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Inflammation and kynurenine pathway dysregulation in post-partum women with severe and suicidal depression

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Abstract

Depression during pregnancy and the post-partum is common, with severe cases resulting in suicidal behavior. Despite the urgent and unmet medical need, the biological underpinnings of peri-partum depression remain unclear. It has been suggested that it is triggered by dynamic

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changes of the immune system during pregnancy and at delivery. Therefore, we investigated whether a pro-inflammatory status in plasma, together with changes in the kynurenine pathway activity, is associated with the development of severe depression and suicidal behavior in the post-partum. Our cross-sectional study targets a unique, understudied population in which the pronounced severity of symptoms required hospitalization.

We analyzed plasma IL-1β, IL-2, IL-6, IL-8, TNF-α, tryptophan, serotonin, kynurenine, nicotinamide, quinolinic- and kynurenic acids in post-partum women diagnosed with peripartum onset depression (PPD) and healthy controls (n=165). We assessed depression severity using the Edinburgh Perinatal Depression Scale and suicidality using the Columbia-Suicide Severity Rating Scale.

We found that increased plasma IL-6 and IL-8 and reductions of serotonin, IL-2 and quinolinic acid were associated with the severity of depressive symptoms and increased the risk for PPD. Moreover, women with lower serotonin levels were at an increased risk for suicidal behavior, even when adjusting for depression severity, psychosocial factors, age BMI, and medication. Our results indicate that severe depression in the post-partum involves dysregulation of the immune response and the kynurenine pathway, with a concomitant reduction in serotonin levels. We propose that inflammatory cytokines and the kynurenine pathway are potential treatment targets in PPD, opening up the possibility of novel therapeutic strategies targeting the peripartum.

1. Introduction

Peripartum depression (PPD), defined as depression with onset during pregnancy and up to four weeks after delivery, affects 15–20% of all pregnant women worldwide (Gavin et al., 2005; Ko et al., 2017; Muzik et al., 2009). The symptoms often start during pregnancy, and the severity is frequently greater after delivery, including cases of severe post-partum psychosis and suicidal behavior (Hirst and Moutier, 2010; Sit et al., 2006). Depression in this vulnerable period leads to significant morbidity and mortality in the mother. Indeed, suicide is the most common cause of death due to illness after childbirth in the industrial world, more common than death due to post-partum hemorrhage or eclampsia (Oates, 2003; Orsolini et al., 2016; Palladino et al., 2011). Suicidal ideation, without any behavioral changes, is by itself a sign of extreme psychological suffering, and is estimated to occur in as many as 14% of pregnant women (Gavin et al., 2011; Lindahl et al., 2005). In addition, PPD is associated with significant morbidity for the infant, in the form of attachment disorders, developmental delay and growth retardation (Muzik and Borovska, 2010). Despite the significance of these problems, few studies have investigated the mechanisms of severe depression and suicidality during the peripartum period.

The maternal immune response undergoes profound changes during pregnancy, first to protect the fetus from rejection, and thereafter to facilitate delivery (Sherer et al., 2018 et al.; Mor and Cardenas, 2010). While some studies indicate that pro-inflammatory changes, implicating several inflammatory mediators, associate with the development of depression during pregnancy, (Christian et al., 2009; Roomruanwang 2018, Osborne et al., 2019, Bränn et al 2017), others did not find such an association (Blackmore et al., 2011; Buglione-Corbett et al., 2018). A likely explanation for the discrepant results could be the inclusion of

women displaying a wide spectrum of depressive symptoms; there is, for example, a lack of studies focusing on the biology of severe, suicidal PPD. The timing of sampling during pregnancy and the post-partum will also greatly influence the biological findings. A healthy, early pregnancy associates with a Th1 driven inflammatory response that subsides during the middle phase of pregnancy. In connection with parturition the inflammatory profile shifts, including an influx of immune cells into the myometrium, promoting the contraction of the uterus, delivery of the baby and expulsion of the placenta (Chavan et al., 2017; Mor and Cardenas, 2010). The physiological time course and resolution of these immunobiological changes during the post-partum, as well as their association with depression and suicidality, are still not completely understood (Guintivano et al., 2018).

In addition to the direct effects that inflammatory cytokines have on the brain (Pape et al., 2019; Raison et al., 2006), they also induce a metabolic pathway which might be critical for depressive and suicidal symptoms, namely the kynurenine pathway (Fig. 1). During the catabolism of tryptophan, the kynurenine pathway generates immunoregulatory and neuroactive metabolites, some of which are modulators of glutamate neurotransmission (Stone et al., 1993; Schwarcz et al., 2012). These mechanisms, down-stream of inflammation, could be specifically important in peripartum depression, since the placenta exhibits high expression and activity of the kynurenine pathway enzymes (Sedelmayr et al., 2014, Keaton et al. 2019). Therefore, if the immune system is dysregulated during pregnancy, there is a risk that this could lead to an altered production of neuroactive metabolites by the placenta, affecting both the mother and the fetus. Finally, in addition to the direct effect on the levels of glutamate modulators through the kynurenine pathway, inflammation may also impact serotonin levels in the peripartum. This could occur because serotonin is also generated by conversion from tryptophan, by tryptophan hydroxylase (TPH), which competes for tryptophan with the kynurenine pathway. Therefore, an increased consumption of tryptophan by the kynurenine pathway during inflammation could potentially also lead to a reduced serotonin synthesis (Fig. 1).

