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DIGITALNI AKADEMSKI ARHIVI I REPOZITORIJI

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A retrospective, case-control study on traditional environmental risk factors in inflammatory bowel disease in Vukovar-Srijem County, north-eastern Croatia, 2010

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Summary
Background Traditional environmental risk factors in inflammatory bowel disease (IBD), ulcerative colitis (UC) and Crohn's disease (CD), were examined as part of the retrospective epidemiologic study conducted in Vukovar-Srijem County, north-eastern Croatia in 2010. The geographical variations in the frequency of IBD in Croatia have been observed, which is also the trend in the Central Eastern European region and Europe as a whole, indicating the influence of environmental and lifestyle factors. However, the data on the spread of environmental IBD risk factors are still limited. The purpose of this study was to analyse the traditional environmental risk factors in IBD on our cohort sample, including measles virus infection and vaccination (MMR vaccine—Mumps, Measles, Rubella), tonsillectomy, appendectomy, current and former cigarette smoking and use of oral contraceptives in women.

Methods This retrospective, case-control study was performed as a part of a wider epidemiologic study aimed at assessing the incidence, prevalence and clinical expression of IBD, in Vukovar-Srijem County (population: 204,768; 2001), which is a lesser developed part of the continental Croatia that experienced deep demographic changes in the recent past. IBD patients were identified according to the hospital's patient records. There were 119 UC patients and 31 CD patients of a total of 150 patients in the cohort. A total of 150 individuals, volunteers, not having a diagnosis of IBD, age- and sex-matched, were used as the control group. Information on examined risk factors were obtained from all subjects in a previously conducted interview. Patients were contacted personally or by phone and interviewed by a gastroenterologist.

Results There were no differences in the number of smokers, former smokers and non-smokers, between UC and CD patients and the controls, nor in the duration of smoking (years), in current smokers and ex-smokers. Only marginally significant longer time of non-smoking, in ex-smokers was found in IBD patients, compared to the controls, more pronounced in CD patients ($p=0.05$). No difference was found in relation to tonsillectomy and risk of IBD. There was no difference in the number of female IBD patients and women from the controls using oral contraceptives. Duration (years) of oral contraceptives use was longer in women from the controls than in female IBD patients ($p<0.001$). Frequency of appendectomy was the lowest in UC patients, compared to the controls and CD patients (3.4, 12.0 and 38.7 %, respectively) ($p<0.001$). No difference was found in relation to measles virus infection and risk of IBD. MMR vaccina-

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tion rates were higher in CD patients (90.3 %), compared to UC patients and the controls (74.8 and 67.3 %, respectively) ($p = 0.026$).

Conclusions No association was found between smoking and tonsillectomy and risk of IBD. Our results do not support the idea of oral contraceptives use as a risk factor for IBD. Frequency of appendectomy was the lowest in UC patients, suggesting that appendectomy decreases the risk of UC, contributing the earlier results. MMR vaccination seemed to be associated with Crohn's disease. These results can add value to our understanding of the increasing incidence of IBD in Croatia and other Central Eastern European countries and can be introductory to future large-scale research.

Keywords Inflammatory bowel disease · Environmental risk factors · Retrospective case-control study · North-eastern Croatia

Introduction

Ulcerative colitis (UC) and Crohn's disease (CD) are two similar chronic intestinal disorders which usually start in adolescence and young adulthood and continue to affect patient's functioning and quality of life across the lifespan. They have similar clinical courses, clinical symptoms and some other features, including the geographic patterns of spread and proposed risk factors, which have been fairly enough to get the common term as inflammatory bowel disease (IBD) [1, 2]. Yet, there are still many differences to give them the status as independent diseases. One of these differences includes the distinct place of inflammation either localized in the intestinal mucosa (UC), or spanning through the entire intestinal wall (CD). Other differences are due to the localization of the disease process in the gastrointestinal tract (with CD primarily affecting terminal ileum and UC primarily affecting the rectum) and the involvement of the genetics and the immune system in pathophysiology and clinical manifestations of these diseases [3, 4].

