

Role of *Salmonella enterica* exposure in Chilean Crohn's disease patients

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Salmonella enterica (SE) and Crohn's disease (CD) and its clinical implications in Chilean patients.

METHODS: Ninety-four unrelated Chilean CD patients from CAREI (Active Cohort Registry of Inflammatory Bowel Disease) presenting to a single inflammatory bowel disease (IBD) unit of a University Hospital were prospectively included in this study. A complete clinical evaluation, including smoking history, was performed at the initial visit, and all the important data of clinical evolution of CD were obtained. Blood samples from these CD patients and 88 healthy sex- and age-matched control subjects were analyzed for exposure to SE and for their *NOD2/CARD15* gene status using the presence of anti-*Salmonella* lipopolysaccharide antibodies [immunoglobulin-G type (IgG)] and polymerase chain reaction (PCR), respectively. We also evaluated exposure to SE in 90 sex- and age-matched patients without CD, but with known smoking status (30 smokers, 30 non-smokers, and 30 former smokers).

RESULTS: CD patients comprised 54 females and 40 males, aged 35.5 ± 15.2 years at diagnosis with a mean follow-up of 9.0 ± 6.8 years. CD was inflammatory in 59 patients (62.7%), stricturing in 24 (25.5%) and penetrating in 15 (15.5%). Thirty cases (31.9%) had lesions in the ileum, 29 (30.8%) had ileocolonic lesions, 32 (34.0%) had colonic lesions and 23 (24.4%) had perianal disease. Sixteen CD patients (17%) were exposed to SE compared to 15 (17%) of 88 healthy control subjects ($P = 0.8$). Thirty-one CD patients (32.9%) were smokers, and 7 (7.4%) were former smokers at diagnosis. In the group exposed to SE, 10 of 16 patients (62.5%) were active smokers compared to 21 of 78 patients (26.9%) in the unexposed group ($P = 0.01$). On the other hand, 10 of 31 smoking patients (32%) were exposed to SE compared to 5 of 56 nonsmoking patients (9%), and one of the seven former smokers (14%) ($P = 0.01$). In the group of 90 patients without CD, but whose smoking status was known, there was no differ-

Abstract

AIM: To study the association between exposure to

ence in exposure to SE [3 of 30 smokers (10%), 5 of 30 non-smokers (16%), and 5 of 30 former smokers (16%); $P = 0.6$]. There were no differences in disease severity between CD patients with and those without anti-SE IgG antibodies, estimated as the appearance of stricturing [2 (12.5%) *vs* 22 (28.2%); $P = 0.2$] or penetrating lesions [2 (12.5%) *vs* 13 (16.6%); $P = 1.0$]; or the need for immunosuppressants [11 (68.7%) *vs* 55 (70.5%); $P = 1.0$], anti-tumor necrosis factor therapy [1 (6.2%) *vs* 7 (8.9%); $P = 1.0$], hospitalization [13 (81.2%) *vs* 58 (74.3%); $P = 0.7$], or surgery [3 (18.7%) *vs* 12 (15.3%); $P = 0.3$], respectively]. No other factors were associated with SE, including *NOD2/CARD15* gene status. Seventeen CD patients (18%) had at least one mutation of the *NOD2/CARD15* gene.

CONCLUSION: Our study found no association between exposure to SE and CD. We observed a positive correlation between SE exposure and cigarette smoking in Chilean patients with CD, but not with disease severity.

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Key words: Crohn's disease; *Salmonella*; Infection; Tobacco; Smoking; Environmental factors

Core tip: The role and clinical implications of *Salmonella enterica* (SE) in Crohn's disease (CD) are controversial and currently unknown. We evaluated the role of exposure to SE in a cohort of Chilean patients suffering from CD. Although our study showed no association between SE exposure and CD, we observed a positive correlation between SE exposure and cigarette smoking in CD patients, but not with disease severity. These data provide evidence that more precisely defines the real role of *Salmonella* infection, an important environmental factor in CD.

