Crohn’s disease environmental factors in the developing world: A case-control study in a statewide catchment area in Brazil

Valéria Cristina Loureiro Salgado, Ronir Raggio Luiz, Neio Boechat, Bianca C Schorr, Isabella S Leão, Tiago Nunes, Cyrla Zaltman

Abstract

AIM
To identify environmental risk factors associated with the development of Crohn’s disease (CD) in order to re-assess the hygiene hypothesis.

METHODS
A hospital-based, case-control study was carried out with CD patients (n = 145) and controls (n = 163)
representing a socioeconomically diverse statewide catchment area in Brazil. Controls were recruited from caregivers of patients seen in different outpatient clinics at the same hospital. A multi-item survey with 94 questions regarding family history of CD, perinatal and childhood circumstances, living conditions, tobacco use and familial socioeconomic status was carried out by interviewers.

RESULTS
On the univariate analysis, predictive variables for CD included being male, under age of 40, a high education level, urban dweller, smaller family size, exposure to enteric pathogens and user of treated water ($P < 0.005$). On the multivariate analysis, variables significantly associated with CD were male gender (OR = 2.09), under age 40 (OR = 3.10), white (OR = 2.32), from a small family in childhood (OR = 2.34) and adulthood (OR = 3.02), absence of viral infections in childhood (OR = 2.23), exposure to enteric pathogens (OR = 2.41), having had an appendectomy (OR = 2.47) and prior or current smoker (OR = 2.83/1.12).

CONCLUSION
Most variables supporting the "hygiene hypothesis" are associated with the development of CD but are not independent predictors of the diagnosis.

Key words: Crohn’s disease; Environment; Hygiene hypothesis; Risk factors

© The Author(s) 2017. Published by Baishideng Publishing Group Inc. All rights reserved.

Core tip: This case-control study aimed to revisit the hygiene hypothesis in inflammatory bowel disease with the inclusion of Crohn’s disease (CD) patients and healthy controls representing a socioeconomically diverse statewide catchment area in Brazil. Subjects completed an extensive 94-item survey regarding perinatal and childhood circumstances, living conditions, smoking and familial socioeconomic status. Most variables supporting the hygiene hypothesis were associated with CD but were not independent predictors of the diagnosis. These findings suggest that, albeit there is an association, the influence that these variables might have on disease development is not as strong as other classic environmental factors (smoking) found to be closely related to disease onset and progression.

INTRODUCTION
The etiology of Crohn’s disease (CD) remains not fully understood, being probably multifactorial, due to a complex interplay between genetic susceptibility and environmental factors. In the United States and Europe, the rise in CD incidence was associated with the effects of industrialization and concomitant environmental and lifestyle changes. These findings point toward the notion that environmental factors might play an important role in CD susceptibility and prevalence in the developed regions of the globe.

In this regard, the hygiene hypothesis postulates that better hygienic conditions would reduce the incidence of infections and favor the development of immune-mediated diseases. In this hypothesis, exposure to different microbial agents could play a protective role in promoting immune system maturation by balancing pro-inflammatory Th1 response and regulatory T cell tolerance. This mechanism would provide protection against subsequent exposure to allergens and antigens and less prevalence of conditions like inflammatory bowel disease (IBD). Lack of experimental evidence, however, persists with regard to the association between the hygiene hypothesis and the increase in CD prevalence.

Incidence and prevalence of CD in Brazil vary according to geographical differences. In the Brazilian State of Rio de Janeiro, the presence of extreme income inequality and the existence of both urban and rural areas provide an interesting case study to further understand the role of environmental factors in CD development. Specifically, the hygiene hypothesis can be tested by taking into consideration these extreme geographical differences present in this relatively small state. Therefore, the objective of the present study is to assess the environmental factors that might be associated with CD development prompted by the great inter-regional differences present in this statewide single center catchment area.

MATERIALS AND METHODS
Study design and patient inclusion
This is a case-control study including CD patients and healthy individuals. Patients were recruited at the IBD outpatient clinic of the Federal University of Rio de Janeiro (UFRJ) Hospital (HUCFF), Brazil. Healthy individuals were recruited from caregivers of patients seen in different outpatient clinics at the same hospital, with no family ties to the cases. The Ethics Committee of the Institute of Public Health Studies/UFRJ approved this study. All patients and control subjects gave written informed consent before enrolment. Data was analyzed anonymously to preserve patient’s privacy.

Patient selection and data collection
All included patients fulfilled the following criteria:
active follow up at the IBD outpatient clinic, established diagnosis of CD by clinical, radiological, endoscopic and histological parameters, 18 to 80 years of age, males and females. Patients and controls with established psychiatric illness or disorders that compromise the level of awareness or understanding were excluded. The sample size was based on a convenience sample according to the number of patients diagnosed with CD recorded in the HUCFF outpatient clinic.

