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ORIGINAL RESEARCH

Microscopic Colitis and Reproductive Factors Related to Exposure to Estrogens and Progesterone

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Abstract: Microscopic colitis (MC) often debuts around or after menopause and is divided into lymphocytic- and collagenous colitis. The aim of this study was to examine whether factors influencing sex hormone levels differed between subgroups of MC as well as between patients and controls. A self-administered questionnaire about parity was completed which included questions surrounding age at first childbirth, menarche and menopause, the use of oral contraceptives, and hormonal replacement therapy. Patients with lymphocytic colitis had children less often compared to those with collagenous colitis (OR = 0.20, 95% CI = 0.05–0.86), however no differences were observed between patients with persistent or transient disease. Patients were less often older than 15 years of age at menarche (OR = 0.48, 95% CI = 0.26–0.91) and were younger at menopause (OR = 0.30, 95% CI = 0.16–0.56) compared with controls. Thus, no obvious association between factors influencing sex hormone levels and presence of MC could be found.

Keywords: microscopic colitis, reproductive factors, estrogen, progesterone

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Introduction

Microscopic colitis (MC) is a chronic disease of unknown etiology with mucosal, colonic inflammation. The disease is divided into collagenous colitis (CC) and lymphocytic colitis (LC), depending on the histopathological picture.[1] Interestingly, MC often debuts in women of mid- or upper middle age, at the time when endogenous sex hormone levels are diminished.[2] Thus, the involvement of sex hormones as well as autoimmunity has been discussed in the pathophysiology of MC.[2] Sex hormones may influence the function of the gastrointestinal tract. Both estrogens and progesterone have been shown to reduce the inflammation in experimentally induced colitis in rats.[3,4] Estrogens exhibit anti-inflammatory and epithelial barrier-enhancing properties in colitic rats, and the protective effect of a fermented soy germ extract are based on protease inhibition and is partly mediated by activity of an estrogen receptor ligand.[5] The assumption among health care professionals is, therefore, that the fall of estrogen- and progesterone levels at menopause could predispose to the debut of a colonic inflammation, triggered by luminal factors. However, associations between factors influencing sex hormone levels and MC have never been examined.

The aim of the present study was to compare parity age at first childbirth, menarche and menopause, as well as the use of oral contraceptives (OC) and hormonal replacement therapy (HRT)—factors that may affect the level of sex hormones during life time —between subgroups of patients suffering from MC and between patients and a population-based control group.

Material and Methods
Patients

Women who had been treated for MC at any outpatient clinic of the Departments of Gastroenterology, Skåne, between 2002 and 2010, were identified through a search for the ICD-10 classification for the two forms CC and LC (K52.8) in outpatient records, as well as in the local register at the Department of Pathology, Skåne University Hospital, Malmö. About one-third of the total number of patients identified were excluded as they were over 73 years of age, since they had many other concomitant diseases and drug therapies, representing secondary MC.[6] Of the patients recognized,

only the 240 patients (median age 63 years, range 22–73 years) who had the diagnoses verified by histopathological examination of colonic biopsies were invited to participate in the present study. Altogether, 159 (median age 63 years, range 22–73 years) of the 240 patients invited accepted to participate and were enrolled in the study. One patient was excluded due to another inflammatory bowel disease (IBD) diagnosis a few weeks after inclusion, leaving 158 patients (66%), and of these, 133 also agreed to provide blood samples. These patients represent the majority of female cases of diagnosed MC in the southernmost districts of Sweden under the age of 73 years.

