Impact of Smoking Cigarette on the mRNA Expression of Cytokines in Mucosa of Inflammatory Bowel Disease

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Summary

It is well known that smoking is the risk factor in the development and clinical course of Crohn's disease (CD), but on the other hand, smoking is a protective factor against ulcerative colitis (UC). The pathways that are influenced by smoking in CD and UC are poorly understood. The aim of our study was to analyse the influence of smoking on the mRNA expression of cytokines in mucosa in patients with CD and UC. We performed a cross-sectional study. The cohort consisted of 86 IBD patients (48 CD patients and 38 UC patients) and took place at the IBD Centre at the University Hospital Bratislava-Ružinov. We took the demographic and clinical data of each patient, including information about their smoking habits. We performed a colonoscopy on each patient and took biopsies from both inflamed and non-inflamed sigma (CD, UC) and terminal ileum (CD). mRNA was extracted from mucosal biopsy samples for each cytokine and was normalized to a housekeeping gene (GAPDH). Finally, we compared the mRNA expression of target cytokines in the mucosa of smokers and non-smokers in IBD patients. Smokers with Crohn's disease have a significantly higher mRNA expression of pro-inflammatory cytokine TNF-α ($p=0.003$) in inflamed mucosa in sigma compared with non-smokers. In smokers with ulcerative colitis, we observed significantly higher mRNA expression of anti-inflammatory cytokine IL 10 ($p=0.022$) in non-inflamed mucosa of sigma. Similarly, smokers with UC have a significantly decreased mRNA expression of cytokine TLR 2 ($p=0.024$) and CCR1 ($p=0.049$) in non-inflamed mucosa of sigma. Based on our results, smoking has a positive influence on cessation and the clinical course of UC due to the stimulation of anti-inflammatory cytokine IL 10 in mucosa. On the other hand, smokers with CD have a higher expression of pro-inflammatory cytokine TNF-α, which could be associated with a worsening of the disease and response to therapy.

Key words

Inflammatory bowel disease • Mucosa • mRNA cytokine • Smoking

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Introduction

Inflammatory bowel diseases (IBDs) are chronic recurring inflammatory gastrointestinal diseases that comprise Crohn's disease (CD) and ulcerative colitis (UC). The etiology of IBD arises from a complex interplay between host genotypes, the immune system and environmental factors (Berkowitz *et al.* 2018).

Smoking is a known risk factor for CD that is

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associated with the worsening of the illness and quality of life, a higher rate of hospitalization (Golovics *et al.* 2015), a higher recurrence after surgery (Yamamoto and Keighley 2000), and a poor response to medical therapy (Parkes *et al.* 2014). The detrimental effects of smoking on CD are ascribed to damaged mucosal barriers due to an increase in intestinal permeability and an alteration of tight junctions (Allais *et al.* 2016, Zuo *et al*. 2014) as well as affected gut microbiota. The dysbiosis of gut microbiota is characterized by an increase in *Firmicutes* and *Actinetobacteria* and a decrease in *Bacteroides* and *Proteobacteria* (Biedermann *et al.* 2014). Smoking also alters microcirculation and significantly reduces blood flow to the gastrointestinal mucosa (Zuo *et al*. 2014). Nevertheless, cigarette smoking causes an influx of neutrophils and CD4+ INF γ^+ T cells into intestinal mucosa (Allais *et al.* 2017, Verschuere *et al*. 2011).

These results cause an injury in the epithelium that leads to the exposure of lumen antigens which can be recognized by immune cells whose recruitment is increased, inducing the development of CD (Ueno *et al.* 2014). Moreover, cigarette smoking upregulates the mRNA expression of cytokines of Th-1 response such as CCL9, CCL20 and IL-1, three important chemokines in the pathogenesis of CD (Lee *et al*. 2017, Oberg *et al*. 2011, Verschuere *et al.* 2011).

