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Different effect of smoking on genders in Crohn's disease

BOWEL

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Original Article

ABSTRACT

Background/Aims: Smoking is a well-established environmental risk factor in Crohn's disease (CD). The study was aimed to investigate possible gender differences in the effect of smoking on the course of CD, with a special focus on selected immune parameters such as Th1/Th2/Th17 cytokines and regulatory T cells (Tregs).

Materials and Methods: A group of 55 adults with CD was enrolled to the study. The analysis of clinical, demographic and immunological characteristics of patients was performed according to their smoking status and gender. Values were considered significant when $p \leq 0.05$.

Results: Patients who smoked, particularly females, more frequently suffered from a moderate-to-severe form of the disease, requiring glucocorticoid and immunosuppressive therapies. Smokers, particularly females, were also hospitalized and underwent surgeries more frequently than non-smokers. Cytometric analysis showed higher levels of serum proinflammatory cytokines and lower levels of peripheral Tregs in female smokers and former smokers, comparing to males from these subgroups.

Conclusion: Presented results demonstrate that in all investigated subgroups, particularly however among current smokers and former smokers, female patients seemed to be more affected by CD. Females developed more severe form of the disease and experienced the onset earlier than men. The imbalance between pro- and anti-inflammatory factors observed in CD patients was also more distinct in female smokers and former smokers, comparing to males, and could substantially contribute to the severity of the disease. Exposure to smoking seems to be one of the environmental factors contributing to the gender differences in CD.

Keywords: Crohn's disease, smoking, gender differences, Th1/Th2/Th17 cytokines, regulatory T cells

INTRODUCTION

Crohn's disease (CD) is a chronic, relapsing inflammatory disease (IBD), originally defined as a disease of the ileum characterized by a granuloma-like immune pathology (1). CD involves the entire alimentary tract, starting from the mouth, however, in most cases can be detected in three locations: solely in the small intestine, solely in the colon, and in both the small and the large bowel. The precise etiology of the disease remains still largely unexplained. Undoubtedly, the disease is of multifactorial nature, involving genetic predispositions, environmental factors, intestinal microbal flora and the immune system (2). Accumulating evidence suggest that CD results from the imbalance between pro- and anti-inflammatory agents and overactivity of Th1 cells (3). Some data suggest that the defect within the regulatory T cell compartment might be responsible for the imbalance (4,5). Several studies have also underlined the importance of nicotine smoking in pathogenesis of CD (6,7), although it is not a universal finding (8,9). Smoking has been demonstrated to have a detrimental effect on the course of the disease by many authors (10-13). The meta-analysis performed by Calkins yielded a pooled odds ratio (OR) 2.0 (1.65-2.47) in current smokers and OR 1.80 (1.33-2.51) in former smokers compared to life-time non-smokers (14). Various reports have been consistent about this effect, showing that about 50% of CD patients are smokers (15). It also seems that smoking might determine disease location, with a higher prevalence of ileal disease and a lower prevalence of colonic involvement in smokers (16). This detrimental effect of smoking on CD might result from its strong immuno-

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modulatory capabilities (17-19). Higher prevalence of females in CD has been also recognized by some authors (20-22). This study was aimed to investigate possible gender differences in the effect of smoking on the course of CD, with a special focus on the selected immune parameters such as proinflammatory mediators and regulatory T cells (Tregs).

MATERIALS AND METHODS

The project was approved by the Ethics Committee at Poznan University of Medical Sciences. A group of 55 adults with CD was enrolled to the study. All patients were Caucasian. The disease had been diagnosed and confirmed by endoscopic and radiologic means. Disease behavior and location were determined according to the Vienna classification (23). Extraintestinal manifestations (EIMs) were also identified. Only patients treated with conventional therapies, including 5-aminosalicylates, prednisolone, budesonide, azathioprine, mercaptopurine, methotrexate and antibiotics, were recruited to the study. Smoking status of patients was assessed based on medical history and questionnaire survey. Patients were categorized into three subgroups: "current smokers", "former smokers", and "non-smokers". Current smokers were defined as patients smoking more than 1 cigarette per day within 6 months prior to the diagnosis. Former smokers were defined as patients who had quitted smoking more than 6 months prior to the diagnosis. Non-smokers were considered to have never smoked. The control group was composed of 15 healthy volunteers, 9 females (60.0%) and six males (40.0%) with a mean age of 31.6±9.2 years who had not been subject to any immunomodulatory therapies. Serum samples were cytometrically tested for concentrations of interleukin (IL-2), IL-4, IL-6, IL-10, tumor necrosis factor (TNF)-a, and interferon (IFN)-g using the Human Th1/Th2/Th17 Cytokine Cytometric Bead Array Kit (BD Biosciences Pharmingen, San Diego, CA). The cytometric analysis was also performed to evaluate the levels of CD4+CD25+FOXP3+ regulatory T cells (BioLegend, San Diego, CA) in peripheral blood of investigated subjects. Descriptive variables are presented as means and medians with range, and categorical variables as frequencies with percentage. Frequencies were compared with χ^2 test and means between 2 groups with the Mann-Whitney test and between 3 or more groups with the Kruskal-Wallis test using SPSS version 15. Values were considered significant when $p \leq 0.05$.