Controls
The Malmö Diet and Cancer Study (MDCS)

The Malmö Diet and Cancer Study (MDCS), a population-based prospective cohort study, invited all women in Malmö born between 1923 and 1950 to participate. Recruitment was carried out between 1991 and 1996, and 41% of eligible subjects participated. In all, 17,035 women completed the baseline examination.[7] The MDCS baseline examination included a dietary assessment, a self-administered questionnaire about marital status, education, employment, smoking- and alcohol habits, parity, age at first childbirth, menarche, and menopause, exposure to OC (ever/never), current use of HRT (yes/no), and medical conditions and medication, as well as anthropometric measurements and the collection of blood samples.[8] Menopausal status was defined using information on previous surgery and menstrual status. The classification of pre-, peri- and postmenopausal women has been described in detail elsewhere.[9] Women selected as controls in a previous study on breast cancer were used in the present study as controls. In all, 737 subjects (median age 56 years, range 45–73 years) were available, the only exclusion criterion was that they should not have had a previous breast cancer at baseline.[10]

Patient recruitment and study design

Between March and June 2011, invitations including information about the study and the questionnaire described above were sent by mail to all 240 women identified with MC. They were also invited to visit the outpatient clinics of the Departments of Gastroenterology, Skåne University Hospital, Malmö or the

Central Hospital in Kristianstad, to provide blood samples. A reminder letter was sent out a month after the invitation letter to those who had not answered. Questionnaires were completed 1–3 weeks before blood samples were collected. Medical records were scrutinized, and age, gastrointestinal symptoms, examinations, and treatments were recorded. The diagnosis of either CC or LC was registered and patients were divided into two groups based on their clinical presentation of MC. One group included patients with at least two episodes of watery diarrhea, and/or their dependence on long-term treatment of corticosteroids to maintain remission and/or two pathological intestinal mucosa biopsies (MC1, n = 78, in the final population for statistical calculation). These criteria are in line with the consensus for diagnosing IBD.[11] The other group included patients who had had only one episode of severe diarrhea or had had a normal biopsy after the initial pathological intestinal biopsy, in combination with clinical remission (MC2, n = 53, in the final population for statistical calculation). Patients with concomitant celiac disease (9 patients) or an acute gastroenteritis briefly prior to the diagnostic colonoscopy (4 patients) were excluded as they had an obvious organic explanation for the intestinal inflammation, and were considered to be suffering from secondary MC.[6] Concomitant diseases and drug treatment in the controls and the patient population have been described previously.[12] In addition to MC, the patient group also suffered from many other diseases, where hypertension (36%), rheumatoid arthritis (24%), and asthma (17%) were the most prevalent. These diseases were more prevalent in the MC group than in the controls.[12]

Patients were compared to controls from the MDSC study.

Statistical analyses

The data were analyzed using the statistical software package SPSS for Windows© (Release 20.0; IBM, NY, USA). The patients were significantly older, with a wider age range than the controls. Therefore, the 12 patients younger and the two patients older than the controls were excluded, as were patients with celiac disease and gastroenteritis (13 patients), leaving 131 of the original 158 patients for statistical analysis of patients compared to controls. Thus, both controls and patients were within the age range 45–73 years.

First, the distribution of continuous variables (age, disease duration, and body mass index (BMI)) was tested using a one-sample Kolmogorov-Smirnov test. All these distributions differed significantly ($P < 0.05$) from a normal distribution. Therefore, the factors studied were categorized and the values were given as median (interquartile range). There were missing values in some variables, which were given a category of their own. Differences between groups were calculated by the 2-tailed Mann-Whitney U-test. Fisher's exact test was used for categorical variables. A P-value < 0.05 was considered statistically significant.

Age was divided into 5-year intervals. Smoking was divided into three categories: subjects who had never smoked, subjects who had stopped smoking, and current smokers, including both regular and occasional smokers. Subjects who denied intake of beer, wine, and alcoholic liquids during the previous year were defined as having no alcohol intake. Subjects were divided into three groups: subjects consuming no alcohol, subjects who had drunk some alcohol in the previous year, but not in the past month, and subjects who had drunk some alcohol in the previous month. Employment was divided into three categories: employed, retired, or other, where other included housewives, students, and the unemployed. Education was divided into having a university education or not.

