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ORIGINAL ARTICLE

Physiological and psychological stress in pregnant women with quiescent inflammatory bowel disease: A pilot study using salivary biomarkers

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Abstract

Background: Pregnant women with inflammatory bowel disease (IBD) are more likely than the general pregnant population to experience adverse maternofetal outcomes, especially if the disease is active at the time of conception and during pregnancy. Elevated stress is often seen in patients with chronic diseases and could account for these outcomes. Salivary cortisol and alpha-amylase (sAA) are novel biomarkers of stress, reflecting the hypothalamic-pituitary-adrenal (HPA) axis and sympathetic nervous system, respectively. Our aim in this pilot study was to assess stress differences between pregnant women with inactive IBD and matched controls using psychometric questionnaires and salivary biomarker measures.
Methods: Thirteen pregnant women with quiescent IBD (6 Crohn's disease, 7 ulcerative colitis) were matched (1:3) to 39 expectant mothers without IBD by parity and gestational age. Participants completed several psychometric questionnaires assessing stress, and salivary cortisol and sAA were collected as objective biomarkers of stress during pregnancy.
Results: Pregnant women with quiescent IBD did not demonstrate significant differences on any psychometric measures of stress or salivary biomarker measures when compared with controls (all $P > 0.05$). Pregnant women with quiescent IBD demonstrated similar cortisol and sAA awakening responses (both $P > 0.05$) and total levels of cortisol and sAA production (both $P > 0.05$) when compared with controls.
Conclusions: Pregnant women with well-controlled IBD do not experience demonstrable differences in psychological stress or dysregulation of salivary stress biomarkers when compared with non-IBD controls. The effect of chronic disease may be evaluated in future studies by including a comparative group of pregnant women with active IBD.

Introduction

Inflammatory bowel diseases (IBD) comprise Crohn's disease (CD) and ulcerative colitis (UC) and are chronic, immune-mediated diseases of the gastrointestinal tract.[1] Their peak onset is between 15 and 35 years of age, thus coinciding with the reproductive years[2] and often raising concerns among patients as to whether their disease could negatively impact fetal development and pregnancy outcomes. Active IBD at the time of conception and during pregnancy is a well-established risk factor for adverse pregnancy outcomes, including spontaneous abortion, low birth weight, and preterm delivery.[3,4] As per international guidelines,[5] physicians should advise patients to conceive while their disease is in remission; however, several studies have demonstrated higher frequencies of adverse pregnancy outcomes compared with the general population independent of disease activity,[6,7] suggesting that other factors, including elevated prenatal maternal stress, may play a role.[8,9]

Stress can be defined as a state of psychological and physiological strain resulting from demanding conditions. Stress has been linked to IBD,[10] as well as many other chronic diseases,

including depression,[11] cardiovascular disease,[12] and HIV/AIDS.[13] Chronic diseases, including IBD, can induce stress by causing patient discomfort, necessitating intrusive diagnostic procedures and hospitalizations and negatively impacting quality of life.[14,15] Furthermore, stress is associated with the IBD course, specifically exacerbation of symptoms, supporting a stress–symptom association.[16] Stress and the IBD course appear to be reciprocal, each contributing to the aggravation of the other.

Excessive prenatal maternal stress is a factor that may negatively impact maternal–fetal outcomes in IBD patients. Excess stress during pregnancy is known to increase levels of various maternal hormones and neurotransmitters, which enter fetal circulation and modulate fetal development.[8] Maternal distress is also linked to poorer obstetric outcomes in healthy pregnant women, including pregnancy complications, preterm labor, and altered fetal well-being.[8,9]

Psychometric assessment of stress is commonly conducted using validated self-report questionnaires, providing personal and subjective quantification of an individual's stress experience. Psychological stress triggers a physiological response, which is complex but is predominately regulated by the hypothalamic-pituitary-adrenal (HPA) axis and sympathetic nervous system (SNS).[10] Salivary alpha-amylase (sAA) is an enzyme that increases with acute stress and correlates well with SNS neurotransmitter release and, as such, is a reliable biomarker for SNS activity.[17] Salivary cortisol concentrations correlate well with HPA axis activity.[18] Normal basal sAA and cortisol levels display diurnal patterns and awakening responses, which exactly mirror each other.[19,20] Salivary biomarkers such as sAA and cortisol enable the objective quantification of stress, have gained popularity for their noninvasive sampling methods and ease of use when compared with blood sampling, and are both now commonly used in stress research.[21–23]

Pregnant patients with even well-controlled IBD may have higher levels of psychological and physiological stress when compared with pregnant women without IBD, and this may contribute to adverse pregnancy outcomes in expectant mothers with IBD. We performed a pilot study to assess stress differences between pregnant women with clinically inactive IBD and non-IBD controls, using psychometric questionnaires and measurement of salivary biomarkers.

