha (TNF-α) suggested to be involved in the pathophysiology of depression (Anisman et al., 1999; Pace et al., 2006) in a group of Greek parturients.

2. Materials and methods

2.1. Subjects

The study was conducted at the Aretaieion University Hospital and its Ethics Committee has approved it. Sixty-two informed consenting native Greek pregnant women, residents of Athens, Greece, physically healthy with gestational ages of 35–38 participated in the study and detailed medical and obstetric history was recorded. Single women and women with previous psychiatric disorders were excluded. Six out of the 62 women were excluded from the study, as a result of having premature labor (< 37 weeks, 5 women), and/or complications during labor (ruptured membranes for more than 12 h, 2 women). None of the remaining 56 women had complicated post-delivery (hospital stay between 3 and 5 days).

2.2. Instruments and rating

For screening and rating mood disturbances, Greek versions of two specific, systematically validated scales were used.

(a) Postpartum Blues Questionnaire (PBQ) (Kennerley and Gath, 1989) completed on admission and on days 1–4 postpartum. Mean value of the consecutive scores for each subject was calculated and the mean of the 56 means (8.2) was selected as the cut-off score for postpartum blues (Kennerley and Gath, 1989; Hapgood et al., 1988).

(b) Edinburgh Postnatal Depression Scale (EPDS) (Cox et al., 1987; Muzik et al., 2000; Teissèdre and Henri Chabrol, 2004) completed on day 4 and sixth week postpartum in order to detect Post-Partum Depression. The cut-off score of the EPDS scale was set to 11, after validation for the Greek population (Leonardou et al., 2009).

2.3. Samples

Two kinds of samples were taken from the parturients: Fifty-six 5 ml blood samples (early in labor) and thirty-three 1 ml CSF sample (right before epidural analgesia was infused). All serum and CSF samples were kept at −80 °C till assayed.

2.4. Methods

TNF-α and IL-6 levels were quantified in 56 serum and 33 CSF samples in the same run and in duplicates with the use of an automated ELISA processing system (DSX four plates, Dynex technologies, Columbia, USA) and commercially available ELISA kits (R&D systems, Minneapolis, USA).

2.5. Statistical analysis

Data statistical analysis was performed by STATA. Due to the small number of patients, we used p-value < .100 to indicate statistically significant results. Normality of the data proved to be problematic, even when trying transformations, so none were included in the final analysis. Under these circumstances, we also tried non-parametric tests. Extremely outlying values were reported for some cytokine factors. We performed comparison analysis both including and excluding these values, and attempted quantitative analysis of our data (multiple regression analysis) for questionnaire scores depending on cytokine levels (attempting to adjust for other factors) not including extreme outliers.

3. Results

3.1. Exploratory data analysis

Clinical and physical characteristics, cytokines’ serum and CSF levels along with psychometric scores for all 56 parturients are summarized in Table 1.
Table 1
Exploratory data.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Range</th>
<th>Proportion %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>32.69</td>
<td>22.00–45.00</td>
<td></td>
</tr>
<tr>
<td>Weight (kgs)</td>
<td>79.33</td>
<td>57.00–128.00</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>163.47</td>
<td>150.00–178.00</td>
<td></td>
</tr>
<tr>
<td>B.M.I.* (kg/m²)</td>
<td>29.55</td>
<td>21.77–45.35</td>
<td></td>
</tr>
<tr>
<td>Menstruation duration</td>
<td>5.01</td>
<td>3.00–8.00</td>
<td></td>
</tr>
<tr>
<td>(days)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Menstruation cycle (days)</td>
<td>28.44</td>
<td>22.00–39.00</td>
<td></td>
</tr>
<tr>
<td>Pregnancy duration (weeks)</td>
<td>38.30</td>
<td>37.00–40.60</td>
<td></td>
</tr>
<tr>
<td>Gravidity (number of</td>
<td>2.21</td>
<td>1.00–9.00</td>
<td></td>
</tr>
<tr>
<td>pregnancies)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parity (number of births)</td>
<td>0.69</td>
<td>0.00–7.00</td>
<td></td>
</tr>
</tbody>
</table>