Parity was dichotomized as nulliparous and parous in order to yield larger groups. Age at first childbirth was also dichotomized as ≤25 years of age and >25 years of age. HRT was defined as non-use, and use of either estrogen or progesterone replacement therapy, or combined estrogen and progesterone therapy. Age at menopause was categorized as ≤45 years, >45 and <53 years, and ≥53 years. These classifications were in accordance with previous classifications.[13] The first category was used as reference. Factors intended to be studied (independent variables), namely, age at menarche, first childbirth, and menopause; parity; exposure to OC (ever/never); exposure to HRT (current/none); or bilateral oophorectomy (yes/no), were initially examined using an unconditional logistic regression to calculate odds ratios with 95% confidence intervals (OR with 95% CI). Analyses were adjusted in a second model for age at baseline, smoking- and alcohol habits, level of education, and employment, as these characteristics

differed by >5 percentage between controls and patients, and between MC1 and MC2. Analyses were also adjusted for age at baseline, smoking- and alcohol habits, employment, and civil status in the calculations between CC and LC. Calculations were first performed on the whole patient group compared with controls, and then separately for patients with CC compared to patients with LC, and patients with MC1 compared to patients with MC2 (dependent variables).

Ethical considerations

The Ethics Committee of Lund University approved the study protocol for patients (Dnr 2009/565 and 2011/209) and controls (Dnr 51-90). All participants gave their written, informed consent to take part in the study.

Results

Patient characteristics

In total, 131 women (median age 63 (59–67) years) with MC were included in the statistical calculations; CC was diagnosed in 82 patients (62.6%) and LC in 49 patients (37.4%) (Table 1). Although identical in age range, the median age was higher in the patient group ($P < 0.001$). The duration of the disease was 7 (3–14) years. Measurements of hemoglobin (Hb) in blood and C-reactive protein (CRP) in plasma were in the majority of patients within reference values, showing that the patients were in an overall inactive phase (data not shown). Of the patients, 91.7% were born in Sweden compared with 90.2% of the controls. The number of married patients did not differ between CC and LC ($P = 0.362$). As patients with

Table 1. Patient and control characteristics.

	Controls N = 737	Microscopic colitis N = 131	*P*-value	CC N = 82	LC N = 49	*P*-value
Age at study (years)	56.16 (50.47–62.36)	63.00 (58.94–67.15)	0.000	62.77 (58.94–67.30)	63.78 (58.74–66.85)	0.761
Age groups (%)			0.000			0.763
45–49	17.1	4.6		4.9	4.1	
50–54	22.3	6.9		4.9	10.2	
55–59	22.3	13.7		15.9	10.2	
60–64	19.5	32.1		34.1	28.6	
65–69	11.7	26.0		24.4	28.6	
70–74	7.2	16.8		15.9	18.4	
Smoking habits (%)			0.076			0.380
Never smoked	42.3	27.5		26.8	28.6	
Former smokers	29.9	36.6		32.9	42.9	
Current smokers	27.8	35.9		40.2	28.6	
Alcohol habits (%)						
Missing value	0.3	3.8	0.206	6.1	0	0.573
Nothing last year	11.0	16.0		13.4	20.4	
Something last year (not last month)	12.3	13.0		13.4	12.2	
Something last month	76.4	67.2		67.1	67.3	
BMI (kg/m^2)	24.84 (22.55–27.79)	24.88 (22.62–29.15)	0.451	24.70 (21.85–29.49)	24.90 (23.10–28.33)	0.977
Missing value (%)	0	44.3		40.2	51.0	
Married women (%)	61.9	58.0	0.542	61.0	53.1	0.362
Level of education (%)						
Missing value	0	2.3	0.002	3.7	0	0.578
≤12 years at school	76.1	67.9		67.1	69.4	
>12 years at school	23.9	29.8		29.3	30.6	
Employment (%)			0.001			0.567
Employed	65.7	44.3		46.3	40.8	
Retired	26.5	49.6		46.3	55.1	
Others*	7.9	6.1		7.3	4.1	

Notes: *Includes housewives, students, and unemployed. Values are given as median (interquartile range). Mann-Whitney U-test or Fischer's exact test were used for statistical calculations. *P* < 0.05 was considered statistically significant.

