Trends in Faecal Calprotectin Levels During Pregnancy in Non-IBD Patients

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Abstract

Background and Aims:

No optimal marker exists to assess activity of inflammatory bowel disease (IBD) during pregnancy, though faecal calprotectin (FCP) is the most commonly used test. However minimal data exists on what a normal calprotectin level is during pregnancy and post-partum in healthy individuals.

Objective:

Our aim is to determine normal FCP levels during pregnancy and post-partum in a healthy population.

Methods:

We performed a prospective analysis of FCP levels from pregnant women at 16- and 34-weeks' gestation and 4- and 12-weeks post-partum. Patient demographics were collected. FCP concentrations were measured with a quantitative ELISA assay.

Results:

98 patients were included in our study. 172 maternal stool samples were collected in total; 62 samples at 16-weeks' gestation, 48 samples at 34-weeks' gestation, 38 samples from 4-weeks post-partum and 24 samples from 12-weeks post-partum. Median age was 33.0 years. 41 patients had a BMI > 25 (41.8%). 16 patients were ex-smokers (16.3 %). The median FCP levels at 16-weeks' gestation was 29.5 µg/g (range 10–476 µg/g), median level from 34-weeks' gestation was 25.6 µg/g (range 10–259 µg/g), from 4-weeks post-partum was 23.4 µg/g (range 10–318 µg/g) and 12-weeks post-partum was 29.4 µg/g (range 10–216 µg/g). There was no significant change in median FCP levels over the course of pregnancy and post-partum ($p = 0.294$).

Conclusion:

Faecal calprotectin levels are not affected by physiological changes in pregnancy or post-partum in normal healthy individuals without IBD. This suggests FCP is a useful tool for identifying flares of colitis during pregnancy.

Summary:

Minimal data exists on normal faecal calprotectin levels in pregnancy and especially in the post-partum period. In this prospective study we found faecal calprotectin levels are not affected by physiological

changes in pregnancy or in the post-partum period in healthy individuals without inflammatory bowel disease. This suggests calprotectin is a useful and safe tool for identifying flares of colitis during pregnancy and in the post-partum period.

Introduction:

Inflammatory bowel disease (IBD) is a lifelong chronic condition, and has a high prevalence in young adults. Many women with IBD are of reproductive age. Having IBD appears to impact women who are contemplating having children as voluntary childlessness is more common in women of child bearing age with IBD {1}. Concerns include fertility, fetal outcomes and the effect of pregnancy on the disease itself {1}.

Women who have disease remission and no previous pelvic surgery have fertility rates similar to women of a similar age without IBD. If conception occurs at a time of quiescent disease, then the risk of relapse is similar to non-pregnant women with IBD. If conception occurs when disease is active two thirds of women will have persistent activity and two thirds of these will deteriorate during pregnancy {2}. Active disease at conception or during pregnancy has been shown to increase adverse fetal outcomes such as low birth weight, preterm birth and fetal loss {3, 4}. One study of 298 pregnancies identified a 19% spontaneous abortion rate in women with IBD {5}. In one large Swedish study an increased risk of preterm birth and low birth weight was identified with IBD, and the risk of these complications was higher with active disease during pregnancy {6}. Stephansson *et al*/ found patients with ulcerative colitis with active disease had increased risks of adverse pregnancy outcomes compared to the general population {7}. Maintaining quiescent disease prior to and during pregnancy is the main determinant of good outcomes for both mother and fetus.

The early identification of flares of IBD is essential to avoid progression of disease activity and symptoms. Symptoms can however be misleading, particularly in pregnancy when gastrointestinal symptoms such as nausea, abdominal cramps and altered bowel movements are common. Non-invasive markers of inflammation such as C-reactive protein (CRP), albumin and haemoglobin can help in the diagnosis of a flare, but are not specific to gastrointestinal inflammation. Endoscopy is the gold standard for identifying and quantifying disease activity in a flare of IBD, and while it is unlikely to be harmful, safety of endoscopy in pregnancy has not been well studied and in general, both endoscopists and pregnant women prefer to avoid performing endoscopy in pregnancy. The American Society for Gastrointestinal Endoscopy guidelines state that endoscopic procedures are only justified in pregnancy when the benefit to both mother and fetus outweighs the risk {8}.