Epidemiologic patterns of spread and clinical behaviour of IBD have been changed during the few past decades [2]. Traditionally, there was an absolute predomination of IBD in developed parts of the world, including northern European countries and North America, with the gradient observed to exist from north to south and from west to east [2, 5]. Recent epidemiologic studies, in contrast, show rapid increase in the incidence and prevalence of IBD in developing countries of Europe and the world, indicating the strong influence of environmental and lifestyle factors for clinical expression of these diseases [6, 7]. Advances in genetic testing have allowed identification of genetic factors in the etiology of IBD, found to be more pronounced in CD, than in UC [4]. Implicated genetic loci identified to date, indicate the distribution of inflammation-promoting functions in the intestinal surface epithelium. The current hypothesis is that in genetically predisposed humans living under certain environmental conditions, the mucosal immune system may become dysregulated, which is the main mechanism in pathogenesis of IBD [8, 9]. Current population studies therefore, in searching for environmental IBD risk factors, are predominantly focused on the role of breastfeeding and other dietary and nutritional factors [10, 11].

Many IBD risk factors classified as environmental, societal and behavioural ones have been identified in early epidemiologic studies, including: socioeconomic status, ethnicity, immigrant status, cigarette smoking, use of oral contraceptives in women, childhood vaccinations and infections, tonsillectomy and appendectomy [10, 12]. They are all known as traditional IBD risk factors. The problem that makes general conclusions on these factors difficult, is in a non-standardized way of subjects' selection into cohort groups and in the variable distribution of these factors in different populations [10]. It is thought nowadays that only by using a combination of different research approaches, including clinical studies, gene association studies and animal laboratory experiments, it is possible to gain substantial new knowledge on the pathogenesis of IBD and the etiologic role of certain environmental risk factors [4].

The geographical variation in the frequency of IBD in Croatia has been observed, which is also the trend in the Central Eastern European region and Europe as a whole, indicating the influence of environmental and lifestyle factors [13]. However, data on the spread of environmental IBD risk factors are still limited. In this retrospective, case-control study, conducted in Vukovar-Srijem County, north-eastern Croatia, in 2010, we examined the traditional environmental risk factors in IBD including those that affect childhood: measles virus infection and vaccination (mostly based on MMR—Mumps, Measles, Rubella vaccine), tonsillectomy and appendectomy, and those that affect adulthood: cigarette smoking and use of oral contraceptives in women. In addition, analysis of the demographic features of IBD patients has provided some notions on the role of certain societal and socioeconomic factors. The results can help understanding the observed increasing incidence of IBD in Central Eastern European countries and provide the clues for future research.

Methods

Patients

The research population included adult (≥ 18) insured persons of the Regional Office of the Croatian Institute for Health Insurance of Vukovar-Srijem County (204,768 citizens; 2001). This region was stricken by the war in Croatia (1991–1997) and experienced deep demographic changes during the war and long post-war period. The population was affected by war traumas that could influence their health status, operating via bad emotions, psycho-social stress and bad eating and life-style habits [14].

The examined sample consisted of patients diagnosed with IBD (UC or CD), identified according to the hospital's patient records. Of a total of 150 patients in the sample, there were 119 patients diagnosed with UC and 31 patients diagnosed with CD. They were identified according to the hospital's patient records. Documentation of the regional hospitals in Vukovar and Vinkovci was used for this purpose. Hospitals in the near surroundings such as Clinical Hospital Centre Osijek and General Hospital Slavonski Brod were also contacted, as some patients were directly referred to these hospitals by their primary care physicians without prior registration in the resident hospitals.

The control group consisted of total of 150 individuals not diagnosed with IBD. They were collected on a voluntary basis, age and sex-matched in order to allow environmental risk factor comparison between patients and non-patients.

Methods

This study on risk factors for IBD was performed as a part of a wider epidemiologic study aimed at assessing incidence and prevalence and clinical expression of IBD in Vukovar-Srijem County, which is a lower developed part of north-eastern continental Croatia [13, 15]. Two 10 year periods (from 2001–2010 and 1991–2000) were used as the periods of surveillance in estimating incidence and prevalence measures. Information on disease characteristics and examined risk factors was obtained from subjects in an interview conducted in 2010.

