Case Control Study

Risk factors associated with inflammatory bowel disease: A multicenter case-control study in Brazil

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BACKGROUND

The etiology of inflammatory bowel disease (IBD) is unknown, but it is believed to be multifactorial. The hygiene hypothesis proposes that better hygiene conditions would lead to less infectious disease during childhood and favor the development of immune-mediated diseases.

AIM

To test the hygiene hypothesis in IBD by assessing the environmental risk factors associated with IBD development in different regions of Brazil with diverse socioeconomic development indices.

METHODS

A multicenter case-control study was carried out with 548 Crohn’s disease (CD) and 492 ulcerative colitis (UC) outpatients and 416 healthy controls, from six IBD centers within different Brazilian states at diverse socioeconomic development stages. A semi-structured questionnaire with 87 socioeconomic and environmental questions was applied. Logistic regression model was created to assess the odds ratio (OR) with P value and 95% confidence intervals (CI).

RESULTS

Predictive variables for both diseases (CD and UC) were women [odd ratios (OR) = 1.31; OR = 1.69], low monthly family income (OR = 1.78; OR = 1.57), lower number of cohabitants (OR = 1.70; OR = 1.60), absence of vaccination (OR = 3.11; OR = 2.51), previous history of bowel infections (OR = 1.78; OR = 1.49), and family history of IBD (OR = 5.26; OR = 3.33). Associated risk factors for CD were age (18-39 years) (OR = 1.73), higher educational level (OR = 2.22), absence of infectious childhood diseases (OR = 1.99). The UC predictive variables were living in an urban area (OR = 1.62), inadequate living conditions (OR = 1.48) and former smokers (OR = 3.36). Appendectomy was a risk factor for CD (OR = 1.58) with inverse association with UC (OR = 4.79). Consumption of treated and untreated water was associated with risk of CD (OR = 1.38) and UC (OR = 1.53), respectively.

CONCLUSION

This is the first examining environmental exposures as risk factors for inflammatory bowel disease in Brazil. Most of the variables associated with disease risk support the role of the hygiene hypothesis in IBD development.

Key words: Crohn’s disease; Ulcerative colitis; Risk factors; Environmental factors; Hygiene hypothesis

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INFORMATION

Inflammatory bowel disease (IBD) encompasses ulcerative colitis (UC) and Crohn’s disease (CD), which are recurrent immune-mediated diseases characterized by a chronic inflammatory process that involves the gastrointestinal tract[1,2]. The etiology of IBD is unknown, but it is believed to be multifactorial. Many theories have been put forward regarding IBD development; these have emphasized interactions between genetic susceptibility (genome), individual immunological factors (immunome), gut microbiota (microbiome), and environmental exposure (exposome), starting at the time of intrauterine life and going through childhood to adulthood and acting as likely triggers for the disease[3-7].

IBD was initially recognized in Europe during the industrial revolution and today has substantially higher incidence and prevalence in developed countries[8]. Although Latin America, and especially Brazil, is considered to be a region of low prevalence and incidence, its incidence has increased over the past few years[9,10]. In Brazil, an incidence increase from 0.68 cases of CD per 100000 inhabitants in 1995 to 5.5 cases in 2015[11] has been reported[12]. Regarding prevalence, Brazilian studies such as the one conducted by Parente et al[3] in 2015 found a prevalence of 12.8 cases per 100000 inhabitants in the northeastern region, while Lima Martins et al[13] reported a prevalence of 38.2 cases per 100000 inhabitants in southeastern Brazil. Until the present study, no Brazilian epidemiological study encompassing the entire national territory had been undertaken[14].

It has been proposed in the hygiene hypothesis that better hygiene conditions would cause less infectious disease during childhood and favor the development of immune-mediated diseases[15]. The hygiene hypothesis could be explained by the impact of the presence or absence of epitope exposure to humans in a critical phase of immune system maturation, having long-lasting effects on immunity regulation. The incidence and prevalence rates of IBD might vary between countries. This can be explained by the interplay between different gene pools and distinct environmental factor exposure[11]. Good hygiene and basic sanitation conditions, high degree of industrial development, and absence of population agglomerations have been considered risk factors for the development of IBD[16].

Brazil is a country with continental dimensions comprising an ethnically diverse population living in different regions with extreme socioeconomic differences. The country is the perfect setting to test the hygiene hypothesis in IBD development. Thus, the aim of this study was to identify IBD environmental risk factors across different geographical regions in Brazil and evaluate if the hygiene hypothesis might explain interregional differences in prevalence/incidence.