LC increased in age, the number of retired persons was greater, but these changes did not reach statistical significance ($P = 0.763$ and $P = 0.567$, respectively). There was no difference between CC and LC whether MC was persistent or transient ($P = 0.273$).

Smoking and drinking habits

More patients than controls were former or current smokers (Table 1). There was no statistically significant difference between CC and LC, or between MC1 and MC2, concerning smoking habits ($P = 0.380$ and $P = 0.128$, respectively). There were only a few patients and controls who consumed beer and stronger alcoholic beverages. Thus, the alcohol intake consisted mainly of wine. The majority of subjects who drank imbibed 1–2 glasses a day. Only a minority of the subjects were non-users of alcohol (Table 1).

Reproductive factors

The only difference between CC and LC was that among patients with LC, where there was a higher percentage of nulliparity (Table 2). Furthermore, patients with LC had fewer children than patients with CC (2 (1–2) and 2 (1–3), respectively), although this did not reach statistical significance ($P = 0.057$). There was no difference in reproductive factors influencing sex hormone levels between those patients who had a transient MC and those with persistent MC (Table 3).

Table 2. Differences between collagenous colitis (CC) and lymphocytic colitis (LC).

	CC N = 82 %	LC N = 49 %	CC/LC Crude OR, 95% CI	OR, 95% CI
Age at menarche (year)				
Missing value	9.8	6.1	"–"	"–"
≤12 (reference)	28.0	18.4	1.00	1.00
>12 to <15	47.6	51.0	1.64 (0.65–4.11)	1.68 (0.62–4.54)
≥ 15	14.6	24.5	2.56 (0.84–7.76)	3.42 (0.96–12.14)
Parity				
Missing value	3.7	2.0	"–"	"–"
Nullipara (reference)	4.9	16.3	1.00	1.00
Parous	91.5	81.6	0.27 (0.08–0.94)	0.20 (0.05–0.86)
Age at first childbirth (year)				
Missing value	4.9	2.0	"–"	"–"
Nullipara (reference)	4.9	16.3	1.00	1.00
≤25	62.2	61.2	0.29 (0.08–1.06)	0.22 (0.05–0.98)
>25	28.0	20.4	0.22 (0.05–0.89)	0.16 (0.03–0.79)
Age at menopause (year)				
Missing value*	25.6	16.3	"–"	"–"
≤45 (reference)	22.0	18.4	1.00	1.00
>45 to <53	28.0	40.8	1.74 (0.64–4.73)	1.92 (0.64–5.81)
≥ 53	24.4	24.5	1.20 (0.41–3.51)	1.09 (0.33–3.64)
Exposure to OC				
Missing value	2.4	0	"–"	"–"
Never (reference)	23.2	24.5	1.00	1.00
Ever	74.4	75.5	0.96 (0.42–2.20)	0.97 (0.40–2.37)
Exposure to HRT				
Missing value	0	0	"–"	"–"
Non (reference)	91.5	91.8	1.00	1.00
Current	8.5	8.2	0.95 (0.26–3.44)	0.91 (0.23–3.59)
Bilateral oophorectomy				
Missing value	7.3	8.2	"–"	"–"
No (reference)	79.3	71.4	1.00	1.00
Yes	13.4	20.4	1.69 (0.65–4.36)	1.68 (0.58–4.88)

Notes: *Missing values includes missing values and premenopausal women. Calculations were adjusted for age, smoking habits, alcohol habits, civil status, and employment.
Abbreviations: HRT, hormonal replacement therapy; CI, Confidence interval; OC, oral contraceptives; OR, Odds ratio.

Table 3. Differences between persistent (MC1) and transient (MC2) microscopic colitis.

