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Mild Depressive Symptoms Are Associated with Elevated C-Reactive Protein and Proinflammatory Cytokine Levels During Early to Midgestation: A Prospective Pilot Study

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Abstract

Background: We examined depressive symptoms, C-reactive protein (CRP), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) levels during early to-midgestation.
Methods: We measured depressive symptoms on the Patient Health Questionnaire-9 (PHQ-9), and serum CRP, IL-6, and TNF-α levels twice in 27 pregnant women.
Results: After adjustment, depressive symptoms prospectively ($\beta = 0.42$, $p < 0.05$ at 16–20 weeks of gestation) and concurrently ($\beta = 0.54$, $p < 0.01$ at 7–10 weeks of gestation) predicted elevated CRP [F (2, 14) = 9.20, $p = 0.003$, $R^2 = 0.57$ and F (3, 15) = 9.08, $p = 0.001$, $R^2 = 0.64$, respectively]. There were similar patterns of results for TNF-α ($\beta = 0.72$, $p < 0.01$) and IL-6 levels ($\beta = 0.39$, $p < 0.05$) at 7–10 weeks of gestation [F (2,19) = 8.84, $p = 0.002$, $R^2 = 0.48$]. Furthermore, the association between depressive symptoms at 7–10 weeks of gestation and increased IL-6 levels at 16–20 weeks of gestation approached statistical significance. We confirmed the findings with the Wilcoxon signed rank test (IL-6: $Z = 2.44$, $p = 0.015$; TNF-α: $Z = 1.94$, $p = 0.05$; CRP: approached statistical significance).
Conclusions: These pilot data suggest that depressive symptoms may be associated with inflammatory markers during early to-midgestation.

Introduction

Pregnancy is a process of hormonal changes that require adjustment, which can be particularly challenging for some women.[1] In Canada, the healthcare-related financial burden of untreated maternal prenatal depression has been estimated to be $14 billion annually.[2] Prenatal depressive symptoms are common[3,4] and particularly high during the first trimester of pregnancy,[5] even when they do not reach clinical levels. An exaggerated maternal C-reactive protein (CRP) response, beyond the normal pregnancy-related increased levels, can increase the risk for pregnancy-related health problems, which could in turn affect fetal growth and even cause premature births.[6]

During pregnancy, CRP is typically elevated. However, Coussons-Read et al.[6] showed that women who reported prenatal high stress levels and low social support are more likely to have higher CRP and cytokine levels. Additionally, Christian et al.[7] examined the relationship between prenatal depressive symptoms and inflammation, including inflammatory responses to an *in vivo* immune challenge among pregnant women. Although some also have observed a relationship between depressive symptoms and increased inflammation, namely, in new mothers,[8] other studies have shown no association. For example, Blackmore et al.[9] studied 145 women at two time points in early and later gestation and found no association between interteukin-6 (IL-6), tumor necrosis factor-α (TNF-α) and depressive symptoms. Therefore, it is essential to determine if depressive symptoms in pregnant women are associated with inflammatory markers.[7]

Our study builds on the Coussons-Reads et al.[6] model of the psychoneuroimmunology of prenatal stress in order to expand it to prenatal depression. Based on their model, we propose to test whether prenatal depression could reduce maternal immune function though the same stress-induced alterations of the immune system. Specifically, this would occur by increased susceptibility to infection and possibly through altered proinflammatory cytokines. Eventually, these inflammatory processes may affect fetal growth, causing reduced neonatal birth weight. This prospective pilot study

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Table 1. Descriptive Data of Participants ($n=27$)

Characteristic	*Mean (SD) or %*
Age, years	24.29 (SD 4.85)
Body mass index (BMI)	29.61 (SD 8.98)
Serum cotinine (in ng/mL)[a]	66.87 (SD 15.70)
Education	48% had some high school education 52% had some college/university education
Gross family income[b]	65% < $40,000 35% > $40,000
Ethnolinguistic background[c]	100% English-speaking
Self-reported psychiatric history	0%

[a]Nine women had cotinine levels above the cutoff for smoking (10 ng/mL).
[b]Most mothers were of low socioeconomic status (SES).
[c]All mothers were Canadian.
SD, standard deviation.

investigated whether maternal prenatal depressive symptoms are related to elevated serum inflammatory markers. We performed two repeated measurements of variables to demonstrate that this is stable and reliable throughout early to midgestation. We hypothesized that there would be significant concurrent and prospective associations among pregnant women's depressive symptoms, CRP, IL-6, and TNF- α levels in early to midgestation.

