

Low hygiene and exposure to infections may be associated with increased risk for ulcerative colitis in a North Indian population

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Abstract

Background Previous studies have attempted to link hygiene hypothesis with IBD. However most of these studies come from developed countries where the level of hygiene is high and universal. Very little data is available from developing countries. The present study explores the truth of hygiene hypotheses and other risk factors for ulcerative colitis (UC) in a North Indian population where the prevalence of UC has been increasing.

Methods A total of 518 patients diagnosed with UC and 188 age-matched controls were included in the study. A structured questionnaire concerning socio-demographics and level of hygiene was completed by all participants. Logistic regression analysis was used to study the association between hygiene-related factors and the risk for UC. Odds ratios and 95% confidence intervals were estimated.

Results There was a higher proportion of females ($P<0.001$), and a higher educational status ($P=0.01$) in UC patients compared with controls. A family history of IBD was present in 7.2% of cases and non-existent in controls. On multivariate analysis, after accounting for potential confounders, having a private bed ($P<0.001$), and having better toilet facilities [(RCA versus none, $P=0.01$; Flush toilet versus none, $P=0.01$), (RCA LATRINE as a toilet technology used in rural areas where no flush facility exists. It was developed under RCA project)] were inversely associated with risk for UC whereas owning a pet ($P=0.01$) and stressful events like a death in the family ($P=0.01$) were associated with greater risks for UC.

Conclusion Our study does not provide definitive evidence to support hygiene hypothesis and rather suggests that the rising incidence of UC in North India may be attributable to inadequate sanitary measures or other as yet unidentified factors.

Keywords Hygiene hypothesis, ulcerative colitis, infections

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Introduction

Inflammatory bowel diseases (IBD), i.e. Crohn's disease (CD) and ulcerative colitis (UC), are chronic, relapsing diseases that affect the gastrointestinal tract. Although their incidence and prevalence rates are the highest among Caucasian populations, recent reports suggest that IBD is on the rise in developing countries among non-Caucasian populations

as well [1,2]. Both diseases are known to have genetic contributions, with that for CD being greater than that for UC, as shown by previous twin studies and more recently genome-wide association studies. Given the smaller contribution of inherited factors to UC susceptibility, it is thought that the onset of UC may be largely related to environmental influences [3]. Many studies have attempted to link the hygiene hypothesis with the UC onset [4]. Based on this hypothesis, the previously observed lower incidence of UC (or CD) in developing populations was attributed to the prevalence of less hygienic practices, suggesting that infections could potentially play a protective role against UC [5]. Likewise, numerous reports mainly from developed populations have shown that variables linked to better hygiene (which are availability and use of hot water, independent bathroom facilities etc.) and lower rate of infections (gastroenteritis, appendicitis, etc.) may enhance the risk for UC. Most previous studies were carried out in developed populations where the level of hygiene is much higher and at times universal [6-8]. A case control study on the

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Conflict of Interest: None

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environmental factors associated with CD has been published from India [9]. Given the smaller variation in hygiene-related exposure among such populations, deciphering the exact role of hygiene in UC is likely to be limited. Moreover, recent worldwide trends in IBD epidemiology also suggest a role for environmental factors in addition to genetic factors. Familial aggregation, smoking habits and appendectomy have been strongly linked to IBD incidence [10].

We have recently observed that the incidence and prevalence of UC among North Indians was high and approached that reported among Caucasian populations [11]. The North Indian population seems an ideal population for investigating the hygiene hypothesis as both economic and industrial growth has altered hygiene practices such that the distribution of these practices across the population has become highly variable. Thus, to further explore the contribution of hygiene level and other risk factors for UC, we carried out a case-control study within this population.

Methods

Design

Consecutive patients diagnosed with UC attending the Gastroenterology outpatient department at a tertiary care hospital (DMC&H, Ludhiana, Punjab) during the time period between January 2005 and December 2009 were recruited. Diagnosis of UC was based on standard criteria that included clinical, colonoscopic, and histopathological criteria.

Controls for the study were recruited from various sources to enhance population representativeness. These included patients' attendants with no gastrointestinal symptoms, replacement blood donors and patients from orthopedic wards with no gastrointestinal symptoms. Patients' relatives were not recruited as controls. Controls were selected concurrently with case recruitment. Based on expected age distribution of the cases, controls were selected so that there were roughly equal proportions within broadly defined case-age groups. Controls were required to be residing within the province of Punjab.