Patients identified as to have the diagnosis of IBD were contacted personally or by phone and interviewed by a gastroenterologist. All participants gave their informed consent prior to their inclusion in the study. The study was approved by the local ethics committee.

Information was obtained from each patient according to two types of protocols standardized for case ascertainment. Protocols were specifically adapted to data collecting either on incidence or prevalence of IBD. The introductory part that included general patient information form was identical for both types of protocols.

The protocol specifically addressing incidence data contained several compartments aimed at gaining information on demographic features (an immigrant, or born in the resident county), education, employment status, family history of IBD (including information on IBD in parents, brothers/sisters and children) and risk factors. Some of the main traditional risk factors for IBD were analysed, including cigarette smoking, oral contraceptives use in women, appendectomy, tonsillectomy and measles virus infection and vaccination. Smoking status was classified into three categories: non-smokers, former smokers (those who stopped smoking before the diagnosis of IBD) and smokers. Duration of non-smoking, in former smokers, is considered the time between cessation of smoking and diagnosis of IBD. Duration of smoking, in current smokers, measures the time of smoking until diagnosis of IBD. Years of smoking/non-smoking, in current and ex-smokers, are presented with median and interquartile range. Duration of oral contraceptives use, in female IBD patients, is considered to be the time period before diagnosis.

A part of the questionnaire specifically addressed the disease characteristics issues, including information regarding: the type and the time of the diagnosis, symptoms and their duration prior to the first medical check-up, body weight at the time of the first physical examination, weight loss in the last 3 months, performed diagnostic procedures and the extent of the disease in the gastrointestinal tract.

The protocol specifically designed to collect data on prevalence of IBD was extended for the part where answers were required on the questions regarding the natural history of a disease, the presence/absence of the intestinal and extraintestinal complications, the number and types of surgical interventions and the results of pathohistological examinations.

Additionally, for the purpose of risk factors analysis, subjects from the control group were asked to fill out the questionnaire on environmental risk factors.

Statistics

Collected data was transformed into a digital database by using *Microsoft Excel 2003, Microsoft Inc.* (PC). All statistical analyses were performed by using statistical software package *Statistica 10.0, StatSoft Inc.* (MFOS licence). Numerical data was presented as median and interquartile range since having the skewed distribution. Categorical data was presented as absolute frequencies and percentages. Differences between groups were assessed by using the student's t-test for parametric distributions and Mann–Whitney U test and Kruskal–Wallis test, appropriate for non-parametric distributions. The categorical data analysis was performed by using the Chi-square or Fisher's exact test where appropriate. The confirmed level of significance of $p<0.05$ was considered statistically significant.

Results

In the sample of 150 patients identified with IBD, there were 119 patients diagnosed with UC and 31 patients diagnosed with CD (Table 1). Demographic data analysis showed significant differences between these two patient groups in the following characteristics: marital status (with higher percentage of married persons among those diagnosed with UC) and resident status by a county of birth (with higher percentage of immigrants to Vukovar-Srijem County among patients diagnosed with UC) (Table 1). Patients diagnosed with CD were on average younger than those diagnosed with UC and showed a greater age variation skewed towards younger age groups (Fig. 1; Table 2, 3, 4 and 5).

Table 1 Demographic features of patients with ulcerative colitis (UC) and Crohn's disease (CD)

	UC—Number/(%)	CD—Number/(%)	*P*
Distribution of subjects by gender			
Male	57 (47.9)	20 (64.5)	=0.099[a]
Female	62 (52.1)	11 (35.5)	
Distribution of subjects by marital status			
Married	90 (75.6)	15 (50.0)	=0.023[b]
Unmarried	17 (14.3)	11 (36.7)	
Divorced	3 (2.5)	1 (3.3)	
Widow(-er)	9 (7.6)	3 (10.0)	
Distribution of subjects by resident status by a county of birth			
Immigrant			
To Vukovar-Srijem County born	46 (38.7)	6 (19.4)	=0.044[a]
In Vukovar-Srijem County	73 (61.3)	25 (80.6)	
Distribution of subjects by the level of education			
Primary school	28 (23.5)	5 (16.1)	=0.710[b]
Secondary school	71 (59.7)	21 (67.7)	
Vocational school	5 (4.2)	2 (6.5)	
University education	15 (12.6)	3 (9.7)	
Distribution of subjects by the employment status			
Employed	39 (32.8)	9 (29.0)	=110[b]
Self-employed	9 (7.6)	1 (3.2)	
Unemployed	17 (14.3)	3 (9.7)	
Retired	39 (32.8)	11 (35.5)	
Housekeepers	12 (10.1)	2 (6.5)	
Students	0 (0)	2 (6.5)	
High school students	3 (2.5)	3 (9.7)	
Total	119 (100)	31 (100)	

