Environmental exposure to trace elements and heavy metals preceding the clinical onset of inflammatory bowel disease

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Author contributions

Study design: IR-L and MB-A.

Collecting and/or interpreting data: All authors.

Drafting the manuscript: IR-L and MB-A.

Revision for relevant intellectual content: JLC and MB-A.

All authors have approved the final version of this manuscript.

Conflicts of interest

IR-L has received financial support for traveling and educational activities from or has served as an advisory board member for Abbvie, Adacyte, Celltrion, Chiesi, Danone, Ferring, Faes Farma, Janssen, Galapagos, MSD, Pfizer, Roche, Takeda, and Tillotts Pharma. Financial support for research: Tillotts Pharma.

MB-A has received financial support for travelling and educational activities from or has served as an advisory board member for Pfizer, MSD, Takeda, AbbVie, Kern, Janssen, Fresenius Kabi, Galapagos, Lilly, BMS, Faes Farma, Chiesi and Adacyte.

The remaining authors declare no conflicts of interest related to this manuscript.
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Abbreviations

CD: Crohn’s disease; IBD: inflammatory bowel disease; UC: ulcerative colitis.

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Abstract

Background

The immune dysregulation underlying inflammatory bowel disease (IBD) can start years before the diagnosis, but the role of triggering factors and environmental exposures during this period is still uncertain.

Methods

This single-center case-control study included asymptomatic subjects with an incidental diagnosis of IBD during the colorectal cancer screening program. Twenty-two minerals and 17 metals were determined at diagnosis in hair samples and compared 1:2 to healthy controls.

Results

Six patients with preclinical IBD (3 ulcerative colitis, 67% left-sided; 3 Crohn’s disease, 100% ileal, 67% inflammatory behaviour) and 13 healthy non-IBD controls were included. No relevant occupational exposures were identified. We found statistically significant higher levels of sodium, potassium and boron among cases compared to controls; while lower levels of zinc, uranium, copper and germanium were observed.

Conclusions

A range of environmental exposures can be identified during the preclinical phase of IBD, but their relationship with the symptomatic onset and disease progression should be further explored.

Keywords: Crohn’s disease; metals; risk factor; environmental exposure; ulcerative colitis.
Lay Summary

In a single-center case-control study, findings demonstrated that the measurement of certain trace elements and heavy metals can suggest the presence of an impaired intestinal barrier in patients with subclinical inflammatory lesions preceding the onset of IBD.
Introduction

Recently, it has been demonstrated that patients with inflammatory bowel disease (IBD) go over a period where the initial inflammatory changes are already present and precede the development of symptoms, defined as the preclinical phase (1). The detection of mucosal abnormalities in otherwise healthy subjects, particularly in the context of colorectal cancer screening programs, has unveiled a new subgroup of patients where this preclinical disease can be also characterized.

In previous studies, investigators have observed that deciduous teeth from patients with IBD have a greater exposure to lead, copper, zinc and chromium during the first months of life, compared to healthy controls (2). Interestingly, the presence of higher fecal calprotectin levels in babies born to mothers with IBD have also suggested the presence of subclinical inflammation in early life, so the interaction with environmental factors during this period can be crucial (3). This process can potentially be influenced by the exposure to certain compounds (e.g. heavy metals), through a modification of gut microbiome or the increase on gut permeability (4, 5). However, there is no information on the role of environmental exposures closer to the preclinical phase, while a more detailed evaluation in this context would help identifying the consequences of a subclinical inflammatory process and possible triggers associated to the onset of the first symptoms. Therefore, our aim was to determine if preclinical IBD is associated to a different exposure to certain heavy metals or minerals.

