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ORIGINAL ARTICLE

Environmental factors associated with Crohn's disease in India

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Abstract
Background The frequency of diagnosis of Crohn's disease (CD) in India is increasing. This case-control study was designed to detect associations of environmental and dietary factors with the diagnosis of CD.
Methods In 200 consecutive patients with CD and 200 control subjects without gastrointestinal disease, environmental hygiene exposures in childhood and in the past one year, and dietary preferences were recorded using a questionnaire. Univariate and multivariate analyses were done.
Results In univariate analysis, CD showed positive association with urban residence (at birth and current), availability of protected drinking water (childhood and current), availability of piped water in the house (childhood and current), and strict vegetarian dietary habit, and negative association with regular fish consumption and presence of cattle in the house compound. Multivariate analysis showed that regular fish consumption (OR 0.52, 95% CI 0.33–0.80, p=0.003), and presence of cattle in the house compound currently (OR 0.57, 95% CI 0.35–0.92, p=0.023) were significant protective associations, whereas use of safe drinking water was positively associated (OR 1.59, 95% CI 1.02–2.47, p=0.042) with the disease.
Conclusion Occurrence of CD was associated with dietary and environmental exposures, which indicate that diet and hygiene may influence the development of this disease.

Keywords Case-control study · Hygiene · Inflammatory bowel disease

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Introduction

Crohn's disease (CD) was described in the first half of the twentieth century, and its incidence increased dramatically in North America and Europe during the twentieth century [1]. The disease is far less common in the developing world [2, 3], though increasing number of cases are being recognized in recent years [4]. The worldwide increase in CD is paralleled by an increase in the incidence of other auto-inflammatory diseases such as multiple sclerosis, type I diabetes and asthma, and has coincided with a steep reduction in infectious diseases. This shift in disease pattern has been explained using the hygiene hypothesis, according to which immune conditioning by childhood infections protects against inflammatory bowel disease in later life [5, 6].

Environmental factors that have been related to the risk of inflammatory bowel disease include infectious agents, smoking and diet [7]. CD has been shown to be associated with markers of better childhood hygiene, such as birth order, urban residence, separate bedroom as a child, and the availability of hot water taps in the house [8, 9]. Smoking also influences disease course and severity in CD [10]. The risk of CD is also elevated soon after appendicectomy, though this probably reflects diagnostic problems in distinguishing between acute appendicitis and incipient CD [11]. Other exposures postulated to be associated with

CD include the use of fast foods, cola drinks, toothpaste, antibiotics and oral contraceptives [12]. Thus, both susceptibility genes and environmental factors appear to be strongly involved in the pathogenesis of CD [13].

The incidence of inflammatory bowel disease has increased in Asian countries [14]. Several studies have shown a high incidence and prevalence of CD in South Asian immigrants to the United Kingdom; in particular, among second generation immigrants, the incidence is similar to that in the people of European origin [15–19]. Similar findings have been reported in South Asian migrants to North America [20]. It is postulated that westernization and industrialization with changes in dietary habits and sanitation have contributed to increasing inflammatory bowel disease in Asians [4]. India is undergoing a rapid industrialization, and changes in diet and hygiene, and these changes have been accompanied by an apparent increase in the diagnosis of inflammatory bowel disease [3, 21]. This case-control study tested the hypothesis that lifestyle and hygiene-related environmental factors would be associated with CD in India.

Methods

Consecutive patients with CD attending the Inflammatory Bowel Diseases Clinic at the Christian Medical College, Vellore between September 2006 and December 2008 were recruited. The diagnosis of CD was based on standard consensus diagnostic criteria [22–24]. Laboratory investigations and imaging studies were done as per the unit's standard clinical protocol. Persons without gastrointestinal disease accompanying patients to the Gastroenterology Clinic of our institution served as control subjects. The controls were not related to the CD patients. For each inflammatory bowel disease (IBD) patient, a person within the same five-year age interval was chosen.