Faecal calprotectin (FCP) is a biochemical marker of inflammation that is specific to the gastrointestinal tract and its use as a non-invasive marker of IBD flares has become more widespread over the past 10 years. Calprotectin is a 36kDa calcium and zinc binding protein expressed by neutrophils. Once a neutrophil migrates to a site of chemoattraction it sets off a cascade of events eventually leading to the release of its cytosolic granules and calprotectin. The amount of calprotectin measured in a stool sample

therefore reflects the number of participating neutrophils in an area of inflammation {9}. FCP is a sensitive and specific non-invasive marker of bowel inflammation and is therefore very useful as a marker of disease activity in patients with known IBD. FCP has been shown to reflect endoscopic disease activity in Crohn's disease {10, 11} and is more sensitive than symptoms or CRP at detecting intestinal inflammation {11, 12}. In many cases, calprotectin levels begin to increase before patients develop symptoms {13}. Amongst various different biological markers, FCP has emerged as the superior non-invasive marker of gastrointestinal inflammation. FCP has become an integral component in the management of IBD and is particularly useful in pregnancy where there is a desire to avoid endoscopy. FCP levels have been studied in pregnant women with known IBD and studies have shown FCP appears to correlate with disease activity in IBD in pregnancy {14} however few data exist on the effects of pregnancy itself on calprotectin levels in patients without IBD or trends in FCP levels during pregnancy and the post-partum period. A single study by Balint *et al*/ compared FCP levels in healthy pregnant and non-pregnant women as well as pregnant and non-pregnant women with IBD and showed no effect of pregnancy on FCP, but did not examine the levels of FCP at different stages of pregnancy and the post-partum period {15}. FCP may also be affected by other factors such as body mass index (BMI) and cigarette smoking. A study of lifestyle factors and FCP as a measure of colorectal cancer risk, found that in healthy individuals there was a 40% increase in FCP levels per increase in BMI by 10 kg/m² and an increase in FCP in cigarette smokers {16}. In order to reliably use FCP as a marker of inflammation in pregnant women with IBD, it is necessary to understand the effect of pregnancy on FCP in healthy women.

In this study we aimed to determine variations in faecal calprotectin levels during pregnancy and the post-partum period in a cohort of healthy women. We also aimed to examine the effect, if any, of BMI, cigarette smoking and parity on FCP levels in pregnant healthy women.

Materials And Methods:

With institutional ethical approval and maternal written consent, a prospective observational study was performed from September 2016 to July 2019. Women were recruited from routine early pregnancy outpatient clinics at the National Maternity Hospital, Dublin. Patients were asked to provide stool samples for analysis at 16- and 34-weeks' gestation and 4- and 12-weeks post-partum. 98 healthy pregnant women were enrolled. All were healthy individuals with no history of any gastrointestinal or other medical disorders. Demographic information and baseline clinical data were collected and included recording of smoking status and measurement of BMI at each of the specified time-points. Stool samples at each time point specified above were collected by patients at home and brought to their routine outpatient appointments. Samples were then stored at - 20°C until analysis. All samples were extracted using the BÜHLMANN CALEX® Cap extraction device (B-CALEX-200, BÜHLMANN Laboratories AG). FCP concentrations were measured using the BÜHLMANN fCAL® ELISA assay (EK-CAL2-WEX) on the Grifols Triturus instrument.

The final calprotectin concentration in $\mu\text{g/g}$ stool in the patient samples was determined using the calibration curve generated from the measured calibrator values. Values below $50 \mu\text{g/g}$ are not indicative of inflammation in the gastrointestinal tract. Values between 50 and $200 \mu\text{g/g}$ can represent mild organic disease and elevated values above $200 \mu\text{g/g}$ are indicative of active inflammation in the gastrointestinal tract.

Statistical analysis was performed using Excel version 2016 and Statistical Package for the Social Sciences version 25. Descriptive statistics were provided to summarise demographic characteristics using means (95% confidence intervals (CI)) or medians for continuous variables and numbers and percentages for categorical variables. A Friedman's test was used to analyse changes in median FCP levels during pregnancy and post-partum. The Mann-Whitney U test followed by post-hoc tests were used to analyse changes in calprotectin concentrations within groups in sub-analysis. A p-value of < 0.05 was considered statistically significant.

Results:

98 women were included in the study. 172 stool samples were collected for analysis of FCP from women from different time points during pregnancy and post-partum. 62 stool samples were collected at 16 weeks' gestation, 48 samples at 34 weeks' gestation, 38 samples at 4 weeks post-partum and 24 samples at 12 weeks post-partum.