[a]c^2test
[b]Fisher's exact test

Analysis and comparison performed on risk factors that affect childhood, including appendectomy, tonsillectomy and measles virus infection and vaccination, between the two patient groups and the control group showed statistically significant differences for appendectomy and measles virus vaccination status (Table 6).

Discussion

In this retrospective, case-control study, we did not find any association between smoking and tonsillectomy and risk of IBD. Interestingly, the result that women in the control group have taken oral contraceptives for a longer period of time than female IBD patients is likely to argue against oral contraceptives as a risk factor for IBD. Frequency of appendectomy was the lowest in UC patients, suggesting that appendectomy decreases the risk of UC, contributing the earlier results. MMR vaccination seemed to be associated with Crohn's disease.

Some conclusions on the risk factors for IBD can also be drawn from the analysis of patients' demographic features, including the statements: (1) married persons are more likely to get UC, than CD and (2) a risk of UC, but not of CD may be increased in the immigrant population (Table 1). The first statement might be partially explained by the fact that patients diagnosed with CD were, on average, younger than those diagnosed with UC (Fig. 1). Comments on the patients' demographic feature analysis have already been provided in our previously published paper [13]. Although these results have only limited value since this analysis is missing in the control (non-patient) group, they nevertheless can shape some directions for future research on societal risk factors for IBD.

In this study, we did not find association between **smoking habit** and risk of IBD (Tables 2, 3 and 4). Only marginally significant longer time of non-smoking, in ex-smokers was observed in CD patients, compared to the control, indicating the possible role of the duration of smoking in the past time for CD, but this statement needs further clarification (Table 3). It has been traditionally accepted that cigarette smoking is an independent risk factor for IBD, however, affecting CD and UC in a different way, so that current smoking increases the risk of CD whereas it provides protection against UC [16, 17]. Similarly, non-smokers and former smokers have been considered as being at a greater risk of developing UC. Although newer studies partially confirm these traditional assumptions, they also indicate that many relationships in this regard remain unresolved [18, 19]. For example, in a recent prospectively conducted study encompassing only women, the authors came to the conclusion that the risk of UC is similar in smokers and non-smokers, but that smoking cessation increases the risk of UC, which continues to persist for a long period of time [20]. A risk of CD was found to be higher in current smokers when compared to former smokers, and higher in former smokers compared to those who have never smoked (non-smokers).

Hormone use in women was found to increase the risk of IBD, although the data is conflicting. According to the meta-analysis from 2008, for women using oral contraceptives the risk of both UC and CD increased, although an association is small [21]. According to the recent large prospective study, hormone replacement therapy in postmenopausal women is likely to increase the risk of UC but not of CD [22]. The risk of UC was found to be proportional to the duration of hormone use and started to decrease from the time of cessation. Apparently, different results were obtained in this study, showing longer use of contraceptives in women from the control group compared to the patient group, indicating against the usage of oral contraceptives as the risk factor for IBD (Fig. 2). However, information on the time of contraceptive therapy cessation is missing, which could also affect the results.