Breastfeeding 93.33

Been pregnant before 67.74

Given birth before 51.61

Caesarian section 70.97

Serum IL-6 levels, pg/ml* | 9.75   | 2.40–292.90     |
| Serum IL-6 levels, pg/ml**| 3.27   | 2.00–14.80      |
| Serum TnF-a levels, pg/ml* | 7.17   | 5.80–14.70      |
| Serum TnF-a levels, pg/ml**| 6.22   | 5.20–8.00       |

| [P.B.Q. ] score for day 1] ≥ 8.2 | 48.39 |
| [P.B.Q. ] score for day 2] ≥ 8.2 | 41.67 |
| [P.B.Q. ] score for day 3] ≥ 8.2 | 41.67 |
| [P.B.Q. ] score for day 4] ≥ 8.2 | 37.74 |
| [Mean P.B.Q. ] score ≥ 8.2 | 46.77 |
| [EPDS* score for first week] ≥ 11 | 24.19 |
| [EPDS* score for sixth week] ≥ 11 | 22.81 |

*B.M.I.= Body Mass Index.

*P.B.Q.= Postpartum Blues Questionnaire.

*EPDS= Edinburgh Postnatal Depression Scale.

*In a total of 56 samples.

**In a total of 33 samples.

3.2. Grouped comparison

Patients were grouped based on psychometric scores (≥ 8.2 for PBQ, ≥ 11 for EPDS) in high and low scorers. Only statistically significant results are presented. P-values in the Wilcoxon–Mann–Whitney (WMW) test for: a) serum IL-6 measurements according to Postpartum Blues for day-1 were 0.09 including and 0.04 excluding extreme outliers, respectively b) CSF IL-6 measurements according to Postpartum Blues for day-2 was 0.07 including extreme outliers c) CSF TNF-a measurements according to Postpartum Blues for day-3 was 0.03. P-values in the T-test for CSF IL-6 measurements according to EPDS for the first week was 0.07 excluding extreme outliers and according to EPDS for the sixth week was 0.09 excluding extreme outliers.

Overall, all significant results were concordant: higher cytokine levels were associated with the ‘high scorers’ group of women, while no results were significant for the reverse effect.

3.3. Regression

The same conclusion as above was reached when our data was analyzed with regression analysis. Specifically, regression analysis for Postpartum Blues scores during the first 4 days was performed in clusters; all Blues measurements were included in the models as correlated ones (according to patient) and all were considered for the final computing of coefficients. This was supported by the fact that there was no significant difference between the scores of these 4 days for each patient (ANOVA for difference in means: p-value=0.53). On the contrary, the two EPDS measurements were separately analyzed (Wilcoxon signed-rank test for paired samples: p-value=0.05, with the sixth week’s EPDS scores tending to be lower). The Mean Postpartum Blues score was treated as an overall measure and was analyzed separately. Multiple regression analysis was performed in terms of forward stepwise regression, separately including each cytokine factor as the initially influential variable, not including extreme values of cytokines, because of leverage problems. We tried to be as parsimonious as possible; only significant factors were included in models. Results are shown in Table 2.

It should be noted that serum TNF-a and CSF IL-6 and TNF-a levels have an almost constant significant effect on psychometric score levels, though CSF TNF-a levels have a quadratic fit on Postpartum Blues and Mean Postpartum Blues scores: higher CSF TNF-a levels predict a decreasing rate of elevation of scores (negative quadratic coefficient; inverse U-shaped curve). Interestingly, out of factors that showed a strong correlation to psychometric scoring (pregnancy duration, duration of menstruation cycle and B.M.I.), pregnancy duration had the most constant (and most of the times adverse) effect on psychometric scores and was included in many of the final regression models.