We designed the current study to assess these immunobiological pathways, for the first time, in women with severe and suicidal depression in the post-partum. We hypothesized that in these women, depression and suicidality would be triggered due to an increased activity of the innate immune system, impacting the activity of the kynurenine metabolites and the levels of serotonin in peripheral blood.

2. Materials and Methods

2.1 Study design

This study was approved by the Michigan State University Institutional Review Board (IRB), East Lansing, Michigan, USA; with collateral review and approval from Spectrum Health IRB and Van Andel Research Institute IRB; both in Grand Rapids, Michigan, USA. Participants signed an informed consent at enrollment, which occurred between 2014 and 2016 at the Mother and Baby program at Pine Rest Christian Mental Health Services and the Obstetrics and Gynecology clinics at Spectrum Health, in Grand Rapids, Michigan. The Mother and Baby Program is a partial hospitalization program for women experiencing

significant symptoms of depression and suicidality, where the women can bring their babies to the day program.

The research visit took place at 8 (6–12) weeks post-partum (median, IQR). Exclusion criteria were: patients with cognitive impairment that interfered with ability to give informed consent or to complete assessments; patients with blood-borne chronic infections including hepatitis B, C, or HIV; patients with schizophrenia spectrum disorder or bipolar disorder type 1 and patients who reported ongoing substance abuse or dependence (in the past 3 months). A total of 168 women were evaluated in the post-partum. Out of these, three were diagnosed with autoimmune disorders: Systemic Lupus Erythematosus (SLE) ($n = 2$) and Myasthenia Gravis ($n = 1$). They received immunosuppressive treatment and were therefore excluded from further analysis. The final cohort consisted of 165 women. The clinical and demographic features of the women are shown in Table 1.

2.2 Psychiatric evaluation

The Edinburgh Postnatal Depression Scale (EPDS) covering depressive symptoms over the past seven days (Cox et al., 1987) and the Columbia Suicide Severity Rating Scale (C-SSRS) (Posner et al., 2011) were completed. “Active suicidal behavior” includes making active preparations and actual suicide attempts, which can be completed, aborted or interrupted, as defined by the CSSRS. The patients also completed a Structured Clinical Interview for Diagnosis in the Diagnostic and Statistical Manual of the American Psychiatric Association (First et al., 2004). 60 women were psychiatrically-healthy and were defined as ‘healthy controls’ for the purpose of this analysis, and 87 women were diagnosed with a *depressive episode with peripartum onset* (PPD) according to the formal DSM-5 criteria (American Psychiatric Association, 2013). There were also 18 women in the cohort who displayed depression but did not fulfill the time criteria for peripartum-onset depression defined by DSM-5, requiring the onset of depression to be during pregnancy or within the first four weeks post-partum. These women were not included in the primary analysis with the clinical diagnosis of PPD as the key target population. All 165 subjects in the cohort were included in the sensitivity analysis of the association of *current depressive symptoms* (total EPDS scores over the past 7 days).

2.3 Somatic evaluation

In conjunction with the psychiatric assessment, the subjects participated in a general physical examination that included height, weight, heart rate, blood pressure and temperature. The subjects reported current symptoms, chronic and active medical comorbidities and ongoing medications. Somatic co-morbidities are listed in Table 2. Subjects in this study were allowed the use of psychotrophic medications due to the severity of symptoms. The medications utilized were; selective serotonin reuptake inhibitors (SSRIs, n=60), serotonin and noradrenaline reuptake inhibitors (SNRIs, n=12), neuroleptics (n=20), anti-epileptic (n=12), benzodiazepines (n=21), bupropion (n=12), lithium (n=5), and tricyclic antidepressants (n=2).

2.4 Blood samples

Blood was drawn by venipuncture of the right or left antecubital vein, in direct conjunction with the psychiatric assessment. Blood sampling was standardized to occur between 9 AM −12 PM. Blood was kept on ice and immediately transported to the laboratory and centrifuged to plasma which was aliquoted and frozen at −80°C until the biological assays.

2.5 Cytokine assays

IL-1β, IL-2, IL-6, IL-8, IL-10, and TNF-α protein levels were measured using the MesoScale Discovery platform and run on a Sector 600 in accordance with the manufacturers' instructions. Samples were run in duplicate and the mean values of the duplicates were used for analysis. Inter-assay coefficients of variation (% CV) were; IL-1β (3.47%), IL-2 (2.98%), IL-6 (2.22%), IL-8 (2.40%), IL-10 (1.78%), and TNF-α (2.84%). Lower detection limits: IL-1β (0.02 pg/ml), IL-2 (0.12 pg/ml), IL-6 (0.07 pg/ml), IL-8 (0.05 pg/ml), IL-10 (0.02 pg/ml), and TNF-α (0.09 pg/ml).