In a majority of formerly performed studies, **appendectomy** was shown to decrease the risk of UC [23–26]. This protective role seems to be stronger if appendectomy

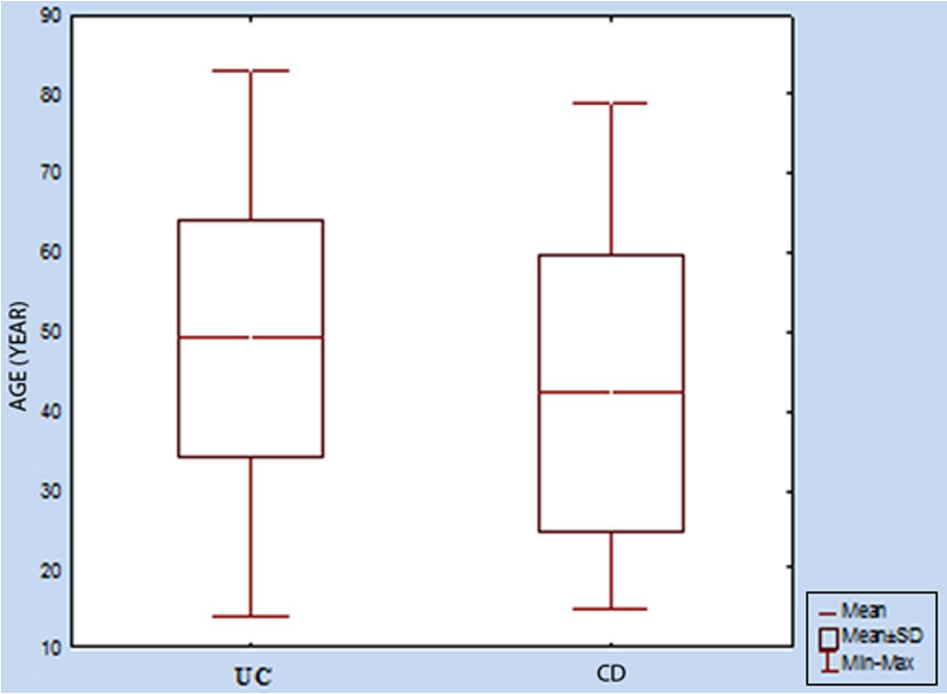

Fig. 1 Distribution by age ($p = 0.024$; Student's t- test)

is performed before the age of 20 [26, 27], although the underlying mechanisms are not yet clear [26]. According to one hypothesis, patients prone to get appendicitis are less prone to get UC. Alternatively, an early appendectomy can modify mucosal immune response, supressing the progression of UC. Our results, showing lower frequency of appendectomy in UC patients, compared to CD patients and the controls (Table 6), are likely to contribute these early results. In general, the association between appendectomy and CD is less clear since correlations that were found are inconsistent and weak [23–26]. An explanation is that it is difficult to realize whether appendectomy is a result of misdiagnosed CD, or whether it really has a causal or modulating role in the development of CD [26]. In line with the first option, it has been found that a number of appendectomies increases close to the time of diagnosis of CD [27]. The percentage of patients in our study who reported appendectomy was higher in CD patients than in UC patients and the controls, but the conclusion on the causal relationships between appendectomy and CD is difficult to draw (Table 6). We did not find an association between **tonsillectomy** and risk of IBD (Table 6), and reports published so far are largely inconsistent on this issue [28, 29].

There is a long-lasting debate in the scientific community and the public domain on association between **measles virus infection and measles virus vaccination** (mostly based on using MMR vaccine) and risk of IBD. A suspicion started to rise in 1995 when Thompson and co-authors announced the results of their prospective cohort study on vaccination outcomes in children vaccinated with MMR vaccine [30]. They found 3-fold and 2.5-fold higher rates for the reported frequency of CD and UC in vaccinated children, compared to the non-vaccinated cohort. Although the accuracy of this study was questioned because of the methodology issues, it potentiated a wave of research in the following period. In a number of studies performed by using a variety of methods, ranging from molecular biology to population-based epidemiologic studies, researchers could not provide evidence to support concerns that arose from this early study on association between measles virus infection/vaccination and IBD [31–33]. However, suspicions remain. Authors of the two recent systematic reviews stated that the studies performed on this issue suffered from a series of methodological limitations to allow definite conclusions, so that "controversy laid to rest" [32, 34]. The results of this study, although can take criticisms for the method-