RESULTS

The relation between smoking behavior and CD outcome is presented in Table 1. The group of CD patients included 55 adults, 32 females (58.2%) and 23 males (41.8%) of a mean age of 31.8±5.8 (median: 33.0) years at diagnosis. Smoking females were the youngest among all subgroups (mean age: 30.8±6.4 years; median: 30.5 years), while non-smoking males were the oldest at diagnosis (mean age: 34.9±3.7 years; median: 34.5 years). In all investigated subgroups females were diagnosed earlier than males, however the differences were not statistically significant. A mean duration of the disease since the diagnosis was 2.4±1.5 (median: 2.0) years. An analysis of medical histories revealed that 33 patients (60.0%) in that group, 21 females (38.2%) and 12 males (21.8%), were smokers; 9 patients (16.4%), 4 females (7.3%) and 5 males (9.1%), were former smokers; 13 patients (23.6%), 7 females (12.7%) and 6 males (10.9%), were non-smokers. Medical history did not show any changes in smoking behavior of patients since a diagnosis. 17 patients (30.9%) in that group had a family history of CD, mostly non-smokers and former smokers. In all investigated subgroups of CD patients, the onset of disease was observed earlier in females than males ($p>0.05$). Analysis showed that current smokers, particularly females, tended to have a more severe course of the disease comparing to former smokers and non-smokers. The highest frequency of hospitalizations during the observation was found in that subgroup (at least 2 hospitalizations: 13; 39.4%), especially among females (8; 24.2%), as well as the highest frequency of surgeries (at least 2 surgeries: 9; 27.3%), including 6 females (18.2%). The highest rate of cases of moderate-to-severe disease measured as CDAI $p \leq 0.05$. was also found among current smokers (13; 24.2%), 9 females (27.3%) and 4 males (12.1%) ($p>0.05$). Current smokers, particularly females, were also prescribed glucocorticoids and immunosuppressants more frequently than patients from other subgroups ($p=0.05$). On the contrary, the highest number of patients with CDAI <150 was observed among former smokers (44.0%) and non-smokers (30.8%), comparing to the current smokers subgroup (9.1%; $p=0.05$). No statistical differences in CD location and behavior were found among the investigated subgroups, except for the presence of disease in the upper GI tract solely in non-smokers (23.1%; $p=0.05$) and rare disease behavior B3 in that subgroup (7.7%; $p=0.05$). Fewer EIMs were experienced by former smokers and non-smokers than current smokers.

A cytometric analysis showed significant differences in serum levels of IL-2, IL-6, IL-17, TNF-a and IFN-g among patients with CD and healthy volunteers (Figure 1). The mean serum cytokine concentration levels in CD vs. Control group were, as follows: IL-2 (10.8±5.7 vs. 4.2±6.4 pg/mL; $p=0.01$), IL-6 (15.2±7.7 vs. 1.1±2.3 pg/mL; $p=0.00000006$), IL-17 (12.3±6.1 vs. 1.9±2.8 pg/mL; $p=0.0000002$), TNF-a (20.7±10.8 vs. 1.1±2.3 pg/mL; $p=0.00000006$), and IFN-g (12.0±4.8 vs. 2.7±3.6 pg/mL; $p=0.0000002$). No significant differences in IL-4 (4.4±7.6 vs. 0.7±1.8 pg/mL) and IL-10 (2.1±3.2 vs. 0.7±1.9 pg/mL) serum levels were observed among the investigated groups ($p>0.05$). The analysis also showed a lower percentage of CD4+CD25+FOXP3+ Tregs in peripheral blood of CD patients compared with the control group (1.7±1.0 vs. 2.4±1.4 % T cells; $p=0.03$) (Figure 2). More detailed analysis of the subgroups categorized according to smoking status did not show any significant differences in serum concentrations of cytokines, although higher levels of proinflammatory mediators, namely IL-2, IL-6, TNF-a AND IFN-g, were found in general in former smokers, followed by current smokers and non-smokers (Figure 3). On the contrary, the highest percentage of peripheral Tregs was found in non-smoking patients, while the lowest percentage