2.6 Detection of tryptophan, serotonin and kynurenine metabolites

Tryptophan and kynurenine were analyzed using high-performance liquid chromatography with ultraviolet/visible wavelength detection. Plasma proteins were precipitated using 10% trichloroacetic acid. After centrifugation, supernatant was filtered through a 0.22 μm PTFE filter and 20 μl was injected into a Thermo Scientific Dionex UltiMate® 3000 (Thermo Scientific, Waltham, MA, USA). Chromatograph separation was achieved on a reverse phase 150×3 mm BDS Hypersil C18 column (Thermo Scientific™) with 3 μm particle size. Column and pre-column tubing were maintained at 35°C with isocratic elution (0.8mL/min) using a mobile phase consisting of 5% methanol in milliQ water containing 50 mM ammonium acetate (pH 4.65 with acetic acid). Results were analyzed using the Chromeleon™ 7.2 Chromatography Data System (Thermo Scientific™ Dionex™). Quinolinic acid was analyzed using gas chromatography-mass spectrometry (GC-MS) as described (Coccaro et al., 2016; Smythe et al., 2002). Ultra-high performance liquid chromatography, coupled to tandem mass spectrometry (UPLC-MS/MS) was used to determine plasma serotonin, kynurenic acid and nicotinic acid concentrations (Cellar et al., 2016). Proteins were precipitated by adding acetonitrile to plasma (3:1, v:v). Following centrifugation, supernatant was evaporated to dryness and then was reconstituted in a diluent containing 200 nM internal standard in 10 mM ammonium formate and 0.1% formic acid in MilliQ water. Samples were filtered through a 0.22 μm PTFE filter and 10uL was injected onto a Waters Acquity BEH C18 UPLC column with a Waters Acquity® UPLC and a Waters Acquity TQ-D mass spectrometer. The inter-assay CVs were: tryptophan (0.95%), kynurenine (1.6%), quinolinic acid (1.1%), serotonin (6.4%), kynurenic acid (6.0%) and nicotinic acid (3.3%). The lower limit of detection was 100 nM (tryptophan and kynurenine), 10 nM (quinolinic acid) and 0.8 nM (serotonin, kynurenic acid and nicotinic acid).

2.7 Statistical Analysis

All statistical analyses were performed using Statistical Package for the Social Sciences (version 23 for Mac). Tests were 2-tailed, $p < 0.05$ was considered significant and $0.05 < p <$

0.1 was interpreted as having modest evidence of an effect (Wood et al., 2014), both are reported with 95% confidence intervals. Raw data for the groups PPD vs healthy were compared using Mann-Whitney's U-tests and reported in Table 3. For regression models, variables with a nonparametrical distribution were transformed into normal distribution using the natural logarithms. All models were adjusted for body mass index (BMI), age, and four socioeconomic factors; marital status (single/in relationship), employment status (employed yes/no), household income and education level. Logistic regression models were used to calculate the effect of the biological variables on a clinical diagnosis of PPD or suicidal status. As a sensitivity analysis, we performed linear regression analyses using the total EPDS score (current depressive symptom depth) as the outcome variable in the entire cohort of 165 women. Next, linear regression models were used to assess the impact of classes of medication (SSRI, SNRI, neuroleptic, antiepileptic, benzodiazepines, lithium, and bupropion) on the biological variables in the PPD cohort. Medications found to have an effect on biomarker levels were excluded as the next step, and regression analyses rerun without these subjects to assess the stability of the models. Pearson's R was used to assess correlations between plasma serotonin and the inflammatory biomarkers in this study.

3. Results

3.1 Depression and suicidality in the cohort

Demographics of the cohort are detailed in Table 1. The population of women with PPD (n = 87) suffered from moderate to severe depression, with a total score on the EPDS scale of 18.5 ± 4.7 (mean ± SD) over the past seven days. Based on the C-SSRS over the past seven days, a substantial number of the women had current suicidal ideation at the time of evaluation (n = 43). There were 13 women with active suicidal behavior during pregnancy and the post-partum, eleven of these had completed a suicide attempt whereas the remaining two had undertaken active preparation for an attempt. Nine of these women displayed active suicidal behavior within seven days of the study assessment. The 60 healthy women had an EPDS score of 4.3 ± 3.6 (mean ± SD), with no reported suicidal ideation or behavior.

3.2 An activated innate immunity in women with PPD

Elevated plasma levels of IL-6 ($p = 0.007$) and IL-8 ($p = 0.009$) both increased the odds of PPD ($OR_{IL\text{-}6} = 3.0$, 95% CI = 1.37 – 6.6; $OR_{IL\text{-}8} = 3.32$, 95% CI = 1.32 – 8.34, per pg/ml increase). Moreover, a decrease in plasma IL-2 increased the risk for PPD (OR = 2.34, 95% CI = 1.35 – 4.05, $p = 0.002$, per pg/ml decrease) (Fig. 2C). The logistic regression models were adjusted for age, BMI, education, economy, marital status and employment as described. Figures 2 A–C show the unadjusted raw data for IL-6, IL-8 and IL-2 in both groups. There was also modest evidence that increased levels of TNF-α increased the odds of PPD (OR = 3.67 per pg/ml, 95% CI: 0.95 – 14.18, $p = 0.066$). The plasma levels of IL-10 and IL-1β were not found to impact the risk for PPD (logistic regression, $p > 0.1$, NS).

3.3 Kynurenine pathway dysregulation and reduced serotonin in women with PPD

Women with lower serotonin in plasma were at significantly increased risk for PPD (OR = 1.43 per nM decrease in serotonin, 95% CI: 1.07 – 1.92, $p = 0.016$) (Fig. 2D). The absolute plasma levels of tryptophan in the blood did not affect the risk for PPD ($p > 0.1$, NS). Since

an elevated kynurenine/serotonin ratio was associated with an increased risk for PPD (OR = 1.35 per unit increase, 95% CI: 1.03 – 1.79, $p = 0.038$), we conclude that relatively more tryptophan was directed towards the kynurenine pathway than to serotonin production in the women with PPD. Decreased levels of the *N*-methyl-D-aspartate receptor (NMDAR) agonist quinolinic acid increased the odds of PPD (OR = 4.48 per nM decrease, 95%CI: 1.41 – 14.25, $p = 0.014$) (Fig. 2E), and the NMDAR-antagonist kynurenic acid had modest evidence of affecting the odds of PPD (OR = 2.64 per nM decrease of kynurenic acid, 95% CI: 0.99 – 7.03, $p = 0.054$). The resulting neurotoxic ratio (quinolinic acid/kynurenic acid) did not significantly affect the risk for PPD (p > 0.1, NS). Plasma nicotinamide also did not significantly impact PPD risk (logistic regression, p > 0.1, NS). The immunobiological data is summarized in Table 3.