Table 2 Smoking status in patients diagnosed with ulcerative colitis or Crohn's disease and the controls

Smoking status	Ulcerative colitis No/(%)	Crohn's disease No/(%)	The controls No/(%)	*p*
Non-smokers	54 (45.4)	15 (48.4)	53 (35.3)	= 0.167[a]
Former smokers	40 (33.6)	6 (19.4)	52 (34.7)	
Smokers	25 (21.0)	10 (32.3)	45 (30.0)	
Total	119 (100)	31 (100)	150 (100)	

[a]Chi-square test

Table 3 Duration of smoking and non-smoking, in former smokers, distribution between patients diagnosed with ulcerative colitis and Crohn's disease and the controls

Duration of smoking/non-smoking in former smokers	Ulcerative colitis ($n=40$)	Crohn's disease ($n=6$)	The controls ($n=52$)	p
Years of non-smoking Median and IQ range (25–75 %)	10.0 (4.3–15.0)	13.0 (9.5–22.5)	7.0 (2.0–12.0)	$=0.052$[a]
Years of smoking Median and IQ range (25–75 %)	15.0 (10.0–23.8)	12.5 (8.8–20.0)	15.0 (8.3–21.5)	$=0.662$[a]
[a]Kruskal-Wallis test *IQ* interquartile range				

Table 4 Duration of smoking in current smokers, distribution between patients diagnosed with ulcerative colitis and Crohn's disease and the controls

Duration of smoking in current smokers	Ulcerative colitis ($n=25$)	Crohn's disease ($n=10$)	The controls ($n=45$)	p
Years of smoking Median and IQ range (25–75 %)	22.0 (10.0–32.5)	20.0 (9.3–23.8)	15.0 (9.0–20.5)	$=0.167$[a]
[a]Kruskal-Wallis Test/*IQ* interquartile range				

Table 5 Oral contraceptives use in women, distribution between patients diagnosed with ulcerative colitis and Crohn's disease and the controls

Oral contraceptives use	Ulcerative colitis No/(%)	Crohn's disease No/(%)	The controls No/(%)	p
Yes	13 (21.0)	1 (9.1)	11 (26.2)	$=0.462$[a]
No	49 (79.0)	10 (90.9)	31 (73.8)	
Total	62 (100)	11 (100)	42 (100)	
[a]Chi-square test				

Table 6 Risk factors that affect childhood, including appendectomy, tonsillectomy, measles virus infection and vaccination, distribution between the patient groups and the controls

Risk factors	Ulcerative colitis No/(%)	Crohn's disease No/(%)	The controls No/(%)	p
Appendectomy				
Yes	4 (3.4)	12 (38.7)	18 (12.0)	<0.001[a]
No	115 (96.6)	19 (61.3)	132 (88.0)	
Tonsillectomy				
Yes	19 (16.0)	9 (29.0)	20 (13.3)	$=0.095$[a]
No	100 (84.0)	22 (71.0)	130 (86.7)	
Measles virus infection in the childhood				
Yes	81 (68.1)	20 (64.5)	101 (67.3)	$=0.932$[a]
No	38 (31.9)	11 (35.5)	49 (32.7)	
Measles virus vaccination				
Yes	89 (74.8)	28 (90.3)	101 (67.3)	$=0.026$[a]
No	30 (25.2)	3 (9.7)	49 (32.7)	
Total	119 (100)	31 (100)	150 (100)	
[a]Chi-square test				