MATERIALS AND METHODS

Study design and inclusion of patients

This was a multicenter case-control study with inclusion of IBD patients from 6 outpatient clinics in different Brazilian states: Federal University of Rio de Janeiro Hospital (Rio de Janeiro), Irmandade Santa Casa da Misericórdia of São Paulo (São Paulo) and Federal University of Juiz de Fora (Minas Gerais) representing the Southeastern region; Federal University of Brasília (Distrito Federal) representing the Central-western region; Federal University of Piauí (Piauí) and Roberto Santos General Hospital (Bahia) representing the Northeastern region. Patients enrolled in the study had an established diagnosis of IBD confirmed by standard clinical, endoscopic, radiologic and histologic criteria[17,18]. Subjects were between 18-80 years of age, either sex. All patients were included in the study between May 2015 and June 2017.

The control group consisted of individuals who were accompanying the patients upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/
seen at the various outpatient clinics of these hospitals, who were healthy and did not have any kinship with the cases. The diagnosis of psychiatric diseases or disorders that would compromise the level of consciousness or comprehension was considered to be exclusion criteria in both groups. The sample size was determined as a convenience sample, according to the number of cases registered in these outpatient units.

**Data collection and definitions**

The semi-structured questionnaire was a translated and modified version of a Canadian questionnaire[13]. It consisted of 87 questions regarding a priori defined risk factors for the development of IBD, such as demographic and socioeconomic characteristics (age at enrolment, age at diagnosis, sex, ethnicity, migration, educational level, monthly income, living conditions, number of cohabitants, and rural or urban origin) and exposure variables (living with domestic animals, breast-feeding for at least 6 mo, consumption of treated water (filtered or boiled), vaccination (complete childhood vaccination card), contact with infectious and parasitic diseases (through laboratory tests), appendectomy, smoking, and family history of IBD with first degree relatives. Some of the variables evaluated referred to both childhood and adulthood before the diagnosis of IBD. Age at enrollment and age at diagnosis (self-reported by the cases) was stratified as follows: 18–39 years (< 40 years) and 40–80 years (> 40 years). The individuals included were divided according to ethnicity into white and non-white. Individuals were considered to be migrants when they moved from other states or country and as non-migrants when they were originated and lived all the time in the state where they were included in the study. In the evaluation of socioeconomic level, the educational level (elementary school, high school, higher education) and monthly family income (up to 3 minimum wages, 3 to 5 minimum wages, more than 5 minimum wages; using the amounts that were currently applicable at the time of the interview) were considered. Living conditions at childhood were defined as adequate when the home was constructed using bricks, had sewage collection, and piped water supply systems; otherwise, the residence was defined as inadequate. The number of cohabitants was evaluated at two times: During the interview (up to 1 cohabitant, 2 to 3, 4 to 8) and during childhood (1 to 3 cohabitants, 4 to 6, 7 or more). According to the 2010 census of the Brazilian Institute for Geography and Statistics[16], living in an urban area refers to state capitals, smaller towns, and more isolated urban areas. If these parameters were not met, the home in childhood was considered to be in a rural area. Exposure to tobacco was characterized into three subgroups: Current smokers (individuals who had been smoking more than 1 cigarette/day for at least 6 mo before the diagnosis), former smokers (individuals who had stopped smoking more than 6 mo before the diagnosis), and non-smokers (those who had never smoked) (World Health Organization Tobacco, United States, 2012)[17]. Appendectomy was taken into account when it had been done before the diagnosis of IBD.

**Ethical aspects**

Free and informed consent was obtained from each participating individual, and all data were analyzed anonymously; thus, preserving the participants’ privacy. The study was approved by the Research Ethics Committee of each participating center and was conducted in accordance with the Declaration of Helsinki and Ordinance No. 196/96 of the Brazilian National Health Council.

**Statistical analysis**

The study design was a case control study that compared cases (CD and UC separately) versus non-IBD control population. Univariate analyses using multinomial logistic regression were employed to assess the association of environmental risk factors with CD and UC and controls. The covariates assessed in univariate analyses included state of residence, age at diagnosis, sex, ethnicity, migration, educational level, family income, living conditions in childhood, cohabitants at enrollment and in childhood, rurality, exposure to domestic animals in childhood, breastfeeding, consumption of treated water at enrollment and in childhood, vaccination, infection during childhood, history of worm disease, previous bowel infection, appendectomy, and smoking status at diagnosis. All variables that met statistical significance in the univariate analyses ($P < 0.05$) were included in the multiple multinomial logistic regression and were expressed as an odds ratio (OR) and $P$ value < 0.05. All variables without statistical significance were excluded in the multiple multinomial logistic regression and the results were expressed as an odds ratio (OR) with 95% confidence interval (CI). The data analysis was conducted using the SPSS software, version 21.
Study was conducted in accordance with the Strengthening of Reporting of Observational Studies in Epidemiology statement[18].

RESULTS

Characteristics of the study population
The study population included 1,456 individuals: 548 with CD (37%), 492 with UC (34%), and 416 controls (29%). Table 1 shows the frequencies of the CD, UC, and control groups in the different states along with the associations shown by the cases (CD and UC) and controls in relation to the various variables analyzed.