Each participant was interviewed by a medical officer, a social worker and a dietician. Clinical and treatment details were ascertained by the medical officer. Socioeconomic status was determined using the modified Kuppuswamy's scale, which is validated for India [25]. This assigns a social score on the basis of education and occupation on a scale of 2 (lowest) to 14 (highest), and an economic score on the basis of family income adjusted for 2007 on a scale of 1 (lowest) to 14 (highest), which are added to yield a composite socioeconomic score (possible range 3–28). Dietary assessment was done using recall of usual dietary practice prior to onset of illness, using food frequency questionnaires relating to foods commonly used in India [26]. A subject was categorized as lacto-vegetarian if he/she never ever ate eggs, meat or fish but consumed milk or milk products, and as a regular consumer of fish or meat, respectively, if he/she ate fish or meat on three or more days every week. Persons using at least ten cigarettes or a packet of *beedi* per week prior to onset of illness were categorized as smokers.

Data on several environmental variables were recorded separately for the childhood period and for the current time. Rural and urban residence was categorized as per the Government of India 1971 census definitions [27]. Nature of the water used for drinking (untreated well water or filtered or boiled water), the availability of water on tap in the house (marking higher level of hygiene than water from a common tap or well located outside the house), the presence of a closed toilet system in the house, the presence of pets in the house, and whether or not the family kept cattle within the compound of the house were recorded and used as surrogate markers of hygiene; these variables were evaluated for the first five years of life (childhood factors) as well as in the past one year (current factors). Although current environmental and dietary factors could have changed in response to the diagnosis of IBD, the data served as a measure of internal consistency. The study protocol was approved by the Research and Ethics Committees of the Christian Medical College, Vellore. Informed written consent was obtained from all participants.

Statistical analysis

Data are reported as frequencies for categorical variables. Sample size of 200 per group (including correction for incomplete data acquisition) was calculated for comparing CD with controls assuming a two-sided probability of 0.05% and 80% study power to identify factors occurring in a third of controls that would confer a relative risk of 2.0 for CD. Continuous variables were expressed as median (interquartile range) and compared between groups using the Mann-Whitney test. Categorical variables were compared using the chi-square test. The categorical variables that were input included gender, strict vegetarianism, fish consumption, current presence of cattle in the house, residence at birth, current residence, use of filtered or boiled water in childhood, use of filtered or boiled water currently, presence of taps in the house in childhood, presence of taps in the house currently. Age and socioeconomic score were input as continuous variables. Factors that were significantly associated with CD on univariate analysis were entered into a conditional forward logistic regression with the diagnosis as the dependent variable. Odds ratios (OR) and confidence intervals (CI) were calculated for the significant associations.

Results

The demographic characteristics of the participants are listed in Table 1. Patients with CD had poorer nutrition and

Table 1 Characteristics of patients with Crohn's disease and control subjects

	Control subjects (n=200)	Crohn's disease (n=200)	p-value
Age (years)	34 (27–44)	33 (24–45)	NS
Male gender (n)	137	117	<0.05
Weight (kg)	55 (49–63)	48 (40–60)	<0.001
Height (cm)	157 (151–163)	158 (150–165)	NS
MUAC (cm)	24 (23–26)	23 (22–24)	<0.001

Data on continuous variables are shown as median (interquartile range)

MUAC mid-upper arm circumference, *NS* not significant

shorter stature than the reference group. Abdominal pain (153 patients), weight loss (149) and diarrhea (135) were the common presenting symptoms. Nearly half the patients each had blood in stools (92) and fever (98). Sixty-two patients (31%) had joint involvement. The extent of involvement was ileocolonic in 80 patients (40%), colonic in 52 (26%), ileal in 51 (25.5%), and upper gastrointestinal in 17 (8.5%). In 47 patients, the disease was categorized as stricturing and in 35 as penetrating; one hundred and eighteen patients had neither strictures nor penetrating disease. Perianal disease was noted in 37 patients. One hundred and eight patients had received aminosalicylates, 72 corticosteroids and 32 azathioprine; 62 patients (31%) had undergone surgery.

Table 2 lists the dietary and personal habits of study participants. Smoking was infrequent and was not associated with CD. Appendicectomy rates were similar in patients with CD and controls (10% vs. 5%, p=0.08). Both groups had only a few lacto-vegetarians but their frequency was higher among patients with CD than in the control group. In contrast, regular consumption of fish was more frequent in the control group (Table 2).