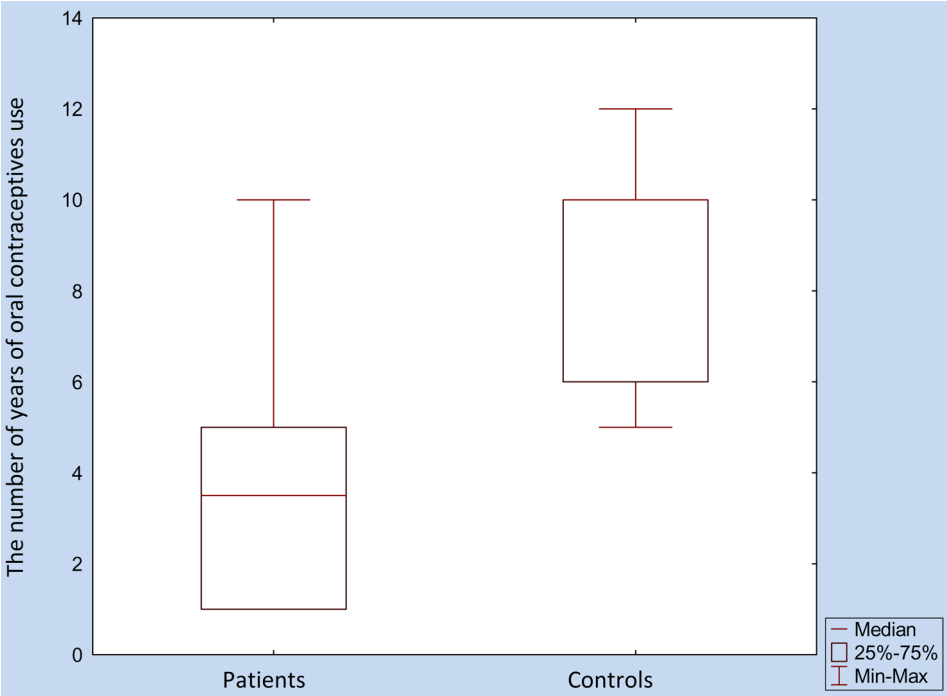

Fig. 2 Women, duration of oral contraceptives use (years) between patients ($N = 14$) and the controls ($N = 11$) ($p < 0.001$; Mann–Whitney U test)

ological shortcomings, are likely to contribute the positive association between measles virus vaccination in the childhood and the development of CD later in life (Table 6).

Although the data is still limited it does show that in the past few decades IBD has become more prevalent in Central Eastern European countries of Hungary, Slovenia, Croatia, Czech Republic, Slovakia, Romania, north eastern Poland and Baltic countries, previously known as low incidence areas of Europe [35]. In some of these parts, including western Hungary, north eastern Slovenia and some counties of Croatia, there is a striking increase in incidence and prevalence of IBD [36–38]. This trend seems to particularly stress highly vulnerable paediatric and adolescent populations [39, 40]. Rapid temporal changes and variation in geographical distribution of IBD contribute to the assumption of the key role of the environmental factors in driving clinical expression of IBD. However, research on this issue in Central Eastern European countries is still insufficient.

One of the best reported risk factor for IBD in general is smoking. Although our results are not concordant with those found in the study from western Hungary where smoking was identified to increase the risk of CD and decrease the risk of UC [36], in both countries, Hungary and Croatia, the average national smoking prevalence rates were high (for Hungary 40.5 %, 1991; for Croatia 30.3 %, 1997). Taking into account the known fact that smoking and other unhealthy lifestyle habits have been imported in Eastern European countries early during the process of globalization, but that their spread might have been alleviated soon after that, by the strict application of public health measures, due to the well-organized public healthcare system in these countries—we can accept a more complex view as an explanation of these non-concordant results. Namely, there could be a complex interplay at the individual level of the effects made by the duration of smoking and the time from quitting, corrected for the prevalence index of smoking in the local population. Alternatively, an average older age at the time of UC diagnosing, in our examined sample, than what is elsewhere cited, might have influenced our results on smoking [14].

An interesting result found in our study is the association between exposure to MMR vaccine in the early childhood and later development of CD. These risk factors have not been assessed in Central Eastern European countries to date, although such study, if performed, might have added to the clarification of the long-lasting dilemma on the accuracy of the proposed association between measles virus vaccination and the risk of CD because measles vaccination is mandatory and universal in these countries [32]. This region would generally be an appropriate arena for checking the accuracy of the hygiene hypothesis because of the good sanitation and public health measure practices at least in the years before the era of globalization. The hygiene hypothesis indicates that high sanitation, wide antibiotics use and mandatory vaccination are the factors that cause poor exposure of the developing immune system of children to antigens and the subsequent breakdown of the immunologic tolerance, leading to the development of immune-mediated and inflammatory mediated diseases, such as allergies, IBD and autoimmune diseases [41]. In contribution to this idea, in the recent case-control study, conducted in Slovakia, the authors found that infrequent contact with animals in the childhood is associated with increased risk for CD, while a small family size seems to increase the risk for UC [42].