Multinomial logistic regression
In univariate multinomial analysis there was a higher predominance of CD in the southeastern region, as observed in the states of Minas Gerais (odd ratios (OR) = 3.33; \( P < 0.001 \)), São Paulo (OR = 2.99; \( P < 0.001 \)) and Rio de Janeiro (OR = 2.18; \( P < 0.001 \)) in comparison to the Federal District, the only reference center of the central-western region. The results from multiple multinomial analysis showed that there was a predominance of CD in southeastern Minas Gerais (OR = 2.61; \( P \) value 0.001) and São Paulo (OR = 2.41; \( P \) value 0.002). In univariate and multiple multinomial analysis, IBD family history was associated with a higher risk for CD (OR = 5.00; \( P \) value < 0.001/OR = 3.03; \( P < 0.001 \) and UC (OR = 5.26; \( P < 0.001 \)/OR = 3.33; \( P < 0.001 \). There was an inverse association for appendectomy, where presence of appendectomy was considered to be a risk factor for CD (OR = 1.78; \( P \) value 0.025) and a protective factor in relation to UC (OR = 3.54; \( P \) value 0.002). The protective factor in UC was confirmed in the multiple multinomial analysis (OR = 4.89; \( P < 0.001 \)) (Table 2 and 3).

Final multiple multinomial analysis
After excluding the variables of ethnicity, migration, number of cohabitants during childhood, exposure to domestic animals, breast-feeding, and history of worm diseases, which were not statistically significant (\( P > 0.05 \)) in the multiple multinomial model, some important associations were detected (Table 4). A higher risk for CD was observed in Minas Gerais (OR = 2.68; 95%CI: 1.56-4.58), São Paulo (OR = 2.52; 95%CI: 1.48-4.28) and Rio de Janeiro (OR = 1.86; 95%CI: 1.11-3.07); thus, showing higher risk of CD in the southeastern region (higher socioeconomic status) compared with the other regions analyzed.

Considering the sociodemographic characteristics, patients with CD were predominantly women, (OR = 1.31; 95%CI: 0.56-1.00) and with age at enrollment below 40 years (OR = 1.73; 95%CI: 1.25-2.39). Socioeconomic risk factors were low monthly family income (OR = 1.78; 95%CI: 0.54-1.55) and higher education (OR = 2.22; 95%CI: 0.27-0.73). In UC, there was predominance of female sex (OR = 1.69; 95%CI: 0.44-0.78) and in socioeconomic aspects there was an association with low monthly family income (OR = 1.57; 95%CI: 0.45-1.35).