Table 1. Characteristic of patients with Crohn's disease according to smoking behavior (n=55)

	Smokers (n=33)			Former smokers (n=9)			Non-smokers (n=13)		
Charactristic	**Females**	**Males**	**Total**	**Females**	**Males**	**Total**	**Females**	**Males**	**Total**
Gender n (%)	21 (63.6%)	12 (36.4%)	33 (100%)	4 (44.4%)	5 (55.6%)	9 (100%)	7 (53.8%)	6 (46.2%)	13 (100%)
Age at diagnosis									
Mean (yrs±SD)	30.8±6.4	31.1±6.0	30.9±6.2	31.0±5.6	31.8±4.4	31.5±4.7	34.0±5.7	34.9±3.7	34.4±4.7
Median (range) yrs	30.5 (19.0-40.0)	29.6 (22.1-43.2)	30.2 (19.0-43.2)	30.3 (26.2-37.3)	30.2 (26.4-38.0)	30.1 (26.2-38.0)	33.3 (28.8-45.0)	34.5 (30.1-41.0)	33.3 (28.8-45.0)
Early onset (<40 yrs) n (%)	20 (60.6%)	11 (33.3%)	31 (93.9%)	4 (44.4%)	5 (55.6%)	9 (100.0%)	6 (46.1%)	5 (38.5%)	11 (84.6%)
Duration of disease									
Mean (yrs±SD)	2.1±1.5	3.1±1.4	2.5±1.5	1.5±1.5	3.5±1.3	2.6±1.7	1.6±0.9	2.4±1.3	2.0±1.1
Median (range) yrs	2.0 (0.0-5.5)	3.5 (0.8-5.2)	2.0 (0.0-5.5)	1.3 (0.0-3.3)	3.6 (1.5-5.0)	3.2 (0.0-5.0)	1.4 (0.7-3.0)	2.5 (0.6-4.0)	1.5 (0.6-4.0)
Family history of IBD n (%)	5 (15.1%)	2 (6.1%)	7 (21.2%)	1 (11.1%)	3 (33.3%)	4 (44.4%)	3 (23.1%)	3 (23.1%)	6 (46.2%)
Hospitalization n (%)									
1 time	7 (21.2%)	5 (15.2%)	12 (36.4%)	1 (11.1%)	2 (22.2%)	3 (33.3%)	3 (23.1%)	4 (30.8%)	7 (53.9%)
At least 2 times	8 (24.2%)	5 (15.2%)	13 (39.4%)	1 (11.1%)	2 (22.2%)	3 (33.3%)	2 (15.4%)	1 (7.7%)	3 (23.1%)
Surgery n (%)									
1 time	6 (18.2%)	4 (12.1%)	10 (30.3%)	1 (11.1%)	1 (11.1%)	2 (22.2%)	3 (23.1%)	1 (7.7%)	4 (30.8%)
At least 2 times	6 (18.1%)	3 (9.1%)	9 (27.3%)	1 (11.1%)	1 (11.1%)	2 (22.2%)	1 (7.7%)	1 (7.7%)	2 (15.4)
CD location n (%)									
Terminal ileum	8 (24.2%)	4 (12.1%)	12 (36.3%)	1 (11.1%)	1 (11.1%)	2 (22.2%)	2 (15.4%)	1 (7.7%)	3 (23.1%)
Colon	6 (18.2%)	4 (12.1%)	10 (30.3%)	2 (22.2%)	3 (33.3%)	5 (55.5%)	3 (23.1%)	2 (15.4%)	5 (38.5%)
Ileocolon	7 (21.2%)	4 (12.1%)	11 (33.3%)	1 (11.1%)	1 (11.1%)	2 (22.2%)	1 (7.7%)	1 (7.7%)	2 (15.4%)
Upper GI tract	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (7.7%)	2 (15.4%)	3 (23.1%) * 0.04
5-ASA	20 (60.6%)	12 (36.4%)	32 (97.0%)	4 (44.4%)	4 (44.4%)	8 (88.9%)	6 (46.1%)	6 (46.1%)	12 (92.2%)
Glucocorticoids	18 (54.6%)	8 (24.2%)	26 (78.8%)	4 (44.4%)	2 (22.2%)	6 (66.7%)	7 (53.8%)	3 (23.1%)	10 (76.9%)
Immunosuppresants	9 (27.3%)	4 (12.1%)	13 (39.4%)	1 (11.1%)	1 (11.1%)	2 (22.2%)	1 (7.7%)	2 (15.4%)	2 (23.1%)
CD behavior n (%)									
B1	9 (27.3%)	6 (18.2%)	15 (45.5%)	2 (22.2%)	2 (22.2%)	4 (44.4%)	3 (23.1%)	4 (30.8%)	7 (53.9%)
B2	2 (6.1%)	1 (3.0%)	3 (9.1%)	1 (11.1%)	1 (11.1%)	2 (22.2%)	3 (23.1%)	2 (15.4%)	5 (38.5%)
B3	10 (30.3%)	5 (15.1%)	15 (45.4%)	1 (11.1%)	2 (22.2%)	3 (33.3%)	1 (7.7%)	0 (0.0%)	1 (7.7%) * 0.05
EIM n (%)	8 (24.2%)	5 (15.1%)	13 (39.3%)	1 (11.1%)	0 (0.0%)	1 (11.1%)	2 (15.4%)	1 (7.7%)	3 (23.1%)
CDAI n (%)									
<150	1 (3.0%)	2 (6.1%)	3 (9.1%)	2 (22.2%)	2 (22.2%)	4 (44.4%) * 0.05	2 (15.4%)	2 (15.4%)	4 (30.8%) * 0.05
150-220	11 (33.3%)	6 (18.2%)	17 (51.5%)	1 (11.1%)	1 (11.1%)	2 (22.2%)	3 (23.1%)	2 (15.4%)	5 (38.5%)
220-450	8 (24.2%)	4 (12.1%)	12 (36.3%)	1 (11.1%)	2 (22.2%)	3 (33.3%)	2 (15.4%)	2 (15.4%)	4 (30.8%)
>450	1 (3.0%)	0 (0.0%)	1 (3.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

