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Risk factors for inflammatory bowel diseases according to the "hygiene hypothesis": A case–control, multi-centre, prospective study in Southern Italy

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Abstract

Background: Ulcerative colitis (UC) and Crohn's disease (CD) are inflammatory bowel diseases (IBD) of unknown aetiology. The 'hygiene hypothesis' (HH) suggests that several hygiene-related factors may have contributed to the increased incidence of IBD. The aim of the study was to evaluate risk factors for IBD related to HH in a cohort of IBD patients from the south of Italy.
Methods: We prospectively performed a one-year, questionnaire-based, case–control, multi-centre study focusing on the principal risk factors for IBD according to HH. We investigated the main surrogate markers of HH (helmintic infections and antibiotics in childhood; breastfeeding; family size/sibship; urban upbringing; personal and domestic hygiene in childhood) in UC and CD patients, in comparison with a control group of healthy subjects. In addition, the traditional risk factors for IBD were also recorded.
Results: The study population included 527 cases of UC, 468 CD and 562 controls. None of the surrogate risk factors of HH was significantly associated with IBD. On the contrary, the traditional risk factors confirmed their statistical significance in this IBD population. Familial aggregation: OR 4.07 for

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UC; OR 4.83 for CD; smoking: OR 0.38 for UC; OR 1.40 for CD; appendectomy: OR 0.28 for UC; OR 1.61 for CD.
Conclusion: Even though risk factors associated to the HH have been proposed as a possible explanation for the increasing calendar trend of IBD incidence, their role does not appear to be statistically significant. Familial aggregation, smoking habits and appendectomy still remain the main risk factors associated with IBD.

1. Introduction

Inflammatory bowel diseases (IBD) – comprising ulcerative colitis (UC), Crohn's disease (CD) and indeterminate colitis – are inflammatory conditions of unknown aetiology.[1–3] Even if many genetic and pathogenetic aspects have been investigated and highlighted in the last 10 years, the cause of the disease still remains unclear.[4–7]

Although many epidemiologic studies have been published only two environmental risk factors (smoking and appendectomy) have been strongly linked to IBD incidence, while conflicting results have been described for others (i.e. use of oral contraceptive, viral or bacterial infections).[8–12] In addition to environmental features, familial aggregation represents the most important risk factor associated with the onset of disease.[13]

The incidence and prevalence of IBD are particularly high in Western countries, with an epidemiological peak in the US and in Northern Europe.[14,15] In particular, when analysing the incidence of IBD in Europe, a clear geographical cut-off can be identified in the Alps mountains, with a 2-fold increase in risk for countries located north of the Alps (~20 vs 10/100,000 inhabitants).[16] Furthermore, data from North America and Europe underline a clear calendar trend of increasing incidence for both UC and CD,[17,18] with a 40–50% increase in prevalence between 1980 and 1990.[19] Interestingly, most studies report an increase in incidence only among IBD patients aged 20–35 years, suggesting that this increase was not exclusively related to the availability of more sensitive diagnostic techniques.[14]

One promising and interesting theory to explain the recent increased incidence of IBD is the "hygiene hypothesis"(HH),[20] which correlates this epidemiological trend with the improvement in general hygienic conditions (i.e. free access to clean water, running hot water, smaller family size, etc.). In these conditions, a decreased antigenic exposure in childhood could be the cause of an immunological over-reaction at the time of a following microbial contact.[21]This hypothesis has been proposed as a possible factor contributing to the increased incidence of inflammatory (e.g. IBD), auto-immune (e.g. thyroid disease) and allergic (e.g. asthma) conditions in the last four decades.[21]

In order to explore this theory, we decided to perform an epidemiological study to investigate the role of risk factors for IBD according to HH in a cohort of patients from Campania, a region of the Southern Italy.

2. Patients and methods

In the course of one calendar year (March 2010–March 2011) we carried out a case–control, multi-centre, prospective, hospital and questionnaire-based survey in the Campania region of Southern Italy.

2.1. Patients and controls

The study included all consecutive IBD patients (UC, CD, indeterminate colitis) observed in 10 regional Gastroenterology Units. The control group comprised physicians, nurses, and support services professionals from the participating sites.