Materials and methods

Participants. Thirteen pregnant women diagnosed with IBD (6 CD, 7 UC) were recruited from the University of Calgary IBD Pregnancy Clinic at a gestational age of 32 ±3 weeks. All 13 women were in disease remission from time of conception through to time of recruitment and data collection, as defined by the Harvey Bradshaw Index (HBI; for CD) or the Simple Clinical Colitis Activity Index (SCCAI; for UC). A comparison sample of pregnant women without IBD was obtained from the Alberta Pregnancy Outcomes and Nutrition (APrON) study.[24] Women reporting concurrent use of corticosteroids, smoking, alcohol or recreational drug use, recent dental work or a tendency for oral bleeding, and/or an acute illness were excluded as any of these factors may affect salivary cortisol levels. Nulliparous and multiparous women have been found to significantly differ in cortisol levels, and cortisol levels increase overall as pregnancy progresses; thus, pregnant women without IBD from the APrON study were matched (3:1) to expectant IBD mothers by parity (nulliparous or multiparous) and gestational age at the time of saliva and psychometric data collection.

Saliva sample collection and analysis. Standardization of saliva collection is necessary for comparability of salivary biomarker data between individuals.[25] We therefore trained participants in proper collection of saliva and emphasized the importance of following protocol. Following training with a research assistant as per standard procedures, participants collected saliva in their own homes over two consecutive days with the aid of a personal digital assistant (PDA) and using oral swabs and saliva tubes (Salimetrics, State College, PA). Saliva was sampled four times each day over two consecutive days to capture basal diurnal patterns of cortisol and sAA and to account for normal day-to-day variation. Samples were collected at waking and 30 min postwaking to assess the cortisol and sAA awakening responses (CAR and sAAR), as well as in the mid-morning and before bed to determine average total daily production of cortisol and sAA (as quantified by the area under the curve from ground, or AUCg).[26] The PDA was programmed to allow a 30-min time window during which participants could respond, after which data were considered missing. Participants were able to prevent the PDA from ringing during inconvenient times.

Participants were instructed to turn the PDA on upon awakening, record their waking time, and collect their first saliva sample. The PDA was programmed to then ring 30 min after the participant awoke, at which time the second saliva sample was collected. This procedure was used to assess awakening responses while allowing for individualized wake times. Each time the PDA signaled for a saliva sample, a unique code was provided that corresponded to a prelabelled saliva tube that the participant was instructed to use. Participants were asked to refrain from consuming food, caffeine, citric drinks, and dairy and to avoid vigorous exercise and/or brushing their teeth in the 30 min prior to saliva collection as these factors could affect salivary cortisol levels. The PDA asked participants to report adherence to these procedures at each sample collection time.

Saliva samples were assayed at Salimetrics (Carlsbad, CA). The enzyme immunoassay for salivary cortisol has a lower sensitivity limit of 0.007 mg/dL, standard curve range from 0.012 to 3.0 mg/dL, and average intra- and interassay coefficients of variation of 3.5 and 5.1%, respectively. Method accuracy determined by serial dilution was 100.8 and 91.7%, and 10% of samples were selected randomly to be assayed in duplicate to confirm reliability; the intraassay coefficient of variation between duplicate tests was 3.5%. Mean values from duplicate samples were used in analyses.

The assay for sAA uses a chromogenic substrate, 2-chloro-r-nitrophenol linked to maltotriose. The enzymatic action of sAA yields 2-chloro-r-nitrophenol, and this can be measured spectrophotometrically at 405 nm using a laboratory plate reader. The amount of sAA activity in the sample is directly proportional to the increase in absorbance at 405 nm, over a 2-min period. Results were computed in U/mL of sAA; intra- and interassay coefficients of variation were 5.5 and 4.7%, respectively. sAA concentrations are affected by salivary flow rate and protein secretion; therefore, we controlled for salivary flow rate

by multiplying raw sAA concentrations (U/mL) by flow rate (computed by dividing sample volume, in mL, by collection time, in minutes). Adjusted sAA concentrations computed using this method were in U/min of sAA.

Psychometric tests. Participants completed several validated psychometric questionnaires, including the Pittsburgh Sleep Quality Index (PSQI), anxiety scale of the Symptom Checklist-90-R (SCL-90-R), Edinburgh Postnatal Depression Scale (EPDS), Stressful Life Events Questionnaire (SLEQ), and Social Support Effectiveness questionnaire (SSE).[27–31] The EPDS has been validated for use during pregnancy.[29]

The PSQI[27] is an index of sleep disturbances and sleep quality over a 1-month interval. The measure comprises 19 individual items within seven components. Each item is weighted on a 0–3 interval scale. All component scores are totaled to provide a global score ranging from 0 to 21, with higher scores indicating greater sleep disturbances.