3.4 Association between serotonin, inflammation and the kynurenine metabolites

A correlation analysis showed that plasma serotonin was closely associated with plasma nicotinamide, one of the furthest downstream metabolites of the kynurenine pathway (Pearson's R 0.49, $p < 0.001$) (Fig. 3A). We also found that the inflammatory cytokine TNF-α was closely associated to the levels of quinolinic acid (Pearson's R 0.56, $p < 0.001$) (Fig. 3B).

3.5 Severity of depressive symptoms

To assess whether the biomarkers were associated with severity of current depressive symptoms, all 165 women were included in the sensitivity analysis using total EPDS score over the past 7 days, adjusted for age, BMI and psychosocial factors. The models for IL-8 (linear regression, Beta 3.9, Standardized Beta 0.22, $p = 0.006$), IL-2 (linear regression, Beta −2.3, Standardized Beta −0.23, $p = 0.005$), serotonin (linear regression, Beta −1.3, Standardized Beta −0.24, $p = 0.003$), serotonin/kynurenine ratio (linear regression, Beta −1.1, Standardized Beta 0.22, $p = 0.009$), and quinolinic acid (linear regression, Beta −4.3, Standardized Beta −0.18, $p = 0.022$), were all significant. IL-6 showed a minor effect (linear regression, Beta 2.3, Standardized Beta 0.17, $p = 0.066$), although when correcting for the effect of neuroleptic treatment (see below) the association was significant (linear regression, Beta 2.7, Standardized Beta 0.20, $p = 0.022$).

3.6 Suicidality

We assessed whether the biomarkers identified above were associated with suicidal behavior and ideation using logistic regression (detailed in Table 4). In summary, lower serotonin was associated with both current suicidal behavior and a history of suicidal behavior. Moreover, lower serotonin significantly increased the odds of a completed suicide attempt during pregnancy (Table 4), and this association remained even when adjusting for EPDS score (69.9% increase in odds per nM decrease in serotonin, 95% CI: 2.1% - 182.8%, $p = 0.044$), indicating that low plasma serotonin might be a unique marker of suicide risk in the post-partum. While quinolinic acid levels significantly impacted the odds of suicidal ideation, they did not affect suicidal behavior.

3.7 Impact of medication

Because the severity of the depressive symptoms necessitated treatment with medication for many of the women, we assessed whether any class of medication affected the plasma levels of biomarkers. Two classes of medication impacted the plasma levels of the biomarkers in this study: neuroleptics, which were associated with reduced levels of IL-6 (linear regression, Beta −0.29, Standardized Beta −0.21, $p = 0.033$), and bupropion, which had a positive effect on IL-8 (linear regression, Beta 0.41, standardized Beta 0.31, $p = 0.01$). We therefore repeated the regression analyses reported above, excluding patients on bupropion (n = 10) and neuroleptics (n = 20) respectively, and the results were still significant. IL-6 was still associated with increased odds of PPD (n = 122, logistic regression, 309.6% increase in OR per pg/ml increase, 95% CI: 69.6% - 889.5%, $p = 0.002$) and with EPDS total score (n = 138, linear regression, beta 0.24, $p = 0.013$). IL-8 was still associated with increased odds of PPD (logistic regression, n = 129, 188.6% increase in OR per pg/ml increase, 95% CI: 12.7% - 639.5%, $p = 0.028$) and EPDS score (n = 146, linear regression, beta 0.18, $p = 0.025$).

3. Discussion

This is the first study of immunobiological and metabolic changes in women with severe and suicidal post-partum depression. These women experienced symptoms of a magnitude that required hospitalization. The majority had current suicidal ideation, and several had recently made a suicide attempt or acted to facilitate an attempt. We found that such severe post-partum depression is characterized by pro-inflammatory changes, a dysregulated kynurenine pathway, and low serotonin levels. Suicidal behavior was specifically associated with reduced serotonin levels, even when adjusting for depression severity. The biological changes in our cohort were robust, since they remained after adjusting for BMI, age, medication and psychosocial elements.