Structured interviews were done by three interviewers trained uniformly using a prepared interview guide aiming to avoid biased information and ensuring compliance with the protocol. The 94-item questionnaire utilized in the present study was a Brazilian Portuguese translation of a previously developed Canadian questionnaire\(^{[8]}\) with a few adaptations to the local reality. The questionnaire is mainly focused on risk factors for the development of CD as demographics aspects (sex, age, ethnicity, economic/social status, household area, family size), living conditions (housing conditions and sanitation, number of cohabitants, contact with pets and quality of water intake), smoking habits, family history (first-degree relatives), vaccinations and diseases (childhood immunizations, worms history, intestinal infections and viral diseases in childhood and appendectomy). Most of these variables were evaluated both in childhood and adulthood, before CD diagnosis. Age refers to current age at the moment the questionnaire was applied. Data was then categorized into two groups: 18 to 39 and 40 to 80 years of age.

Study subjects were racially stratified into two categories: white and non-white. Economic/social status was categorized based on the educational level and family income. Educational level was divided into three categories: elementary, high school and college degree. Family income was organized taking into consideration the household’s gross monthly income according to multiples of the minimum wage: group 1 covered monthly household income of 0 to 3 minimum wages (up to about US $285 per household per month); group 2 covered household income of 3 to 5 minimum wages (US $855 to US $1425 per month) and group 3 covered increments up to more than 5 minimum wages (above US $1425 per month).

The household area was classified as urban or rural according to the Brazilian Institute of Geography and Statistics (IBGE). Definition of urban areas included cities (municipal seats), villages (district headquarters) or isolated urban areas; the areas outside of these parameters were considered rural (Ministry of Planning, Budget and Management Brazilian Institute of Geography and Statistics - IBGE 2010 Census). The assessment of housing conditions and adequate sanitation included: the presence of garbage collection, running water, and sewage drainage. Family size was characterized according to the number of inhabitants: 1 inhabitant, 2 to 3 inhabitants, and 4 to 8 inhabitants.

Exposure to tobacco also was considered and three classes were defined: never (never consumed tobacco daily), previous (currently non-smokers/ex-smokers) or current tobacco user (current daily smokers) (Global Adult Tobacco Survey - GATS, 2a Edition. Atlanta, United States, 2011).

**Ethical statement**

The study was approved by the Ethics Committee of the University Hospital Clementino Fraga Filho of the Federal University of Rio de Janeiro, Brazil (HUCFF-UFRJ). Informed consent was obtained from all subjects prior their enrollment.

**Statistical analysis**

To verify differences between the two study groups (CD and controls), we used Pearson’s $\chi^2$. Univariate and multivariate logistic regression analyses were performed to identify variables associated with the development of CD. In the first multivariate analysis, all variables of interest were included in the model (analysis 1). A second multivariate analysis was then performed in which the model comprised only variables that (A) reached statistical significance or (B) had an OR higher than 2 at the first multivariate analysis (analysis 2). Significance level was set at $P < 0.05$. Statistical analyses were performed using Package for the Social Sciences (SPSS) for Windows version 17.0.

**RESULTS**

The study population included 308 individuals: 145 (47%) CD patients and 163 (53%) controls. Significant differences between groups were evident when analyzing variables comprising demographic characteristics, hygiene, and others environmental factors before the diagnosis as summarized in Table 1.

**Univariate analysis**

The variables associated with CD are shown in Table 2. In this analysis, risk factors such as being male, under 40 years old, high educational level, urban living, smaller family size in childhood, exposure to enteric pathogens and user of treated water were significantly associated with CD ($P < 0.05$).

**Multivariate analysis**

The logistic regression analysis was performed initially on all the variables studied (multivariate analysis 1) demonstrating that males, being under 40 years of age and white are associated with a greater likelihood to develop CD compared with controls (Table 3). Smaller families in childhood and adulthood, exposure to enteric pathogens and appendectomy prior to CD diagnosis were confirmed to be risk factors for CD development. Prior or current tobacco exposure were also identified as risk factors for developing the
In this regard, having a higher educational level or but the same might not be true for the other factors. to treated water and less infections is straightforward, at a later stage years of life and more immune-mediated conditions associated with less infectious diseases during the first years of life and more immune-mediated conditions at a later stage[5,6]. The relationship between access to treated water and less infections is straightforward, but the same might not be true for the other factors. In this regard, having a higher educational level or disease. Considering only variables reaching statistical significance or having an OR higher than 2 in the first multivariate analysis (multivariate analysis 2), a strong risk association was found for male sex, under age of 40, white, from smaller families in childhood and adulthood, absence of viral infections in childhood, exposure to enteric pathogens, appendectomy and prior and current smoking.

**DISCUSSION**

There is a slow but steady increase in CD prevalence worldwide, mainly in developing countries from Eastern Europe, Latin America and Asia, although the prevalence remains lower in comparison with Western Europe and North America[12-18]. The hygiene hypothesis has been proposed as a possible explanation for the significant increase in CD incidence in the last decades[19]. The present report assessed the hygiene hypothesis and other environmental factors in a cohort of CD patients and control subjects living in a statewide single center catchment area with great social and environmental inter-regional differences.