5-ASA: 5-aminosalicylates; B1: nonconstricturing, nonpenetrating; B2: stricturing; B3: penetrating; CD: Crohn's disease; CDAI: Crohn's disease Activity Index; EIM: extraintestinal manifestations; GI tract: gastrointestinal tract; SD: standard deviation; yrs: years

*p≤ 0.05 vs. smokers

in current smokers, although the observed differences were not statistically significant (Table 2). Further analysis showed gender differences in the investigated immune parameters in all subgroups of CD patients (Table 2). In general, higher levels of proinflammatory cytokines were found in females in all subgroups comparing to males. These differences were more distinct among current smokers and former smokers, although they were not statistically significant except for IL-6 in female

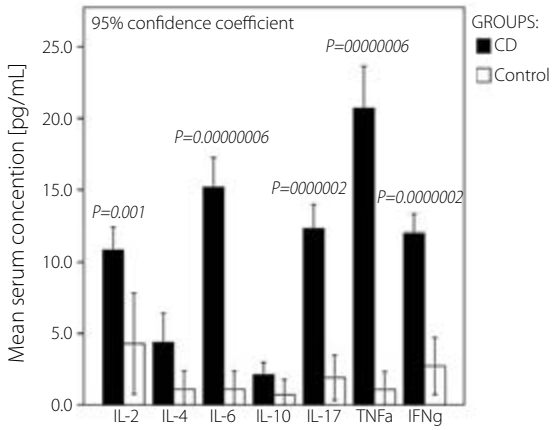

Figure 1. Mean concentrations of cytokines in the serum of patients with Crohn's disease (CD) and healthy volunteers (CONTROL). Values are presented as mean [95% confidence coefficient]. p≤0.05 vs. Control.

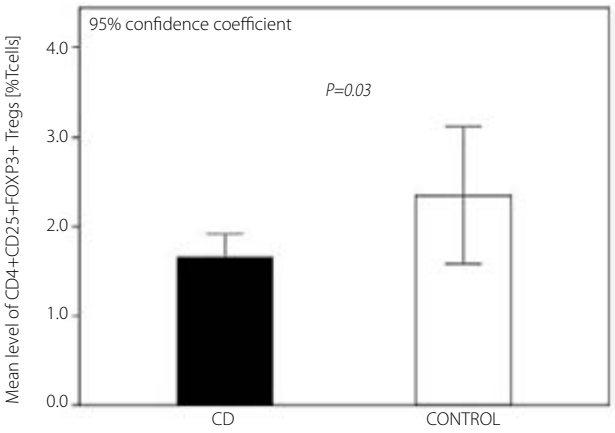

Figure 2. Mean level of CD4+CD25+FOXP3+ regulatory T cells (Tregs) in peripheral blood of patients with Crohn's disease (CD) and healthy volunteers (CONTROL) [values shown as % T cells]. p≤0.05 vs. Control.