Exposure ascertainment

In order to acquire information on hygiene-related exposure prior to the diagnosis of the cases (and date of interview for the controls), a structured questionnaire developed by the investigators was provided to the participants. The questionnaire was personally handed to the participants by a trained medical officer. Both interviewers and participants were blinded to the main study objectives. The focus of the questionnaire was to acquire information on socio-demographics (age at diagnosis, gender, education) and exposure to infections. To reduce the potential for recall bias, only hygiene-related exposure that would entail minimal recall was enquired, including the number of siblings, availability of a private bed (always versus

never/sometimes), type of toilet facilities (none versus RCA or flush toilets), availability of personal towels for bathing (always versus never/sometimes), hand-washing prior to meals (always versus never/sometimes), pet ownership (no versus yes) and sources of drinking water (municipal, tube wells versus others that included well water, river water etc.). In addition to hygiene-related exposure, information was acquired with respect to family history of IBD (any relative), smoking (ever versus never), death in the family preceding the diagnosis and illness in the family preceding the diagnosis.

In most circumstances the questionnaire was handed to the participant. For participants <18 years of age, the questionnaire was given to the parent/guardian. Each participant provided informed consent and the study was approved by the Institutional Ethics committee.

Statistical analysis

After completion of the interview, each questionnaire was immediately checked for completeness, inconsistencies and missing data. Data were entered in a web-based database specifically constructed for the study. The database allowed for correction of inconsistent entries at the time of data entry. Data entry was carried out by personnel independent of those who carried out the interviews. Quality checks included identification of outliers, coding errors and rates of missing entries. These were corrected where possible. In the initial analysis the distribution of hygiene-related variables, potential confounders [such as age, gender, family history of IBD, socio-economic status (education) and other variables of interest (smoking, triggers of stress such as death or illness in the family)] were compared between the cases and controls using either t-tests or chi-square tests. Subsequent analysis was carried out by fitting an unconditional logistic regression model to the data. In the first step, univariate analysis (single variable) was carried out. To examine associations after accounting for potential confounding variables, a multivariate logistic regression model was fit. A stepwise regression procedure was used setting $P=0.20$ for variables to enter into the model. Model adequacy and fit was evaluated using the Hosmer and Lemeshow test. Odds ratios (OR) and 95% confidence intervals (95% CI) were estimated.

Results

Between 2005 and 2009, approximately 616 UC cases were registered with the Gastroenterology unit at the study hospital. Of these we were able to successfully recruit 513 cases for participation (83.3%). A total of 225 controls were approached for participation, of which 188 (83.5%) agreed to participate. Complete data on all the relevant study variables was available for 674 of the 701 (96.1%) participants.

The socio-demographic characteristics of the cases and controls are listed in Table 1. The median age at diagnosis

for the cases was 35.6 years and at interview for the controls was 38.1 years (P=0.33). There was a greater proportion of males (73.4% versus 54.6%, P<0.001) among the controls. Overall the distribution of educational level differed between cases and controls (P=0.01). These differences were the result of a greater proportion of UC cases having higher levels of education (graduation and beyond, 40.5% versus 29.6%). The majority of our cases were Sikhs (55%), followed by Hindus (44.7%), which is in accordance with the religion-wise population distribution in the State. Family history of IBD was present in 7.2% of the cases (Table 2), while none of the controls reported having a relative diagnosed with IBD resulting in a strong association with UC (P<0.001). The prevalence of smoking prior to disease diagnosis or interview was low both in the cases and controls and with no apparent differences (P=0.88).

Hygiene-related exposure

Univariate analysis (Table 2) showed that in comparison with controls, cases had less access to private beds (P<0.001), were less likely to wash hands prior to meals (P=0.002), were more likely to have a personal towel for bathing (P=0.008), owned a pet more often (P<0.001), and had less access to toilets (RCA versus none, P=0.003; Flush toilet versus none, P=0.006). No differences with regards to the sources of available drinking water were evident between the comparison groups. Cases reported stressful event like a death in the family more often than the controls (P<0.001). On multivariate analysis (Table 3), after accounting for potential confounders, having a private bed (P<0.001), and having better toilet facilities were inversely associated with risk for UC (RCA versus none, P=0.001; Flush toilet versus none, P=0.01). On the other hand, owning a pet (P=0.01) and having a death in the family (P=0.01) were associated with greater risks for UC. There were suggestions that regular hand-

washing was associated with inverse risk for UC (P=0.09); this relationship did not achieve statistical significance. No associations with smoking were evident but this was based on small numbers in lieu of custom and religious practices in Punjab state where women and the Sikh population are not supposed to smoke. Similarly, a family history of IBD could not be included in the regression models as none of the controls reported such a history.