Regarding the environmental exposure variables for CD, 2–3 cohabitants in adulthood (OR = 1.70; 95%CI: 1.22-2.35), absence of vaccination during childhood (OR = 3.11; 95%CI: 1.82-5.29), absence of infectious childhood diseases (OR = 1.99; 95%CI: 1.20-3.26), consumption of treated water (OR = 1.38; 95%CI: 0.51-0.98) and history of bowel infections (OR = 1.78; 95%CI: 0.42-0.73) were risk factors for disease development. For UC, inadequate living conditions (OR = 1.48; 95%CI: 1.01-2.16), 2–3 cohabitants in adulthood (OR = 1.60; 95%CI: 1.15-2.21), living in an urban area (OR = 1.62; 95%CI: 1.16-2.25), consumption of untreated water (OR = 1.53; 95%CI: 1.11-2.11), absence of vaccination during childhood (OR = 2.51; 95%CI: 1.49-4.21), and history of bowel infections (OR = 1.49; 95%CI: 0.50-0.89) were risk factors for developing UC. Regarding exposure to tobacco, an association of risk between former smokers and CD could be observed (OR = 1.81; 95%CI: 1.06-3.05), but with considerably more significant risk when associated with UC (OR = 3.36; 95%CI: 1.91-5.90). Although not statistically significant, this analysis indicated an association between the risk of development of CD and appendectomy (OR = 1.58; 95%CI: 0.36-1.09) and higher risk of UC when individuals had not undergone appendectomy (OR = 4.79; 95%CI: 2.05-11.11). Family history of IBD showed a significant association with CD (OR = 5.26; 95%CI: 0.20-1.51) and UC (OR = 3.33; 95%CI: 0.14-1.23).
| Characteristics                        | CD (n = 548) | UC (n = 492) | Controls (n = 416) |
|---------------------------------------|-------------|-------------|-------------------|
|                                       | n           | %           | n               | %       |
|                                       | n           | %           | n               | %       |
|                                       | n           | %           | n               | %       |
| State of Brazil                       |             |             |                  |         |
| Rio de Janeiro                        | 145         | 26.5        | 100             | 20.3    |
| São Paulo                             | 102         | 18.6        | 78              | 15.9    |
| Minas Gerais                          | 98          | 7.9         | 57              | 11.6    |
| Bahia                                 | 59          | 0.8         | 69              | 14.0    |
| Piauí                                 | 82          | 5.0         | 92              | 18.7    |
| Federal District                      | 62          | 1.3         | 96              | 19.5    |
| Age at enrollment (yr)                |             |             |                  |         |
| < 40                                  | 267         | 8.7         | 159             | 32.3    |
| > 40                                  | 281         | 1.3         | 333             | 67.7    |
| Age at Diagnosis (yr)                 |             |             |                  |         |
| < 40                                  | 462         | 4.3         | 299             | 60.8    |
| > 0                                   | 86          | 5.7         | 193             | 39.2    |
| Sex                                   |             |             |                  |         |
| Male                                  | 227         | 1.4         | 168             | 34.1    |
| Female                                | 321         | 8.6         | 324             | 65.9    |
| Ethnicity                             |             |             |                  |         |
| White                                 | 263         | 8.0         | 189             | 38.4    |
| Non-white                             | 285         | 2.0         | 303             | 61.6    |
| Migration                             |             |             |                  |         |
| No                                    | 348         | 3.5         | 297             | 60.4    |
| Yes                                   | 200         | 6.5         | 195             | 39.6    |
| Educational level                     |             |             |                  |         |
| Elementary school                     | 182         | 3.2         | 228             | 46.3    |
| High school                           | 264         | 8.2         | 202             | 41.1    |
| Higher education                      | 102         | 8.6         | 62              | 12.6    |
| Monthly family income (minimum wages) |             |             |                  |         |
| Up to 3                               | 244         | 4.5         | 253             | 51.4    |
| 3 to 5                                | 135         | 4.6         | 113             | 23.0    |
| 5 or more                             | 121         | 2.1         | 89              | 18.1    |
| Not stated                             | 48          | 8.8         | 37              | 7.5     |
| Living condition                      |             |             |                  |         |
| Inadequate                            | 336         | 61.3        | 359             | 73.0    |
| Adequate                              | 212         | 8.7         | 133             | 27.0    |
| Cohabitants (n)                        |             |             |                  |         |
| Up to 1                               | 116         | 1.2         | 110             | 22.4    |
| 2 to 3                                | 299         | 4.6         | 252             | 51.2    |
| 4 to 8                                | 133         | 4.3         | 130             | 26.4    |
| Rural living                          |             |             |                  |         |
| No                                    | 356         | 5.0         | 266             | 54.1    |
| Yes                                   | 192         | 5.0         | 226             | 45.9    |
| Exposure to domestic animal            |             |             |                  |         |
| No                                    | 75          | 3.7         | 64              | 13.0    |
| Yes                                   | 473         | 86.3        | 428             | 87.0    |
| Breastfeeding (for at least 6 mo)     |             |             |                  |         |
| No                                    | 42          | 7.7         | 21              | 4.3     |
| Yes                                   | 472         | 86.1        | 421             | 85.6    |
| Consumption of treated water          |             |             |                  |         |
| No                                    | 223         | 40.7        | 316             | 64.2    |
| Yes                                   | 325         | 59.3        | 176             | 35.8    |
| Vaccination | No | 62 | 11.3 | 71 | 14.4 | 26 | 6.3 |
|-------------|----|----|------|----|------|----|-----|
|             | Yes| 486| 88.7 | 421| 85.6 | 390| 93.8|
| Infectious diseases | No | 65 | 11.9 | 41 | 8.3 | 29 | 7.0 |
|             | Yes| 483| 88.1 | 451| 91.7 | 387| 93.0|
| Worm disease | No | 193| 35.2 | 141| 28.7 | 140| 33.7|
|             | Yes| 355| 64.8 | 351| 71.3 | 276| 66.3|
| Bowel infection | No | 225| 41.1 | 218 | 44.3 | 229| 55.0|
|             | Yes| 323| 58.9 | 274| 55.7 | 187| 45.0|
| Appendectomy | No | 496| 90.5 | 484| 98.4 | 393| 94.5|
|             | Yes| 52 | 9.5 | 8 | 1.6 | 23| 5.5 |
| Exposure to tobacco at diagnosis | Never | 353| 64.4 | 273| 55.5 | 280| 67.3|
|             | Former| 145| 26.5 | 191| 38.8 | 91| 21.9|
|             | Current| 50 | 9.1 | 28 | 5.7 | 45| 0.8 |
| Familial IBD | No | 438| 79.9 | 430| 87.4 | 393| 94.5|
|             | Yes| 80 | 14.6 | 46 | 9.3 | 14| 3.4 |
|             | Not stated| 30 | 5.5 | 16 | 3.3 | 9| 2.2 |

1Childhood.
2Adulthood. IBD: Inflammatory bowel disease; CD: Crohn’s disease; UC: Ulcerative colitis.