Conversely, smoking appears protective against ulcerative colitis and UC smokers are less likely to need hospitalization, potent drugs or require a colectomy (Mahid *et al*. 2006). A beneficial effect of nicotine in UC is explained by the activation of the nicotinic receptors α7 (α7-nAChR) in immune cells such as macrophages and dendritic cells (de Souza and Fiocchi 2016). The stimulation of these receptors α7 (α7-nAChR) by nicotine leads to the decreased production of pro-inflammatory cytokines TNF-α and IL-2 and suppresses the function of $CD4^+$ $CD25^+$ regulatory T cells (Lakhan and Kirchgessner 2011, Gomes *et al.* 2018). Additionally, the α7-nAChR receptor is also expressed by endothelial cells, where its activation decreases the production of chemokines and the expression of adhesion molecules in the endothelium. This mechanism can help to modulate leukocyte migration and inflammation (Saeed *et al.* 2005).

Consistently, a French study performed by Seksik *et al.* (2009) has shown that CD non-smokers spent less time with the active disease compared to CD smokers. In a cohort of UC patients, a reduction in oral corticosteroid use and colectomy rates in UC smokers was found. Two further studies published similar results, showing that continued smoking is a risk factor for worsening the disease based on the Montreal Classification (disease progression from B1 to B2/B3) (Lakatos *et al.* 2013, Lawrance *et al.* 2013).

Tabaco smoke contains more than 4500 chemicals, among these nicotine, carbon monoxide (CO), and nitrogen oxide are known to have immunomodulatory capacities as well as to interact with intestinal immunity and gut function. The first tissues that interact directly with cigarette smoke are in the respiratory and gastrointestinal tracts, where it can affect local immune response (Talhout *et al.* 2011). The pathways by which cigarette smoking exerts the opposite effect in CD and UC have not been fully clarified yet.

Aim of the study

The aim of this study was to identify whether and how smoking affects the mucosal immune system, especially the mRNA expression of cytokines in the mucosa of IBD patients.

Methods

Study design

Consecutive patients with inflammatory bowel disease who underwent a colonoscopy between February and October 2013 were included in this cross-sectional study. All of these IBD patients were followed-up with at the 5th Department of Internal Medicine, Sub-department of Gastroenterology and Hepatology of the University Hospital in Bratislava Ruzinov and the Faculty of Medicine of Comenius University in Bratislava. Clinical information including smoking habits was obtained from each patient at the time of the colonoscopy. During the colonoscopy, biopsies were taken from inflamed and non-inflamed mucosa. Mucosal samples were collected for an extraction of mRNA for each cytokine. Finally, we compared the mRNA expression of cytokines in the intestinal mucosa between smoking and non-smoking IBD patients.

Cohort

Mucosal biopsy samples were collected from 86 patients with IBD (48 with CD, 38 with UC) during the colonoscopy. The diagnosis of CD as well as UC was established by standard clinical, endoscopic and histological criteria and classified according the Montreal Classifications. (22) Among CD patients, 38 were

non-smokers and 10 were smokers. The UC cohort consisted of 30 non-smokers and 8 UC smokers. Additional data of IBD patients is included in Table 1.

Tobacco use

The smoking status of all patients was categorized into "*never being smokers*" and "*former smokers*" as non-smokers, which comprised answers in a questionnaire and "*current smokers"* as smokers. From *"current smokers,"* the average number of cigarettes per day was recorded.

Colonoscopy and biopsies

In CD patients, during the colonoscopy we took biopsy specimens from the terminal ileum and sigma from the inflamed mucosa and if applicable also from the non-inflamed mucosa. Biopsy specimens from the sigma were collected in the same manner from UC patients. Inflamed mucosa was defined as the presence of marked hyperaemia, friability, erosions or ulcers. The obtained mucosal biopsies were immediately immersed in an RNA stabilizing solution (RNA *later*, Qiagen) until processed and sent for mRNA cytokines analysis the same day.