The socioeconomic score was marginally higher in the patients with CD (Table 2). Urban residence (birth and current), safe drinking water (childhood and current), and availability of piped water in the house (childhood and current) were all positively associated with a diagnosis of CD (Table 3). Interestingly, the presence of cattle in the house compound currently was negatively associated with diagnosis of CD (Table 3).

On multivariate analysis, protective associations were noted with a history of regular fish consumption (OR 0.52, 95% CI 0.33–0.80, p=0.003) and presence of cattle in the house compound currently (OR 0.57, 95% CI 0.35–0.92, p=0.023), while the use of treated drinking water was positively and significantly associated with CD (OR 1.59, 95% CI 1.02–2.47, p=0.042) (Table 4).

Discussion

This study tried to identify environmental and dietary factors associated with a diagnosis of CD in India. On univariate analysis, several variables related to better hygiene, including urban residence, use of treated drinking water and presence of piped water in the house, were associated with CD. On multivariate analysis, only two hygiene-related variables and one dietary variable were associated with CD. Although these results have to be interpreted with caution since the study was a hospital-based case-control study, these findings suggest that environmental variables, particularly those in adult life, are associated with CD and may explain the recent increase in diagnosis of CD in India.

The clinical presentation and extent of involvement in patients with CD in the present study were similar to those described in a recent multi-center study of 182 Indian patients with CD [28]. The colon was involved in about two-thirds (along with the ileum in 40%) of our patients. The higher prevalence of ileocolonic involvement in Asian patients with CD has been previously commented upon [4]. Isolated ileal disease was seen in only about a quarter of patients, an observation consistent with the rarity of commonly described NOD2 mutations in the Indian population [29, 30].

Table 2 Socioeconomic, past medical history, dietary preferences and personal habits of study participants

	Controls (n=200)	CD (n=200)	p-value
Socioeconomic score (median [interquartile range])	20 (16–24)	22 (18–26)	0.0110
Pulmonary tuberculosis in past (n)	3	11	NS
Appendicectomy (n)	10	20	NS
Smoking history (n)	32	25	NS
Lacto-vegetarian (n)	8	19	0.0440
Regular fish consumption (n)	144	109	0.0004
Regular meat consumption (n)	185	175	NS

NS not significant

Table 3 Environmental variables related to hygiene in patients with Crohn's disease

Variable		Crohn's disease	Control	Odds ratio	95% CI	*p*-value
Urban residence	At birth	88/112	63/136	1.69	1.12–2.55	0.013
	Past 1 year	126/74	97/103	1.80	1.21–2.69	0.004
Treated drinking water	Childhood	24/174	8/191	3.29	1.44–7.52	0.003
	Past 1 year	83/115	57/143	1.81	1.19–2.74	0.006
Piped water in house	Childhood	69/130	47/151	1.70	1.10–2.64	0.020
	Past 1 year	124/72	98/101	1.77	1.18–2.65	0.006
Closed toilet in house	Childhood	101/83	97/88	1.10	0.73–1.66	NS
	Past 1 year	186/13	182/17	1.33	0.63–2.83	NS
Tooth cleanser use	Childhood	149/49	146/54	1.12	0.71–1.76	NS
	Past 1 year	198/2	191/9	4.66	0.99–21.87	NS
Pets in house	Childhood	44/151	53/145	0.79	0.50–1.26	NS
	Past 1 year	30/167	27/173	1.15	0.65–2.01	NS
Cattle in house compound	Childhood	91/106	111/89	0.68	0.46–1.02	NS
	Past 1 year	38/156	66/134	0.49	0.31–0.78	0.002

Data are numbers of participants answering "yes"/"no" to each question. Missing numbers indicate that participants were uncertain of the answer and responded "do not know"

NS not significant

Interestingly, smoking, which is consistently associated with risk of CD in other populations, was not associated with CD in our patients. Smoking is associated with more severe illness and continuing inflammation in CD patients. In a Dutch cohort, the NOD2 (R702W) mutation (along with several other SNPs - rs17234657, rs2165047 and rs10883365) was associated with CD only in smokers [31]. Whereas the prevalence of the latter SNPs in the Indian population is not known, the NOD2 (R702W) mutation is rare or absent [29, 30]. This may explain the lack of association between CD and smoking in our patients.