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INTRODUCTION

Crohn's disease (CD) is an immunological bowel disorder of unknown etiology, characterized by chronic relapsing inflammation of the gastrointestinal tract^[1]. CD is a complex and heterogeneous disorder^[2], which is determined by, among other factors, the interaction between diverse environmental and genetic factors^[3]. Studies on migrants have highlighted the importance of environmental factors in CD, and it is likely that there are important geographical differences^[4,5]. Among the risk factors associated with CD are infections and smoking^[6-12], and both are major public health problems^[13-15] (<http://www.ispch.cl/sites/>

[default/files/Vigilancia_Salmonella_spp_0](http://www.ispch.cl/sites/default/files/Vigilancia_Salmonella_spp_0)). Preliminary studies have suggested a correlation between CD and infectious gastroenteritis^[16,17]. A previous report showed that, in some populations, the incidence of CD increases over time after an acute gastroenteritis episode caused by *Salmonella enterica* (SE)^[18]. However, a recent report did not find an association between SE and CD^[19]. Different gene-environmental interactions and gene-gene interactions have been studied in CD and are frequently related to the *NOD2/CARD15* gene^[20-25]. Polymorphisms in this gene have been the most consistent and significant genetic factor implicated in CD susceptibility^[26]. However, studies of environmental-environmental interaction and their implications are scarce^[27,28].

The reason for the possible association between SE and CD is unknown, but it is possible that mucosal immunity changes after SE exposure, promoting immune modification of the intestinal mucosa^[29]. Thus, the intestinal mucosa may develop an enhanced inflammatory response when other factors affecting the immune response (genetic or environmental) are present. Moreover, the protein encoded by the *NOD2/CARD15* gene acts as an intracellular receptor that recognizes bacteria-derived molecules, including SE^[30,31]. In addition, the clinical implications of SE in CD are unknown.

The aim of this study was to explore whether there is an association between SE exposure and CD and its implications in Chilean patients with CD.

MATERIALS AND METHODS

CD patients and clinical definitions

Ninety-four unrelated Chilean CD patients from CAREI (Active Cohort Registry of Inflammatory Bowel Disease) visiting a single inflammatory bowel disease (IBD) unit of a university referral center between December 2010 and June 2012 were included in this study. All CD patients gave written informed consent to participate in the study, and the local ethics committee approved the project. The study was performed in accordance with the principles stated in the declaration of Helsinki.

The diagnosis of CD was based on standard clinical, radiological, endoscopic, and histological criteria^[32]. A complete clinical evaluation was performed at the initial visit, and all the important data of the clinical evolution of CD were obtained from a prospective clinical database of our unit. This evaluation included gender, age at diagnosis of CD, length of follow-up, location and behavior of CD, family history of IBD, previous appendectomy, smoking habit, oral contraceptive use, extra-intestinal manifestations of CD, and history of gastroenteritis caused by SE and/or vaccine for SE. The following characteristics were also documented: need for surgery or hospitalization for CD, use of steroids, use of immunosuppressants (azathioprine, 6-mercaptopurine and methotrexate) and use of anti-tumor necrosis factor (TNF) therapy.

Smokers were defined as patients who smoked more than seven cigarettes per week (one cigarette daily). A for-

mer smoker was defined as a patient who quit smoking at least 1 year before diagnosis. Nonsmokers were defined as patients who had never smoked or who smoke less than seven cigarettes per week^[9]. The location and behavior of CD were determined according to X-ray and endoscopic findings; all patients underwent these procedures at least once at our center. The location and behavior of CD were defined according to criteria of the Montreal classification^[2]; however, behaviors were registered as non-exclusive categories if intestinal stricturing occurred at a different time from the penetrating lesions; therefore, patients could belong to both categories. The severity of the disease was estimated as the appearance of stricturing or penetrating lesions or the need for immunosuppressants, anti-TNF therapy, hospitalization or surgery.