The theory most frequently used to explain the observed rapid increase in incidence rates of IBD in Hungary, Croatia and Slovenia correspond with the loss of traditional dietary habits and the acquisition of more "westernised" types of diet, including higher intake of sugar and fats and lower content of fibre and fresh fruits in the diet [35, 40]. This theory is concordant with the current understanding of the pathogenesis of IBD in which the emphasis is put on the role of commensal bacteria and the mucosal immune system [43]. Moreover, it is believed that a complex range of environmental factors operating through the "westernised" standards of living was necessary to potentiate changes in the epidemiologic patterns of IBD in Central Eastern European countries. This assumption is now being confirmed by the results of the recently published large prospective population-based study aimed at identifying differences in environmental risk factors for IBD between Western and Eastern European countries [44]. According to this study, Eastern European countries, when compared to Western European countries, were higher in sugar intake, daily consumption of fast food, appendectomy before the age of 20 and vaccination rates, and lower in the number of childhood infections and fibre intake, all these factors known to be included in the Western lifestyle. We came to the same conclusion although indirectly, upon our study on incidence and prevalence of IBD in Zadar County (the Adriatic Sea coastal area, southern position). Oppositely to what might have been expected for this area, according to the traditional view on the existence of the north to south gradient, we found there the pattern of IBD which is approaching that of the developed countries in Europe, probably due to the fast economic grow of this county [38].

Limitations

Methodological limitations of this study include small number of cases, lack of power analysis and lack of the frequency estimation for the examined risk factors in the general population. In addition, this study was performed in the restricted area of north-eastern Croatia, characterized with low incidence and prevalence rates of IBD. The results, thus, do not reflect the global situation in Croatia, where some counties have been identified as areas with rapidly increasing incidence rates of IBD. Future systematic and large-scale studies would be necessary in order to make more consistent conclusions on the influence of certain environmental risk factors on changes in the epidemiologic patterns of IBD in Croatia, as well as in the wider region of Central Eastern Europe. For these future studies, as already noted in the Discussion chapter, it would be of the utmost importance to have information on the distribution (frequency) of the examined risk factors in the general population. Nevertheless, results of this study indicate that the traditional environmental risk factors for IBD, identified in a number of previous studies, can also be a driving force in our local epidemiologic context. This conclusion indicates that some risk factors are universal and present in different populations. As the group of researchers wrote: "Environmental risk factors of individual, familial, community-based, country based and regionally based origin may all contribute to the pathogenesis of IBD. The geographical variation of IBD provides clues for researchers to investigate possible environmental aetiological factors" [45].

Conclusions

Heterogeneity in distribution of IBD, within a wider region (Central Eastern Europe), or within one country (Croatia), suggests that multiple and heterogeneous risk factors operate in a local area and have to be studied there, in order to understand their causal relationships with changes in IBD epidemiologic patterns. In addition, as already mentioned group of authors stated: "Changes in socioeconomic status might occur differently in different geographical areas and populations and, consequently, it is important to consider the heterogeneity of risk factors applicable to the individual patients" [45]. Thus, in some areas, fast demographic changes take place (Vukovar-Srijem County), operating through risk factors such as the immigrant status and psycho-social stress [14]. Some other areas experience fast economic grow (Zadar County) and rapid acceptance of "westernised" lifestyles [38]. Even more, it is suggested that the bulk of identified risk factors need to be integrated with the results of the genetic and migrant population studies in order to improve prediction and prevention strategies related to IBD [36]. For example, recent findings indicate a greater frequency of the NOD2 gene, known genetic marker of CD, in central areas of Europe, than in other parts of Europe. This finding is coincidental with the increase in incidence of CD in some countries of Central Eastern Europe [46]. The question is whether changes in environmental factors can provide clues for this relationship.

Conflict of interest

The authors declare no conflict of interest.

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