Table 1. Clinical characteristics of IBD smokers and non-smokers according Montreal classification

IBD		**CD non-smokers**	**CD smokers**	**UC non-smokers**	**UC smokers**
(n=86)		(n=38)	(n=10)	(n=30)	(n=8)
Sex (male / female)		26/12	8/2	15/15	6/2
Median age (range) yrs		44 (24-68)	43 (27-53)	44 (23-68)	48 (28-70)
Average cigarettes per/day		-	10.1 (20-1)	-	9.4 (20-2)
Location:	L1	15 (39 %)	4 (40 %)	E1 3 (10 %)	0 (0 %)
	L2	4 (11 %)	1 (10 %)	E2 12 (40 %)	5 (62 %)
	L3	18 (47 %)	5 (50 %)	E3 15 (50 %)	3 (38 %)
	L4	1 (3 %)	-	-	-
Behavior:	B1	13 (34 %)	2 (20 %)	-	-
	B2	14 (37 %)	3 (30 %)	-	-
	B3	11 (29 %)	5 (50 %)	-	-
IBD surgery		18 (47 %)	5 (50 %)	0 (0 %)	1 (12.5 %)
Vitamin D		24	30	29	31,5
Median ng/ml (Range)		(5-59)	(7-68)	(6-60)	(12-44)
Calprotectin		144	113	240	57
Median ug/g (Range)		(1-1141)	(8-990)	(1-1408)	(48-179)

Table 2. Colonoscopy biopsies samples of IBD patients

Analyzed tissue samples (n=179)	**Sigma (n=116)**		**Terminal ileum (n=63)**	
	Non-inflamed (n=78)	Inflamed (n=38)	Non-inflamed (n=37)	Inflamed (n=26)
Ulcerative colitis	44	24	-	-
Crohn's disease	34	14	37	26

Real time PCR (RT-qPCR)

Tissue RNA was isolated by RNeasy Mini Kit (QIAGEN) for each cytokine (TNF-α, FOXP3, IL-6, IL8, IL-10, IL-12, IL-17, IL-23, TLR2, TLR4, TLR5 CCR1, CCR2, CCR5, CCR9, CCL5, CD207, CD 206) according to the manufacturer's instructions. RNA samples of the

Table 3. Comparison of the mRNA expression of cytokines between CD smokers and CD non-smokers in sigma

Non inflamed sigma					
mRNA cytokine	**Mean smokers ΔC1**	**Std. deviation**	**Mean non-smokers ΔC2**	**Std. deviation**	**P**
IL 6	-10.958	3.844	-11.841	2.658	0.301
IL 8	-8.949	3.123	-9.533	2.770	0.750
IL10	-11.457	3.967	-11.982	2.423	0.182
IL12	-11.431	3.852	-12.212	2.513	0.252
IL 17	-11.537	3.846	-12.493	2.560	0.279
IL 23	-11.025	3.841	-11.231	2.337	0.169
TLR 2	-10.321	3.663	-11.133	2.252	0.121
TLR 4	-11.440	2.914	-12.384	1.867	0.846
TLR 5	-10.166	3.465	-10.812	1.988	0.209
CD 206	-8.401	2.690	-8.656	1.509	0.195
CD 207	-9.228	2.340	-10.097	1.832	0.537
CCL 5	-6.615	2.323	-7.002	1.179	0.105
CCR 1	-8.546	2.061	-9.448	1.762	0.703
CCR 2	-10.524	5.110	-10.660	2.442	0.051
CCR 5	-8.053	2.676	-8.707	1.610	0.189
CCR 9	-7.980	1.593	-9.096	1.768	0.244
FOXP3	-8.487	2.183	-9.318	1.681	0.849
TNF α	-8.643	1.195	-8.938	1.480	0.482
Inflamed sigma					
mRNA cytokine	**Mean smokers ΔC1**	**Std. deviation**	**Mean non-smokers ΔC2**	**Std. deviation**	**P**
IL 6	-12.590	0.192	-11.434	2.487	0.093
IL 8	-8.415	1.993	-7.467	3.231	0.440
IL10	-12.503	0.880	-11.852	1.413	0.325
IL12	-12.386	0.401	-12.348	0.964	0.170
IL 17	-11.590	0.192	-12.358	0.819	0.233
IL 23	-11.770	0.741	-10.238	1.645	0.164
TLR 2	-10.642	2.313	-9.884	2.157	0.922
TLR 4	-12.372	1.676	-12.886	1.209	0.747
TLR 5	-10.185	1.486	-10.725	0.940	0.552
CD 206	-9.284	1.141	-8.570	1.200	0.976
CD 207	-11.071	0.652	-10.620	0.773	0.845
CCL 5	-7.397	0.528	-7.008	0.651	0.529
CCR 1	-9.689	1.191	-7.940	2.029	0.486
CCR 2	-10.803	0.773	-10.569	1.157	0.426
CCR 5	-9.130	1.286	-7.953	1.195	0.849
CCR 9	-9.054	2.016	-10.022	1.327	0.563
FOXP3	-9521	1.773	-8.300	1.500	0.681
TNF α	-7.860	1.392	-9.927	0.526	0.003