Control patients

Eighty-eight unrelated Chilean healthy blood donors, matched by sex and age, were studied as control subjects to determine if there were any differences in the frequency of exposure to SE, independent of *NOD2/CARD15* gene status. In addition, we studied the frequency of SE exposure in a sample of patients without CD who were visiting a respiratory disease unit at our university center. These patients were differentiated by their smoking status and matched by sex and age. Thus, we studied 90 patients who were distributed as follows: 30 smokers, 30 non-smokers, and 30 former smokers. Smoking status was defined in the same way as in CD patients^[9].

Determination of *Salmonella* exposure

For each subject, a venous blood sample was extracted to obtain serum, which was stored at -70 °C freezer until analysis. To evaluate previous exposure to *Salmonella*, serum samples were tested for the presence of anti-*Salmonella* lipopolysaccharide (LPS) antibodies [immunoglobulin-G type (IgG)] using an enzyme-linked immunosorbent assay (ELISA)^[33]. The sensitivity and specificity of this methodology have been evaluated in a previous study^[34]. Briefly, ELISA plates (Nalgene-Nunc®, Thermo Scientific, Waltham, MA, United States) were activated overnight at 4 °C with 500 ng of *Salmonella typhimurium* LPS (L7261-25MG SIGMA-ALDRICH, St. Louis, MO, United States) in 50 µL 0.1 mol/L bicarbonate buffer (NaHCO₃, Merck, Whitehouse Station, NJ, United States) and blocked with 100 µL of PBS-BSA 3% for 1 h at room temperature. Then, 50 µL of serum diluted in PBS at 1/64, 1/128, and 1/256 was added to each well and incubated for 2 h at room temperature. Next, three washes with 200 µL of PBS-Tween 0.05% were performed. After the washes, 50 µL of an IgG conjugated with peroxidase (clone G18-145, Becton Dickinson, Franklin Lakes, NJ, United States) was added to each well (diluted 1/2000 in PBS) and incubated for 2 h at room temperature. Three washes with PBS-Tween 0.05% were then performed, and the positive reaction was developed using 3-3'-5'-tetramethyl-benzidine at a final concentration 100 µg/mL (Sigma-

Aldrich) as a colorimetric substrate. The enzymatic reaction was stopped with 2 mol/L H₂SO₄, and absorbance was recorded at 450 nm in an ELISA plate reader (Thermo Scientific). As a positive control, a group of subjects who had a previous history of gastroenteritis caused by SE or typhoid fever were included in this study and were used to standardize the methodology. As negative controls, a group of control subjects that had not been exposed to *Salmonella* were included, which comprised a serum of a pool of nine subjects up to 19 years old with no previous clinical history of gastroenteritis or typhoid fever. Both positive and negative controls were included in all the assays performed. Patients exposed to SE were defined as those patients with OD values higher than 2 SD over the value obtained for negative controls in the three dilutions tested in each determination. Those subjects not exposed to SE were all the other CD patients. As described before, this test allows the identification of patients that have been infected with either *Salmonella Typhimurium* or *Salmonella Enteritidis*^[34]. These two serovars account for 80% of the cases of gastroenteritis caused by *Salmonella* in Chile (http://www.ispch.cl/sites/default/files/Vigilancia_Salmonella_spp_0.pdf).