Considering the demographic aspects of CD, the literature presents controversial results regarding gender, although some studies have reported a slight predominance in males, which was confirmed in our study[20]. We identified a predominance of the disease in young individuals with a peak prevalence between 18 and 39 years of age, which was also comparable to previous reports[16,18,19]. The Brazilian population is characterized by a mixed race ancestry, with a genetic background originated from three main parental populations - Europeans, Brazilian Native Amerindians and Africans[21]. Interestingly, our study showed a predominance of white individuals in the CD group; this finding is in keeping with the higher incidence of IBD found in people with European ancestry.

In the univariate analysis, several important variables supporting the hygiene hypothesis were associated with CD development in the present study: access to treated water, higher educational level, smaller family size and being an urban dweller. Theoretically, the presence of these variables could be associated with less infectious diseases during the first years of life and more immune-mediated conditions at a later stage[5,6]. The relationship between access to treated water and less infections is straightforward, but the same might not be true for the other factors. In this regard, having a higher educational level or

| Table 1 Demographics and environmental factors from Crohn’s disease and control groups (n = 308) n (%) |
| --- |
| Groups CD (n = 145) | Controls (n = 163) | P value¹ |
| Sex | | | |
| Male | 62 (42.8) | 43 (26.4) | 0.002 |
| Female | 83 (57.2) | 120 (73.6) |  |
| Age (yr) | | | |
| 18-39 | 79 (54.5) | 50 (30.7) | < 0.0001 |
| 40-80 | 66 (45.5) | 113 (69.3) |  |
| Race | | | |
| White | 74 (51) | 65 (39.9) | 0.050 |
| Non-white | 71 (49) | 98 (60.1) |  |
| Educational level | | | |
| Elementary | 45 (31) | 79 (48.5) |  |
| High school | 79 (54.5) | 70 (42.9) | 0.006 |
| Graduate school | 21 (14.5) | 14 (8.6) |  |
| Family income² | | | |
| < 3 | 46 (31.7) | 54 (33.1) |  |
| 3-5 | 44 (30.3) | 55 (33.7) | 0.730 |
| > 5 | 40 (27.6) | 36 (22.1) |  |
| No information | 15 (10.3) | 18 (11) |  |
| Rural area | | | |
| No | 114 (78.6) | 106 (65) | 0.008 |
| Yes | 31 (21.4) | 57 (35) |  |
| Housing conditions | | | |
| Inadequate | 12 (8.3) | 12 (7.4) | 0.765 |
| Adequate | 133 (91.7) | 151 (92.6) |  |
| Family size in adulthood (n) | | | |
| Until 1 | 23 (15.9) | 47 (28.8) |  |
| 2-3 | 85 (58.6) | 83 (50.9) | 0.024 |
| 4-8 | 37 (25.5) | 33 (20.2) |  |
| Family size in childhood (n) | | | |
| 1-3 | 45 (31) | 35 (21.5) |  |
| 4-6 | 61 (42.1) | 56 (34.4) | 0.006 |
| > 7 | 39 (26.9) | 72 (44.2) |  |
| Pets | | | |
| No | 30 (20.7) | 21 (12.9) | 0.066 |
| Yes | 115 (79.3) | 142 (87.1) |  |
| Breastfeeding | | | |
| No | 12 (8.3) | 13 (8) |  |
| Yes | 125 (86.2) | 133 (81.6) | 0.289 |
| Unknown | 8 (5.5) | 17 (10.4) |  |
| Exposure to untreated water | | | |
| No | 43 (29.7) | 71 (43.6) | 0.012 |
| Yes | 102 (70.3) | 92 (56.4) |  |
| Vaccine (childhood) | | | |
| No | 12 (8.3) | 20 (12.3) | 0.252 |
| Yes | 133 (91.7) | 143 (87.7) |  |
| Viral diseases (childhood) | | | |
| No | 17 (11.7) | 11 (6.7) | 0.129 |
| Yes | 128 (88.3) | 152 (93.3) |  |
| Helminthic infections | | | |
| No | 45 (31) | 45 (27.6) | 0.509 |
| Yes | 100 (69) | 118 (72.4) |  |
| Exposure to enteric pathogens | | | |
| No | 67 (46.2) | 106 (65) | 0.001 |
| Yes | 78 (53.8) | 57 (35) |  |
| Previous appendectomy | | | |
| No | 133 (91.7) | 153 (93.9) | 0.466 |
| Yes | 12 (8.3) | 10 (6.1) |  |
| Tobacco exposure | | | |
| Never | 86 (59.3) | 106 (65) |  |
| Prior | 42 (29) | 33 (20.2) | 0.190 |
| Current | 17 (11.7) | 24 (14.7) |  |

¹χ² test; ²Minimum wage. CD: Crohn’s disease.
a smaller family size might function as a surrogate marker for a higher socioeconomic status, with subsequent less exposure to infectious diseases in early childhood. Likewise, being an urban dweller might lead to less exposure to livestock, decreasing the risk of parasitic infections that could modulate the immune system\cite{10,22-24}. Despite these univariate associations supporting the hygiene hypothesis in this case-control setting, only having a smaller family size maintained statistical significance in the multivariate analysis.