Methods

A single-centre, cross-sectional, and observational study was conducted at Hospital Universitario de Galdakao (Spain). All consecutive patients with an incidental diagnosis of IBD during the colorectal cancer screening program were invited to participate. The Basque colorectal cancer screening program invites all persons between 50 to 69 years to perform a fecal immunochemical test, followed by a complete colonoscopy in those with a positive
result (cut-off 20 µg Hb/g). In this study, we included all patients with a combination clinical, endoscopic and histologic data, requiring the presence of chronic infiltrate and absence of any enteropathogen or alternative diagnosis, all of them suggesting a diagnosis of Crohn’s disease (CD) or ulcerative colitis (UC) and following the same criteria as in previous reports from this cohort (6, 7). A sample containing 0.25 grams from the scalp were collected from each individual with a recent (<2 months) diagnosis of preclinical IBD. Patients receiving regular non-steroidal anti-inflammatory drugs were excluded. Samples consisted in 3-4 cm of hair more proximal to the scalp, therefore representing recent exposure to these compounds around the date of the index colonoscopy. A control population was also recruited from the same geographical region including subjects without a diagnosis of IBD and not receiving regular medical treatment for any other condition. We used a commercial kit (Doctor’s Data, Inc., St Charles, Illinois) that allows the investigation of 17 metals (aluminum, antimony, arsenic, barium, beryllium, bismuth, cadmium, lead, mercury, platinum, thallium, thorium, uranium, nickel, silver, tin, and titanium) and 22 minerals (calcium, magnesium, sodium, potassium, copper, zinc, manganese, chromium, vanadium, molybdenum, boron, iodine, lithium, phosphorus, selenium, strontium, sulfur, cobalt, iron, germanium, rubidium, zirconium) through inductively coupled plasma mass spectrometry (ICP-MS) as described in previous reports (8). Descriptive statistics and non-parametric tests were used for comparisons between both groups. The protocol was approved by the local Ethics Committee (No 01/21) and all subjects signed an informed consent before undergoing any study procedure.

**Results**

We included 6 patients with preclinical IBD (median age 58 years [IQR, 54-62]; 67% women; 83% former smokers) and 13 healthy non-IBD controls (median age 35 years [IQR, 33-39]; 69% women; 92% never smokers). Their main characteristics are summarized on Table 1.
Disease extent was limited to the ileum in all cases with CD (N=3), and among those with UC (N=3) the disease was mainly classified as pancolitis (67%) followed by left-sided colitis (33%). Most patients and controls lived on urban areas, without statistical differences between patients and controls. No relevant occupational exposures were identified or reported by patients, and no imbalance between patients and controls was observed.

We found statistically significant higher levels of sodium (Na), potassium (K), boron (B) among cases compared to controls (Figure 1). The lower limit of detection among them was 6, 3, and 0.07, respectively. On the contrary, they demonstrated lower levels of zinc (Zn), uranium (U), copper (Cu), and germanium (Ge). The lower limit of detection was 57, 0.0, 7.8, and 0.03, respectively. The remaining minerals and trace elements showed similar levels among cases and controls.

**Discussion**

Our study shows that patients with subclinical inflammatory lesions observed within the colorectal cancer screening programme are associated with significant differences in certain elements, including sodium, potassium, boron, zinc, uranium, copper, and germanium. This finding provides new data on environmental factors associated to subclinical inflammatory changes at the mucosal level before the symptomatic onset of IBD. The differential exposure to these components might be associated with the early events in the pathophysiology of the disease but they could be also a consequence of an altered gut permeability. Other factors, including the role of industrialization, gut microbiome function and composition, or nutritional deficiencies should be also considered regarding these findings.

The most common micronutrient deficiencies in patients with IBD include iron, vitamin B12, vitamin D, folic acid, selenium, zinc, and vitamin B6, and this is expected to be even more prominent in recently-diagnosed patients (9, 10). However, exposure to minerals and other trace elements has not been properly explored in IBD. Hair concentrations of different
compounds have been assessed in children with newly-diagnosed IBD and adults, and they are considered reliable markers of their serum concentrations (8, 11). In adults with CD, the hair concentrations of iron, chromium and cobalt have been found significantly higher as compared to control subjects, and particularly high concentrations were found in CD patients with low serum ferritin (8). Though, the exact role of many trace elements is still unclear and their relationship with IBD remains to be determined. More aspects still need to be further evaluated, including their biological effects, their relationship with an impaired gut barrier function or their potential influence on clinical disease activity (4, 5). For instance, boron has not been identified a clear biological function, but some studies suggest that it may have health benefits if consumed in the daily diet through the reduction of certain cytokines and also in some functions as reproduction and development, calcium metabolism, bone formation, brain function, insulin and energy substrate metabolism, immunity, and the function of steroid hormones (12, 13). Other elements as zinc deficiency has been already associated with an increased risk of CD but not UC (14, 15). Moreover, patients with IBD and low levels of zinc have a six-fold increase in the risk of relapse in patients in clinical and biochemical remission (16). The association between zinc deficiency and an increased gut permeability has been considered as a possible explanation for this finding, and some studies have suggested that zinc supplementation can resolve this gut barrier dysfunction (17). Therefore, the low zinc exposure observed in our study is in line with these findings and it may suggest potential targets for intervention through diet and supplementation.