NOD2/CARD15 genotyping

To detect *NOD2/CARD15* gene variants, genomic DNA from whole blood samples was isolated using standard molecular biology techniques. Specific sequences of exon 4 (missense mutation R702W), exon 8 (missense mutation G908R) and exon 11 (frameshift mutation L1007fsinsC) of the *Nod2/CARD15* gene were amplified by a polymerase chain reaction (PCR, using primers 5'- CCT TCA GAT CAC AGC AGC CTT C -3' and 5'- GGG ATG GAG TGG AAG TGC TTG -3' for exon 4; 5'- TCT AAG TCT GTA ATG TAA AGC CAC -3' and 5'- AGC TCC TCC CTC TTC ACC TGA -3' for exon 8, and 5'- CTG AGC CTT TGT TGA TGA GCT C -3' and 5'- ATT CTT CAA CCA CAT CCC CAT TC -3' for exon 11. PCR amplifications were performed in a MaxiGene Gradient Thermocycler (Axygen, Union City, CA, United States), using 250 ng of DNA, 1 nmol/mL of each primer, 0.2 mmol/L deoxynucleoside triphosphates, 1.5 mmol/L Magnesium Chloride and 50 U/mL of Taq DNA polymerase (Invitrogen, Carlsbad, CA, United States), using standard PCR amplification cycles. The PCR products obtained for exon 4 and exon 8 were digested with the restriction enzymes *Hpa* II and *Hba* I (New England Biolabs, Ipswich, MA, United States), and the digested PCR products were resolved by electrophoresis in 1% agarose gels containing 0.5 µg/mL ethidium bromide and visualized under a UV light transilluminator (UVP, Inc., Upland, CA, United States). PCR products obtained for exon 11 were gel purified and sequenced in an ABI 3100 automatic sequencer by Macrogen (<http://www.macrogen.com>). Frameshift mutations were analyzed for each sample, using the Vector NTI software (Invitrogen). CD patients positive for at least one of the

Table 1 Characteristics of Crohn's disease patients *n* (%)

| Characteristic | <i>n</i> = 94 |
|--------------------------------------|----------------|
| Gender | |
| Female | 54 (57) |
| Age at diagnosis (yr) | |
| Median (range) | 31 (9-80) |
| Early onset (< 40 yr) | 61 (64.0) |
| Follow-up (yr) | |
| Median (range) | 7.0 (2.0-34.0) |
| Smoking at diagnosis | 31 (32.0) |
| History of gastroenteritis due to SE | 5 (5.0) |
| Previous appendectomy | 20 (21.0) |
| Disease localization | |
| Ileum | 30 (31.9) |
| Ileocolonic | 29 (30.8) |
| Colon | 32 (31.9) |
| Upper gastrointestinal tract | 3 (3.1) |
| Disease behavior | |
| Non-stricturing, non-penetrating | 59 (62.7) |
| Stricturing | 24 (25.5) |
| Penetrating | 15 (15.9) |
| Perianal disease | 23 (24.4) |
| Extraintestinal manifestation | 49 (52.1) |
| Family history of IBD | 14 (14.8) |

SE: *Salmonella enterica*; IBD: Inflammatory bowel disease.

NOD2/CARD15 gene polymorphisms were categorized as gene variant carriers.

Statistical analysis

CD patients were compared to control subjects in relation to the presence of anti-SE IgG. Among CD patients, we compared those exposed to SE *vs* unexposed in relation to the most important clinical and genetic characteristics associated with CD. Categorical variables were compared using the Fisher's exact test. Continuous variables were expressed as the mean ± SD, and compared using Student's *t* test. A *P* value of less than 0.05 was considered to indicate statistical significance. Analysis was carried out using the StatView software package (SAS Institute Inc., Cary, NC, United States).

RESULTS

Patient population

The study population consisted of 94 patients with CD; 54 females and 40 males. Age at diagnosis was 35.5 ± 15.2 years. Mean follow-up was 9.0 ± 6.8 years, with a median of 7.0 years. Behavior of CD was inflammatory in 59 patients (62.7%), stricturing in 24 patients (25.5%) and penetrating in 15 patients (15.5%). Thirty cases (31.9%) had lesions in the terminal ileum, 29 (30.8%) were ileocolonic, 32 (34.0%) had colonic lesions and 23 (24.4%) had perianal disease. Five patients (5%) reported previous gastrointestinal infection caused by *Salmonella*, and only one patient (1%) had received a vaccine for *Salmonella*. Thirty-one CD patients (32.9%) were smokers, and seven (7.4%) were former smokers at diagnosis. The clinical characteristics of CD are shown in Table 1.