Even though previous reports have indicated that pet ownership during childhood could be a risk factor for infectious diseases with subsequent reduction of immune-mediated conditions, we did not observe this association\cite{10,14}. In fact, the association between having a pet during childhood and CD development is not entirely clear. In this regard, a Canadian case-control study has observed that contact with pets during childhood might have the opposite expected effect. In this study, pet ownership was associated with an increased risk to develop CD later on\cite{25}. Further studies are still needed to establish a clear association between pet ownership in early childhood and an increased risk to develop CD.

The hygiene hypothesis suggests that improved sanitation and reduced exposure to enteric organisms during childhood might lead to inappropriate immunological responses later in life and higher risk of CD\cite{26}. We observed, however, that a greater exposure to enteric pathogens was associated with a higher risk for the development of CD, a finding also confirmed by others\cite{27}. These authors have shown that the increased risk occurs mainly in the first year after the diagnosis of infection, suggesting the existence of a detection bias. Another possible explanation for these results could be an incorrect diagnosis of intestinal infection at CD onset\cite{27}. The same might explain the association between CD susceptibility and appendectomy. A significant association between previous appendectomy at diagnosis and the presence of CD was observed in this study. This finding, however, can be due to a misdiagnosis of appendicitis in initial cases of CD, especially in disease phenotypes with appendicular or ileocecal involvement.

The current study and several other reports have implicated smoking as a risk factor for the development of CD\cite{28}. The pathophysiology behind the effects of smoking on CD is not well understood, but it is hypothesized that there are influences from nicotine and the participation of increased oxidative stress in the

### Table 2 Univariate analysis of associations between demographics, environmental factors in Crohn’s disease patients

| Characteristics                          | Logistic univariate models | 95%CI | P value <sup>2</sup> |
|-----------------------------------------|---------------------------|-------|----------------------|
| Sex                                     |                           |       |                      |
| Male                                    | 2.09                      | 1.29-3.37 | 0.003               |
| Female                                  | 1.00                      |        |                      |
| Age (yr)                                |                           |       |                      |
| 18-39                                   | 2.71                      | 1.70-4.31 | < 0.001             |
| 40-80                                   | 1.00                      |        |                      |
| Race                                     |                           |       |                      |
| White                                   | 1.57                      | 1.00-2.47 | 0.050               |
| Non-white                               | 1.00                      |        |                      |
| Educational level                       |                           |       |                      |
| Elementary                              | 1.00                      |        |                      |
| High school                             | 1.98                      | 1.22-3.22 | 0.006               |
| Graduate school                         | 2.63                      | 1.21-5.68 | 0.014               |
| Family income<sup>1</sup>               |                           |       |                      |
| < 3                                     | 1.00                      |        |                      |
| 3-5                                     | 0.94                      | 0.54-1.64 | 0.826               |
| > 5                                     | 1.30                      | 0.72-2.37 | 0.384               |
| No information                          | 0.98                      | 0.44-2.15 | 0.957               |
| Rural area                              |                           |       |                      |
| No                                      | 1.98                      | 1.19-3.29 | 0.009               |
| Yes                                     | 1.00                      |        |                      |
| Housing conditions                      |                           |       |                      |
| Inadequate                              | 1.14                      | 0.49-2.61 | 0.765               |
| Adequate                                | 1.00                      |        |                      |
| Family size in adulthood (<i>n</i>)     |                           |       |                      |
| Until 1                                  | 1.00                      |        |                      |
| 2-3                                     | 2.09                      | 1.17-3.75 | 0.013               |
| 4-8                                     | 2.29                      | 1.15-4.54 | 0.018               |
| Family size in childhood (<i>n</i>)     |                           |       |                      |
| 1-3                                     | 2.37                      | 1.31-4.27 | 0.004               |
| 4-6                                     | 2.01                      | 1.18-3.42 | 0.010               |
| > 7                                     | 1.00                      |        |                      |
| Pets                                    |                           |       |                      |
| No                                      | 1.76                      | 0.96-3.24 | 0.068               |
| Yes                                     | 1.00                      |        |                      |
| Breastfeeding                           |                           |       |                      |
| No                                      | 1.00                      |        |                      |
| Yes                                     | 1.02                      | 0.45-2.31 | 0.966               |
| Unknown                                 | 0.51                      | 0.16-1.61 | 0.251               |
| Exposure to untreated water             |                           |       |                      |
| No                                      | 1.00                      |        |                      |
| Yes                                     | 1.83                      | 1.14-2.93 | 0.012               |
| Vaccine (childhood)                     |                           |       |                      |
| No                                      | 1.00                      |        |                      |
| Yes                                     | 1.55                      | 0.73-3.29 | 0.254               |
| Viral diseases (childhood)              |                           |       |                      |
| No                                      | 1.84                      | 0.83-4.06 | 0.134               |
| Yes                                     | 1.00                      |        |                      |
| Helminthic infections                   |                           |       |                      |
| No                                      | 1.18                      | 0.72-1.95 | 0.509               |
| Yes                                     | 1.00                      |        |                      |
| Exposure to enteric pathogens           |                           |       |                      |
| No                                      | 1.00                      |        |                      |
| Yes                                     | 2.16                      | 1.37-3.42 | 0.001               |
| Previous appendectomy                   |                           |       |                      |
| No                                      | 1.00                      |        |                      |
| Yes                                     | 1.38                      | 0.57-3.29 | 0.468               |
| Tobacco exposure                        |                           |       |                      |
| Never                                   | 1.00                      |        |                      |
| Prior                                   | 1.57                      | 0.92-2.60 | 0.101               |
| Current                                 | 0.87                      | 0.44-1.73 | 0.697               |