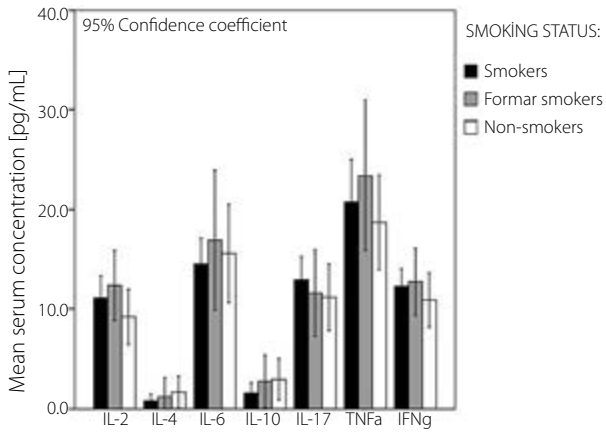

Figure 3. Mean concentrations of cytokines in the serum of CD patients according to their smoking status (smokers vs. former smokers vs. non-smokers). Values are presented as mean [95% confidence coefficient]. p≤0.05 vs. Smokers.

smokers (p=0.05). Higher levels of proinflammatory cytokines in female smokers and former smokers were also accompanied by lower levels of peripheral Tregs comparing to males (p=0.05 in smokers).

DISCUSSION

Presented results demonstrate that smoking has a deleterious effect on the course of CD. Current smokers constituted the majority of investigated CD patients (60.0%), which is consistent with other reports (14,24). Patients who smoked, particularly females, more frequently suffered from a moderate-to-severe form of the disease (CDAI ≥220), requiring glucocorticoid and immunosuppressive therapies (p=0.05). The increased need for immunosuppressants among smokers is supposedly dose-dependent, however this aspect was not analyzed in the study (13). Smokers, particularly females, were also hospitalized and underwent surgeries more frequently than non-smokers. Cottone et al have shown that macroscopic lesions on the ileal site of the anastomosis were observed 1 year after surgery in 70% of smokers vs. 35% of non-smokers and 27% of former smokers (11). However, due to the relatively short period of observation (mean: 2.4±1.5 yrs; median: 2.0 yrs) this effect was not observed in the study. The study showed that smoking was preferentially associated with ileal disease, and non-smoking with colonic disease, however the observed differences were not statistically significant. It is worth noting that all instances of disease location in the upper GI tract were found among non-smokers (p=0.04). Current smokers also experienced EIM more often than non-smokers and former smokers.

All CD patients, particularly however former smokers and current smokers, were also characterized by significantly higher levels of serum proinflammatory cytokines, such as IL-2, IL-6, IL-17, TNF-a and IFN-g comparing to the healthy control. This reflected the ongoing, more severe Th1-driven inflammatory processes (3). It is believed that inflammation in CD is caused by an IL-12-driven Th1 response, which results in the generation of IFN-g, the key inflammatory mediator (3). The predominance of Th1 response is consistent with the presence of granulomas in CD patients, which is a well described outcome of Th1-related immune response. Higher levels of proinflammatory cytokines in these subgroups were accompanied by lower percentages of peripheral Tregs, the cells responsible for maintenance of immune tolerance and prevention of inflammation (4,5). The lower percentage of peripheral Tregs in CD might have resulted from the impaired function and/or homeostasis of these cells, which have been also implicated in the development of several autoimmune diseases. This imbalance between pro- and anti-inflammatory factors observed in CD patients was more distinct in females than males among current smokers and former smokers and could substantially contribute to the severity of the disease.

Presented results show that in all investigated subgroups, particularly however among current smokers and former smokers,

Table 2. Selected peripheral immune parameters of patients with Crohn's disease according to smoking behavior and gender (n=55)