Table 2 Comparison between Crohn's disease patients exposed and those not exposed to *Salmonella enterica*

| | IgG SE (+) (<i>n</i> = 16) | IgG SE (-) (<i>n</i> = 78) |
|------------------------------|-----------------------------|-----------------------------|
| Age at diagnosis (yr) | 38.6 ± 15.6 | 34.9 ± 15.1 |
| Female | 6 (37.5) | 48 (61.5) |
| Disease duration, years (yr) | 11.2 ± 9.5 | 8.5 ± 6.1 |
| Disease site | | |
| Ileum | 5 (31.2) | 25 (32.0) |
| Ileocolonic | 6 (37.5) | 23 (29.4) |
| Colonic | 5 (31.2) | 27 (34.6) |
| Proximal | 0 (0.0) | 3 (3.8) |
| Perianal disease | 1 (6.2) | 22 (28.2) |
| Disease Behaviour | | |
| Inflammatory | 12 (75) | 47 (60.2) |
| Stricturing | 2 (12.5) | 22 (28.2) |
| Penetrating | 2 (12.5) | 13 (16.6) |
| Family history of IBD | 3 (18.7) | 11 (14.1) |
| Active smokers | 10 (62.5) | 21 (26.9) ^b |
| Previous appendectomy | 6 (37.5) | 14 (17.9) |
| Oral contraceptive use | 1 (6.2) | 4 (5.1) |
| Extraintestinal involvement | 7 (43.7) | 42 (53.8) |
| Immunosuppressants use | 11 (68.7) | 55 (70.5) |
| Steroid use | 13 (81.2) | 67 (85.8) |
| Anti TNF therapy | 1 (6.2) | 7 (8.9) |
| Hospitalization due to IBD | 13 (81.2) | 58 (74.3) |
| Bowel Surgery | 3 (18.7) | 26 (33.3) |
| <i>NOD2/CARD15</i> Variant | 5 (31.2) | 12 (15.3) |

Data are expressed as absolute numbers (percentage) or mean ± SD. ^b*P* < 0.01 *vs* IgG *Salmonella enterica* (SE) (+). IBD: Inflammatory bowel disease; TNF: Tumor necrosis factor.

Exposure to SE and analysis of factors influencing exposure to SE

Sixteen CD patients (17%) were exposed to SE, as determined by the presence of anti SE-IgG in the serum. Among the 88 sex- and age-matched healthy blood donors, 15 patients (17%) were exposed to SE. There was no difference in exposure to SE between CD and control subjects (*P* = 0.8).

Comparison of the clinical characteristics between those who had been exposed to SE and those who had not, are shown in Table 2. With the exception of smoking, there were no significant differences in the clinical variables studied between the group of patients exposed to SE and those not exposed. Exposure to SE was significantly associated with cigarette smoking; in the group exposed to SE, 10 of 16 patients (62.5%) were active smokers compared to 21 of 78 patients (26.9%) in the group that was not exposed (*P* = 0.01). On the other hand, 10 of 31 smokers (32%) had exposure to SE compared with 5 of 56 nonsmokers (9%) and 1 of 7 former smokers (14%) (*P* = 0.01). No other factors were associated with exposure to SE. We also analyzed whether *NOD2/CARD15* gene variations were associated with exposure to SE, and the result was negative (*P* = 0.2, Table 2).

To assess whether increased exposure to SE was specific to CD patients who smoked, we determined the frequency of exposure to SE in smoking patients without CD. In these patients, there was no difference in exposure

to SE, defined by 3 of 30 smokers (10%) with detectable levels of anti-SE IgG compared to 5 of 30 non-smokers (16%) and 5 of 30 former smokers (16%), and the *P* value was non-significant (*P* = 0.6).

Age at diagnosis of CD, sex distribution, duration of CD, CD location, family history of IBD, and extraintestinal manifestations were similar in both groups of patients (Table 2), whereas other environmental clinical characteristics, such as use of oral contraceptives and previous appendectomy, were similar among carriers and non-carriers of anti-SE IgG (Table 2).

CD severity according to presence of anti-SE IgG

The prevalence of several indicators of disease severity, including stricturing or penetrating lesions, the need for immunosuppressants, anti-TNF therapy, hospitalization or surgery was similar in CD patients with and those without anti-SE IgG antibodies (Table 2).