Table 2 Hygiene-related exposure and other risk factors for ulcerative colitis. Results from univariate logistic regression analysis

Characteristic	Cases N=513	Controls N=188	Univariate OR (95% CI)	P value
Family history of IBD†				
No	476 (92.8)	188 (100.0)	Reference	-
Yes	37 (7.2)	0 (0.0)	Reference	-
Smoking				
Never	487 (94.9)	179 (95.2)	Reference	
Ever	26 (5.1)	9 (4.8)	1.06 (0.49-2.31)	0.88
Private bed (%)				
Never/sometimes	373 (72.7)	94 (50.0)	Reference	
Always	140 (27.3)	94 (50.0)	0.37 (0.26-0.53)	<0.001*
Use of personal towel (%)				
Never/sometimes	217 (42.5)	101 (53.7)	Reference	
Always	294 (57.5)	87 (46.3)	1.57 (1.12-2.20)	0.008*
Owning a pet				
No	394 (76.8)	168 (89.4)	Reference	
Yes	119 (23.2)	20 (10.6)	2.54 (1.53-4.21)	<0.001*
Hand-washing				
Never/Sometimes	295 (57.5)	83 (44.2)	Reference	
Always	218 (42.5)	105 (55.6)	0.58 (0.42-0.82)	0.002*
Toilet facilities				
None	98 (19.1)	18 (9.6)	Reference	
RCA	106 (20.7)	49 (26.1)	0.40 (0.22-0.73)	0.003*
Flush	309 (60.2)	121 (64.4)	0.47 (0.27-0.81)	0.006*
Water source				
Municipal	169 (33.3)	54 (29.0)	Reference	
Tube well	318 (62.6)	126 (67.7)	0.81 (0.56-1.17)	0.25
Other	21 (4.1)	6 (3.2)	1.12 (0.43-2.91)	0.74
Death in family				
No	387 (75.4)	171 (91.0)	Reference	
Yes	126 (24.6)	17 (9.0)	3.27 (1.91-5.61)	<0.001*
Illness in family				
No	484 (94.4)	180 (95.7)	Reference	
Yes	29 (5.7)	8 (4.3)	1.35 (0.61-3.00)	0.46
Number of siblings (%)				
Q1 (≤2)	178 (34.7)	49 (26.1)	Reference	
Q2 (3)	89 (17.3)	76 (40.4)	0.32 (0.21-0.50)	<0.001
Q3 (4-5)	164 (32.0)	46 (24.5)	0.98 (0.62-1.54)	0.94
Q4 (>6)	82 (16.0)	17 (9.0)	1.33 (0.72-2.45)	0.91

*P significant value

IBD, Inflammatory bowel disease; RCA, as a toilet technology used in rural areas where no flush facility exists. It was developed under RCA project

Table 1 Socio-demographic features of the study population

Characteristic	Cases N=513	Controls N=188	P value
Age at diagnosis/interview (years) (%)			
Q1 (≤28.7)	137 (26.7)	39 (20.74)	
Q2 (28.74-36.34)	129 (25.1)	47 (25.0)	
Q3 (36.5-46.8)	121 (23.6)	54 (28.7)	
Q4 (>46.9)	126 (24.6)	48 (25.5)	0.33
Median age at diagnosis/interview	35.61	38.11	
Gender (%)			
Female	233 (45.4)	50 (26.6)	
Male	280 (54.6)	138 (73.4)	<0.001
Education (%)			
None	28 (5.5)	19 (10.8)	
Primary (up to grade 5)	75 (14.8)	23 (13.1)	
Secondary (up to grade 10)	198 (39.1)	82 (46.6)	
Graduation and beyond	205 (40.5)	52 (29.6)	0.01

Table 3 Multivariate analysis of the association between hygiene-related characteristics and risk for ulcerative colitis*

Characteristic	Odds ratio	95% CI	P value
Smoking			
No	Reference		
Yes	1.27	0.54-3.0	0.58
Private bed (%)			
No	Reference		
Yes	0.25	0.16-0.39	<0.001**
Use of personal towel (%)			
No	Reference		
Yes	1.24	0.73-2.09	0.43
Owning a pet			
No	Reference		
Yes	2.02	1.14-3.59	0.01**
Hand-washing			
No	Reference		
Yes	0.64	0.39-1.07	0.09
Toilet facilities			
None	Reference		
RCA	0.29	0.14-0.60	0.001**
Flush	0.43	0.23-0.82	0.01**
Water source			
Municipal	Reference		
Tube well	1.26	0.79-1.98	0.32
Other	1.77	0.59-5.33	0.31
Death in family			
No	Reference		
Yes	2.19	1.18-4.07	0.01
Illness in family			
No	Reference		
Yes	0.88	0.36-2.2	0.80
Number of siblings (%)			
Q1 (≤2)	Reference		
Q2 (3)	0.33	0.19-0.57	<0.001**
Q3 (4-5)	0.95	0.55-1.64	0.86
Q4 (>6)	1.40	0.67-2.92	0.36