**DISCUSSION**

In Brazil, studies on the incidence and prevalence of inflammatory bowel diseases have been regional, and studies investigating risk factors involved in the development of IBD are lacking. Historically, the incidence and prevalence of IBD in Brazil have been low as compared with Western countries in Europe, North America, and Australia. However, at the turn of the 21st century, the incidence and prevalence of IBD in Brazil have steadily risen; paralleling the incidence of IBD in the West during the second half of the 20th century. Our study indicates that environmental IBD risk factors may be driving the rising incidence of IBD in Brazil, some of which share similarities to IBD in the West, whereas others are clearly different. More importantly, the results confirm that the hygiene hypothesis might explain key differences in IBD epidemiology among states with diverse socioeconomic statuses.

This was the first multicenter study in Brazil and Latin America that evaluated potential risk factors associated with IBD development in geographical regions that present very different population and socioeconomic characteristics. For example, the population in the northeastern region presents the worst socioeconomic conditions, with low human socioeconomic development indexes (HDI) compared with the southeastern and central-western regions. Piauí, a state in northeastern Brazil, presents the lowest HDI (0.646 in 2010), in comparison with the southeastern states (HDI 0.783) and central-western states (HDI 0.824). The distribution of IBD in Brazil was heterogeneous with predominance of CD in the southeastern region (Minas Gerais, São Paulo and Rio de Janeiro), compared with the northeastern states (Piauí and Bahia), and a central-western state (Federal District). Brazil is a continental country, so this can be correlated with important climatic, sociocultural, and economic variations among different geographical regions.

In the West, sex predominance varies by age at diagnosis such that males have a higher risk of CD during childhood; however, in adolescence and adulthood women are more likely to be diagnosed with CD than men. In contrast, the incidence of UC does not differ between men or women until after the age of 45 whereas men have had a higher incidence of UC than women. In Brazil, female sex was a risk factor both in CD and in UC in the present study. These data are contrary to Chinese and Hungarian studies yet consistent with Latin American studies and other few Brazilian regional studies. Brazil is an ethnically diverse country, which makes it a unique country to study race within Latin America and its impact on disease. For example, the Northeastern region is a geographical area where African-descendant individuals...
Table 2: Significant variables obtained from the comparative univariate and multiple multinomial logistic analysis between Crohn’s disease and ulcerative colitis, with healthy control as reference

| Characteristics               | Univariate multinomial logistic model | Multiple multinomial logistic model |
|-------------------------------|--------------------------------------|-----------------------------------|
|                               | CD          | UC          | CD          | UC          |
|                               | OR          | P value     | OR          | P value     |
| State of Brazil               |             |             |             |             |
| Rio de Janeiro                | 2.18        | < 0.001     | 0.97        | 0.881       |
| São Paulo                     | 2.99        | < 0.001     | 1.48        | 0.107       |
| Min Minas Gerais              | 3.33        | < 0.001     | 1.25        | 0.388       |
| Bahia                         | 1.00        | 0.994       | 0.76        | 0.215       |
| Piauí                         | 1.15        | 0.539       | 0.83        | 0.388       |
| Age at enrollment (yr)        |             |             |             |             |
| < 40                          | 1.49        | 0.005       | 0.75        | 0.038       |
| > 40                          | 1           | 1           | 1           | 1           |
| Sex                           |             |             |             |             |
| Male                          | 0.71        | 0.006       | 0.52        | < 0.001     |
| Female                        | 1           | 1           | 1           | 1           |
| Ethnicity                     |             |             |             |             |
| White                         | 1.76        | < 0.001     | 1.19        | 0.208       |
| Non-white                     | 1           | 1           | 1           | 1           |
| Educational level             |             |             |             |             |
| Elementary school             | 0.57        | 0.004       | 1.16        | 0.471       |
| High school                   | 0.79        | 0.225       | 1.00        | 0.989       |
| Higher education              | 1           | 1           | 1           | 1           |
| Monthly family income         |             |             |             |             |
| (minimum wages)               |             |             |             |             |
| Up to 3                       | 1.19        | 0.304       | 1.68        | 0.004       |
| 3 to 5                        | 1.03        | 0.884       | 1.17        | 0.435       |
| 5 or more                     | 1.19        | < 0.001     | 1.79        | 0.007       |
| Not stated                    | 0.61        | 0.038       | 0.64        | 0.081       |
| Living condition              |             |             |             |             |
| Inadequate                    | 0.89        | 0.404       | 1.52        | 0.004       |
| Adequate                      | 1           | 1           | 1           | 1           |
| Cohabitants (n)               |             |             |             |             |
| Up to 1                       | 1.55        | 0.020       | 1.50        | 0.032       |
| 2 to 3                        | 1.78        | < 0.001     | 1.53        | 0.006       |
| 4 to 8                        | 1           | 1           | 1           | 1           |
| 1 a 3                         | 1.91        | 0.001       | 0.89        | 0.569       |
| 4 a 6                         | 1.26        | 0.109       | 0.83        | 0.201       |
| Rural living                  |             |             |             |             |
| No                            | 1.61        | < 0.001     | 1.02        | 0.890       |
| Yes                           | 1           | 1           | 1           | 1           |
| Consumption of treated water  |             |             |             |             |
| No                            | 0.65        | 0.001       | 1.70        | < 0.001     |
| Yes                           | 1           | 1           | 1           | 1           |
| Vaccination                   |             |             |             |             |
| No                            | 1.91        | 0.006       | 2.53        | < 0.001     |
| Yes                           | 1           | 1           | 1           | 1           |
| Infectious disease            |             |             |             |             |
| No                            | 1.80        | 0.012       | 1.21        | 0.444       |
| Yes                           | 1           | 1           | 1           | 1           |
| Bowel infection                |             |             |             |             |
| No                            | 0.57        | < 0.001     | 0.65        | 0.001       |
| Yes                           | 1           | 1           | 1           | 1           |
| Appendectomy                  |             |             |             |             |
| No                            | 0.56        | 0.025       | 3.54        | 0.002       |
| Yes                           | 1           | 1           | 1           | 1           |
Table 3 Non-significant variables obtained from the comparative univariate and multiple multinomial logistic analysis between Crohn’s disease and ulcerative colitis, with healthy control as reference