Materials and Methods

Participants

The sample consisted of the first 27 pregnant women recruited at the Cumberland Regional Health Care Centre (CRHCC) prenatal clinic (Amherst, Nova Scotia, Canada). This sample size corresponds to 13.5% of the subsequent study sample size (with 25 of its participants being enough to detect a large effect, based on a power analysis with a desired statistical power of 0.8). The recruitment was based on the inclusion criteria of being pregnant and at the first prenatal visit. The exclusion criteria were multiple gestation and such systemic diseases as hypertension, preeclampsia, and lupus or gross placental abnormalities. All potential participants approached agreed to participate in this pilot study. Seventy-six percent were nulliparous (never having given birth). Table 1 shows mean cotinine levels along with demographic data. Participants were on average 24.29 years old (standard deviation [SD] 4.85) and had an average of 12.41 years of education (SD 2.01). They were all Caucasian and English-speaking Canadians. Most women were married/living with their partner and from low to medium socioeconomic status (SES) (65% had a gross family income < $40,000). Almost half of the sample (48%) had some high school education, and the rest of the sample (52%) had some college/university education. Their mean body mass index (BMI) was 29.61 (SD 8.98). None had a self-reported psychiatric history. Nine women were smokers (serum cotinine > 10 ng/mL), and none were taking a prescription drug for depression.

The study received ethics approval from the local health authority and Mount Allison University's Research Ethics Boards. Participants provided written informed consent at the onset of the study. They were assessed twice during pregnancy to demonstrate the stability/reliability of the relationship between depressive symptoms and inflammatory markers: (1) early gestation: at time 1 (T1) during the first prenatal visit, which was between 7 and 10 weeks of pregnancy, and (2) midgestation: at T2 during a follow-up visit, which was between 16 and 20 weeks of pregnancy. There were no participant dropouts from T1 to T2.

Inflammatory markers

According to Meier-Ewert et al.,[10] there is no diurnal variation of CRP concentrations. To avoid any potential diurnal variation of cytokines, we collected all blood samples in the morning (0830 and 1230 hours). The collected serum was immediately stored at −80°C until assayed. We measured serum CRP, high-sensitivity (hs)IL-6, and hsTNF-α twice, at T1 and again at T2, with the sandwich enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Inc., Minneapolis, MN). Serum samples were assayed on the same day by the same technician and in duplicate. Repeated assays at T1 and at T2, respectively, were highly correlated, $r=0.99$, $p<0.00$. The intra-assay coefficient of variation (CV) was 5.5% for CRP, 7.4% for IL-6, and 5.3% for TNF-α.

Depressive symptoms

We assessed depressive symptoms using the PHQ-9, a widely used depression screening module of the full Patient Health Questionnaire, which is a self-administered version of the Primary Care Evaluation of Mental Disorders (PRIME-MD).[11] We chose the PHQ-9 because it has been validated in ethnically diverse populations in primary care and has a reliable and valid French version (some of the participants in the larger study will be French-speaking). The PHQ-9 consists of questions based on the DSM-IV criteria for a major depressive episode. The PHQ-9 has high internal reliability as well as criterion and construct validity[11] and is short and easy to use (< 3 minutes). Each of the questions asks patients to select the frequency of the depressive symptoms they have experienced in the past 2 weeks. Scores from each item range from 0 to 3 (e.g., not at all to nearly every day). We used the continuous PHQ-9 scores to represent symptom severity.

Data analysis

We performed statistical analyses using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL). We set statistical significance at $p<0.05$. There were no extreme scores in the depressive data. Because of positively skewed distributions, we log-transformed the CRPs and cytokine data. All the $\log_{10}$ transformations resulted in normal distributions, with no outliers (> 3 SD above the mean). Based on previous literature,[6,12] we specified *a priori* covariates that could potentially influence inflammation in order to adjust for them in the analyses. These potential confounders were maternal age, smoking (measured with serum cotinine, orasure-1124E kit), BMI, and trait anxiety (measured with the State-Trait Anxiety Inventory). Before running the regression analysis, we examined correlations between potential covariates and the PHQ-9 depression total scores. These scores are the sum of scores for the nine items for each woman. Only covariates that were significantly related to the outcome measure were entered into our model. The

