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

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Abstract

Background

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

Methods

A prospective analysis of FCP levels from pregnant women and post-partum women. Patient demographics were collected. FCP concentrations were measured with a quantitative ELISA assay.

Results

98 patients were included. 172 maternal stool samples were collected; 62 samples at 16-weeks' gestation, 48 at 34-weeks' gestation, 38 at 4-weeks post-partum and 24 at 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 %). Median FCP levels at 16-weeks' gestation was 29.5 µg/g (range 10-476 µg/g), at 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 ($p = 0.29$).

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.

Keywords: Faecal calprotectin, pregnancy, post-partum, variation in faecal calprotectin levels

Introduction

Inflammatory bowel disease (IBD) is a lifelong chronic condition, with a high prevalence in young adults. Many women with IBD are of reproductive age and this can impact a women's choice to reproduce.[1] 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 the risk of relapse is similar to non-pregnant women with IBD. If conception occurs when disease is active two thirds of women have persistent activity and two thirds deteriorate during pregnancy.[2] Active disease at conception or during pregnancy increases adverse fetal outcomes such as low birth weight, preterm birth and fetal loss[3, 4] with rates of spontaneous abortion quoted at 19%.[5] Increased risk of preterm birth and low birth weight has been reported with IBD. The risk of these complications appears higher with active disease during pregnancy.[6]

The early identification of flares of IBD in pregnancy is essential to avoid progression of disease activity and escalate treatment when needed. Symptoms can however be misleading, particularly in pregnancy when gastrointestinal symptoms 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 IBD flares, however the safety of endoscopy in pregnancy has not been well studied. In general, both endoscopists and pregnant women prefer to avoid performing endoscopy in pregnancy. The American Society for Gastrointestinal Endoscopy guidelines state endoscopic procedures are only justified in pregnancy when the benefit to both mother and fetus outweighs the risk.[7]

Faecal calprotectin (FCP) is a biochemical marker of inflammation that is specific to the gastrointestinal tract and used as a non-invasive marker of IBD flares. 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 correlates with disease activity in IBD in pregnancy[8] however limited 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.

In this study we aimed to determine variations in FCP levels during pregnancy and the post-partum period in a cohort of healthy women without IBD. We also aimed to examine the effect of BMI, cigarette smoking and parity on FCP levels during pregnancy.

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 provided stool samples for analysis at 16- and 34-weeks' gestation and 4- and 12-weeks post-partum. 98 healthy pregnant women were enrolled 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 μg/g stool in the patient samples was determined using the calibration curve generated from the measured calibrator values. Values below 50 μg/g are not indicative of inflammation in the gastrointestinal tract. Values between 50 and 200 μg/g can represent mild organic disease and elevated values above 200 μg/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 with ranges 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- 30 and 7 (7.14%) were obese with a BMI > 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.

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 Overweight Obese	41 34 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 1: Demographics for 98 enrolled subjects.

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 µg/g (IQR 12.7–46.0 µg/g). The median FCP level during pregnancy (n = 110) was 28.2 µg/g (IQR 12.7–47.5 µg/g), while the median FCP level post-partum (n=62) was 25.9 µg/g (IQR 13.8–39.8 µg/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 µg/g (IQR 17.7–49.5 µg/g) and the median FCP level at 34 weeks' gestation was 25.6 µg/g (IQR 10.0–47.3 µg/g) (Figure 1). The median FCP levels at 4 weeks post-partum was 23.4 µg/g (IQR 11.4–40.6 µg/g) and the median FCP level at 12 weeks post-partum was 29.4 µg/g (IQR 15.7–42.5 µg/g) (Figure 1). Median FCP level did not significantly change during pregnancy or in the post-partum period (p = 0.29, Z score 3.7) (Table 2).