Uranium is a naturally occurring heavy metal, and trace amounts of uranium can be detected. As a heavy metal, it exhibits potential chemical toxicity, similar to that of lead, and this effect is of much greater concern than its radiotoxicity. In our study, patients with preclinical CD showed lower levels of uranium compared to controls, and the biological effect of this finding needs further confirmation and to exactly determine its relationship with gastrointestinal diseases. Copper is involved in energy production, iron metabolism, neuropeptide activation, connective tissue and neurotransmitter synthesis. Among them, ceruloplasmin is the most important, carrying more than 95% of the total copper in human plasma. Copper deficiency
is rare, and it can include anemia, hypopigmentation, hypercholesterolemia, connective tissue disorders, osteoporosis and other bone defects, abnormal lipid metabolism, ataxia, and increased risk of infection. Lower levels of copper have been described in celiac disease, Menkes disease and people taking zinc supplements. Other components like germanium are classified as a metalloid or semi-metal, and they have no known biological role.

Despite some positive aspects of our approach, including the recruitment of asymptomatic patients with confirmed mucosal lesions from the same geographical region and clinical setting, an evaluation of a wide range of compounds in hair samples, and considering potential confounding factors as occupational exposures (18), we should also acknowledge some limitations. Mainly, the different age and proportion of smoking habits between cases and controls may have influenced our findings. Despite the different distribution of them between both cohorts, we consider that smoking might probably be more important than age in our findings (19). Active smoking has been associated with an impaired gut barrier function, that will ultimately lead to increased permeability (19), but its influence among former smokers and at different segments of the gastrointestinal tract is still unclear. Regarding some patient characteristics (e.g. age), our cohort with incidental IBD findings may correspond to a subgroup of patients with a slightly different phenotype. Despite this consideration, no other target population undergoes endoscopic examinations while being asymptomatic and would bring the opportunity to capture the subclinical inflammatory process in a relevant number of patients.

In conclusion, our findings suggest that non-invasive biomarkers can reflect the integrity of the intestinal barrier in patients with subclinical inflammatory lesions preceding the onset of IBD. However, their exact relationship and prognostic impact would need to be determined in future studies.
References


Table 1. Patient characteristics.

<table>
<thead>
<tr>
<th></th>
<th>Cases N=6</th>
<th>Non-IBD controls N=13</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, median (IQR)</strong></td>
<td>58 years (54-62)</td>
<td>35 years (33-39)</td>
</tr>
<tr>
<td><strong>Gender, women, n (%)</strong></td>
<td>4 (67%)</td>
<td>9 (69%)</td>
</tr>
<tr>
<td><strong>Type of IBD, n (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td>3 (50%)</td>
<td></td>
</tr>
<tr>
<td>Crohn's disease</td>
<td>3 (50%)</td>
<td></td>
</tr>
<tr>
<td><strong>UC extent, n (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left-sided colitis</td>
<td>1 (33%)</td>
<td></td>
</tr>
<tr>
<td>Pancolitis</td>
<td>2 (67%)</td>
<td></td>
</tr>
<tr>
<td><strong>CD location, n (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ileal</td>
<td>3 (100%)</td>
<td></td>
</tr>
<tr>
<td><strong>CD behaviour, n (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-stricturing non-penetrating (B1)</td>
<td>2 (67%)</td>
<td></td>
</tr>
<tr>
<td>Stricturing (B2)</td>
<td>1 (33%)</td>
<td></td>
</tr>
<tr>
<td>Penetrating (B3)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><strong>Smoking habits, n (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Former</td>
<td>5 (83%)</td>
<td>1 (8%)</td>
</tr>
<tr>
<td>Never</td>
<td>1 (17%)</td>
<td>12 (92%)</td>
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<tr>
<td><strong>Place of residence, n (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>6 (100%)</td>
<td>12 (92%)</td>
</tr>
<tr>
<td>Rural</td>
<td>0</td>
<td>1 (8%)</td>
</tr>
</tbody>
</table>
Figure legends

Figure 1. Hair concentrations of trace elements and minerals in patients with preclinical IBD and healthy controls.

Data availability

Original data from this study will be provided upon reasonable request to the corresponding author.
Figure 1

### Sodium

- Concentration (µg/g)
- Case: X ± Y
- Control: X ± Y
- p=0.022

### Potassium

- Concentration (µg/g)
- Case: X ± Y
- Control: X ± Y
- p=0.046

### Zinc

- Concentration (µg/g)
- Case: X ± Y
- Control: X ± Y
- p=0.036

### Uranium

- Concentration (µg/g)
- Case: X ± Y
- Control: X ± Y
- p=0.001

### Boron

- Concentration (µg/g)
- Case: X ± Y
- Control: X ± Y
- p=0.009

### Copper

- Concentration (µg/g)
- Case: X ± Y
- Control: X ± Y
- p=0.003

### Germanium

- Concentration (µg/g)
- Case: X ± Y
- Control: X ± Y
- p=0.046