Table 2. Mean Patient Health Questionnaire-9 Total Scores and Median C-Reactive Protein, Interleukin-6, and Tumor Necrosis Factor-α of Pregnant Women in Sample ($n = 27$)

	T1 (7–10 weeks of gestation)	*T2 (16–20 weeks of gestation)*
PHQ-9 total score[a]	6.60 (±3.30)	6.62 (±4.02)
Median CRP (mg/L)[b]	4.27 (1.95–6.52)	5.95 (3.69–12.66)
Median IL-6 (pg/mL)[b]	1.17 (0.75–1.69)	1.55 (0.96–3.01)
Median TNF-α (pg/mL)[b]	0.95 (0.88–1.14)	1.08 (0.95–1.33)

[a]Mean PHQ-9 total score (±SD).
[b]Median value (Q25-Q75: lower-upper quartiles).

univariate correlations of maternal age and trait anxiety with the inflammatory markers were in the moderate range ($r = 0.5$, $p < 0.05$). As cotinine and BMI were associated with neither inflammatory markers (outcome measures) nor depressive scores, there was no need to adjust for these variables. We included depressive symptoms at T1 and T2 in the same model to predict the outcomes (inflammatory markers). Finally, using a paired *t* test analysis, we explored whether within-woman changes (increases) in depressive symptoms from T1 to T2 prospectively predicted changes in any of the inflammatory markers.

Results

Mean PHQ-9 scores were 6.60 (SD 3.30) at T1 and 6.62 (SD 4.02) at T2. These mean values represent mild depression and are below clinically significant cutoffs.[11] Specifically, 63% of the women in the sample fell into categories corresponding to mild depressive symptoms, and only 19% of them had moderately severe depressive symptoms. Depressive symptoms at T1 (7–10 weeks of gestation) and depressive symptoms at T2 (16–20 weeks of gestation) were moderately correlated ($r = 0.534$, $p = 0.019$). Table 2 shows the median levels of CRP, IL-6, and TNF-α at early and midgestation. None of the values for the inflammatory markers was below the limit of detection. The inflammatory markers at T1 and depressive symptoms at T1 and T2 were moderately correlated, with correlations ranging from 0.44 to 0.53.

Table 3 shows the results of the regression analysis, after adjusting for the covariates of maternal age and trait anxiety. We found that depressive symptoms prospectively ($\beta = 0.42$, $p < 0.05$ at T1) and concurrently ($\beta = 0.54$, $p \leq 0.01$ at T2) predicted elevated CRP levels during midgestation [$F(2, 14) = 9.20$, $p = 0.003$, $R^2 = 0.57$ and $F(3, 15) = 9.08$, $p = 0.001$, $R^2 = 0.64$, respectively]. Prospectively refers to the association between depressive symptoms at early gestation and inflammatory markers at midgestation. Concurrently refers to the association depressive symptoms-inflammation at a specific time point (either early or midgestation). Table 3 shows there were similar patterns of results for maternal depressive symptoms and increased concurrent IL-6 levels ($\beta = 0.39$, $p < 0.05$ at T2) as well as increased TNF-α at T1 ($\beta = 0.72$, $p < 0.01$) and T2 ($\beta = 0.52$, $p < 0.05$) [$F(2,19) = 8.84$, $p = 0.002$, $R^2 = 0.48$]. Stated differently, these results indicate that for every 1 point increase in the PHQ-9 score at T1, we found a 0.42 point increase in CRP levels at T1, a 0.54 point increase in CRP levels at T2, a 0.39 point increase in IL-6 levels at T2, a 0.72 point increase in TNF-α levels at T1, and a 0.52 point increase in TNF-α levels at T2.