<sup>1</sup>Minimum wage, <sup>2</sup>χ<sup>2</sup> test. CD: Crohn’s disease.
### Table 3  Multiple linear regression to assess independent factors associated with the development of Crohn’s disease

| Characteristics                  | Multivariate analysis 1 | Multivariate analysis 2 |  
|----------------------------------|-------------------------|-------------------------|  
|                                  | OR<sub>a</sub>  | 95%CI | P value | OR<sub>a</sub>  | 95%CI | P value |  
| **Sex**                          |                      |                  |          |                      |                  |          |  
| Male                             | 2.08                  | 1.18-3.68        | 0.011    | 2.09                  | 1.22-3.59        | 0.007    |  
| Female                           | 1.00                  |                  |          | 1.00                  |                  |          |  
| **Age (yr)**                     |                      |                  |          |                      |                  |          |  
| 18-39                            | 2.83                  | 1.51-5.32        | <0.001   | 3.10                  | 1.71-5.63        | <0.001   |  
| 40-80                            | 1.00                  |                  |          | 1.00                  |                  |          |  
| **Race**                         |                      |                  |          |                      |                  |          |  
| White                            | 2.30                  | 1.31-4.02        | 0.004    | 2.32                  | 1.36-3.97        | 0.002    |  
| Non-white                        | 1.00                  |                  |          | 1.00                  |                  |          |  
| **Educational level**            |                      |                  |          |                      |                  |          |  
| Elementary                       | 1.00                  |                  |          | 1.00                  |                  |          |  
| High school                      | 1.44                  | 0.75-2.75        | 0.266    | 1.45                  | 0.52-4.04        | 0.470    |  
| Graduate school                  | 1.00                  |                  |          |                      |                  |          |  
| **Family income¹**               |                      |                  |          |                      |                  |          |  
| < 3                              | 1.00                  |                  |          | 1.00                  |                  |          |  
| 3-5                              | 0.83                  | 0.42-1.64        | 0.609    | 0.90                  | 0.40-2.00        | 0.807    |  
| > 5                              | 1.74                  | 0.67-4.51        | 0.254    |                      |                  |          |  
| No information                   | 1.32                  | 0.65-2.68        | 0.432    | 1.00                  |                  |          |  
| **Rural area**                   |                      |                  |          |                      |                  |          |  
| No                               | 1.36                  | 0.49-3.79        | 0.548    | 1.00                  |                  |          |  
| Yes                              | 1.00                  |                  |          | 1.00                  |                  |          |  
| **Housing conditions**           |                      |                  |          |                      |                  |          |  
| Inadequate                       | 1.45                  | 0.69-3.05        | 0.323    | 1.00                  |                  |          |  
| Adequate                         | 1.00                  |                  |          | 1.00                  |                  |          |  
| **Family size in adulthood (n)**|                      |                  |          |                      |                  |          |  
| Until 1                          | 1.00                  |                  |          | 1.00                  |                  |          |  
| 2-3                              | 3.26                  | 1.59-6.68        | 0.001    | 3.02                  | 1.52-5.99        | 0.002    |  
| 4-8                              | 3.16                  | 1.36-7.34        | 0.007    | 2.87                  | 1.26-6.50        | 0.012    |  
| **Family size in childhood (n)**|                      |                  |          |                      |                  |          |  
| 1-3                              | 1.28                  | 0.57-2.84        | 0.541    | 1.66                  | 0.81-3.40        | 0.159    |  
| 4-6                              | 2.08                  | 1.06-4.08        | 0.032    | 2.34                  | 1.27-4.32        | 0.006    |  
| > 7                              | 1.00                  |                  |          | 1.00                  |                  |          |  
| **Pets**                         |                      |                  |          |                      |                  |          |  
| No                               | 1.45                  | 0.69-3.05        | 0.323    | 1.00                  |                  |          |  
| Yes                              | 1.00                  |                  |          | 1.00                  |                  |          |  
| **Breastfeeding**                |                      |                  |          |                      |                  |          |  
| No                               | 1.00                  |                  |          | 1.00                  |                  |          |  
| Yes                              | 0.77                  | 0.28-2.09        | 0.616    | 0.48                  | 0.12-1.89        | 0.296    |  
| Unknown                          | 0.48                  | 0.12-1.89        | 0.296    |                      |                  |          |  
| **Exposure to untreated water**  |                      |                  |          |                      |                  |          |  
| No                               | 1.00                  |                  |          | 1.00                  |                  |          |  
| Yes                              | 1.54                  | 0.80-2.93        | 0.188    | 0.48                  | 0.12-1.89        | 0.296    |  
| Vaccine (childhood)              |                      |                  |          |                      |                  |          |  
| No                               | 1.00                  |                  |          | 1.00                  |                  |          |  
| Yes                              | 0.57                  | 0.20-1.58        | 0.284    | 0.48                  | 0.12-1.89        | 0.296    |  
| Viral diseases (childhood)       |                      |                  |          |                      |                  |          |  
| No                               | 2.42                  | 0.90-6.48        | 0.078    | 2.23                  | 0.88-5.62        | 0.088    |  
| Yes                              | 1.00                  |                  |          | 1.00                  |                  |          |  
| **Helmintic infections**         |                      |                  |          |                      |                  |          |  
| No                               | 0.91                  | 0.51-1.65        | 0.776    | 0.91                  | 0.51-1.65        | 0.776    |  
| Yes                              | 1.00                  |                  |          | 1.00                  |                  |          |  
| **Exposure to enteric pathogens**|                      |                  |          |                      |                  |          |  
| No                               | 1.00                  |                  |          | 1.00                  |                  |          |  
| Yes                              | 2.89                  | 1.62-5.17        | <0.001   | 2.23                  | 1.41-4.10        | 0.001    |  
| **Previous appendectomy**        |                      |                  |          |                      |                  |          |  
| No                               | 1.00                  |                  |          | 1.00                  |                  |          |  
| Yes                              | 2.71                  | 0.93-7.83        | 0.065    | 2.47                  | 0.89-6.83        | 0.080    |  
| **Tobacco exposure**             |                      |                  |          |                      |                  |          |  
| Never                            | 1.00                  |                  |          | 1.00                  |                  |          |  
| Prior                            | 3.13                  | 1.57-6.26        | 0.001    | 2.83                  | 1.46-5.47        | 0.002    |  
| Current                          | 1.13                  | 0.50-2.54        | 0.768    | 1.12                  | 0.51-2.44        | 0.775    |  
| **Family history**               |                      |                  |          |                      |                  |          |  
| No                               | 1.00                  |                  |          | 1.00                  |                  |          |  
| Yes                              | 1.27                  | 0.45-3.53        | 0.644    |                      |                  |          |  