	Smokers (n=33)			Former smokers (n=9)			Non-smokers (n=13)		
Charactristic	**Females**	**Males**	**Total**	**Females**	**Males**	**Total**	**Females**	**Males**	**Total**
IL-2 (mean±SD) [pg/mL]	12.6±5.8	8.4±6.7	11.1±6.4	14.5±3.9	10.6±4.7	12.4±4.6	9.0±5.3	9.4±3.9	9.2±4.6
IL-4 (mean±SD) [pg/mL]	1.0±2.2	0.4±1.5	0.8±2.0	1.3±2.6	1.2±2.7	1.2±2.5	0.7±2.0	2.8±3.1	1.7±2.7
IL-6 (mean±SD) [pg/mL]	16.4±6.3	11.3±7.9*0.05	14.5±5.3	17.6±12.6	16.3±7.0	16.9±9.1	15.8±8.9	15.3±8.1	15.6±8.2
IL-10 (mean±SD) [pg/mL]	1.6±3.1	1.7±2.9	1,6±3.0	1.3±2.5	4.0±3.8	2.8±3.4	2.9±3.7	3.1±3.5	2.9±3.5
IL-17 (mean±SD) [pg/mL]	13.8±6.7	11.3±6.2	12.9±6.6	11.5±6.8	11.6±5.4	11.6±5.7	10.9±5.9	11.5±5.6	11.2±5.6
TNF-α (mean±SD) pg/mL]	22.9±12.6	17.0±10.5	20.8±12.0	21.3±10.7	25.0±9.9	23.4±9.8	18.3±7.3	19.2±9.3	18.7±7.9
IFN-γ (mean±SD) [pg/mL]	13.2±4.2	10.6±6.0	12.3±5.0	12.9±5.4	12.5±4.1	12.7±4.4	11.5±5.2	10.1±4.0	10.0±4.5
Tregs [% T cells]	1.3±0.8	1.8±0.9*0.05	1.5±0.8	1.3±1.0	1.9±0.9	1.7±1.1	2.0±1.2	1.9±1.2	2.0±1.2

IFN: interferon; IL: interleukin; SD: standard deviation; TNF: tumor necrosis factor; Tregs: regulatory T cells

*p≤0.05 versus females

female patients seemed to be more affected by CD. Females developed more severe form of the disease and experienced the onset earlier than men. This observation is consistent with other reports (25,26). The precise mechanism responsible for the gender differences in CD is however not known. Exposure to smoking seems to be one of the factors contributing to this unequal distribution among genders. It is believed that cigarette smoking strongly affects both cell-mediated and humoral immune response, leading to both the release and inhibition of various pro- and anti-inflammatory mediators (27). It seems that the molecular mechanism at least partially responsible for immunomodulating capabilities of smoking involves activation of inhibitor of IκB kinase (IKK), fosforylation of IκB (inhibitor of nuclear factor NF-κB), NF-κB nuclear translocation and histone acetylation (17,18,28). NF-κB is a key transcription factor regulating the expression of various proinflammatory cytokines and numerous studies have linked its activation to elevated cytokine expression in smokers (29). 20 other transcriptional factors regulated by smoking have been identified so far, including GATA, PAX5, Smad 3/4, AP-1, ISRE, ICSBP (30,31). There is evidence supporting the idea that females are more vulnerable to the immunomodulatory effect of smoking, and easier disrupt the normal immune balance, which results in significantly increased production of IFN-g, not accompanied by release of Th2 cytokines (32). A few possible explanations of this phenomenon have been proposed, like the negative effect of estrogen exerted on proinflammatory cytokine gene regulation and immune cell interactions (33) or differences in smoking habits among genders, since females use more filter cigarettes and more light cigarettes resulting in higher relative exposure to smoke than nicotine (34). According to some authors, gender differences in CD development vary geographically, are strongly influenced by attained age, and are present after the mid-second decade of life (35). This, in turn, leaves the possibility opened for speculations regarding other environmental factors that might contribute to the gender differences in CD. Importantly, similar biological mechanism by which smoking interferes with the pathogenesis of CD might be involved in the development of different autoimmune diseases, such as multiple sclerosis (36), rheumatoid arthritis (37), systemic lupus erythematous (38), psoriasis (39) and others, particularly, since they are also characterized by a strong prevalence of females (40-44).

The presented study had some limitations. A retrospective nature of the study may have led to a bias in the interpretation of data. A relatively small number of patients in investigated subgroups could have contributed to unexpected and/or non-significant results. Another limitation to the study was a relatively short period of observation (2.4±1.5 years, median: 2.0 years), which might have affected the clinical findings. The analysis did not also cover the effect of nicotine daily dose (13), passive smoking effect (9) and oral contraceptive use (45), which potentially may affect the severity of the disease.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Poznan Univesrity of Medical Sciences (number 27/10)

Informed concent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Authors contribution: Concept - J.K.; Design - J.K.; Supervision - J.K.; Resource - J.K.; Materials - J.K.; Data Collection &/or Processing - J.K., B.P., P.R., M.M.; Analysis &/or Interpretation - J.K., B.P., P.R.; Literature Search - J.K., B.P., P.R., M.M.; Writing - J.K., B.P., P.R.; Critical Reviews - J.K., B.P., P.R., M.M.