The appendicectomy rate was 5% in our control group (compared to a rate of 1.3% in Sweden [32]) and showed a trend to be higher (10%, p=0.08) in patients with CD. A recent meta analysis showed a positive association of appendicectomy with CD in the early stages of the illness, possibly because some patients with early CD undergo surgery with a mistaken diagnosis of acute appendicitis [11].

Diet may play a significant role in the genesis of CD, probably through effects on the gut microbiota. In our study, the number of strict lacto-vegetarians though small, was somewhat higher in patients with CD than in controls. Regular consumption of fish, on the other hand, was negatively associated with CD. In the multivariate analysis, only the latter association was observed. Though the interviewer in our study did try to specifically target dietary preference prior to the diagnosis of CD, we cannot be certain that these associations could not have resulted from dietary changes following the diagnosis of CD. Our findings parallel the finding of a protective association of diets containing fish with CD in children in a previous study [33]. Fish oils are rich in omega-3 long chain polyunsaturated fatty acids which can get incorporated into cell membranes, reducing the availability of arachidonic acid for the synthesis of pro-inflammatory eicosanoids. Indeed, administration of fish oils to patients with inflammatory bowel diseases has been shown to alter the profile of inflammatory mediators in the gastrointestinal mucosa [34]. On the other hand, a meta analysis of intervention trials did not support a role for fish oils in the maintenance of remission in CD [35].

Surrogate markers of better hygiene in childhood were positively associated with CD in univariate analysis; however, on multivariate analysis, the only significantly associated factors pertained to adult life including the use of

Table 4 Multivariate analysis of association between environmental variables and Crohn's disease

Variable	Odds ratio	95% confidence interval	*p*-value
Consumption of fish more than once a week	0.515	0.333–0.798	0.003
Presence of cattle in the compound of the house currently	0.572	0.354–0.924	0.023
Current use of filtered or boiled water	1.585	1.018–2.470	0.042

treated drinking water, presence of cattle in the house compound, and regular consumption of fish. Interestingly, a recent investigation in Spain also identified adult-life hygiene factors (urban residence, social status and education) rather than childhood hygiene factors to be associated with CD [36]. The original hygiene hypothesis proposed the association of CD with hygiene-related variables during early childhood, leading to loss of oral tolerance [7, 9]. According to a recent alternative explanation, hygiene variables are related to alterations in the intestinal microbiota, which tilt the balance away from tolerance and towards intestinal inflammation [6]. In the present study, presence of cattle within the house compound was inversely associated with CD. A previous study reported that residence on a farm was negatively associated with CD [37]. We have previously shown that the fecal flora of rural children and adults living in close proximity to cattle contain the ruminal microbe, *Butyrivibrio fibrisolvens* [38], which is a major producer of butyrate and other short chain fatty acids. This microbe may exert probiotic effects in the human gastrointestinal tract, helping maintain immune homeostasis and preventing bowel inflammation.

Several limitations of the study design need to be recognized. There were significant gender differences between cases and controls, with more males among the controls than among the cases. However, the multivariate analysis showed that there were associations that were independent of gender. The fact this was a hospital-based study was a limitation. Ideally, epidemiological associations should use community-based cases and controls with an excess of controls. In other countries, these are done using population-based registries. However, CD continues to be rare in our country, there is no population-based registry, and community sampling would require enormous numbers to be screened. Under the circumstances, it appears reasonable to conduct a hospital-based case-control study as any epidemiological associations would be best understood during the process of health transition that India is undergoing presently. The issue of recall bias troubles all case-control studies. We asked for recall of childhood practices as well as current practices. There was reasonable concordance between childhood practices (e.g., eating fish, use of safe drinking water) and current practices which provided a degree of internal validation of the findings. While the study findings must be interpreted within these limitations, we believe that these are moderately robust, because these appear biologically plausible.

In conclusion, our study documents the environmental associations of CD that relate predominantly to hygiene and diet in adult life. These findings are consistent with the recent suggestions that alterations in intestinal microbiota in adult life, rather than changes in immune conditioning in early childhood, explain the hygiene hypothesis in relation to causation of CD.

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Author contributions SP contributed to supervision and data analysis; MKS contributed to data collation and analysis; VS to design of study and data collection; AP to histological diagnosis, and BSR to design, overall supervision, data analysis, and writing up the paper.

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