The diagnosis of IBD was made and reviewed in accordance with the European Crohn's and Colitis Organisation (ECCO) guidelines.[22,23]

2.2. Questionnaire

The questionnaire focused on the principal risk factors for development of IBD according to HH. In particular, we investigated the main surrogate markers of HH: 1) helmintic infections in childhood; 2) owning pets in childhood; 3) use of antibiotics in childhood; 4) breastfeeding; 5) family size (number of brothers/sisters); 6) sibship; 7) urban upbringing; 8) dental care history; 9) compulsory and optional vaccinations; 10) allergies in childhood.

In addition, the traditional risk factors for IBD (familial aggregation, smoke, appendectomy) were also recorded.

All the queries and variables in the questionnaire were simplified as far as possible and presented in a dichotomic way (yes/no). In addition, in case of doubt also the option of answer "I don't remember" was offered. Dental care and family size were analysed as dichotomic variables (≤ 1 vs ≥ 2).

The study was approved by the local Ethics Committee (Prot. 92/09). All patients and controls gave their written consent to participate in the study.

2.3. Statistical analysis

Statistics were performed by using Pearson's chi-square and odds ratio values (OR). The analysis was two-tailed; $p<0.05$ was considered statistically significant.

3. Results

The study population included 527 cases of UC, 468 cases of CD and 562 controls. The main characteristics of the patient studied are summarised in Table 1.

In the IBD group no case of indeterminate colitis was present.

No significant difference was observed between the IBD group and the control group in terms of demographic features (Table 1).

Table 2 shows the results of the statistical analysis of the main risk factors related to the hygiene hypothesis in our cohort of patients. None of the surrogate risk factors of HH was significantly associated with IBD. On the contrary, as shown in the same table, the well-known risk factors such as smoking, history of appendectomy and familial aggregation confirmed their statistical significance in our IBD patients (Table 2). Familial aggregation was the main risk factor associated with IBD (OR 4.07 for UC; OR 4.83 for CD). In the same way, both smoking and appendectomy confirmed their divergent risk profile in the two IBD (smoking habit: OR 0.38 for UC; OR 1.40 for CD; appendectomy: OR 0.28 for UC; OR 1.61 for CD) (Fig. 1A–C).

4. Discussion

The hygiene hypothesis has been proposed as a possible explanation for the significant increase of IBD incidence in the last decades, although studies exploring this theory are still scarce in the literature.[24]

The present report investigated this topic in a cohort of consecutive IBD patients observed in ten centres from the South of Italy; this is one of the first studies aimed to verify this hypothesis in a European population.

Our study analysed the main surrogate markers of HH in patients suffering from IBD (childhood and helmintic infections; antibiotics in childhood; breastfeeding; family size/-sibship; urban upbringing; personal and domestic hygiene) and in a control group of healthy subjects.

In our study none of the HH risk factors appeared to be significantly associated with UC or CD. On the other hand, familial aggregation, smoking habits and appendectomy confirmed to be risk factors for IBD in our population. In accordance with previous reports, familial aggregation resulted to be the most important variable in terms of risk for IBD (OR 4), while smoking habits and appendectomy confirmed the different risk profile in UC and CD patients (smoking habit: OR 0.38 for UC; OR 1.40 for CD; appendectomy: OR 0.28 for UC; OR 1.61 for CD).[9–12] The fact that these well-defined risk factors for IBD in North America, Europe and Italy[25,26] were confirmed in our cohort clearly indicate that this is an unselected population of patients mirroring the general population of IBD cases found in Western countries, thus reinforcing the negative results on HH risk factors.

Our data are in contrast with those reported in a previous paper by Bernstein et al., showing in a population-based, case–control study on risk factors for IBD in Canada, a slightly reduced risk for Crohn's disease in subjects coming from large families and in those owning pets in childhood (13% and 34% reduced risk, respectively).[24] Out of about twenty variables, only a few were significantly associated with IBD among a large series of possible risk factors, many of which potentially referable to the hygienic theory (family size, number of brother/sisters, urban vs rural area, birth order, pets prior to age 5). Drawing upon Bernstein et al.'s study, we investigated variables such as family size and pets in childhood as potential risk factors for IBD, but we could not demonstrate any statistical significance in our population, which was numerically wider than the Canadian's one.