The median age of participants was 33 years (IQR 31–36). 13 (13.3%) women were multiparous and 85 (86.7%) were nulliparous. At 16 weeks' gestation, 34 (34.7%) participants were overweight with a BMI between 25 and 30 and 7 (7.14%) were obese with a BMI over 30. The median weight of participants at 16 weeks' gestation was 67 kg (IQR 63.9–73.4). The median BMI at 16 weeks' gestation was 24.4 (IQR 22.7–26.2). 16 (16.3%) patients were ex-smokers and one patient was an active smoker. Basic demographics are summarised in Table 1.

Median FCP levels were within normal levels throughout the study at each individual time point. The median FCP level in all samples collected was $27.5 \mu\text{g/g}$ (IQR $12.7\text{--}46.0 \mu\text{g/g}$). The median FCP level during pregnancy ($n = 110$) was $28.2 \mu\text{g/g}$ (IQR $12.7\text{--}47.5 \mu\text{g/g}$), while the median FCP level post-partum ($n = 62$) was $25.9 \mu\text{g/g}$ (IQR $13.8\text{--}39.8 \mu\text{g/g}$). There was no statistically significant rise in FCP levels during pregnancy or the post-partum period. The median FCP level at 16 weeks' gestation was $29.5 \mu\text{g/g}$ (IQR $17.7\text{--}49.5 \mu\text{g/g}$) and the median FCP level at 34 weeks' gestation was $25.6 \mu\text{g/g}$ (IQR $10.0\text{--}47.3 \mu\text{g/g}$) (Fig. 1). The median FCP levels at 4 weeks post-partum was $23.4 \mu\text{g/g}$ (IQR $11.4\text{--}40.6 \mu\text{g/g}$) and the median FCP level at 12 weeks post-partum was $29.4 \mu\text{g/g}$ (IQR $15.7\text{--}42.5 \mu\text{g/g}$) (Fig. 1). The median FCP level did not significantly change during pregnancy or in the post-partum period ($p = 0.29$, Z score 3.7) (Table 2).

A subgroup analysis to examine the effect of BMI, smoking history and gravity on FCP levels during pregnancy and the post-partum period was performed. Only one enrolled subject was actively smoking during pregnancy. There was no significant difference in median FCP levels between non-smokers and ex-

smokers at any time point (16 weeks gestation $p = 0.7$, 34 weeks' gestation $p = 0.4$, 4 weeks post-partum $p = 0.4$, 12 weeks post-partum $p = 0.2$). There was no significant difference in median calprotectin levels during pregnancy or post-partum depending on patient's BMI (16 weeks gestation $p = 0.7$, 34 weeks' gestation $p = 0.4$, 4 weeks post-partum $p = 0.3$, 12 weeks post-partum $p = 0.7$) or parity (16 weeks gestation $p = 0.07$, 34 weeks' gestation $p = 0.07$, 4 weeks post-partum $p = 0.25$, 12 weeks post-partum $p = 0.15$) at any timepoint (Fig. 2).

Discussion:

In this prospective study we aimed to determine faecal calprotectin levels during pregnancy and the post-partum period in healthy women in order to further our understanding of this biomarker in pregnant women with IBD. We found that faecal calprotectin levels are within normal range during pregnancy in healthy women and that they remain stable at the examined time-points during pregnancy and the post-partum period.

The management of active IBD in pregnancy is challenging. Many of the non-invasive markers of inflammation used to monitor flares of IBD may be altered during pregnancy. Iron deficiency anaemia is common in pregnancy due to haemodilution. CRP is commonly used as a biomarker for active disease in clinical practice, but has been shown to be affected by complications associated with pregnancy such as pre-eclampsia {17}. Maternal serum albumin levels fall as pregnancy progresses limiting the use of this biomarker in the assessment of inflammation {18}. Several studies have looked at the utility of calprotectin as a marker of disease activity in pregnancy for patients with established IBD. One small study of 17 women found patients with active disease pre-conception and during pregnancy had higher FCP levels than women in remission {19}. A separate prospective study of 57 women found a positive correlation between FCP levels and clinical scores such as the Harvey Bradshaw Index or the Partial Mayo Score {20}. Kanis et al also showed calprotectin had a sensitivity of 82% and specificity of 80% for diagnosing flares during pregnancy {21}.