	MC1 N = 78 %	MC2 N = 53 %	MC1/MC2 Crude OR 95% CI	OR 95% CI
Age at menarche (year)				
Missing value	10.3	5.7	"–"	"–"
≤12 (reference)	24.4	24.5	1.00	1.00
>12 to <15	47.4	50.9	0.94 (0.40–2.22)	0.94 (0.37–2.37)
≥15	17.9	18.9	0.96 (0.33–2.81)	0.94 (0.29–3.04)
Age at first childbirth (year)				
Missing value	3.8	3.8	"–"	"–"
Nullipara (reference)	10.3	7.5	1.00	1.00
≤25	64.1	58.6	0.81 (0.22–2.90)	1.40 (0.33–5.97)
>25	21.8	30.2	0.53 (0.13–2.11)	0.67 (0.15–3.05)
Parity				
Missing value	3.8	1.9	"–"	"–"
Nullipara (reference)	10.3	7.5	1.00	1.00
Parous	85.9	90.6	0.70 (0.20–2.45)	1.03 (0.26–4.14)
Age at menopause (year)				
Missing value*	23.1	20.8	"–"	"–"
≤45 (reference)	19.2	22.6	1.00	1.00
>45 to <53	34.6	30.2	1.35 (0.51–3.59)	1.50 (0.52–4.30)
≥53	23.1	26.4	1.03 (0.37–2.88)	1.42 (0.45–4.42)
Exposure to OC				
Missing value	2.6	0	"–"	"–"
Never (reference)	19.2	30.2	1.00	1.00
Ever	78.2	69.8	1.76 (0.78–3.97)	2.24 (0.91–5.55)
Exposure to HRT				
Missing value	0	0	"–"	"–"
Non (reference)	88.5	96.2	1.00	1.00
Current	11.5	3.8	3.33 (0.69–16.06)	3.48 (0.68–17.73)
Bilateral oophorectomy				
Missing value	7.7	7.5	"–"	"–"
No (reference)	17.9	13.2	1.00	1.00
Yes	74.4	79.2	1.45 (0.54–3.90)	1.43 (0.49–4.15)

Notes: *Missing values includes missing values and premenopausal women. Calculations were adjusted for age, smoking habits, alcohol habits, level of education, and employment.
Abbreviations: HRT, hormonal replacement therapy; CI, Confidence interval; OC, oral contraceptives; OR, Odds ratio.

More of the patients had their menarche before the age of 15 years. Many controls reached menopause between 45–53 years of age, whereas more patients with MC were younger than 45 years when they reached menopause (Table 4). There was no difference in parity or age at first childbirth between the groups (Table 4). The majority of MC patients had used OC at some period of their life. In contrast, fewer patients than controls were on current treatment with HRT (Table 4).

Discussion

The present study showed no differences in the influence of sex hormones between the subgroups, except

that patients with LC were more often nulliparous than patients with CC. This is interesting as some studies have shown a female predominance in both CC and LC,[2] whereas others have not been able to confirm this in LC.[14,15] Patients with MC differed from the external control group as they had reached menarche and menopause earlier, but there was no difference in parity or age at first childbirth. More of the patients than controls had been exposed to OC at any time during their lives, and fewer were exposed to current HRT.

Although the debut of MC often occurs in predominantly middle-aged women, the role of sex hormones has never been examined in this entity. MC is

Table 4. Differences between controls and patients with microscopic colitis (MC).