More recently, Lopez-Serrano et al. have shown an increased risk of IBD in Spanish subjects with high educational achievement and high social level (RR 1.83 and 1.68, respectively), underlining the potential role of HH in this geographical context.[27] We decided not to include this type of variables in our study because, in our opinion, they are not directly indicative of a better hygienic condition. Furthermore, socioeconomic circumstances can fluctuate thus introducing a potential bias. The same paper also reported a 4-fold increase in IBD risk in subjects living in urban areas; this result was not confirmed either in our or in the Canadian experience. This discrepancy could be explained by the different size of the sample population (about half the number of patients compared to the present study) or by geographical features.

The aim of our study was to investigate the risk factors for IBD according to the HH avoiding other possible confounding variables. We recorded the traditional risk factors for IBD (family history, smoking and appendectomy) only in order to generate an epidemiological and statistical "internal control" for our sample population. A strength of the present study compared to the previously published paper is the quite large sample size for both IBD patients and controls. In effect, our hospital-based, multi-centre study approach allowed us to recruit a number of patients similar to those usually reported in population-based or registry-based epidemiological studies. Population-based and registry-based studies are usually considered the best option for epidemiological investigations. However, an IBD registry was established only recently in Italy, thus limiting the possibility of such an approach, and making our methodological procedure the only one effective to get this kind information in our country.

Finally, our results do not support the hygiene hypothesis as a potential explanation for the increased incidence of IBD.

One can hypothesise that the negative findings of this study may be related to methodological biases. First, even if the inclusion of the population was performed prospectively, the questionnaire included items referred mainly to the

Table 1 Features of IBD patients and control group.

Variable	UC	CD	Controls	*p*
Number	527	468	562	
Median age (years)	37 (16–63)	36 (18–61)	39 (18–66)	n.s.
Gender (M/F)	285/242	263/205	310/252	n.s.
Extension (E1–E2–E3)	175–201–151	–	–	–
Location (L1–L2–L3–L4)	–	180–145–141–2	–	–
Behaviour (B1–B2–B3)	–	231–146–91	–	–

Table 2 Risk factors for IBD in accordance with HH.

Variable	UC # 527 (%)	CD # 468 (%)	Controls # 562 (%)	*p*	OR	I.C.
Animals in childhood	242 (45.9)	202 (43.1)	247 (43.9)			
UC vs controls				n.s.	1.08	0.85–1.37
CD vs controls				n.s.	0.96	0.75–1.24
Helmintic infections in childhood	51 (9.7)	58 (12.3)	63 (11.2)			
UC vs controls				n.s.	0.84	0.57–1.25
CD vs controls				n.s.	1.12	0.76–1.63
Antibiotics in childhood	231 (43.8)	212 (45.2)	260 (46.2)			
UC vs controls				n.s.	0.90	0.71–1.15
CD vs controls				n.s.	0.96	0.75–1.23
Number of brothers/sisters (≤ 1 vs ≥ 2)	112 (21.2)	105 (22.4)	124 (22.0)			
UC vs controls				n.s.	0.95	0.71–1.27
CD vs controls				n.s.	1.02	0.76–1.37
Sibship (first-born)	190 (36.0)	161 (34.4)	191 (33.9)			
UC vs controls				n.s.	1.09	0.85–1.40
CD vs controls				n.s.	1.01	0.78–1.31
Urban upbringing	445 (84.4)	381 (81.4)	481 (85.5)			
UC vs controls				n.s.	0.91	0.65–1.27
CD vs controls				n.s.	0.73	0.52–1.02
Breastfeeding	416 (78.9)	353 (75.4)	419 (74.5)			
UC vs controls				n.s.	1.27	0.96–1.69
CD vs controls				n.s.	1.04	0.78–1.39
Dental care (≤ 1 vs ≥ 2 times)	339 (64.3)	312 (66.6)	358 (63.7)			
UC vs controls				n.s.	1.02	0.80–1.31
CD vs controls				n.s.	1.13	0.88–1.47
Compulsory vaccinations	488 (92.5)	425 (90.8)	512 (91.1)			
UC vs controls				n.s.	1.22	0.78–1.99
CD vs controls				n.s.	0.96	0.62–1.48
Optional vaccinations	101 (19.1)	97 (20.7)	104 (18.5)			
UC vs controls				n.s	1.04	0.77–1.41
CD vs controls				n.s.	1.15	0.84–1.56
Allergy in childhood	131 (24.8)	117 (25.0)	129 (22.9)			
UC vs controls				n.s.	1.11	0.84–1.46
CD vs controls				n.s.	1.11	0.83–1.49
Familial aggregation	72 (13.6)	74 (15.8)	21 (3.7)			
UC vs controls				<0.001	4.07	2.46–6.73
CD vs controls				<0.001	4.83	2.92–7.99
Smoking	88 (16.6)	198 (42.3)	193 (34.3)			
UC vs controls				0.008	0.38	0.28–0.51
CD vs controls				<0.001	1.40	1.08–1.80
Appendectomy	49 (9.2)	172 (36.7)	149 (26.5)			
UC vs controls				<0.001	0.28	0.20–0.40
CD vs controls				<0.001	1.61	1.23–2.10