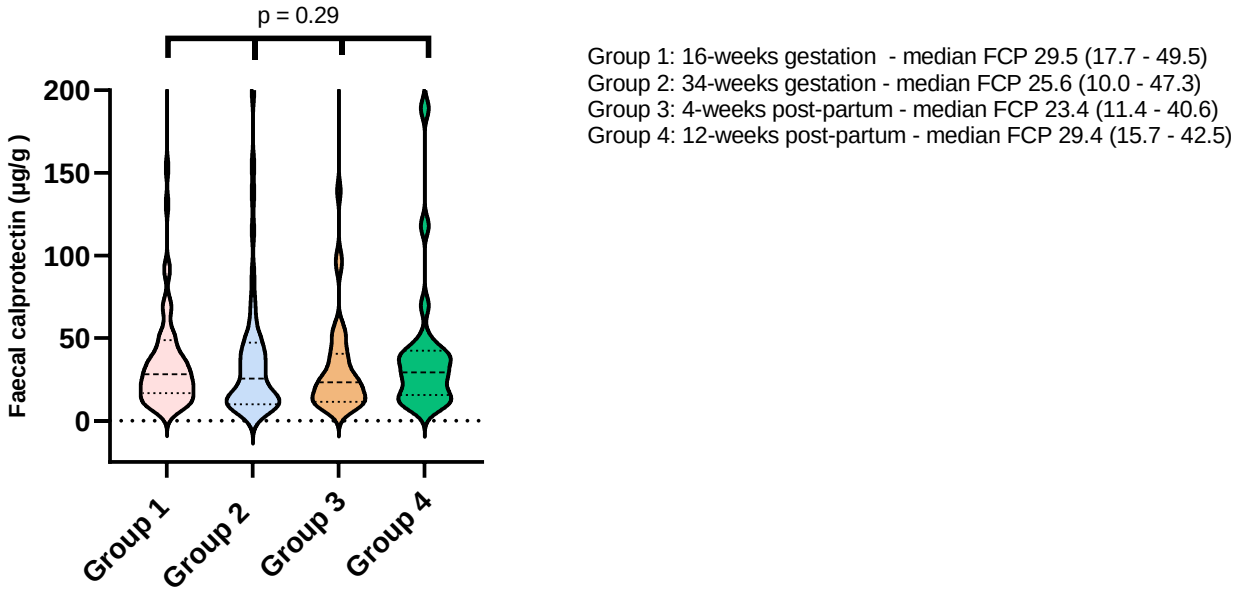


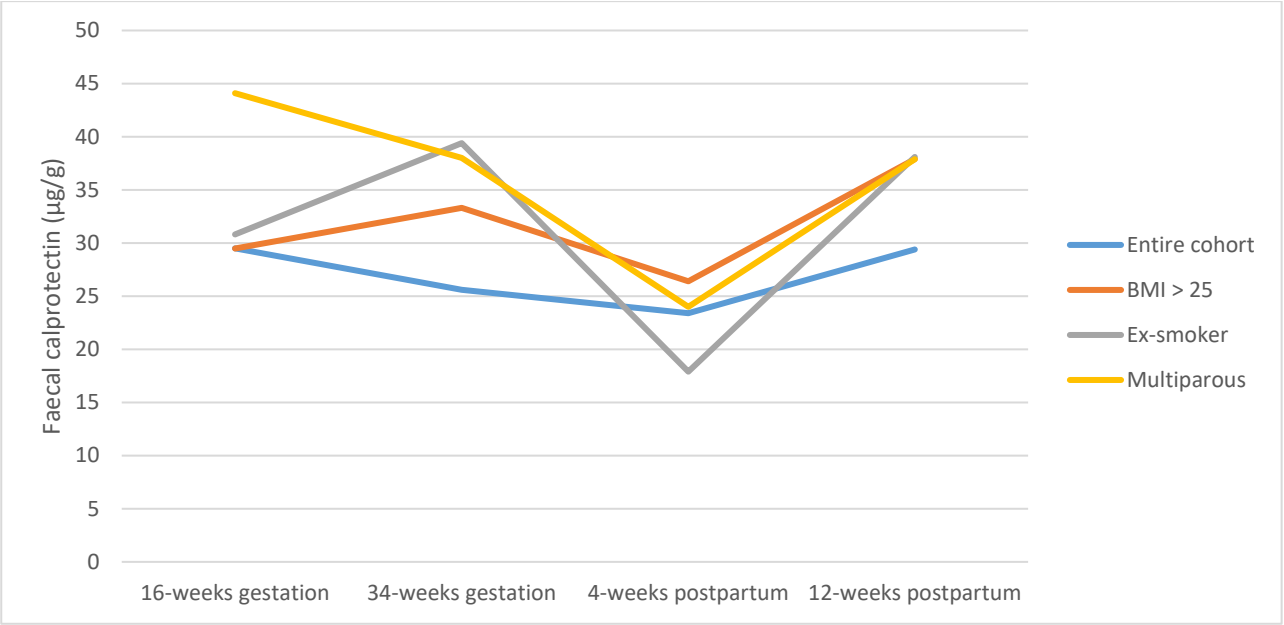
Figure 1: Median faecal calprotectin levels at four time points in pregnancy and post-partum (n= 172).