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REFERENCES

1. Tersigni R, Prantera C. Crohn's disease. A multidisciplinary approach. Milan: Springer-Verlag Italia; 2010.
2. Baumgart DC, Carding SR. Inflammatory bowel disease: cause and immunobiology. Lancet 2007; 369: 1627-40. [CrossRef]

3. Strober W, Fuss IJ. Proinflammatory cytokines in the pathogenesis of inflammatory bowel diseases. Gastroenterology 2011; 140: 1756-67. [CrossRef]
4. Allez M, Mayer L. Regulatory T cells. Peace keeprs in the gut. Inflammatory Bowel Disease 2004; 10: 666-76. [CrossRef]
5. Maul J, Loddenkemper C, Mundt P, et al. Peripheral and intestinal regulatory CD4+CD25high T cells in inflammatory bowel disease. Gastroenterology 2005; 128: 1868-78. [CrossRef]
6. Benoni C, Nilsson A. Smoking habits in patients with inflammatory bowel disease. Scand J Gastroenterol 1984; 19: 824-30.
7. Yamamoto T, Keighley MR. The association of cigarette smoking with a high risk of recurrence after ileocolonic resection for ileocecal Crohn's disease. Surgery Today 1999; 29: 579-80. [CrossRef]
8. Fidder HH, Avidan B, Lahav M, et al. Clinical and demographic characterization of Jewish Crohn's disease patients in Israel. J Clin Gastroenterol 2003; 36: 8-12. [CrossRef]
9. van der Heide F, Wassenaar M, van der Linde K, et al. Effects of active and passive smoking on Crohn's disease and ulcerative colitis in a cohort from a regional hospital. Eur J Gastroenterol Hepatol 2011; 23: 255-61. [CrossRef]
10. Moum B, Ekbom A, Vatn MH, et al. Clinical course during the 1st year after diagnosis in ulcerative colitis and Crohn's disease. Results of a large, prospective population-based study in southeastern Norway, 1990-93. Scand J Gastroenterol 1997; 32: 1005-12. [CrossRef]
11. Cottone M, Rosselli M, Orlando A, et al. Smoking habits and recurrence in Crohn's disease. Gastroenterology 1994; 106: 643-8.
12. Lindberg E, Järnerot G, Huitfeldt B. Smoking in Crohn's disease: effect on localisation and clinical course. Gut 1992; 33: 779-82. [CrossRef]
13. Cosnes J, Carbonnel F, Beaugerie L, et al. Effects of cigarette smoking on the long-term course of Crohn's disease. Gastroenterology 1996; 110: 424-31. [CrossRef]
14. Calkins BM. A meta-analysis of the role of smoking in inflammatory bowel disease. Dig Dis Sci 1989; 34: 1841-54.
15. Birrenbach T, Böcker U. Inflammatory bowel disease and smoking: a review of epidemiology, pathophysiology, and therapeutic implications. Inflamm Bowel Dis 2004; 10: 848-59. [CrossRef]
16. Cosnes J. What is the link between the use of tobacco and IBD? Inflamm Bowel Dis 2008; (14 Suppl): S14-5. [CrossRef]
17. Hasnis E, Bar-Shai M, Burbea Z, Reznick AZ. Mechanisms underlying cigarette smoke-induced NF-kappaB activation in human lymphocytes: the role of reactive nitrogen species. J Physiol Pharmacol 2007; 58: 275-87.
18. Yang SR, Chida AS, Bauter MR, et al. Cigarette smoke induces proinflammatory cytokine release by activation of NF-kappaB and posttranslational modifications of histone deacetylase in macrophages. Am J Physiol Lung Cell Mol Physiol 2006; 291: L46-57. [CrossRef]
19. Verschuere S, De Smet R, Allais L, Cuvelier CA. The effect of smoking on intestinal inflammation: what can be learned from animal models? J Crohns Colitis 2012; 6: 1-12. [CrossRef]
20. Bernstein CN, Blanchard JF, Rawsthorne P, et al. Epidemiology of Crohn's disease and ulcerative colitis in a central Canadian province: a population-based study. Am J Epidemiol 1999; 15: 916-24. [CrossRef]
21. Haug K, Schrumpf E, Halvorsen JF, et al. Epidemiology of Crohn's disease in western Norway. Study group of Inflammatory Bowel Disease in Western Norway. Scand J Gastroenterol 1989; 24: 1271-5. [CrossRef]
22. Munkholm P, Langholz E, Nielsen OH, et al. Incidence and prevalence of Crohn's disease in the county of Copenhagen, 1962-87: a sixfold increase in incidence. Scand J Gastroenterol 1992; 27: 609-14. [CrossRef]
23. Gasche C, Scholmerich J, Brynskov J, et al. A simple classification of Crohn's disease: report of the Working Party for the World Congresses of Gastroenterology, Vienna 1998. InflammBowel Dis 2000; 6: 8-15. [CrossRef]
24. Somerville KW, Logan RFA, Edmond M, et al. Smoking and Crohn's disease. Br Med J (Clin Res Ed) 1984; 289: 954-6. [CrossRef]
25. Sutherland LR, Ramcharan S, Bryant H, et al. Effect of cigarette smoking on recurrence of Crohn's disease. Gastroenterology 1990; 98: 1123-8.
26. Russel MG, Nieman FH, Bergers JM, et al. Cigarette smoking and quality of life in patients with inflammatory bowel disease. South Limburg IBD Study Group. Eur J Gastroenterol Hepatol 1996; 8: 1075-81. [CrossRef]
27. Arnson Y, Shoenfeld Y, Amital H. Effects of tobacco smoke on immunity, inflammation and autoimmunity. J Autoimmun 2010; 34: J258-65. [CrossRef]
28. Szulakowski P, Crowther AJ, Jiménez LA, et al. The effect of smoking on the transcriptional regulation of lung inflammation in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2006; 174: 41-50. [CrossRef]
29. Gonçalves RB, Coletta RD, Silvério KG, et al. Impact of smoking on inflammation: overview of molecular mechanisms. Inflamm Res 2011; 60: 409-24. [CrossRef]
30. Zhao J, Harper R, Barchowsky A, et al. Identification of multiple MAPK-mediated transcription factors regulated by tobacco smoke in airway epithelial cells. Am J Physiol Lung Cell Mol Physiol 2007; 293: L480-90. [CrossRef]
31. Li YT, He B, Wang YZ. Exposure to cigarette smoke upregulates AP-1 activity and induces TNF-alpha overexpression in mouse lungs. Inhal Toxicol 2009; 21: 641-7. [CrossRef]
32. Whetzel CA, Corwin EJ, Klein LC. Disruption in Th1/Th2 immune response in young adult smokers. Addict Behav 2007; 32: 1-8. [CrossRef]
33. Rider V, Abdou NI. Gender differences in autoimmunity: molecular basis for estrogen effects in systemic lupus erythematosus. Int mmunopharmacol 2001; 1: 1009-24. [CrossRef]
34. Zeman MV, Hiraki L, Sellers EM. Gender differences in tobacco smoking: higher relative exposure to smoke than nicotine in women. J Womens Health Gend Based Med 2002; 11: 147-53. [CrossRef]
35. Molinié F, Gower-Rousseau C, Yzet T, et al. Opposite evolution in incidence of Crohn's disease and ulcerative colitis in Northern France (1988-1999). Gut 2004; 53: 843-8. [CrossRef]
36. Jafari N, Hintzen RQ. The association between cigarette smoking and multiple sclerosis. J Neurol Sci 2011; 311: 78-85. [CrossRef]
37. Klareskog L, Malmström V, Lundberg K, et al. Smoking, citrullination and genetic variability in the immunopathogenesis of rheumatoid arthritis. Semin Immunol 2011; 23: 92-8. [CrossRef]
38. Freemer MM, King Jr TE, Criswell LA. Association of smoking with dsDNA autoantibody production in systemic lupus erythematosus. Ann Rheum Dis 2006; 65: 581-4. [CrossRef]
39. Li W, Han J, Choi HK, et al. Smoking and risk of incident psoriasis among women and men in the United States: A combined analysis. Am J Epidemiol 2012; 175: 402-13. [CrossRef]