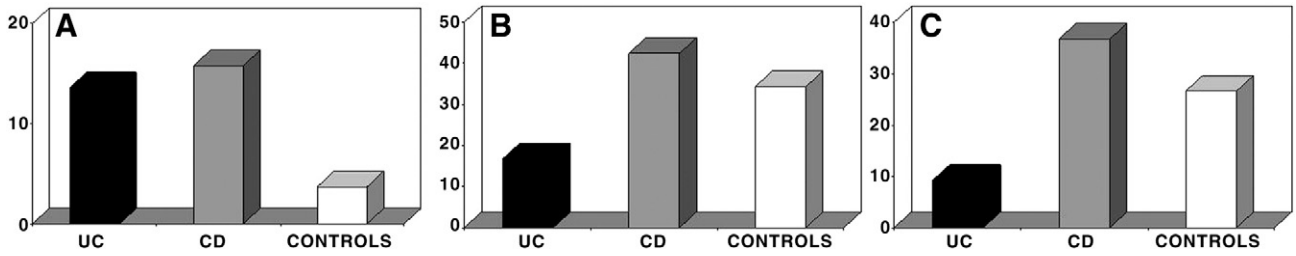

Figure 1 Percentage of patients with familial aggregation (A), smoking habit (B) and appendectomy (C) in IBD.

participants' past, exposing the study to a "recall bias". For some variables such as family history, family size, urban upbringing, vaccinations or having pets in childhood, this approach should not affect the results. For other variables, such as use of antibiotics in childhood, helmintic infections and dental care the recall bias could be considered a critical matter. This has been suggested as a possible factor justifying the conflicting results reported in the literature. However, with a large control group as in our study any recall bias should influence in the same manner IBD and control groups, thus having only a limited negative effect. Based on these considerations, it has been proposed that studies exploring etiologic hypotheses should be performed prospectively, preferentially examining paediatric cohorts of patients. This type of epidemiological approach has been described in two recent papers by Shaw et al. and Hviid et al. reporting a slight increased risk for IBD in paediatric patients exposed to antibiotics in their first year of life[28,29]; with the limits of our retrospective analysis this finding was not evident in our population. In any case, this methodology cannot be applied today to investigate changes in habits that occurred about 3–4 decades ago according to the HH hypothesis.

It is possible to argue that the different results found in our study compared to previous reports may be partially related to the different questionnaires used. However, our questionnaire, although not validated, is very similar in nature to those used in other studies.[24,27]

In conclusion, although HH has been proposed as a possible explanation for the increasing calendar trend of IBD incidence, its actuarial role in our population does not appear to be significant.

Family history, smoking habits and appendectomy were confirmed as main risk factors associated with IBD.

Conflict of interest

The authors have no conflicts of interest.

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