Our results support the hypothesis that a pro-inflammatory state in the blood is associated with depressive symptoms and increased risk for severe peripartum depression. IL-6 is a regulatory and pro-inflammatory cytokine, consistently linked to depression (Dowlati et al., 2010; Haapakoski et al., 2015), which activates both monocytes and microglia, as well as other cell types of the innate immune system. In a previous study of women with affective disorders, we found that increased blood levels of IL-6 were associated with a cellular immune response in subjects assessed to be at suicide risk (Keaton et al., 2019). We have also observed IL-6 to be increased in CSF and blood of male and female suicide attempters (Janelidze et al., 2011; Lindqvist et al., 2009). Our results, showing an increased risk for PPD in women with elevated IL-6, are also in line with a recent study that found elevations of CRP and IL-6 in women diagnosed with depression during the 6 first months of the post-partum (Liu et al., 2016). IL-8 is another pro-inflammatory mediator, known to stimulate macrophage and granulocyte mediated mechanisms in the periphery. One previous study showed that the plasma IL-8/IL-10 ratio together with salivary cortisol predicted EPDS scores indicative of depression in the post-partum (Corwin et al, 2015), which is in line with our data. Interestingly, both IL-8 levels and genotypes have been linked to suicidality in clinical cohorts (Janelidze et al., 2015; Isung et al., 2012, Noorozi et al., 2018, Knowles et

al., 2019). At the same time, we found here that decreased levels of IL-2 also increased the risk for peripartum depression, which to the best of our knowledge has not been reported previously. IL-2 has a major role in the immune response as an activator of T-cells, inducing regulatory and effector T-cells into their respective subtypes. Altogether, a reduction in IL-2 and the concomitant increase in IL-6 and IL-8 indicate that, in women with depression in the post-partum period, the innate immune response was activated rather than T-lymphocyte mediated immune mechanisms.

Moreover, our data show that the peripheral inflammation induces the kynurenine pathway enzymes to metabolize tryptophan, shunting it away from serotonin production in the women with peripartum depression. Even though the plasma tryptophan concentration was similar in women with and without depression, there was a higher ratio of kynurenine produced in the women with PPD, relative to the amount of serotonin produced. Low plasma serotonin concentration increased the risk for depression, and these changes were pronounced in women with suicidal behavior. In fact, low serotonin increased the odds for suicidal behavior also when adjusting for severity of depressive symptoms. Thus, plasma serotonin could be a potential indicator of suicide risk in the post-partum. The large part of peripheral serotonin is produced by enterochromaffin cells and released into the bloodstream, where it is either bound by platelets or remains as free serotonin (El-Merahbi et al., 2015). There are reports showing that plasma serotonin correlates with central levels of serotonin (Audhya et al., 2012; Liu et al., 2010), although this connection is not yet thoroughly established. Based on our results in the current study, we believe that peripheral serotonin might be a more relevant biomarker for neuropsychiatric disease than previously thought, and that it warrants future investigation. We also observed a close association between serotonin and nicotinamide, a downstream kynurenine metabolite. Interestingly, nicotinamide has been linked to the release of serotonin from cultured neurons (Tian et al., 2013). This possible mechanism of action for nicotinamide should also be explored further, as it might represent a biological link by which the kynurenine pathway exerts a feedback loop on serotonin production.

We have previously shown quinolinic acid levels to be increased by around 300% in the CSF of patients who recently attempted suicide (Erhardt et al., 2013). By contrast, a post-mortem study found reduced quinolinic acid in post-mortem brain tissue from depressed individuals (Clark et al., 2016). In the current study, we found that reduced quinolinic acid levels in plasma were associated with depressive symptoms in the post-partum. These studies illustrate that the dynamics of quinolinic acid changes in depression and suicidality are still not fully understood. It is important to note that in this study, the inflammatory cytokines TNF-α and IL-6 still correlate positively with quinolinic acid levels in the blood, as expected when the kynurenine pathway is activated by inflammation. Therefore, the absolute levels of quinolinic acid produced under inflammatory conditions could be compromised in women with PPD. The regulation of the individual enzymes of the pathway depends on several factors, including both inflammatory conditions and hormones, such as corticosteroids and estrogen (Jayawickrama et al., 2017, Sorgdrager et al., 2018; Dostal et al, 2018). In our longitudinal study during pregnancy in women with peripartum depression, we observed that plasma quinolinic acid levels first increased steeply during pregnancy, then to decline after delivery (unpublished data). Thus, hormonal changes associated with

parturition and the delivery of the placenta are likely implicated in the regulation of quinolinic acid levels. In addition, we also observed a modest reduction in the glutamate receptor antagonist kynurenic acid was associated with peripartum depression. In line with this, we have previously observed reduced levels of kynurenic acid in the blood of suicide attempters (Bay-Richter et al., 2015). To summarize, the regulation of molecules acting on glutamate receptors remains of critical interest for the understanding of the interplay between inflammation, depression and suicide risk. A limitation to our current study is that the plasma levels of the kynurenine metabolites we assayed might not fully mirror the levels in the brain. Future mechanistic studies should therefore attempt to monitor these mediators in the CSF of subjects with peripartum depression.

In conclusion, women with severe PPD and suicidality constitute an understudied population, likely to exhibit unique, pronounced biological changes underpinning their symptoms. We detected distinct immunobiological changes in women with severe peripartum depression and suicidality. Both depressive symptoms and suicidal behavior were linked to inflammation and low levels of serotonin in peripheral blood samples. Our results suggest a dysregulated immunity in women with PPD, with activation of the innate immune response that causes a preferential shunting of tryptophan along the catabolic kynurenine pathway instead of towards serotonin production. These findings are particularly important considering that inflammation as well as the kynurenine pathway can be targeted pharmacologically, possibly leading to novel therapeutic strategies in women with PPD. Our study warrants further study of this patient group, including central measures of inflammation and kynurenine pathway activity, with the goal of leading up to clinical biomarkers of risk for peripartum depression and the initiation of future clinical trials targeting this critical pathway.