* Adjusted for other variables in the table and gender and smoking,

** P value<0.05,

Discussion

The 'IBD hygiene hypothesis' has been proposed as a possible explanation for the changing disease epidemiology worldwide. However, there is scanty data exploring this hypothesis from the Asian population. We carried out a case-control study in a North Indian population to examine the association between hygiene-related exposure and the risk for UC. In our study hygiene-related variables that are potential proxies for higher rates of infections (owning a pet, higher number of siblings) were positively associated with UC, whereas those proxies for lower rates of infections (having a private bed, hand-washing prior to meals and better toilet facilities) were inversely associated with UC. Our results indicate an increased risk for UC in people exposed to infections.

The hygiene hypothesis alludes to the potential for low hygiene or exposure to infections to confer risk for chronic immunologically mediated disease [12]. First proposed in the context of childhood asthma, it has been implicated in various other diseases, in particular, autoimmune phenomena, such as IBD. A number of epidemiological observations, in particular ecological observations such as increasing incidence of IBD concurrent with a fall in rates of infections in Caucasian populations and the historic lower incidence of IBD in developing populations where hygiene standards are comparatively lower, have mooted the hypothesis [13-15]. A number of observational studies have been carried out both among children and adults, to dissect these potential associations. The majority of these studies have been carried out in developed populations. Evidence across studies has been quite inconsistent. Many of these inconsistencies could be attributed to the retrospective nature of most studies, difficulty in measuring infection-related exposures, limited variability in exposure and/or limited power [6-10]. To overcome some of these limitations, we carried out this study in a developing population where hygiene-related exposure varies and where the incidence of UC appears to be on the rise. We also focused on measuring exposure parameters less prone to recall bias. Our findings by and large do not support the hygiene hypothesis. In contrast they suggest that poor hygiene could entail a higher risk for UC in this population.

In accordance with other studies, familial aggregation was strongly associated with risk for IBD. Both exposure to common environmental factors and higher genetic predisposition are likely to be associated with increased risk in the family [16]. We did not observe any association between smoking and UC. Numerous studies have shown an inverse association, although underlying mechanisms are unclear [17]. The reported prevalence of smoking in our population was quite low (~5.0%). This low frequency is consistent with the prevalent cultural and religious beliefs of the predominantly Sikh population that was part of the study, for whom smoking is forbidden according to the Sikh religion; low prevalence precluded the adequate examination of this association.

In addition to association with hygiene-related exposure we observed that psychosocial factors may be associated with risk for UC [18]. Towards this end we observed that a major stressful event like demise of a family member was associated with higher risk for UC. These findings are consistent with the known associations between "stressful life events" and risk for both CD and UC. The reliability of the current findings is reliant on the potential for bias in the study. Although recall bias cannot be completely ruled out, notably, all associations were noted with some hygiene-related variables, no associations were noted with other variables such as source of drinking water and use of a personal towel. These findings provide reasonable assurance that there was no generalized over or under reporting by the participants. We would like to mention that our study has some shortcomings; unequal case-control numbers and significant gender differences although the analysis was independent of gender variable. Moreover, this was a hospital-based study and true epidemiological associations cannot be ensured. However,

Summary Box**What is already known:**

- The hygiene hypothesis alludes to the potential for low hygiene or exposure to infections conferring risk for chronic immunologically mediated diseases
- In inflammatory bowel disease (IBD) a number of observational studies have been carried out both among children and adults in developed populations studying the association of hygiene hypothesis and IBD but evidence across studies has been quite inconsistent
- Very little data is available from developing countries

What the new findings are:

- Our study in a developing area does not provide definitive evidence to support the hygiene hypothesis and on the contrary suggests that the rising incidence of ulcerative colitis in North India may be attributable to inadequate sanitary measures or other as yet unidentified factors

due to very low incidence of disease this study was planned as an institutional based study.

In conclusion, our study adds to the growing literature that has been at best equivocal with regards to the role of hygiene and UC. Our study does not provide support for the hygiene hypothesis and suggests that the rising incidence of UC observed in India may be attributed to inadequate sanitary measures or other factors which are yet to be identified.

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