ΔC1 represents the difference in the number of PCR cycles needed to express the mRNA of the house keeping gene GAPDH and the number of PCR cycles needed to express the mRNA of the target cytokine of smokers with IBD, ΔC = (ΔC house-keeping gene - ΔC cytokines), ΔC2 represents the difference in the number of PCR cycles needed to express the mRNA of the house keeping gene GAPDH and the number of PCR cycles needed to express the mRNA of the target cytokine of non- smokers with IBD, ΔC = (ΔC house-keeping gene - ΔC cytokines), P: two-tailed p-value evaluating the null hypothesis against an alternative hypothesis

cytokines were converted to cDNA using Thermo Scientific Maxima H Minus First Strand cDNA Synthesis K. DNA was isolated with a DN-easy Blood & Tissue Kit (QIAGEN). The quality and quantity of RNA was screened by an Agilent RNA 6000 Nano Kit. The gene expression of cytokines was analyzed by a custom array gene expression kit (QIAGEN) including house-keeping gene (Glyceraldehyde 3-phosphate dehydrogenase, GAPDH). Data were analyzed by RT2 Profiler PCR Array Data Analysis v3.5 software (QIAGEN). Next, we analyzed the difference between the expression of mRNA of the target cytokine and the expression of mRNA of the housekeeping gene, GAPDH. The difference was expressed as ΔC, which represents the difference in the number of PCR cycles needed to express the mRNA of the house keeping gene GAPDH ($\Delta C_{\text{house-keeping gene}}$) and the number of PCR cycles needed to express the mRNA of the target cytokine ($\Delta C_{\text{cytokine}}$). $\Delta C=(\Delta C_{\text{house-keeping gene}} - \Delta C_{\text{cytokine}})$.

Statistical analysis

Statistical analyses were performed using SPSS 19.0 (IBM SPSS Inc., Chicago, Illinois, United States). Nominal and ordinal variables such as clinical characteristics were analyzed using the Chi square test with Yate′s correction. If any cell of the contingency table contained a value of less than 5, Fisher's exact test was used instead. The Kolmogorov-Smirnov test was used to analyze the normality of the distribution of measured parameters (age, duration of the disease, VD serum concentration). We compared the mRNA expression of cytokines (ΔC, see above) between smokers and non-smokers in inflamed and non-inflamed mucosa using a paired sample – T test in patients with ulcerative colitis (sigma) as well as in patients with Crohn′s disease (sigma/terminal ileum). Statistical significance was considered at a level of $P<0.05$.

Ethical considerations

The study was approved by the local ethical committee. All the subjects gave their written informed consent to participate in the study.

Results

Study population

Study cohort consists of 86 IBD patients, 48 patients with Crohn′s disease and 38 patients with ulcerative colitis. One patient with ulcerative colitis has left hemicolectomy prior to entering the study, we took biopsy samples from non-inflamed sigma.

Analyses of the expression of mRNA of cytokines between smokers and non-smokers in CD patients

Firstly, we found out a significantly higher the mRNA expression of cytokines TNF-α (ΔC_1=-7.860±1.392 smokers vs. $\Delta C_{2=}$ -9.927±0.526 non-smokers, p=0.003) in inflamed mucosa of sigma of CD smokers (Table 4). Another our analyses did not show any differences between smokers and non-smokers in non-inflamed terminal ileum as well as inflamed terminal ileum (Table 3).