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.

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

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 (range) FCP levels between non-smokers and ex-smokers at any time point (16 weeks gestation 28.2 µg/g (10 – 476) versus 30.8 µg/g (10 – 215), p = 0.7, 34 weeks' gestation 24.5 µg/g (10 – 259) versus 39.4 µg/g (10 – 49.1), p = 0.4, 4 weeks post-partum 22.0 µg/g (10 – 99.6) versus 17.9 µg/g (10 – 318), p = 0.4, 12 weeks post-partum 27.7 µg/g (10 – 216) versus 38.1 µg/g (37.5 – 38.6) (p = 0.2) (Figure 2). There was no significant difference in median (range) calprotectin levels during pregnancy or post-partum for participants with BMI < 25 versus those with BMI > 25 (16 weeks gestation 31.2 µg/g (10 – 304) versus 29.5 µg/g (10 – 476), p = 0.7, 34 weeks' gestation 21.7 µg/g (10 – 155) versus 33.3 µg/g (10 – 259), p = 0.4, 4 weeks post-partum 19.3 µg/g (10 – 93) versus 36.4 µg/g (10 – 318), p = 0.3, 12 weeks post-partum 26.1 µg/g (10 -216) versus 37.9 µg/g (16.8 – 189), p = 0.7) (Figure 2). We found no difference in median FCP levels for participants who nulliparous versus multiparous (16 weeks gestation 25.7 µg/g (10 – 476) versus 44.1 µg/g (28.2 – 219), p = 0.07, 34 weeks' gestation 21.7 µg/g (10 – 199) versus 38.0 µg/g (10 – 259), p = 0.07, 4 weeks post-partum 23.9 µg/g (10 – 318) versus 24.0 µg/g (12.3 – 57.8), p = 0.25, 12 weeks post-partum 27.8 µg/g (10 – 216) versus 37.9 µg/g (10 – 118), p = 0.15) at any timepoint (Figure 2).



Median FCP (range)	16-weeks gestation (µg/g)	34-weeks gestation (µg/g)	4-weeks postpartum (µg/g)	12-weeks postpartum (µg/g)
Entire cohort	29.5 (10 – 476)	25.6 (10 – 259)	23.4 (10 – 318)	29.4 (10 – 216)
BMI > 25	29.5 (10 – 476)	33.3 (10 – 259)	26.4 (10 – 318)	37.9 (17 – 189)
Ex-smoker	30.8 (10 -215)	39.4 (10 – 49.1)	17.9 (10 – 318)	38.1 (37.5 – 38.6)
Multiparous	44.1 (28.2 – 219)	38.0 (10 – 259)	24.0 (12.3 - 57.8)	37.9 (10 – 118)

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).

Discussion

In this prospective study we found pregnancy and the post-partum period itself has no impact on faecal calprotectin levels in healthy individuals without IBD. Faecal calprotectin levels are within normal range during pregnancy in healthy women and remain stable during pregnancy and the post-partum period.