¹OR<sub>a</sub> = Odd Ratio adjusted. In the final model only the variables included were statistically significant (95%CI not including value 1). P value Hosmer-Lemeshow statistic for setting the final model = 0.406.
intestinal mucosa\textsuperscript{29}. Smoking cessation and smoking prevention might positively influence the development and evolution of CD\textsuperscript{30}. Interestingly, the studies on smoking are not consistent across all ethnic groups, demonstrating the potential for interactions between smoking and others environmental or genetic factors to influence disease occurrence, course or phenotype\textsuperscript{28}.

Importantly, interpretation of the present results should be performed with the perspective that there are some methodological limitations, including the possibility of reporting bias, which are common in case-controlled studies. Nonetheless, information obtained through structured questionnaires collected by graduate students, who were trained in a uniform protocol, tend to minimize this possibility. It was not possible, however, to control the recall bias, as some variables addressed issues related to early life.

Overall, in this case-control study, several factors associated with the development of CD in the univariate analysis (high education level, urban dweller, smaller family size, user of treated water) support the role of the hygiene hypothesis in the pathogenesis of CD. However, more importantly, most variables were not found to be independent predictors for the development of CD. This suggests that, albeit there is an association, the influence that these variables might have on disease development is not as strong as other classic environmental factors (smoking) found to be closely related to disease onset and progression.

**COMMENTS**

**Background**

Developing countries currently face an increase in the incidence of Crohn’s disease (CD) in parallel with greater urbanization. Brazil, therefore, represents a good case study to revisit the hygiene hypothesis.

**Research frontiers**

The hygiene hypothesis and the contribution of each environmental factor to disease development and progression need further investigation. A better understanding of the environmental factors associated with CD can potentially have preventive or therapeutic implications.

**Innovations and breakthroughs**

Most case-control studies looking at environmental factors of CD took place in developed countries with a less diverse population regarding its socio-economic status and hygienic conditions. The present study used a socioeconomically diverse Brazilian population to adequately assess the influence of key factors related to the hygiene hypothesis on disease development.