Analyses of the expression of mRNA of cytokines between smokers and non-smokers in UC patients

Next, we analyzed the mRNA expression of cytokines in non-inflamed mucosa of sigma in UC smokers and non-smokers patients (Table 3). We observed a significantly higher mRNA expression of cytokines IL 10 (ΔC_1= -10.862±5.174 smokers vs. $\Delta C_{2=}$ -12.576±1.688 non-smokers, p=0.022) in UC smokers. There was also significant lower expression of mRNA of cytokines TLR 2 (ΔC_1= -12.240±0.873 smokers vs. ΔC_2= -11.10 7±1.621 non-smokers, p=0.024) and CCR1 (ΔC_1= -10.197±0.572 smokers vs. ΔC_2= -9.662±1.071 non-smokers, p=0.049) in smokers (Table 5).

In the same group of patients, biopsy samples could not be obtained from inflamed mucosa of sigma, as the patients had only a non-inflamed mucosa of sigma.

Discussion

Presented results showed a significant increase in the mRNA expression of TNF-α in the inflamed mucosa of sigma in the subgroup of CD smokers compared to CD non-smokers. This explains that smoking might cause the increased recruitment of CD4+ and CD8+ T cells as well as CD11b+ dendritic cells in the mucosa of the colon (Allais *et al*. 2017, Arnson *et al.* 2010). These immune cells produce a large number of Th1/Th17-associated pro-inflammatory cytokines such as IFN-γ, IL-17A, and TNF-α (Lackeyram *et al.* 2017). The highest mRNA expression of TNF-α is in the first stage of the establishment of the disease (Zorzi *et al.* 2013, Baumgart and Sandborn 2012). The molecular mechanism at least could be partially responsible for the immunomodulation capabilities of smoking involves the activation of NF-κB nuclear translocation. According to recent studies, NF-κB is a key transcription factor regulating the mRNA expression of various pro-inflammatory cytokines and its activation leads to a higher mRNA expression of pro-inflammatory cytokines, especially TNF-α, in smokers (Hasnis *et al.* 2007).

Table 4. Comparison of the mRNA expression of cytokines between CD smokers and CD non-smokers in terminal ileum

Non inflamed terminal ileum					
mRNA cytokine	**Mean smokers ΔC^1**	**Std. deviation**	**Mean non-smokers ΔC^2**	**Std. deviation**	**P**
IL 6	-12.588	0.917	-11.860	2.675	0.195
IL 8	-9.172	2.958	-9.010	2.245	0.469
IL10	-12.013	3.318	-12.255	2.087	0.290
IL12	-12.172	3.358	-12.142	2.160	0.340
IL 17	-12,157	3.992	-12.783	2.322	0.174
IL 23	-11.537	3.846	-12.493	2.176	0.471
TLR 2	-9.731	1.981	-9.550	1.738	0.486
TLR 4	-11.919	2.048	-12.225	1.424	0.236
TLR 5	-10.439	2.647	-10.818	1.621	0.130
CD 206	-8.746	1.800	-8.356	1.054	0.491
CD 207	-9.353	1.662	-9.382	1.681	0.923
CCL 5	-5.902	1.369	-5.903	1.280	0.656
CCR 1	-8.857	1.141	-8.670	1.067	0.825
CCR 2	-10.775	2.952	-10.569	2.074	0.615
CCR 5	-7.633	1.974	-7.727	1.665	0.147
CCR 9	-9,972	2.249	-10.688	2.047	0.567
FOXP3	-8.987	2.182	-9.107	1.717	0.553
TNF α	-8.104	1.259	-8.257	1.319	0.778
Inflamed terminal ileum					
mRNA cytokine	**Mean smokers ΔC^1**	**Std. deviation**	**Mean non-smokers ΔC^2**	**Std. deviation**	**P**
IL 6	-8.951	3.274	-9.587	3.556	0.822
IL 8	-4.264	4.182	-5.462	3.005	0.114
IL10	-10.754	1.629	-10.915	2.760	0.364
IL12	-12.604	0.877	-12.127	2.932	0.193
IL 17	-12.843	0.725	-12.943	2.746	0.245
IL 23	-9.992	1.646	-9.686	2.649	0.343
TLR 2	-8.203	2552	-8.421	2.462	0.121
TLR 4	-11.957	1.404	-12.471	2.225	0.482
TLR 5	-10.559	1.379	-10.383	2.116	0.554
CD 206	-7.421	1.148	-7.960	1.690	0.609
CD 207	-10.332	0.834	-10.196	1.881	0.351
CCL 5	-7.461	1.508	-6.967	1.547	0.981
CCR 1	-6.398	2.009	-6.908	2.209	0.743
CCR 2	-9.403	1.451	-9.685	2.505	0.264
CCR 5	-7.204	1.222	-7.430	1.815	0.420
CCR 9	-10.812	0.974	-10.328	1.916	0.634
FOXP3	-7.633	1.616	-7.946	1.726	0.741
TNF α	-7.487	1.611	-7.250	2.213	0.489