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Highlights

- Severe peripartum depression (PPD) with suicidality is an understudied disorder
- Severe depression in the post-partum involves dysregulation of the immune response
- Tryptophan is shunted towards the kynurenine pathway instead of serotonin production
- Women with reduced serotonin are at an increased risk for suicidal behavior
- Inflammation and the kynurenine pathway may be treatment targets in severe PPD

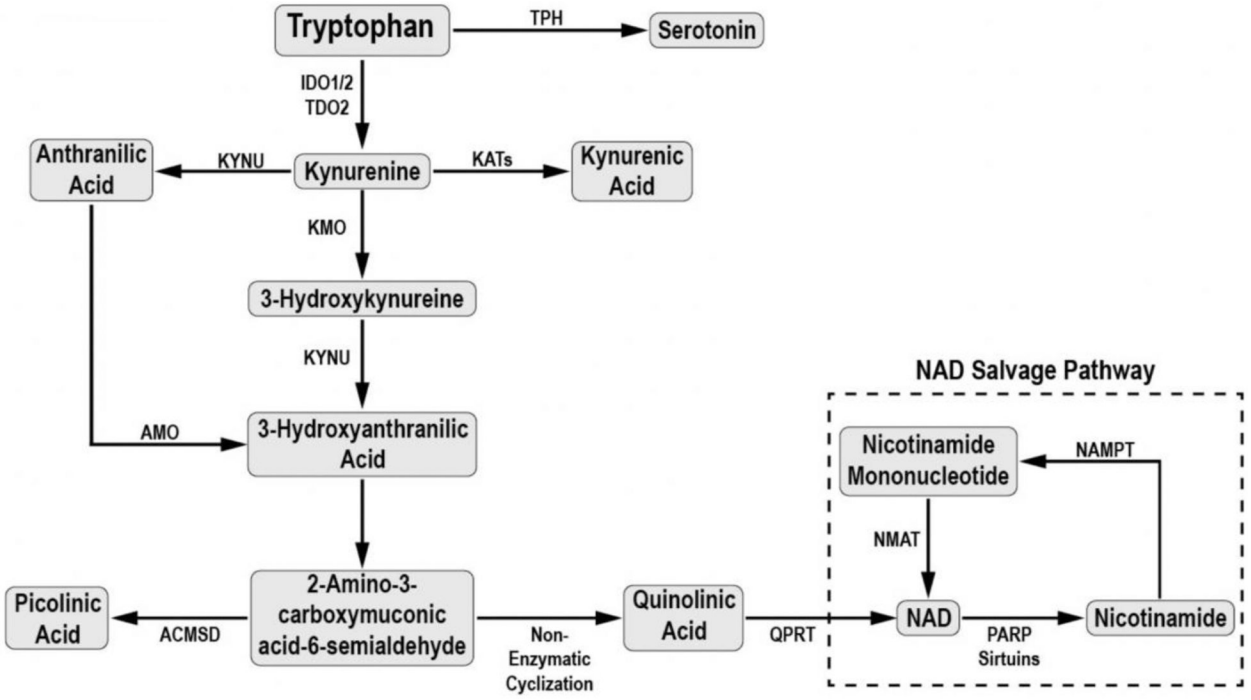

Figure 1. Schematic of the Kynurenine Pathway.

TPH = tryptophan hydroxylase, IDO = indoleamine 2,3-dioxygenase, TDO = tryptophan 2,3-dioxygenase, KAT = kynurenine amino transferase, KYNU = kynureninase, KMO = kynurenine 3-monooxygenase, 3-HAO = 3-hydroxyanthranilic acid 3,4-dioxygenase, ACMSD = α-amino-β-carboxymuconate-ε-semialdehyde decarboxylase, QPRT = quinolinate phosphoribosyl transferase, NAD = nicotinamide dinucleotide, PARP = poly (ADP-ribose) polymerase, NMAT = nicotinamide-nucleotide adenylyltransferase, NAMPT = nicotinamide phosphoribosyltransferase.