NOD2/CARD15 genotype

Seventeen CD patients (18%) had at least one mutation of the *NOD2/CARD15* gene. All were heterozygous for these variants. The distribution of the three variants was as follows: 11 (11%) for R702W, one (1%) for G908R and five (5%) for L1007fsinsC. This frequency was higher than in control subjects, with 3 of 88 healthy blood donors (3%) displaying least 1 *NOD2/CARD15* gene mutation (*P* = 0.003).

DISCUSSION

This study evaluated the role of *Salmonella* in CD and its interactions with genetic (*NOD2/CARD15* gene) and environmental risk factors in Chilean patients. This is a step towards unraveling the importance of environmental factors in CD and their association with other risk factors in a new population. In CD, an important part of the disease pathogenesis could be related to environmental factors and their interaction with other patient factors^[1,3,11,22,27]. Our study showed no difference in the level of previous exposure to *Salmonella* between CD patients and controls. On the other hand, we observed a striking correlation between smoking and exposure to SE in CD, independent of *NOD2/CARD15* gene variants, implying a particular pattern of environment-environment interaction.

The role of microbes in CD is supported by the fact that most mouse models of IBD develop colitis only in the presence of intestinal bacteria^[35], and several human studies have shown remissions in CD patients after antibiotic therapy^[36]. A growing body of evidence suggests a correlation between CD and infectious gastroenteritis^[16,17] (http://www.ispch.cl/sites/default/files/Vigilancia_Salmonella_spp_0.pdf). Although a previous study implicated *Salmonella* as a risk factor for CD, in which patients with gastroenteritis caused by *Salmonella* had a higher risk for developing IBD compared to age- and sex-matched controls^[18], our study showed no difference

in previous exposure to *Salmonella* between CD patients and controls. The design of our study differs from other studies because the diagnostic criterion was based on a cross serological test to determine previous exposure to SE using the presence of anti-*Salmonella* IgG. Given the relatively low rate of history of previous typhoid fever or gastroenteritis caused by SE in our CD patient population, it is possible that independent factors of gastrointestinal infections may be more relevant in our population, including non-*NOD2/CARD15* genes, given the low frequency of mutations in this gene in our CD patients. However, our findings agree with a recent report that did not find an association between SE and CD, and this study strongly suggests that the positive associations observed in the earlier studies were the result of a detection bias^[19]. The study of Jess *et al*^[19] showed that the temporal risk patterns for IBD are not different following negative and positive stool tests for SE. This observation strongly suggests that increased occurrence of *Salmonella* around the time of diagnosis results from detection bias resulting from increased rates of stool testing. Moreover, the occurrence of *Salmonella* infection in patients with nonspecific gastrointestinal symptoms compatible with CD could represent a “by chance” finding and should not exclude the patients from subsequent clinical examination if gastrointestinal symptoms persist^[19].

In other autoimmune diseases, such as rheumatoid arthritis (RA), smoking is a well-established environmental risk factor^[15,37,38]. In CD, smoking is also a major risk factor, and it is associated with several complications over the course of CD^[9,10,39]. A meta-analysis supports the view that current smoking is associated with a significantly higher risk of CD^[40]. Recent studies have reinforced the importance of smoking, suggesting that studies of risk factors for IBD should be stratified for smoking behavior, especially in cohorts of limited sample size, as in the current study^[41].