The SCL-90-R[28] is a self-report measure evaluating a broad range of psychological problems and symptoms of psychopathology; the anxiety subscale utilized in the present study comprises 10 items that assess general anxiety symptoms. SCL-90-R anxiety scores range from 0 to 40, with higher scores indicating more anxiety.

The EPDS[29] is a 10-item screening questionnaire designed specifically to assess depression during the perinatal period and has been validated for use during pregnancy. Scores range from 0 to 30, with higher scores indicating more depressive symptomatology.

The SLEQ[30] is a measure of exposure to stressful life events, adapted from a previous scale[32]; the present study utilized a seven-item 'yes' or 'no' questionnaire designed for use with pregnant women.[30] Scores on the SLEQ range from 0 to 7, with higher scores indicating exposure to a greater number of stressful life events.

The SSE[31] is a 35-item questionnaire assessing the effectiveness of emotional, informational, and task-related support provided by a partner. Respondents report on the effectiveness of support received from their partner in the preceding 3 months and rate the extent to which they perceive that support as negatively infringing on their own efficacy and/or self-esteem. Total scores range from 0 to 80, with higher scores indicating more effective social support.

Participants either completed the questionnaires in the laboratory at the time of saliva collection training or took them home with saliva kits and mailed back completed questionnaires with their saliva samples.

Table 1 Baseline demographic and clinical characteristics of inflammatory bowel disease pregnant cases and noninflammatory bowel disease controls

	Non-IBD (N = 39)	IBD (N = 13)	P value
Age (years)	31.5 ± 4.1	30.7 ± 3.2	0.57
Gestational age at data collection (weeks)	32.6 ± 1.1	31.8 ± 2.1	Matched
Marital status (%)			0.41
Single	2.6	7.7	
Common law or married	97.4	92.3	
Employment (%)			0.58
Full time	76.9	69.2	
Part time	23.1	30.8	
Income (%)			0.11
Less than $100 000	41.0	15.4	
$100 000 or more	59.0	84.6	
Born in Canada (%)			0.06
Yes	65.8	92.3	
No	34.2	7.7	
Education (%)			0.86
Less than university degree	28.2	30.8	
Completed university degree or greater	71.8	69.2	
Ethnicity (%)			0.02
Caucasian	69.2	100.0	
Other	30.8	0.0	
Parity (%)			Matched
Nulliparous	53.8	53.8	
Multiparous	46.2	46.2	
Gravida (%)			0.81
1	35.9	38.5	
>1	64.1	61.5	
Prepregnancy body mass index (%)			0.41
Underweight	5.1	0.0	
Normal weight	53.8	30.8	
Overweight	28.2	53.8	
Obese	12.8	15.4	

Data are % or mean ± SD.
IBD, inflammatory bowel disease.

Statistical analysis. All statistical analyses were performed using Stata (StataCorp, College Station, TA, USA. 2015. Stata Statistical Software: Release 14). A two-sided $P \leq 0.05$ was considered statistically significant. Parametric data were summarized as mean ± standard deviation. Participant characteristics between IBD and non-IBD groups (matched for parity and gestational age at time of data collection) were compared using the Cochran–Mantel–Haenszel χ^2 test for categorical data and a two-sample Wilcoxon rank sum test for continuous data.

Ethical considerations

Written informed consent was obtained from all study participants prior to any data collection. Ethics approval was obtained from the Conjoint Health Research Ethics Board at the University of Calgary.

Results

Baseline demographic and clinical characteristics of pregnant women with IBD and controls are summarized in Table 1. Baseline characteristics were similar between groups apart from ethnicity as all IBD participants were Caucasian. Within the IBD sample, three patients were on dual therapy with anti-TNF and azathioprine, four patients were on biologic monotherapy, one

patient was on azathioprine monotherapy, two patients were on 5-ASA therapy, and one patient was not taking any medications.

Average scores on psychometric questionnaires and comparisons between groups are summarized in Table 2. Pregnant women with IBD had lower mean scores on the SSE and higher mean scores on all other psychometric measures compared to controls; however, differences did not reach statistical significance. Lower mean scores on the SSE indicate lower levels of perceived social support in pregnant IBD women, while higher mean scores on all other questionnaires indicate higher levels of psychological stress. The largest between-group differences were observed on the sleep disturbances measure (PSQI 7.5 *vs* 5.9, $P = 0.08$) and stressful life events measure (SLEQ 2.3 *vs* 0.9, $P = 0.11$), indicating greater sleep disturbance and greater exposure to stressors in women with IBD; however, these did not reach statistical significance.