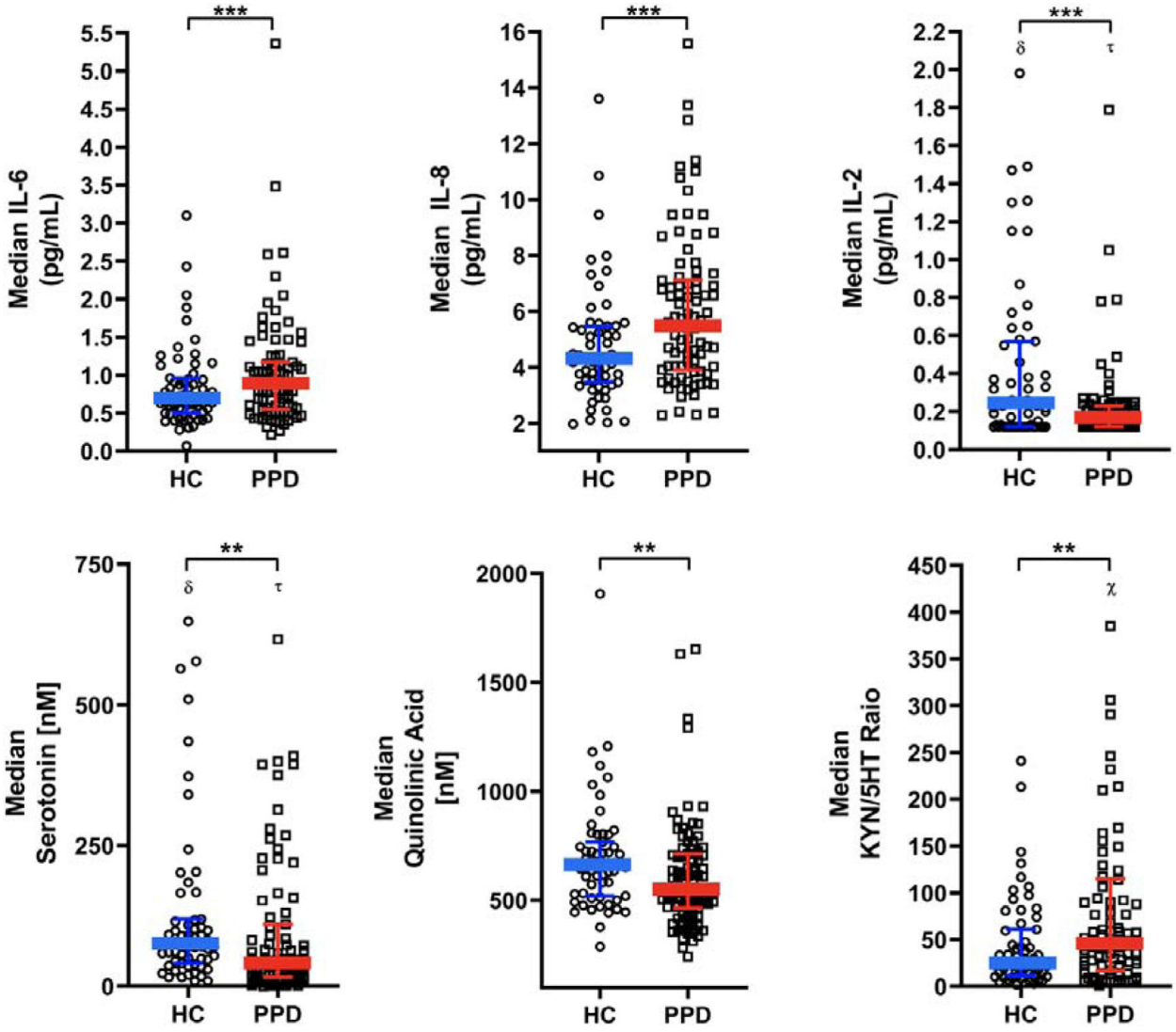

Figure 2. A-F.

Plasma levels of inflammatory markers and serotonin (raw data, unadjusted). Plasma **A**. IL-6 (pg/mL), **B**. IL-8 (pg/mL), **C**. IL-2 (pg/mL), **D**. serotonin [nM], **E**. QUIN [nM] and F. The kynurenine/serotonin (5HT) ratio. The median and interquartile range (IQR) is indicated for all analytes. Significance level indicated in the figures was determined based on the logistic regression models described in the results and Table 3. ** $p < 0.05$, *** $p < 0.01$.

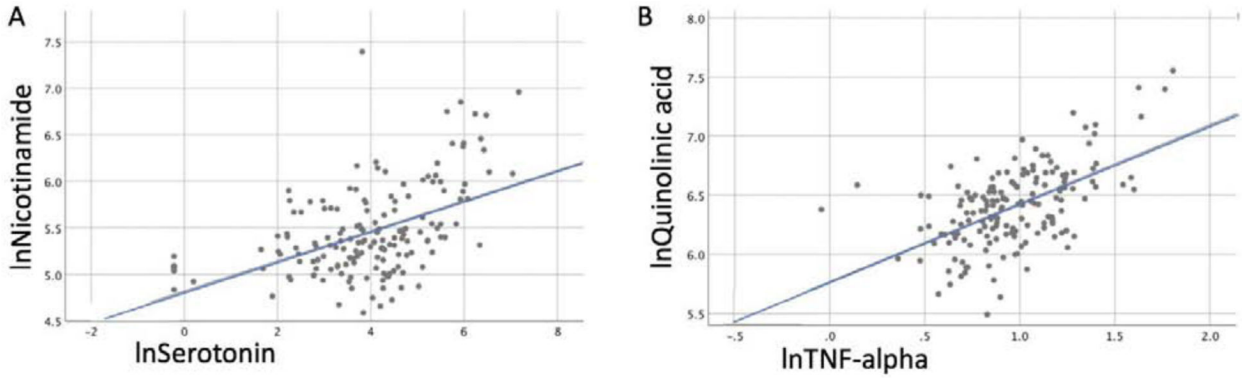

Figure 3A.

Scatterplot of all individuals in the study (n=165), showing the correlation between the levels of nicotinamide, one of the most down-stream products of the kynurenine pathway, and serotonin. **B**. Scatterplot showing the correlation between quinolinic acid and TNF- α in the cohort. The natural logarithms (ln) were used to transform the data into normal distribution.

Table 1.

Patient Demographics

	PPD N=87	Healthy N=60	EPDS >13 N=95	EPDS <13 N=70
Age (mean ± SD)	26.7 ± 5.2	28 ± 6.3	26.9 ± 5.5	27.5 ± 5.9
BMI (mean ± SD)	29.6 ± 7.1	30.5 ± 7.9	30.5 ± 8.0	30.9 ± 7.6
Marital status percentages	36% single	43% single	35% single	41% single
	64% in relation	57% in relation	65% in relation	59% in relation
EPDS score (mean ± SD)	18.5 ± 4.7	4.2 ± 3.6	18.8 ± 4.1	4.7 ± 3.6
Employed percentages	37% unemployed	38% unemployed	37% unemployed	40% unemployed
	63% employed	62% employed	63% employed	60% employed

Table 2.