4. Discussion

Immune tolerance is a key factor for pregnancy maintenance originating from the mother’s immune system diversion as to permit cohabitation with the “foreign” body (Hunt et al., 2006). This is further supported by clinical observations showing that many women suffering from autoimmune diseases experience a remission when carrying a baby (Buyon, 1998; Da Silva
Ordinary least squares (OLS) estimations were calculated for Mean Blues and EPDS (first, sixth week); robust clustered estimations were calculated for each letter (w=Pregnancy duration in weeks, c=Menstruation cycle in days, b=B.M.I.). Indicators imply statistical significance (p-value).

Clinical paradigm for this “reaction” could be postpartum thyroiditis: the rise in titres of autoantibodies to thyroid peroxidase postpartum in asymptomatic, during pregnancy, women (Muller et al., 2001) probably allow a chronic covert autoimmune process to be manifested into a fulminate disease state.

Our study was conducted in order to explore a possible correlation between immune mechanisms and mood shifts after childbirth. Therefore we measured proinflammatory cytokines, IL-6 and TNF-a, that have been involved in the pathophysiology of depression and anxiety, both in serum and CSF. This is the first time—to the best of our knowledge—on CSF cytokine measurements either at delivery or in the early puerperium, although CSF investigation is considered to be the most sensitive approach to the central nervous system in clinical medicine (Bechter and Bogerts, 2007).

Our data demonstrates a positive correlation between CSF TNF-a and IL-6 as well as serum TNF-a levels with the scoring levels in two mood scales (Postpartum Blues scale, EPDS) in the early puerperium. Given that several studies have demonstrated a positive link between blues symptoms and postpartum depression (Henshaw, 2003) as well as with major depression this finding could be relevant to postpartum major depression. The question now rises on how elevated IL-6 and TNF-a might affect mood. These proinflammatory cytokines in the periphery are produced mainly by macrophages and T cells (Abbas et al., 2000) and within the CNS they derive from astrocytes and microglia (Breder et al., 1993). Peripherally produced cytokines can be passively transported from the blood into the brain (Watkins et al., 1995; Dantzer et al., 1999) through regions where the Blood Brain Barrier (BBB) is less restrictive. Moreover, Chandler et al. (1997), suggested that TNF-a can promote BBB degeneration enabling its entrance into the brain parenchyma. Additionally, both TNF-a and IL-6 have been shown to be able to enter the brain by active transport through saturable transport mechanisms (Banks et al., 1995). After production, proinflammatory cytokines could act as neuromodulators and mediate the behavioural, neuroendocrine, or neurochemical features of depressive disorders (Schiepers et al., 2005). One of the best described mechanisms possibly underlying cytokine induced depression involves the kynurenine pathway and the consequent tryptophan (TRP)

Table 2

Stepwise multiple regression analysis of psychometric test scores depending on Serum and CSF levels of IL-6 and Serum levels of TNF-a, examining squared values of the cytokines and other factors possibly influencing psychometric scores.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>PBQ (day 1, 2, 3, 4) ¹</th>
<th>Mean Blues (days 1–4) ¹</th>
<th>EPDS (first week) ¹</th>
<th>EPDS (sixth week) ¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum IL-6 (pg/ml)</td>
<td>(p=0.946) -0.022 [-0.688, 0.643]</td>
<td>0.257 [-0.643, 1.156]</td>
<td>-0.126 [-1.148, 0.896]</td>
<td>0.078 [-0.879 to 1.035]</td>
</tr>
<tr>
<td>CSF IL-6 (pg/ml)</td>
<td>(p=0.035)* 4.908 [0.367, 9.448]</td>
<td>3.818 [-0.350, 7.986]</td>
<td>7.361 [0.419, 14.303]</td>
<td>6.474 [1.529, 11.419]</td>
</tr>
<tr>
<td>Serum TNF-a (pg/ml)</td>
<td>(p=0.023)* 1.551 [0.219, 2.882]</td>
<td>1.332 [-0.009, 2.674]</td>
<td>1.695 [-0.034, 3.425]</td>
<td>0.988 [-0.431, 2.407]</td>
</tr>
<tr>
<td></td>
<td>Quadratic:*−2.708 [−5.124, −0.292]</td>
<td>Quadratic:*−2.866 [−5.388, −0.344]</td>
<td>(p=0.099)*</td>
<td>(p=0.072)*</td>
</tr>
</tbody>
</table>

[95% C.I.], p-p-value.