The management of active IBD in pregnancy is challenging. Stephansson *et al* found patients with ulcerative colitis with active disease have increased risks of adverse pregnancy outcomes compared to the general population.[9] Maintaining quiescent disease prior to and during pregnancy is the main determinant of good outcomes for mother and fetus. Unfortunately, many 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.[10] Maternal serum albumin levels fall as pregnancy progresses limiting the use of this biomarker.[11]

Calprotectin is a calcium and zinc binding protein expressed by neutrophils. Once neutrophils migrate to a site of chemoattraction they set off a cascade of events leading to the release of 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.[12] FCP is a sensitive and specific non-invasive marker of bowel inflammation and is very useful as a marker of disease activity in patients with IBD. FCP has been shown to reflect endoscopic disease activity in Crohn's disease[13, 14] and is more sensitive than symptoms or CRP at detecting intestinal inflammation.[14, 15] In many cases, calprotectin levels begin to increase before patients develop symptoms.[16]. Amongst various different markers, FCP has emerged as the superior non-invasive marker of gastrointestinal inflammation.

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 found patients with active disease pre-conception and during pregnancy had higher FCP levels than women in remission.[17] A separate larger prospective study found a positive correlation between FCP levels and clinical scores such as the Harvey Bradshaw Index or the Partial Mayo Score.[18]. Kanis et al found calprotectin had a sensitivity of 82% and specificity of 80% for diagnosing flares during pregnancy.[19] However limited 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. 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.

A single study by Balint *et al* compared FCP levels in healthy pregnant and non-pregnant women and showed no effect of pregnancy on FCP and a recent study by Kim et al found similar results however neither looked at calprotectin levels in the post-partum period.[20, 21]

Our data confirm the results of the two available studies to date[20, 21] highlighting pregnancy does not alter faecal calprotectin, and also highlights that calprotectin does not fluctuate during pregnancy or in the post-partum period in healthy women.[20] To our knowledge, these data are the first to examine calprotectin level is in the post-partum period. There is good evidence 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. In confirming 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.

FCP can also be affected by other factors such as body mass index (BMI) and cigarette smoking. A study of the impact of lifestyle factors on FCP found 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.[24]

Our sub-analysis on the effects of BMI and parity on calprotectin levels during pregnancy demonstrated calprotectin is not affected by these factors. The analysis of cigarette smoking was limited by the fact only one single patient was an active smoker during pregnancy, but we did not demonstrate any effect of previous smoking on calprotectin levels. 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.[24]

Strengths of our study include the prospective, observational nature of the study and 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.

In summary our data proves 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 and in the post-partum period are due to intestinal inflammation and disease activity rather than pregnancy-related factors.

Declaration of Conflicts of Interest:
The authors have no conflicts of interest.

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References:

1. Selinger CP, Eaden J, Selby W, Jones DB et al. Inflammatory bowel disease and pregnancy: lack of knowledge is associated with negative views. J Crohns Colitis. 2013; 7:206-213.