| Characteristics          | Univariate multinomial logistic model | Multiple multinomial logistic model |
|--------------------------|--------------------------------------|-------------------------------------|
|                          | CD | UC | P value | OR | CD | UC | P value | OR | CD | UC | P value |
| Migration                | No | 0.98 | 0.888 | 0.86 | 0.269 | 0.83 | 0.258 | 1.04 | 0.802 |
|                          | Yes | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Exposure to domestic animal | No | 0.92 | 0.666 | 0.87 | 0.471 | 0.88 | 0.542 | 0.94 | 0.786 |
|                          | Yes | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Breastfeeding            | No | 1.40 | 0.210 | 0.79 | 0.435 | 1.14 | 0.661 | 0.60 | 0.116 |
|                          | Yes | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| History of worm disease  | No | 1.07 | 0.613 | 0.79 | 0.105 | 0.99 | 0.946 | 0.81 | 0.187 |
|                          | Yes | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |

1 Category of reference = healthy control.
2 Childhood. CD: Crohn’s disease; UC: Ulcerative colitis; OR: Odd ratios.

predominate. Historically, epidemiological studies have reported higher prevalence of the disease in Caucasians than in black and Asian people[23-25]. In the present study, univariate analysis showed that white individuals predominated in the CD group; thus, corroborating a previous study conducted by this team in the state of Rio de Janeiro[19]. However, the results from the multivariate analysis did not show any statistical significance for CD and UC.

A positive association between higher educational and income levels and the development of IBD has already been verified[26]. However, this association was only partially confirmed in this study. Individuals who had higher educational levels, had also higher risk of developing IBD. However, individuals with lower family monthly incomes (up to 3 minimum wages)[27] had higher risk of developing IBD. In the present study, several proxy factors of the hygiene hypothesis were associated with the development of IBD. For example, low number of cohabitants during adulthood, living in an urban area, and absence of infectious childhood diseases showed a significant positive association with the development of CD and UC. Also, consumption of treated water during both phases of life was associated with CD, but not in patients with UC. These results corroborate the hygiene hypothesis, which may explain the lower incidence of IBD in South America, Africa, and Asia compared with developed countries[12,28].