**Applications**

These results highlight the importance of key environmental factors associated with the hygiene hypothesis but also suggest that these factors are not disease predictors, being very unlikely that they have an independent causative role.

**Terminology**

The hygiene hypothesis postulates that better hygienic conditions would reduce the incidence of infections and favor the development of immune-mediated diseases. In this hypothesis, exposure to different microbial agents could play a protective role in promoting immune system maturation.

**Peer-review**

This manuscript offers new evidence to support the hygiene hypothesis, an interesting topic which has drawn increasing attention in recent years considering this potential role in the development of CD. This case-control study was well carried out, especially the well-designed questionnaire containing most of interested environmental factors, making the data analysis reliable and convincing. The relationship between several important variables supporting the hygiene hypothesis and CD development has been clearly demonstrated in the discussion section, even taking into consideration the extreme geographical differences in Brazil. Meanwhile, the structure of this manuscript is complete and the language is perfect.

**REFERENCES**

1. Strober W, Fuss I, Mannon P. The fundamental basis of inflammatory bowel disease. J Clin Invest 2007; 117: 514-521 [PMID: 17332878 DOI: 10.1172/JCI30587]
2. Chamberlin WM, Nauer SA. Integrating theories of the etiology of Crohn’s disease. On the etiology of Crohn’s disease: questioning the hypotheses. Med Sci Monit 2006; 12: RA27-RA33 [PMID: 16449960]
3. Shanahan F. Crohn’s disease. Lancet 2002; 359: 62-69 [PMID: 11809204 DOI: 10.1016/S0140-6736(02)07284-7]
4. Ko Y, Butcher R, Leong RW. Epidemiological studies of migration and environmental risk factors in the inflammatory bowel diseases. World J Gastroenterol 2014; 20: 1238-1247 [PMID: 24574798 DOI: 10.3748/wjg.v20.i5.1238]
5. Ruyssers NE, De Winter BY, De Man JG, Loukas A, Herman AG, Pelckmans PA, Moreels TG. Worms and the treatment of inflammatory bowel disease: are molecules the answer? Clin Dev Immunol 2008; 2008: 567314 [PMID: 18509490 DOI: 10.1155/2008/567314]
6. Lakatos PL. Environmental factors affecting inflammatory bowel disease: have we made progress? Dig Dis 2009; 27: 215-225 [PMID: 19786744 DOI: 10.1159/000228553]
7. García Rodríguez LA, González-Pérez A, Johansson S, Wallander MA. Risk factors for inflammatory bowel disease in the general population. Aliment Pharmacol Ther 2005; 22: 309-315 [PMID: 16097997 DOI: 10.1111/j.1365-2036.2005.02564.x]
8. Bernstein CN, Rawsthorne P, Cheang M, Blanchard JF. A population-based case control study of potential risk factors for IBD. Am J Gastroenterol 2006; 101: 993-1002 [PMID: 16696783 DOI: 10.1111/j.1572-0241.2006.00381.x]
9. Lashner BA, Lofus EV Jr. True or false? The hygiene hypothesis for Crohn’s disease. Am J Gastroenterol 2006; 101: 1003-1004 [PMID: 16696784 DOI: 10.1111/j.1572-0241.2006.00563.x]
10. Koloski NA, Brett L, Radford-Smith G. Hygiene hypothesis in inflammatory bowel disease: a critical review of the literature. World J Gastroenterol 2008; 14: 165-173 [PMID: 18186549 DOI: 10.3748/wjg.v14.i165]
11. Zaltman C. Inflammatory bowel disease: how relevant for Brazil? Cad Saude Publica 2007; 23: 992-993 [PMID: 17486222 DOI: 10.1590/S0102-311X2007000500001]
12. Molodecky NA, Kaplan GG. Environmental risk factors for inflammatory bowel disease. Gastroenterol Hepatol (N Y) 2010; 6: 339-346 [PMID: 20567592]
13. Ponder A, Long MD. A clinical review of recent findings in the epidemiology of inflammatory bowel disease. Clin Epidemiol 2013; 5: 237-247 [PMID: 23922566]
14. Ananthakrishnan AN. Epidemiology and risk factors for IBD. Nat Rev Gastroenterol Hepatol 2015; 12: 205-217 [PMID: 25732745 DOI: 10.1038/nrgastro.2015.34]
15. Victoria CR, Sassaik LY, Nunes HR. Incidence and prevalence rates of inflammatory bowel diseases, in midwestern of São Paulo State, Brazil. Arq Gastroenterol 2009; 46: 20-25 [PMID: 19466305 DOI: 10.1590/S0004-28032009000100009]
16. Parente JM, Coy CS, Campelo V, Parente MP, Costa LA, da Silva RM, Stephan C, Záitune JM. Inflammatory bowel disease
in an underdeveloped region of Northeastern Brazil. World J Gastroenterol 2015; 21: 1197-1206 [PMID: 2556193 DOI: 10.3748/wjg.v21.i4.1197]