Salivary biomarker findings are reported in Table 3. Total daily biomarker production was based on average AUCg values over two consecutive sampling days. Awakening responses are based on average area under the curve from increase (for CAR) or decrease (for sAAR) over two consecutive sampling days (i.e., the area under the curve from baseline waking level to the peak or trough at 30 min postwaking). There were no statistically significant differences between groups in total daily production of cortisol or sAA. Pregnant women with IBD had an attenuated mean CAR compared with non-IBD controls (0.94 *vs* 2.85 ug/dL, $P = 0.08$); however, this did not reach statistical significance.

Neonatal measures assessed at time of delivery, including birth weight and length, were within normal clinical parameters for both groups and showed no significant differences between mothers with and without inactive IBD.

Discussion

Our pilot study found that pregnant women with quiescent IBD did not experience demonstrable differences in levels of prenatal stress compared with non-IBD controls. Stress is a multifaceted condition and has traditionally been assessed using a range of psychometric tests and questionnaires. We found that pregnant women with inactive IBD had scores similar to controls on all completed psychometric questionnaires, although IBD women reported more symptomatology in all cases, with the largest between-group differences observed on the SLEQ (measuring exposure to stressful life events) and PSQI (measuring sleep disturbances). These differences did not reach statistical significance but are consistent with the expectation that those with chronic disease would report more stress and stress-related conditions (such as depression, anxiety, and poorer sleep quality) and a lowered quality of life.[33]

Stress has been linked to IBD[10] and many other chronic diseases, including onset; relapse and clinical course in depression[11]; increased risk of exacerbation, morbidity, and mortality in cardiovascular disease[12]; and the progression of HIV/AIDS.[13] Self-report measures are those in which individuals report on their own conditions or qualities. Self-report questionnaires provide an overview of the stress experienced by an individual but may not always be comparable between individuals as scores may be affected by variation in thoughts and mood at the time of completion and/or exaggeration or minimization of the actual condition by the self-reporter. Self-report methods rely on participants to reliably report on their own conditions; individual variability in reporting and disclosure may lead to inaccurate assessments of true or comparable stress levels.

Psychological stress triggers physiological changes through activation of the SNS and HPA axis.[10] The SNS responds to an acute stressor by elevating the "fight or flight" response, mediated through the release of catecholamines from the adrenal medulla. Here, we used sAA as a noninvasive biomarker for SNS activity. Normal basal sAA displays a diurnal pattern in which levels are relatively low at waking, decrease rapidly over the first 30 min after waking (referred to as the sAA awakening response), and then increase steadily over the course of the day. This pattern is exactly opposite to the diurnal pattern observed in salivary cortisol.[19,20]

The HPA axis releases a cascade of hormones in response to the perception of stress, beginning with the release of corticotrophin-releasing hormone (CRH) and culminating in a rise in cortisol.[10] Normal basal cortisol displays a diurnal pattern in which concentrations at waking are relatively high, increase rapidly to peak at 30 min postwaking (referred to as the cortisol awakening response, or CAR), and then gradually decline over the course of the day.[20]

Most molecules in blood such as cortisol transfer into saliva; thus, salivary levels of these molecules are an index of corresponding bioactive components of blood markers of stress system activity.[34] sAA is produced in the oral mucosa itself as a marker of SNS activity. Salivary biomarkers such as cortisol and sAA are a noninvasive approach to the objective measurement of psychological stress and have been widely used within psychological research.[23] To the best of our knowledge, this is the first study reported in the literature using salivary biomarkers to assess stress in pregnant IBD patients. Ease of collection and noninvasive sampling methods make salivary biomarkers a promising tool for future research in stress with IBD patients. Although we took care to ensure that none of our participants had poor oral health (which can affect saliva sampling), it should be noted for future studies that pan-gastrointestinal tract involvement in certain IBD patients, including oral ulcers, may affect salivary cortisol levels; for example, significant blood contamination of the saliva has the potential to falsely elevate salivary

Table 2 Mean psychometric questionnaire scores for inflammatory bowel disease pregnant cases and noninflammatory bowel disease controls

Questionnaire	IBD	Non-IBD	*P* value
Stressful life events	2.3 ± 2.6	0.9 ± 0.9	0.11
Edinburgh Postnatal Depression Scale	6.5 ± 4.5	6.2 ± 5.2	0.77
Pittsburgh Sleep Quality Index	7.5 ± 2.9	5.9 ± 2.9	0.08
Social Support Effectiveness	53.1 ± 16.5	56.7 ± 14.1	0.55
Symptom Checklist-90-R (anxiety subscale)	4.6 ± 4.0	4.0 ± 4.5	0.58

Data are expressed as mean ± SD.
IBD, inflammatory bowel disease.