	Controls N = 737 %	MC N = 131 %	Controls/microscopic colitis	
			Crude OR, 95% CI	OR, 95% CI
Age at menarche (year)				
Missing value	0	8.4	"–"	"–"
≤12 (reference)	19.1	24.4	1.00	1.00
>12 to <15	54.3	48.9	0.70 (0.44–1.12)	0.65 (0.38–1.09)
≥15	26.1	18.3	0.55 (0.31–0.98)	0.48 (0.26–0.91)
Parity				
Missing value	2.6	3.1	"–"	"–"
Nullipara (reference)	11.4	9.2	1.00	1.00
Parous	86.0	87.8	1.27 (0.67–2.40)	1.21 (0.61–2.40)
Age at first childbirth (year)				
Missing value	2.6	3.8	"–"	"–"
Nullipara (reference)	11.1	9.2	1.00	1.00
≤25	53.6	61.8	1.40 (0.73–2.69)	1.46 (0.72–2.96)
>25	32.4	25.2	0.94 (0.46–1.91)	0.79 (0.37–1.69)
Age at menopause (year)				
Missing value*	32.8	22.1	"–"	"–"
≤45 (reference)	11.0	20.6	1.00	1.00
>45 to <53	39.9	32.8	0.44 (0.26–0.75)	0.30 (0.16–0.56)
≥53	16.0	24.4	0.81 (0.45–1.46)	0.53 (0.28–1.03)
Exposure to OC				
Missing value	0	1.5	"–"	"–"
Never (reference)	50.2	23.7	1.00	1.00
Ever	49.8	74.8	3.19 (2.08–4.89)	7.49 (4.46–12.43)
Exposure to HRT				
Missing value	0.4	0	"–"	"–"
Non (reference)	79.5	91.6	1.00	1.00
Current	20.1	8.4	0.36 (0.19–0.69)	0.42 (0.22–00.83)
Bilateral oophorectomy				
Missing value	66.5	7.6	"–"	"–"
No (reference)	29.0	76.3	1.00	1.00
Yes	4.2	16.0	1.45 (0.79–2.65)	1.19 (0.60–2.35)

Notes: *Missing values includes missing values and premenopausal women. Calculations were adjusted for age, smoking habits, alcohol habits, level of education, and employment.
Abbreviations: HRT, hormonal replacement therapy; CI, Confidence interval; OC, oral contraceptives; OR, Odds ratio.

sometimes characterized as a subgroup of IBD,[16] and autoimmunity is assumed to be involved in the pathogenesis of IBD.[2] Autoimmune diseases are often in remission during pregnancy, since the elevated estrogen levels during pregnancy influence the cytokine profile in general, not only the cytokine profile in the gut.[17] Prior studies on hormonal influences have been performed in patients with IBD, and studies have shown an association between OC and a risk to develop IBD, especially Crohn's disease, among younger, premenopausal women. This increased risk of IBD reverts to that of the non-exposed population when the women stop the use of OC.[18] In a large prospective study, postmenopausal hormone therapy was associated with an increased risk of ulcerative colitis (UC), but not Crohn's disease.[19] When scrutinizing all papers written about colonic toxicity of administered drugs and chemicals, a hypercoagulable state with ischemic colitis due to mesenteric vein thrombosis was the only association found between OC and the gastrointestinal tract.[20] Both estrogen- and progesterone receptors are expressed in the gastrointestinal tract under normal conditions,[21,22] with predominance of estrogen receptor β in the colon, mainly located in epithelial cells.[21] Sex steroids have been shown to influence colonic transit time,[23] chloride ion secretion,[24] and epithelium formation.[25] One important function of the intestinal epithelium is to provide a

protective barrier for the internal milieu against luminal factors. The physical barrier is dependent on intercellular tight junctions sealing the intercellular spaces between the epithelial cells.[26] Increased intercellular permeability has been implicated in the pathogenesis of chronic, mucosal inflammation.[27,28] There is a physiological link between circulating estrogens and estrogen receptor β-mediated increase in tight junction proteins, with pivotal functions in the maintenance of intercellular spaces in female rats.[29] The protective role of estradiol in decreasing paracellular permeability enhances its beneficial effects on intestinal barrier function.[4] In recent years, progesterone has been reported to suppress inflammatory responses to reduce lipid peroxidation and cell membrane damage due to free oxygen radicals in clinical- and experimental studies.[3] Although sex hormones strenghten the epithelial barrier in experimental trials in rats,[4,29] pharmacological levels of estrogens and progesterone in OC and HRT taken over a long time span seem to increase the risk of IBD.[18,19]