Our data confirm the results of Balint *et al* study in highlighting that pregnancy does not alter faecal calprotectin, but also shows that calprotectin does not fluctuate during pregnancy or in the post-partum period in healthy women {15}. To our knowledge, these data are the first to examine calprotectin level in the post-partum period. There is good evidence that patients with IBD are at risk of disease flare in the post-partum period. This can be due to the disease itself, but may also be contributed to by the practice of withholding doses of biologic therapy at various points during the third trimester to avoid placental transfer {22, 23}. Although endoscopy is a viable method for identifying disease flares in the post-partum period, a reliable non-invasive marker of disease inflammation is more accessible and acceptable to patients. In confirming that FCP is not affected by systemic changes in the puerperium, we can have greater confidence in its ability to accurately identify intestinal inflammation in IBD and facilitate early and effective treatment.

The sub-analysis of the effects of BMI and parity on calprotectin levels during pregnancy demonstrated that calprotectin is not affected by these factors. The analysis of cigarette smoking was limited by the fact that only a single patient was an active smoker during pregnancy, but we did not demonstrate any effect of previous smoking. The single subject that was actively smoking had the highest calprotectin level of all the subjects examined. Although no previous studies have looked at the effects of smoking status on calprotectin levels during pregnancy, smoking has been associated with mild elevations in calprotectin levels in non-pregnant healthy individuals {16}.

This was a prospective, observational study designed to answer a clinical question of importance to those who use faecal calprotectin in the management of women with IBD during pregnancy and post-partum. The collection of stool samples for calprotectin at two points during pregnancy and a further two points in the post-partum period allowed each subject to act as their own control and is a particular strength. The study is limited by incomplete samples at some study time points. This was unavoidable as the study was dependant on subjects bringing samples to their scheduled obstetric visits. This increases the risk of a type 2 statistical error for the post-partum analysis, but the very stable and normal levels of FCP in the study subjects throughout would suggest this risk is low.

Conclusion:

These data suggest that faecal calprotectin is not affected by pregnancy or the post-partum period in healthy women and provides reassurance that elevations in calprotectin in pregnant women with IBD are due to intestinal inflammation and disease activity rather than pregnancy-related factors.

Declarations

Disclosures:

The authors have no conflicts of interest

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Authors Contribution:

Doherty: study design, data collection, manuscript writing, review and revision of manuscript.

Moore: study design, sample and data collection, review and revision of manuscript.

Byrne: study design, sample and data collection, review and revision of manuscript.

Yelverton: study design, sample and data collection, review and revision of manuscript.

Kivlehan: data analysis, review and revision of manuscript. Twomey: data analysis, review and revision of manuscript.

Cullen: study design, data collection, manuscript writing, review and revision of manuscript.

McAuliffe: study design, data collection, review and revision of manuscript.

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Tables

Table 1: Demographics for 98 enrolled subjects

Basic Demographics (n = 98)	
Median age (years) (IQR)	33.0 (31–36)
Multiparity (already has one baby)	13
Median BMI (16 weeks gestation)	24.4 (22.7– 26.2)
BMI > 25	41
Overweight	34
Obese	7
Smoker	1
Ex-smokers	16
Previous appendicectomy	11
Previous LLETZ	5
Previous tonsillectomy	10

IQR = interquartile range, BMI = body mass index, LLETZ = large loop excision of the transformation zone.

Table 2: Variation in faecal calprotectin levels in pregnancy and post-partum

Variance in calprotectin levels in pregnancy and post-partum (ANOVA)					
	16 weeks gestation (n = 62)	34 weeks gestation (n = 48)	4 weeks post-partum (n = 38)	12 weeks post-partum (n = 24)	P - value
Median FCP levels (µg/g) (IQR)	29.5 (17.7 – 49.5)	25.6 (10.0 – 47.3)	23.35 (11.4 – 40.6)	29.35 (15.7 – 42.5)	0.29
Mean Rank	3.2	2.5	1.8	2.6	

FCP = faecal calprotectin.

Figures

Figure 1

Median faecal calprotectin levels at four time points in pregnancy and post-partum (n= 172)

FCP = faecal calprotectin, BMI = body mass index

Figure 2

Variation in calprotectin levels dependant on BMI, smoking status and parity at four time points in pregnancy and post-partum (n= 172)