Medical Diagnoses of Patients

	PPD N=87	**Healthy Total N=60**
Somatic diagnoses (n)	Migraine (8)	Migraine (1)
	Asthma / allergy (8)	Asthma / allergy (8)
	Endometriosis (4)	Thyroid disease (5)
	Thyroid disease (3)	Reflux / GERD (3)
	Reflux / GERD (3)	Diabetes (5)
	Clotting disorder (3)	Anemia (2)
	Arthritis (2)	Gall stones (2)
	Kidney stones (2)	Celiac disease (1)
	Gall stones (1)	High blood pressure (4)
	Celiac disease (1)	Epilepsy (1)
	Dermatitis (1)	
	Psoriasis (1)	
	Diabetes (1)	
	Glaucoma (1)	

Table 3.

Biomarkers listed by the increase in odds for a diagnosis of PPD by unit increase of the marker. The fully adjusted model is shown in column 6, adjusting for age, BMI and psychosocial factors as described in the text. column 5 shows the *p* value from Mann-Whitney camparisons of the unadjusted data in the two groups. Median and interquartile range (IQR) for each unadjusted biomaker in the groups are shown in columns 3 (PPD group) and 4 (healthy group).

BIOMARKER	Data display	PDD diagnosis	Healthy	Mann-Whitney(raw data)	Odds ratio(fully adjusted model)
IL-8	Median	5.5	4.3		**OR = 3.32**
	IQR	3.9–7.1	3.5–5.5	*p = 0.002*	95% CI = [1.32, 8.34]
					p=0.009
IL-6	Median	0.89	0.70		**OR = 3.0**
	IQR	0.55–1.17	0.50–0.96	*p* = 0.08	95% CI = [1.37, 6.60]
					p=0.007
IL-2	Median	0.17	0.23		**OR = 2.34**
	IQR	0.12–0.23	0.12–0.57	*p* = 0.012	95% CI = [1.35, 4.05]
					p=0.002
Serotonin	Median	41.2	72.4		***OR = 1.43***
	IQR	16.4–110.0	40.6–119.3	*p* = 0.003	95% CI = [1.07, 1.92]
					p=0.016
Quinolinic acid	Median	553.5	654		***OR = 1.35***
	IQR	464–713	519–768	*p* = 0.011	95% CI = [1.03,1.79]
					p=0.014
TNF-α	Median	2.7	2.4		***OR = 3.67***
	IQR	2.29–3.3	2.0–2.90	*p* = 0.032	95% CI = [0.95, 14.18]
					p=0.066
Kynurenic acid	Median	54.9	59.5		***OR = 2.64***
	IQR	43.4–66.6	44.2–82.8	*p* = 0.084	95% CI = [0.99, 7.03]
					p=0.054
Tryptophan	Median	34980	36020		OR = 1.00
	IQR	30930–39100	32390–42290	*p* = 0.11	95% CI = [>0.99, <1.01]
					p>0.1
Kynurenine	Median	1962	2113		OR = 1.00
	IQR	1486–2193	1800–2374	*p* = 0.038	95% CI = [>0.99, <1.01]
					p>0.1
Nicotinamide	Median	210.4	210.4		OR = 1.00
	IQR	168.4–343.4	160.9–295.5	*p* = 0.49	95% CI = [>0.99, <1.01]
					p>0.1
IL-10	Median	0.31	0.3		OR = 1.57
	IQR	0.26–0.50	0.23–0.38	*p* = 0.27	95% CI = [0.83, 2.99]
					p>0.1

BIOMARKER	Data display	PDD diagnosis	Healthy	Mann-Whitney(raw data)	Odds ratio(fully adjusted model)
IL-1β	Median	0.094	0.081		OR = 1.06
	IQR	0.066–0.14	0.056–0.12	$p = 0.22$	95% CI = [0.58, 1.96]
					$p > 0.1$

Table 4.

Suicidal ideation and behavior. The effects of biomakers on suicidality were determined using logistic regression, adjusting for age, BMI and psychosocial factors, as described in the text.

N displaying each type of suicidality / those without	**Active suicidal ideation** *43/114*	**Suicidal behavior in peripartum** *13/144*	**Current suicidal behavior** *9/148*	**Completed attempt in peripartum** *11/146*	**Current Attempt (past 7 days)** *5/152*
Serotonin 95% CI	OR: 0.77 [0.56,1.06] *p* = 0.11	OR: 0.51 [0.32,0.81] ***p* = 0.005**	OR: 0.50 [0.29, 0.87] ***p* = 0.013**	OR: 0.51 [0.31, 0.84] ***p* = 0.007**	OR: 0.44 [0.22,0.87] ***p* = 0.019**
QUIN 95% CI	OR: 0.1 [0.02,0.51] ***p = 0.006***	OR: 0.1 [0.09,0.36] *p* = 0.11	OR: 0.09 [0.01,0.64] *p* = 0.07	OR: 0.14 [0.02,1.17] *p* = 0.07	OR: 0.06 [0.002, 2.07] *p* = 0.12
QUIN/KYNA 95% CI	OR: 0.71 [0.25,2.01] *p* = 0.52	OR: 0.65 [0.17,2.42] *p* = 0.52	OR: 0.70 [0.13, 3.73] *p* = 0.68	OR: 0.49 [0.11, 2.16] *p* = 0.34	OR: 0.33 [0.03,3.5] *p* = 0.38