ΔC1 represents the difference in the number of PCR cycles needed to express the mRNA of the house keeping gene GAPDH and the number of PCR cycles needed to express the mRNA of the target cytokine of smokers with IBD, ΔC = (ΔC house-keeping gene - ΔC cytokines), ΔC2 represents the difference in the number of PCR cycles needed to express the mRNA of the house keeping gene GAPDH and the number of PCR cycles needed to express the mRNA of the target cytokine of non- smokers with IBD, ΔC = (ΔC house-keeping gene - ΔC cytokines), P: two-tailed p-value evaluating the null hypothesis against an alternative hypothesis

Table 5. Comparison of the mRNA expression of cytokines between UC smokers and non-smokers in non-inflamed sigma

Non inflamed sigma					
mRNA cytokine	**Mean ΔC1 in smokers**	**Std. deviation**	**Mean ΔC2 in non-smokers**	**Std. deviation**	**p**
IL 6	-13.132	1.736	-12.968	2.108	0.609
IL 8	-11.208	1.015	-10.922	2.370	0.232
IL10	-10.862	5.714	-12.576	1.688	0.022
IL12	-13.104	1.514	-12.846	0.985	0.413
IL 17	-14.100	2.033	-13.474	2.043	0.940
IL 23	-11.776	1.078	-11.900	1.658	0.409
TLR 2	-12.240	0.873	-11.107	1.621	0.024
TLR 4	-11.730	1.022	-11.910	1.218	0.977
TLR 5	-11.245	1.318	-10.733	1.031	0.683
CD 206	-9.571	0.602	-9.630	0.824	0.220
CD 207	-11.640	1.293	-10.799	0.994	0.437
CCL 5	-7.773	0.725	-7.552	1.084	0.262
CCR 1	-10.197	0.572	-9.662	1.071	0.049
CCR 2	-11.680	1.506	-10.968	1.953	0.446
CCR 5	-9.384	1.003	-9.503	0.860	0.868
CCR 9	-8.856	1.290	-8.804	1.457	0.827
FOXP3	-10.093	0.694	-10.313	0.875	0.589
TNFα	-10.013	1.220	-9.634	0.933	0.181

ΔC1 represents the difference in the number of PCR cycles needed to express the mRNA of the house keeping gene GAPDH and the number of PCR cycles needed to express the mRNA of the target cytokine of smokers with IBD, ΔC = (ΔC house-keeping gene - ΔC cytokines), ΔC2 represents the difference in the number of PCR cycles needed to express the mRNA of the house keeping gene GAPDH and the number of PCR cycles needed to express the mRNA of the target cytokine of non- smokers with IBD, ΔC = (ΔC house-keeping gene - ΔC cytokines), P: two-tailed p-value evaluating the null hypothesis against an alternative hypothesis

According to our results, smoking has a positive effect on the mucosa of sigma in patients with ulcerative colitis. UC smokers were characterized by a higher mRNA expression of anti-inflammatory cytokine IL-10.

In this context, several inherent pharmacological properties of cigarette smoke could explain this. Carbon monoxide (CO) as a component of the gas phase of cigarette smoke is associated with anti-inflammatory properties. CO reduces antigen presentation by APCs and prevents the maturation of dendritic cells (DCs) (Sheikh *et al.* 2011, Riquelme *et al*. 2016, Mackern-Oberti *et al*. 2015). CO-treated DCs retained the capacity to secrete anti-inflammatory cytokine IL-10 despite losing their ability to produce pro-inflammatory cytokine IL-12 (Riquelme *et al.* 2016).