40. Jawaheer D, Lum RF, Gregersen PK, et al. Influence of male sex on disease phenotype in familial rheumatoid arthritis. Arthritis Rheum 2006; 54: 3087-94. **[CrossRef]**
41. Brennan P, Silman A. Why the gender difference in susceptibility to rheumatoid arthritis? Ann Rheum Dis 1995; 54: 694-5. **[CrossRef]**
42. Eikelenboom MJ, Killestein J, Kragt JJ, Uitdehaag BM, Polman CH. Gender differences in multiple sclerosis: cytokines and vitamin D. J Neurol Sci 2009; 286: 40-2. **[CrossRef]**
43. Sellner J, Kraus J, Awad A, et al. The increasing incidence and prevalence of female multiple sclerosis--a critical analysis of potential environmental factors. Autoimmun Rev 2011; 10: 495-502. **[CrossRef]**
44. Wasef Y. Gender differences in systemic lupus erythematosus. Gender Med 2004; 1: 12-7. **[CrossRef]**
45. Timmer A, Sutherland LR, Martin F. Oral contraceptive use and smoking are risk factors for relapse in Crohn's disease. The Canadian Mesalamine for Remission of Crohn's Disease Study Group. Gastroenterolo 1998; 114: 1143-50. **[CrossRef]**