Table 3 Salivary biomarker results for inflammatory bowel disease pregnant cases and noninflammatory bowel disease controls

Biomarker measure	IBD	Non-IBD	*P* value
Total cortisol (ug/dL min)	284.42 ± 91.89	259.09 ± 67.43	0.51
Cortisol awakening response (ug/dL min)	0.94 ± 3.19	2.85 ± 2.53	0.08
Total alpha-amylase (U/ml min)	112 415.30 ± 53 559.80	104 308.85 ± 68 450.96	0.36
Alpha-amylase awakening response (U/ml min)	−402.76 ± 998.91	−415.99 ± 1 020.81	0.92

Data are expressed as mean ± SD.
IBD, inflammatory bowel disease.

cortisol levels. Here, we visually screened samples to ensure there were no obvious signs of blood contamination.

Following a stressful episode, the HPA axis returns circulating cortisol concentrations to baseline through counterregulatory mechanisms.[35] Chronic stress exposure can lead to HPA axis dysregulation or hyporesponsiveness, wherein cortisol responses to further stressors become blunted, including an attenuation of the CAR. A blunted or attenuated CAR is a marker of HPA axis dysregulation, and several studies have shown associations between a blunted CAR and each of chronic stress, anxiety, insomnia, depression, and poorer self-perceived general health.[36–38]

Our study observed similar levels of total cortisol and sAA production in pregnant women with IBD when compared with non-IBD controls, suggesting similar levels of activity of both the SNS and HPA axis between groups. An attenuated mean CAR was observed in pregnant women with IBD, but this difference was statistically nonsignificant. This suggests that pregnant women with IBD whose disease is clinically inactive may not have demonstrable differences in stress levels nor salivary biomarker dysregulation when compared with controls; this lack of difference may be related to the present pregnant IBD cohort being in clinical remission.

Our psychometric and biomarker findings are consistent with research in a nonpregnant sample which found that IBD poses little to no detriment to psychological functioning in those whose disease is quiescent, but those with active IBD demonstrate lower psychological well-being, both when compared with matched non-IBD controls.[33] It has been found that nonpregnant IBD patients with active disease demonstrate higher levels of distress, perceived stress, and health-related anxiety and lower levels of social support and well-being when compared with those with inactive disease, although general quality of life is decreased even by inactive disease in the nonpregnant IBD population.[33] Similarly, a recent systematic review of over 170 articles found that IBD patients with active disease had a higher prevalence of anxiety and depressive symptoms when compared with those in remission.[39]

Excessive maternal stress is linked to poorer obstetric outcomes in healthy pregnant women, including pregnancy complications, preterm labor, and altered fetal well-being.[8,9] Pregnant women with inactive IBD who are found to display increased levels of stress may benefit from stress management counselling, intended to control stress and improve social and personal competence. Training in stress management has been found in a nonpregnant sample of Crohn's disease patients with inactive disease to significantly reduce tiredness, constipation, abdominal pain, and distended abdomen when compared with conventional medical treatment.[40] These results were found to persist upon follow up at 12 months post-treatment.[40]

In the present study, all pregnant IBD women were in clinical remission from time of conception through to time of data collection; thus, results may have been different if an additional comparison group of pregnant women with active IBD had been included. A limitation of using salivary biomarkers to assess stress during pregnancy is the lack of standardized reference ranges consistent with different levels of disease activity. The current literature only describes common patterns and associations between stress and diurnal patterns of biomarker production.

Another limitation of the present study was the small sample size of IBD cases (N = 13), which reduced power and increased the possibility of type II errors. Based on our sample, there were no significant associations between quiescent IBD and psychological and physiological stress during pregnancy.

Conclusions

Our pilot study supports the use of salivary biomarkers as an easily obtainable, noninvasive measure of stress in research settings, as well as clinical practice for patients with chronic diseases, including IBD. No differences were demonstrated in subjective stress nor dysregulation of salivary stress biomarkers by pregnant women with quiescent IBD when compared with controls, although our small sample size suggests that replication is required. Because maternal distress is linked to poorer obstetric outcomes, pregnant women with IBD who are found to display increased levels of stress may benefit from stress management counselling, intended to increase personal and social competence. The effect of chronic disease may be evaluated in future larger studies by including a comparison group of pregnant women with IBD whose disease is clinically active.

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