(⁎⁎) statistical significance (p-value < 0.100).

w, c, b Indicates imply statistical significance (p-value < 0.100) of other factors (apart from cytokines) in multiple regression model, as indicated by each letter (w=Pregnancy duration in weeks, c=Menstruation cycle in days, b=B.M.I.).

¹Ordinary least squares (OLS) estimations were calculated for Mean Blues and EPDS (first, sixth week); robust clustered estimations were calculated for Blues (days 1 through 4), clustering for each patient (repeated measuring of Postpartum Blues during the first postpartum days). Factors included in each model were selected according to statistical significance (only terms corresponding to p-value <0.100 were entered). No extremely outlying values of cytokines were included (Serum IL-6<9.35 ng/ml [3 patients excluded], CSF IL-6<9.8 ng/ml [3 patients excluded], CSF IL-6<4.3 ng/ml [1 patient excluded]).

²Linear corresponds to the linear term of the cytokine, while ‘Quadratic’ corresponds to the quadratic (squared) term of the cytokine in the regression model. Quadratic estimates are reported only in regression models where quadratic terms reached statistical significance (p-value<0.100).
depletion. Briefly, IFN-γ mainly, and possibly other TH-1 cytokines as well (Weiss et al., 1999) enhance the activity of indoleamine 2,3-dioxigenase (IDO), the first enzyme in the kynurenine pathway that degrades and converts tryptophan, the precursor of serotonin to kynurenic and then quinolinic acid. Thus, the induction of IDO results in the decreased availability of serotonin and possibly depressive symptoms (Schroocksadl et al., 2006). Interestingly, Maes et al. (2002), showed that depressive and anxiety symptoms in the early puerperium were related to increased degradation of tryptophan into kynurenic.

The number of studies on immune variables and mood disorders in the early puerperium is limited. Maes et al. (2000), showed that depressive symptoms on the first and third day postpartum were associated with elevated IL-6 and IL6 receptors (IL6-R) plasma levels whereas anxiety symptoms were associated with elevated IL-6 and IL-1R antagonist (IL-1RA) plasma levels. No CSF measurements were included in this study. Our findings of elevated IL-6 levels are in accordance with those findings, although CSF samples were collected at delivery rather than the early puerperium.

Our study also revealed an adverse association between pregnancy duration and the psychometric scores which may well are in accordance with the reported prenatal depression positive association with spontaneous preterm birth (Dayan et al., 2002, 2006).

At this point it will be worth mentioning the some of the limitations of our study: the study group and the number of immune variables measured was rather small, while there was a lack of structured interview for diagnosis of depression and only self-rated measures were used. However, there was no previous database to permit larger scale investigation and CSF sampling was of limited amount, (1 ml) in order to avoid any side effects, experimental and usually, it was not easy to obtain consent. In addition, above cut-off scores in self-rated measures, particularly EPDS postpartum corresponds to 95% accuracy in SCID ascertainment major depression by second postnatal month (Leonardou et al., 2009).

In conclusion, we have demonstrated an association between elevated serum and CSF TNF-a and IL-6 levels at delivery with depressive mood shifts in the early puerperium. This finding should be further investigated by measuring more immune variables relevant probably to tryptophan metabolism and the kynurenine pathway. Moreover, in order to answer the intriguing question rising, whether a “rebound” reaction of the maternal immune system postnatal could underlie postpartum mood disorders, multiple measurements, before and after delivery is needed. These findings could probably help us elucidate the underlying mechanisms involved in the pathophysiology of mood disorders not only postpartum but also, in general.

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Conflict of interest
All authors report no biomedical financial interests or potential conflicts of interest.

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