Farrukh A, Mayberry JF. Inflammatory bowel disease in Hispanic communities: a concerted South American approach could identify the aetiology of Crohn’s disease and ulcerative colitis. Arg Gastroenterol 2014; 51: 271-275 [PMID: 2559153 DOI: 10.1590/ S0004-28032014000400002]

Guo AY, Stevens BW, Wilson RG, Russell CN, Cohen MA, Sturgeon HC, Thornton A, Giallourakis C, Khalili H, Nguyen DD, Saik J, Yajnik V, Xavier RJ, Ananthakrishnan AN. Early life environment and natural history of inflammatory bowel diseases. BMC Gastroenterol 2014; 14: 216 [PMID: 25510175 DOI: 10.1186/s12876-014-0216-8]

Castiglione F, Diaferia M, Morace F, Labianca O, Meucci C, Cuomo A, Panarese A, Romano M, Sorrentini I, D’Onofrio C, Caporaso N, Rispo A. Risk factors for inflammatory bowel diseases according to the “hygiene hypothesis”: a case-control, multi-centre, prospective study in Southern Italy. J Crohns Colitis 2012; 6: 324-329 [PMID: 22405169 DOI: 10.1016/j.crohns.2011.09.003]

Zeng Z, Zhu Z, Yang Y, Ruan W, Peng X, Su Y, Peng L, Chen M, Hu P. Incidence and clinical characteristics of inflammatory bowel disease in a developed region of Guangdong Province, China: a prospective population-based study. J Gastroenterol Hepatol 2013; 28: 1148-1153 [PMID: 23432198 DOI: 10.1111/j.1440-1746.2013.07070.x]

Lins TC, Vieira RG, Abreu BS, Grattapaglia D, Pereira RW. Genetic composition of Brazilian population samples based on a set of twenty-eight ancestry informative SNPs. Am J Hum Biol 2010; 22: 187-192 [PMID: 19639555]

Weinstock J, Summers R, Elliott DE. Helminths and harmony. Gut 2004; 53: 7-9 [PMID: 14684567 DOI: 10.1136/gut.53.1.7]

Frohlich A, Delamere L, Barlow LA, Panaccione R, Gutsch S, Fedorak RN, Madsen K, Kaplan GG; Alberta IBD Consortium. Environment and the inflammatory bowel diseases. Can J Gastroenterol 2013; 27: e18-e24 [PMID: 23516681 DOI: 10.1155/2013/102859]

Berg AM, Dam AN, Farraye FA. Environmental influences on the onset and clinical course of Crohn’s disease-part 2: infections and medication use. Gastroenterol Hepatol (N Y) 2013; 9: 803-810 [PMID: 24772046]

Amre DK, Lambrette P, Law L, Krupoves A, Chotard V, Costea F, Griman G, Israel D, Mack D, Seidman EG. Investigating the hygiene hypothesis as a risk factor in pediatric onset Crohn’s disease: a case-control study. Am J Gastroenterol 2006; 101: 1005-1011 [PMID: 16573775 DOI: 10.1111/j.1572-0241.2006.00526.x]

Porter CK, Tribble DR, Aliaga PA, Halvorson HA, Riddle MS. Infectious gastroenteritis and risk of developing inflammatory bowel disease. Gastroenterology 2008; 135: 781-786 [PMID: 18640117 DOI: 10.1053/j.gastro.2008.05.081]

Jakobsen C, Paerregaard A, Munkholm P, Wewer V. Environmental factors and risk of developing paediatric inflammatory bowel disease -- a population based study 2007-2009. J Crohns Colitis 2013; 7: 79-88 [PMID: 22748696 DOI: 10.1016/j.crohns.2012.05.024]

To N, Gracie DJ, Ford AC. Systematic review with meta-analysis: the adverse effects of tobacco smoking on the natural history of Crohn’s disease. Aliment Pharmacol Ther 2016; 43: 549-561 [PMID: 26749371 DOI: 10.1111/apt.13511]

Parkes GC, Whelan K, Lindsay JO. Smoking in inflammatory bowel disease: impact on disease course and insights into the aetiology of its effect. J Crohns Colitis 2014; 8: 717-725 [PMID: 24636140 DOI: 10.1016/j.crohns.2014.02.002]

Nunes T, Etchevers MJ, Garcia-Sánchez V, Girard D, Martí E, Barreiro-de Acosta M, Gomollón F, Arroyo M, Bastida G, Gonzalez B, Monfort D, Garcia-Planella E, Figueroa C, Panés J, Sans M. Impact of Smoking Cessation on the Clinical Course of Crohn’s Disease Under Current Therapeutic Algorithms: A Multicenter Prospective Study. Am J Gastroenterol 2016; 111: 411-419 [PMID: 26856753 DOI: 10.1038/ajg.2015.401]

P- Reviewer: Garcia-Olmo D, Zhu WM S- Editor: Qi Y L- Editor: A E- Editor: Zhang FF