However, the anti-inflammatory effect of cigarette smoke prevails in UC but not in ileal CD. This could be explained by the different expression of Aryl hydrocarbon (AhR) receptors. Stimulation by dioxide as a product of tobacco combustion induces an anti- inflammatory and protective response (Chinen *et al.* 2015). Monteleone *et al.* (2011) reported in their study that the expression of AhR is downregulated in inflamed CD tissue in contrast with inflamed UC tissue.

Our finding of an increased mRNA expression of IL-10 is in line with the results of Neisner and Volk (1995) who reported an elevated mRNA expression of IL-10 in biopsies from UC patients.

In our study, we found a significantly decreased mRNA expression of Toll-like receptor 2 (TLR2) in UC smokers in comparison with UC non-smokers in non-inflamed mucosa of sigma. Yuing Fan and colleagues in their study observed an overexpression of TLR2, TLR4, and TLR9 in the colonic mucosa of patients with UC (Fan and Liu 2015). The purpose of this receptor upregulation may be to increase the antigenic stimulation of inflammatory and immune pathways activated by ligand binding (Lee *et al.* 2006). Canto *et al.* (2006) identified an upregulated expression of TLR2 in peripheral blood monocytes which was associated with elevated circulating TNF-α concentrations in active UC and CD. Our study showed a significantly lower mRNA

expression of TLR2, as well as a lower mRNA expression of TNF-α, but in non-inflamed mucosa of sigma.

Finally, all of our UC smokers were also characterized by a significantly decreased mRNA expression of chemokine receptor CCR1 in non-inflamed mucosa in sigma compared to UC non-smokers. As for CCR1, the ligands for this receptor include CCL3, CCL5 (RANTES), CCL7, and CCL23 (Danese and Gasbarrini 2005). Several studies reported the increased expression of CCL3 and CCL5, mainly in intraepithelial lymphocytes and in the subepithelial lamina propria in colonic biopsies of UC and CD patients (Ajuebor *et al.* 2001, Mazzucchelli *et al.* 1996). This suggests that the role of CCL5/RANTES during chronic colitis appears to be attracting CCR1- and CCR5-bearing inflammatory cells into colonic tissue where the activation of these cells leads to tissue ulceration (Gunaltay *et al*. 2015, Andres *et al*. 2000). In our opinion, this reflected that ongoing tobacco (smoking) might suppress the mRNA expression of CCL5 and result in a decrease in the mRNA expression of its chemokine receptor CCR1. There is a lack of studies about the influence of smoking on the mRNA expression of chemokine receptors in patients with IBD.

To conclude, CD smokers had a significantly higher mRNA expression of the pro-inflammatory cytokine TNF-α in the inflamed mucosa of sigma compared to CD non-smokers. UC smokers had a significantly higher mRNA expression of anti-inflammatory cytokine IL-10 in the non-inflamed mucosa of sigma compared to non-smokers. In the same group, we observed a significantly lower mRNA expression of TLR2 and CCR1 in the non-inflamed mucosa of sigma.

Although, nicotine showed positive effect on the expression mRNA cytokines in colonic mucosa of UC patients, it is not sufficient to treat UC. A number of clinical studies, that tested nicotine as a treatment, reported that nicotine lacks efficacy in treating disease relapses and remission (Pullan *et al.* 1994, Sanborn *et al.* 1997, Ingram *et al.* 2005). In addition, they also reported sides effects as a result of the high systemic nicotine concentrations required (Nikfar *et al.* 2010).

Limitations

The first limitation of this study is the moderate size of the cohort, which influenced the statistical power of the study. The second limitation is that our analyses did not cover the effects of therapy, which potentially may affect the mRNA expression of target cytokines. Thirdly, all of the possible confidential clinical data that might have influenced the results was not collected (oral contraceptive use, passive smoking effect, daily dose of nicotine).

Conflict of Interest

There is no conflict of interest.

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