We found a significant association between *Salmonella* exposure and active smoking in a group of Chilean CD patients. These two factors may have an additive effect on the development of the disease in a subset of CD patients. To date, there is no clear biological explanation for why smoking is associated with an increased risk of CD^[42]. In smokers with CD, an increased immune response against *Salmonella* could be developed, based on a greater presence of anti-*Salmonella* IgG. SE may produce a change in mucosal immunity^[29], and this change could be exacerbated in smoking patients. This could lead to tissue damage and the onset of IBD in susceptible hosts. *Salmonella* triggers an inflammatory response categorized as Th1^[43], and given that intestinal immune response in CD is classically recognized as Th1, it is conceivable that invasive bacteria, such as *Salmonella*, could trigger this abnormal intestinal immune reaction. In addition, inflammation is required by SE to colonize intestinal mucosa and compete with resident microflora^[44]. In experimental studies, prolonged exposure to concentrated smoke leads to decreased expression and activity of the anti-inflam-

matory enzyme heme oxygenase-1^[45], and an increase in cell autophagy in the bowel^[46]. It is possible that chronic smoking may cause a pro-inflammatory state, and *Salmonella* infection could be facilitated in smoking patients. *Salmonella* infection and tobacco consumption are a major public health problem in Chile and worldwide^[9,13-15] (http://www.bcn.cl/carpeta_temas/tema_as_portada.2006-09-25.0806013222/documentospdf-sobre-obesidad/VIGIA20.pdf and http://www.redsalud.gov.cl/portal/docs/page/minsalcl/g_home/submenu_portada_2011/ens2010.pdf), and there should be a greater emphasis on the importance of food safety and smoking avoidance.

Notably, we did not observe any association with a higher severity in patients exposed to SE compared to those not exposed, defined as the presence of stricturing or penetrating lesions or the need for immunosuppressants, anti-TNF therapy, hospitalization or surgery.

A limitation of our study is the relatively small sample population; therefore, future larger studies will be needed to confirm the relationship between smoking and infection with SE. However, the absence of this association in a control sample without CD supports the notion that this is a specific feature of CD patients.

In conclusion, our study found no association between exposure to SE and CD in a new well-defined population of Chilean CD patients. We observed a positive correlation between SE exposure and cigarette smoking in patients with CD, but not with disease severity. This research more precisely defines the real role of *Salmonella* exposure, an important environmental factor in CD, and how risk factors combine to trigger CD in a given patient.

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COMMENTS

Background

Different environmental factors have been studied in Crohn's disease (CD). An important environmental factor that has been implicated as a possible trigger of CD is infectious gastroenteritis. There is a major controversy concerning the role of *Salmonella enterica* (SE) as a risk factor for CD. It is possible that there are important geographical differences; however, to date, there are still no data addressing the interactions between SE exposure and other environmental factors and the clinical implications of these interactions in CD.

Research frontiers

Different gene-environment and gene-gene interactions have been studied in CD. However, environment-environment interaction studies and their clinical implications are scarce. There may be important ethnic variations. The focus of this research was to establish the real importance of an environmental factor as infectious gastroenteritis caused by SE in Chilean CD patients.

Innovations and breakthroughs

Results of previous studies on the role of SE in CD have been controversial. The present study was designed to evaluate the role of SE in CD and its interactions with genetic and environmental risk factors in Chilean patients. The authors determined previous exposure to SE defined by the presence of anti-*Salmonella* IgG. Although their study showed no difference in previous exposure to *Salmonella* between CD patients and controls, they observed that smoking was

associated with exposure to SE in CD. If they analyze a key element, such as clinical implication of SE exposure, they did not observe a correlation between SE exposure and CD severity. This research more precisely defines the role of *Salmonella* infection, an important environmental factor, in CD and how risk factors combine to trigger CD in a given patient.

Applications

The study results suggests that SE exposure is not associated with CD; however, the authors observed that smoking was associated with exposure to SE in Chilean CD patients, independent of other factors, implying a particular pattern of environment-environment interaction.

Terminology

Gene-gene, gene-environment, or environment-environment interactions: In complex diseases, such as CD, there are different implications or consequences for the disease when two factors (gene and/or environmental) are present in the same patient.

Peer review

This paper represents a considerable amount of good work, and it is a well-done and controlled study. It is a study in a well-defined population. This manuscript aimed to study the association between exposure to SE and CD. In addition, they also analyzed the involvement of cigarette smoking. They showed no association for the SE status and CD, but positive association between SE and smoking in CD.

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