In Brazil, disease prevention and health promotion policies for the population have obtained satisfactory results, with reduction of the prevalence of infectious diseases during childhood. Reassuringly, the results from the present study showed that there
| Characteristics                              | Final multiple multinomial logistic modelCD | Final multiple multinomial logistic modelUC |
|---------------------------------------------|------------------------------------------|------------------------------------------|
| **Characteristics**                         | **OR** | **95% CI** | **OR** | **95% CI** |
| State of Brazil                             | 1.86   | 1.11-3.07  | 0.85   | 0.52-1.37  |
|               Rio de Janeiro                  | 2.52   | 1.48-4.28  | 1.27   | 0.75-2.14  |
|               São Paulo                       | 2.68   | 1.56-4.58  | 1.30   | 0.75-2.25  |
|               Minas Gerais                    | 0.86   | 0.51-1.45  | 0.71   | 0.43-1.16  |
|                Bahia                         | 0.88   | 0.54-1.43  | 0.84   | 0.53-1.31  |
|               Federal District               | 1      | 1           |        |            |
| Age at enrollment (yr)                      | 1.73   | 1.25-2.39  | 1.09   | 0.78-1.52  |
| 18 - 39                                      | 1      | 1           |        |            |
| 40 - 80                                      | 1      | 1           |        |            |
| Sex                                          | 0.76   | 0.56-1.00  | 0.59   | 0.44-0.78  |
| Male                                         | 1      | 1           |        |            |
| Female                                       |        |            |        |            |
| Educational level                           | 0.45   | 0.27-0.73  | 0.67   | 0.39-1.12  |
| Elementary school                           | 0.70   | 0.45-1.09  | 0.84   | 0.52-1.33  |
| High school                                  | 1      | 1           |        |            |
| Higher education                             |        |            |        |            |
| Monthly family income (minimum wages)       | 1.78   | 0.54-1.55  | 1.57   | 0.45-1.35  |
| Up to 3                                      | 1      | 1           |        |            |
| 3 to 5                                       | 1.21   | 1.16-2.70  | 1.14   | 1.02-2.40  |
| 5 or more                                    | 1      | 1           |        |            |
| Not stated                                   | 0.92   | 0.78-1.86  | 0.78   | 0.72-1.77  |
| Living condition                             | 1.15   | 0.79-1.66  | 1.48   | 1.01-2.16  |
| Inadequate                                   | 1      | 1           |        |            |
| Adequate                                     |        |            |        |            |
| Cohabitants (n)                              | 1.09   | 0.72-1.65  | 1.24   | 0.82-1.87  |
| Up to 1                                      | 1      | 1           |        |            |
| 2 to 3                                       | 1.70   | 1.22-2.35  | 1.60   | 1.15-2.21  |
| 4 to 8                                       | 1      | 1           |        |            |
| Rural living                                 | 1.33   | 0.95-1.86  | 1.62   | 1.16-2.25  |
| Yes                                          | 1      | 1           |        |            |
| No                                           |        |            |        |            |
| Consumption of treated water                 | 0.72   | 0.51-0.98  | 1.53   | 1.11-2.11  |
| Yes                                          | 1      | 1           |        |            |
| No                                           | 3.11   | 1.82-5.29  | 2.51   | 1.49-4.21  |
| Vaccination                                  |        |            |        |            |
| Yes                                          | 1      | 1           |        |            |
| No                                           | 1.99   | 1.20-3.26  | 1.37   | 0.80-2.32  |
| Yes                                          | 1      | 1           |        |            |
| Infectious disease                           | 1.99   | 1.20-3.26  | 1.37   | 0.80-2.32  |
| Yes                                          | 1      | 1           |        |            |
| No                                           | 0.56   | 0.42-0.73  | 0.67   | 0.50-0.89  |
| Bowel infection                              |        |            |        |            |
| Yes                                          | 1      | 1           |        |            |
| No                                           | 0.63   | 0.36-1.09  | 0.79   | 0.20-1.51  |
| Appendectomy                                 |        |            |        |            |
| Yes                                          | 1      | 1           |        |            |
| Never                                        |        |            |        |            |
| Exposure to tobacco                          | 1.08   | 0.66-1.76  | 1.50   | 0.87-2.57  |
| Former                                       | 1.81   | 1.06-3.05  | 3.36   | 1.91-5.90  |
| Current                                      | 1      | 1           |        |            |
| Exposure to tobacco                          | 1.08   | 0.66-1.76  | 1.50   | 0.87-2.57  |
| Never                                        |        |            |        |            |
| Former                                       | 1.81   | 1.06-3.05  | 3.36   | 1.91-5.90  |
| Current                                      | 1      | 1           |        |            |
| Familial IBD                                 | 0.19   | 0.20-1.31  | 0.30   | 0.14-1.23  |

1. CD = Crohn’s disease, UC = ulcerative colitis
2. IBD = inflammatory bowel disease
was no association between vaccination and higher risk of developing IBD, regardless of the geographical area analyzed. These findings confirm the results from a meta-analysis on 11 studies (with 2400 IBD cases and 34000 controls), which did not show any significant increase in IBD risk after immunization against BCG, diphtheria, tetanus, varicella, pertussis, measles, mumps, and rubella.[2] Despite improvements in hygiene conditions throughout Brazil, “favelas” (slum dwellings) are still present in urban centers. These “favelas” comprise marginal communities with precarious infrastructure and basic sanitation conditions, especially those in the interior areas of some states. These places favor occurrences of infectious gastroenteritis (Campylobacter sp, Salmonella sp, Shigella sp, Yersinia sp), which are possible triggers of IBD.[3] In the present study, a strong association was detected between infectious gastroenteritis and IBD, especially in relation to CD. However, since intestinal infections are not always confirmed through diagnostic methods, these episodes might possibly represent early symptoms of IBD.

Appendectomy prior to the diagnosis and smoking are considered to be risk factors that impact CD and UC differently. Appendectomy, especially if conducted before 10 years of age, has a negative association with UC.[4,5]. This was confirmed in the present study. It has been postulated that appendices are bacterial reservoirs that are involved in regulation of immunological responses to the hosts’ microbiota, and that the presence of environmental and microbial factors would promote higher activation of the Th1 pathway, with consequent development of appendicitis. This would explain the lower risk of developing UC, because the predominating immunological pathway is Th2.[6]. Regarding CD, the results from the present study showed that there was a significant positive association with appendectomy. This finding, however, could be due to interpretation bias, since a diagnosis of CD that is made during the short-term postoperative period may lead to an erroneous initial diagnosis of appendicitis in cases of CD involving the appendicular or ileocecal region[7].