Severe MC has to be treated by a derivation of fecals from the colonic mucosa, which heals after diversion.[30] This has raised the hypothesis that luminal factors trigger the mucosal inflammation. The fall in levels of estrogens and progesterone at menopause could theoretically impair the epithelial barrier function, and the mucosa could be influenced to a greater extent by luminal factors, e.g. drugs. At the same time, the older the person, the more drugs are used, and the combination of different drugs may have a synergetic effect on the mucosa. As there was no difference in sex hormone influences between MC1 and MC2, it can be suggested that other factors are further involved in the pathophysiology and maintenance of inflammation. However, one limitation in this study is that we have not measured the sex hormone levels directly, only registered factors that indirectly affect sex hormone levels. Another limitation is the small cohort in the study. Nevertheless, in light of the current knowledge on MC, a colonic, epithelial dysfunction seems more pertinent in the pathophysiology of MC than autoimmunity.[31] Intestinal ischemia, drugs, and environmental factors, such as smoking, may trigger mucosal changes which could represent an intestinal reaction to diverse irritants rather than being a specific entity.[12,32]

There are several differences which affect sex hormone levels between patients in our study and controls.

These differences must be interpreted with caution as the controls are an external group. It is very difficult to recruit healthy volunteers to clinical studies. The response rate of our control group was 41%, and therefore it can be assumed that these subjects are healthier than those who did not agree to participate. However, it is a strength for the first time to compare patients with MC with such a well-defined control group.[10] Furthermore, the data concerning smoking, overweight, and level of education were similar to a study with 80% participation of the same population.[7] The difference in OC consumption may be explained by the fact that the external control group was recruited two decades previously, and therefore may have not been exposed to OC to the same extent as the women born later. In the same way, the use of HRT was highest during the nineties, at the time when the control group was recruited. Later on, when the side effects were better known, the consumption of HRT had diminished.[33] A higher use of OC earlier in life should not be important as previous reports have shown that the increased risk of Crohn's disease during OC treatment is reversed after cessation.[18] In addition, it would have been useful to know the past use of HRT, not only the current use. However, as the response rate to this question was very low, we chose not to calculate with this parameter. Due to severe side effects of HRT, prevention of MC by prescribing HRT is not an option,[33] and thus, of no clinical interest. The study is cross-sectional, and a prospective study is necessary to determine the time of initiation of MC in relation to hormonal and environmental changes. Furthermore, few controls may also suffer from MC. However, as the prevalence of MC in the population is around 1–12 per 100000^2 this could not affect the results.

In conclusion, there were no differences in exposure to factors influencing sex hormones between CC and LC, or between MC1 and MC2, excepting that more patients with LC were nulliparous. Patients with MC reach menarche and menopause earlier and have been exposed to OC to a greater extent, and to a lesser extent to HRT, compared with the previously recruited controls. Since we found no differences in exposure to hormonal treatments between patients with transient and persistent MC, factors other than hormonal levels may affect the susceptibility to develop colonic, mucosal

inflammation to a greater extent, and luminal factors should be further examined for causality in prospective studies.

Author Contributions

Conceived and designed the experiments: BR, JM, BO. Analyzed the data: BO. Wrote the first draft of the manuscript: BO. Contributed to the writing of the manuscript: BR, JM. Agree with manuscript results and conclusions: BR, JM, BO. Jointly developed the structure and arguments for the paper: BR, JM, BO. Made critical revisions and approved final version: BR, JM. All authors reviewed and approved of the final manuscript.

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Competing Interests

Author(s) disclose no potential conflicts of interest.

Disclosures and Ethics

As a requirement of publication the authors have provided signed confirmation of their compliance with ethical and legal obligations including but not limited to compliance with ICMJE authorship and competing interests guidelines, that the article is neither under consideration for publication nor published elsewhere, of their compliance with legal and ethical guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copyrighted material. This article was subject to blind, independent, expert peer review. The reviewers reported no competing interests.

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