2. Caprilli R, Gassull M A, Escher J C, Moser G et al. European evidence-based consensus on the diagnosis and management of Crohn's Disease: special situations. Gut, 2006; 55 (1): 36-58.
3. Abdul Sultan A, West J, Ban L, Humes D et al. Adverse Pregnancy Outcomes Among Women with Inflammatory Bowel Disease: A Population-Based Study from England. Inflamm Bowel Dis 2016; 22: 1621-1630.
4. Van der Woude CJ, Ardizzone S, Bengtson MB, et al. The second European evidenced-based consensus on reproduction and pregnancy in inflammatory bowel disease. J Crohns Colitis. 2015;9(2):107–24. https://doi.org/10.1093/ecco-jcc/jju006.
5. de Lima-Karagiannis A, Zelinkova-Detkova Z, van der Woude CJ. The Effects of Active IBD During Pregnancy in the Era of Novel IBD Therapies. *Am J Gastroenterol*. 2016;111(9):1305-1312. doi:10.1038/ajg.2016.254
6. Bröms G, Granath F, Linder M, et al. Birth outcomes in women with inflammatory bowel disease: effects of disease activity and drug exposure. Inflamm Bowel Dis 2014; 20:1091–1098.
7. American Society for Gastrointestinal Endoscopy. Guidelines for endoscopy in pregnant and lactating women. ASGE 2012. Available from: https://www.asge.org/docs/default-source/education/practice_guidelines/doc-5c7150fd-910a-4181-89bf-bc697b369103.pdf?sfvrsn=d23a4a51_6
8. Tandon P, Leung K, Yusuf A, Huang VW. Noninvasive methods for assessing inflammatory bowel disease activity in pregnancy: a systematic review. J Clin Gastroenterol. 2019;53(8):574-581.
9. Stephansson O, Larsson H, Pedersen L, et al. Congenital abnormalities and other birth outcomes in children born to women with ulcerative colitis in Denmark and Sweden. Inflamm Bowel Dis 2010; 17: 795–801.
10. Hwang HS, Kwon JY, Kim MA, Park YW, Kim YH. Maternal serum highly sensitive C-reactive protein in normal pregnancy and preeclampsia. Int J Gynaecol Obstet 2007; 98: 105-109.
11. Maher JE, Goldenberg RL, Tamura T, et al. Albumin levels in pregnancy: a hypothesis--decreased levels of albumin are related to increased levels of alpha-fetoprotein. *Early Hum Dev*. 1993;34(3):209-215. doi:10.1016/0378-3782(93)90178-w
12. Bjarnason I. The Use of Fecal Calprotectin in Inflammatory Bowel Disease. Gastroenterol Hepatol (N Y). 2017;13(1):53–56.
13. D'Haens G, Ferrante M, Vermeire S, et al. Fecal calprotectin is a surrogate marker for endoscopic lesions in inflammatory bowel disease. Inflamm Bowel Dis 2012; 18:2218–2224.
14. Sipponen T, Savilahti E, Kolho KL, et al. Crohn's disease activity assessed by fecal calprotectin and lactoferrin: correlation with Crohn's disease activity index and endoscopic findings. Inflamm Bowel Dis 2008; 14:40–46.
15. Vieira A, Fang CB, Rolim EG, et al. Inflammatory bowel disease activity assessed by fecal calprotectin and lactoferrin: correlation with laboratory parameters, clinical, endoscopic and histological indexes. BMC Res Notes 2009; 2:221.
16. Wright, E. K. et al. Measurement of fecal calprotectin improves monitoring and detection of recurrence of Crohn's disease after surgery. Gastroenterology 2015; 148**:**938–947.
17. Huang V, Bal J, Foshaug RR, et al. Su1255 Fecal Calprotectin Is Elevated with Clinical Disease Activity During Pregnancy in Women with Inflammatory Bowel Disease. Gastroenterology 2015; 148(Suppl 1): S452

18. Schweistein H, Adar T, Shteingart S, Raveh1 A, Granovsky- Grisaru S, Goldin1 E, Shitrit A. P135 Serum Chitinase 3-like-1 (CHI3L1) and faecal calprotectin levels for non-invasive disease activity assessment in inflammatory bowel disease patients during pregnancy. Gastroenterology 2016;150(Suppl 1): S987.
19. Kanis S, de Lima A, Van Oorschot V, Van der Woude C. Fecal calprotectin is a poor predictor of IBD relapse during pregnancy. Gastroenterology 2016;150(Suppl 1): S556.
20. Balint, A., Berenyi, A., Farkas, K., Pallagi-Kunstar, E et al. Pregnancy does not affect fecal calprotectin concentration in healthy women. Turkish Journal of Gastroenterology, 2017; *28*: 171-175.
21. Kim E, Tarassishin L, Eisele C, Barre A, Nair N, Rendon A et al. Longitudinal Changes in Fecal Calprotectin Levels Among Pregnant Women With and Without Inflammatory Bowel Disease and Their Babies. Gastroenterology. 2021;160(4):1118-1130.e3.
22. Pedersen N, Bortoli A, Duricova D, et al. The course of inflammatory bowel disease during pregnancy and postpartum: a prospective European ECCO-EpiCom Study of 209 pregnant women. Aliment Pharmacol Ther. 2013;38(5):501–512.
23. Kane S, Lemieux N. The role of breastfeeding in postpartum disease activity in women with inflammatory bowel disease. Am J Gastroenterol. 2005;100(1):102–105.
24. Poullis A, Foster R, Shetty A, et al. Bowel inflammation as measured by fecal calprotectin a link between lifestyle factors and colorectal cancer risk. Cancer Epidemiol Biomarkers Prev. 2004;13(2):279–284.