Additionally, the association between depressive symptoms during early pregnancy (T1) and increased IL-6 at T2 approached statistical significance ($\beta = 0.41$, $p = 0.056$). Although it remains possible that the somatic questions of the PHQ-9 may have influenced the results, the same patterns of results remained when the somatic items were removed from the total score of the PHQ-9 scale. Given our small sample size, we confirmed the findings with the Wilcoxon signed rank test in order to minimize any possibility that sampling error may have driven these findings. Results of this nonparametric test showed significant changes in the proinflammatory cytokine levels from T1 to T2 ($Z = 2.44$, $p = 0.01$ for IL-6; $Z = 1.94$, $p = 0.05$ for TNF-α), and changes approached statistical significance for CRP levels ($Z = 1.44$, $p = 0.1$). Finally, when we explored whether within-woman increases in depressive symptoms from T1 to T2 predicted changes in inflammation, a paired *t* test analysis showed the same patterns of results: there was a significant association between increases in depressive symptoms and IL-6 levels ($p < 0.006$). Similar associations approached statistical significance in the case of CRP and TNF-α. Interestingly, this pattern of results was even stronger in women whose PHQ-9 scores reached the

Table 3. Multiple Linear Regression Analysis of Relationship Between C-Reactive Protein and Proinflammatory Cytokines During Midgestation (T2) as Dependent Variables and Maternal Prenatal Depressive Symptoms During Early (T1) and Midgestation (T2) as Predictors

Dependent variable	*Predictor*	β	*SE*	t	p
CRP (T2)	Depressive symptoms (T1)[a]	0.42	0.026	2.36	0.03
	Depressive symptoms (T2)[b]	0.54	0.026	3.09	0.01
IL-6 (T2)	Depressive symptoms (T1)	0.41	0.022	2.09	0.056[c]
	Depressive symptoms (T2)	0.39	0.022	2.26	0.04
TNF-α (T2)	Depressive symptoms (T1)	0.72	0.006	4.18	0.00
	Depressive symptoms (T2)	0.52	0.009	2.17	0.047

The regression analysis was adjusted for maternal age and trait anxiety (the latter was positively correlated with depressive symptoms, $r = 0.60$, $p = 0.003$).
[a]T1, between 7 and 10 weeks of gestation.
[b]T2 between 16 and 20 weeks of gestation.
[c]Despite the small sample size, this result approached statistical significance.
SE, standard error.

clinical level at T2 (PHQ-9 total score ≥ 10, which corresponds to a major depression).

Discussion and Conclusions

This pilot study suggests that mild to moderate prenatal depressive symptoms may be associated with increased inflammatory markers in early and midgestation. Recent literature has shown that depression, regardless of pregnancy, is accompanied by elevated CRP in women[11] as well as increased IL-6 and TNF-α concentrations.[13] Compared to other studies with pregnant women,[6,7] our results mostly support an association between the same proinflammatory markers and prenatal depressive symptoms, which often did not even reach clinical levels. This finding is particularly meaningful, as women with a history of depression who would suffer from postpartum depressive symptoms may have an amplified sensitized inflammatory response.[14]

Strengths and limitations

Although promising, our pilot findings should be interpreted with caution because of the small sample size and the high effect sizes, which could have been due to nonrandom sampling. As there was no outlier in the depressive scores, however, it is less likely that the observed effects were driven by extreme values. In comparison to earlier studies with both pregnant and nonpregnant women,[12] the absence of a comparison group (e.g., nonpregnant women) may have limited the findings. Although none of the participant had medical conditions or complications (a strength as opposed to earlier studies with pregnant women and postpartum women), we must bear in mind that many of these conditions may not be detectable at the time points assessed. Additionally, the relationship among depressive symptoms, CRP, and IL-6 could be bidirectional.[12] Nevertheless, the longitudinal design of our pilot study allowed us to investigate the relationship between maternal depressive symptoms and inflammation in early to midgestation both concurrently and prospectively. The multiple measurements demonstrated that the relationship between depressive symptoms and inflammatory markers is stable and reliable from early to midgestation. Although it remains possible that the somatic items of the PHQ-9 may have been an influence, the same patterns of results remained when they were removed from the total score of the PHQ-9 scale. Finally, our pilot results are further strengthened by the following observations: (1) when we looked for any longitudinal associations between increases in depressive symptoms and changes in inflammatory levels, we found similar patterns of results (significant for cytokines and approaching statistical significance for CRP), and (2) we observed a stronger pattern of results in women whose depressive symptoms reached clinical levels.

Future directions

We found a dose-response relationship between depressive symptoms and inflammatory markers during pregnancy. Interestingly, this finding corroborates the results of a recent meta-analysis with clinical and community samples of men and nonpregnant women.[15] Can such a statistically significant relationship translate into a clinically significant result that could affect fetal growth? This deserves further investigation, which is in progress in a larger study.

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Disclosure Statement

No competing financial interests exist.

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