Smoking is a well-established environmental risk factor for IBD in the West. Smoking increases the risk of developing CD, whereas never smokers and those who quit smoking are more likely to develop UC. Smoking may modulate the risk of IBD by promoting epigenetic alterations that modify the genic expression that occurs in innate and adaptive immune responses and in alterations to the gut microbiome composition[8]. In the present study, smoking was negatively associated with UC, with a higher association among former smokers, which is consistent with data from the West[9] and studies from Asia[10]. In contrast, former and current smokers were not associated with the development of CD. This finding is inconsistent with studies from the West; however, an environmental risk factor study from the Asia-Pacific Crohn’s and Colitis Epidemiology Study also showed that smoking was not associated with CD in Asian countries[10]. Taken together smoking may not be associated with CD in newly industrialized countries outside the Western world. Future studies are necessary to corroborate these findings in other developing countries.

An inherent methodological limitation of epidemiological studies is the information bias, a common occurrence in case-control studies. In this study, however, information on the exposure to risk factors was obtained through a structured questionnaire, with data collection standardization by the researchers. These practices tend to minimize this possibility. Regarding variables related to childhood, recall bias needs to be taken into consideration. Additionally, numerous environmental risk factors were studied and thus, the findings are limited by multiple comparison errors. The study was carried out in public IBD centers located in urban areas with cases that are usually more severe and are more difficult to diagnose. These cases do not represent the totality of the population with IBD that may be undergoing treatment in the private setting, in secondary care units, and in other regions of the country that were not included.

The IBD burden in South America, including Brazil, is increasing at a rate possibly even greater than other developing regions around the world. However, there is a paucity of high-quality epidemiological studies being necessary more powerful and

| Variable | Yes | 1 | 0.10-0.34 | 0.16-0.56 |
|----------|-----|---|-----------|-----------|
| Not stated | 0.56 | 0.42 |

1 After excluding the variables that were not statistically significant (P > 0.05) in the multiple model (category of reference = healthy controls).
2 Childhood.
3 Adulthood. CD: Crohn’s disease; UC: Ulcerative colitis; OR: Odd ratios.
representative data to further explore modifiable risk factors and disease phenotypes[38].

In conclusion, this multicenter study from the southeastern, central-western, and northeastern regions of Brazil supports the hygiene hypothesis. Interestingly, several environmental risk factors are consistent with established risk factors within the West, whereas other environmental factors have distinctly different associations. Brazil is a large heterogeneous region that differs by demography, socioeconomic status, ethnicity, and healthcare support. Therefore, future studies are necessary to confirm the risk factor associations observed in the present study.

ARTICLE HIGHLIGHTS

Research background
The etiology of inflammatory bowel disease (IBD) is unknown, but it is believed to be multifactorial. The hygiene hypothesis proposes that better hygiene conditions would lead to less infectious disease during childhood and favor the development of immune-mediated diseases.

Research motivation
Brazil is a country with continental dimensions comprising an ethnically diverse population living in different regions with extreme socioeconomic differences. The country is the perfect setting to test the hygiene hypothesis in IBD development.

Research objectives
The aim of this study was to identify IBD environmental risk factors across different geographical regions in Brazil and evaluate if the hygiene hypothesis might explain interregional differences in prevalence/incidence.

Research methods
A multicenter case-control study with 548 Crohn’s disease (CD), 492 ulcerative colitis (UC) outpatients, and 416 healthy controls. A semi-structured questionnaire with 87 socioeconomic and environmental questions was applied.

Research results
Predictive variables for both diseases (CD and UC) were women [odd ratios (OR) = 1.31; OR = 1.69], low monthly family income (OR = 1.78; OR = 1.57), lower number of cohabitants (OR = 1.70; OR = 1.60), absence of vaccination (OR = 3.11; OR = 2.51), previous history of bowel infections (OR = 1.78; OR = 1.49), and family history of IBD (OR = 5.26; OR = 3.33). Associated risk factors for CD were age (18-39 years) (OR = 1.73), higher educational level (OR = 2.22), absence of infectious childhood diseases (OR = 1.99). The UC predictive variables were living in an urban area (OR = 1.62), inadequate living conditions (OR = 1.48) and former smokers (OR = 3.36). Appendectomy was a risk factor for CD (OR = 1.58) with inverse association with UC (OR = 4.79). Consumption of treated and untreated water was associated with risk of CD (OR = 1.38) and UC (OR = 1.53), respectively.

Research conclusions
Most of the variables associated with disease risk support the role of the hygiene hypothesis in IBD development.

Research perspectives
Brazil is a large heterogeneous region that differs by demography, socioeconomic status, ethnicity, and healthcare support. Therefore, future studies are necessary to confirm the risk factor associations observed in the present study.

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