Humoral Immune Response And Luminal Microorganisms In Patients With Indeterminate Colitis

B Mahdi, W Salih, B Kadum, R Hasan, M Ameen

Citation

Abstract

Background: Microorganisms that directly interact with the intestinal mucosa are obscured by fecal flora. Some members of the endogenous faecal microflora have a clear detrimental role in most animal models of colitis and enteritis. This is strongly suspected to be the cause of indeterminate colitis (IC).

Patients and methods: The study consisted of two groups: 75 patients groups with IC and control group consisted of 30 healthy volunteers. Sigmodoscope and colonoscope examination were done for the patients group and biopsy were also taken from suspicious lesion for histopathological examination for confirming the diagnosis. Blood samples were collected from them and serum were collected for immunoglobulins (IgG, IgM and IgA) levels and complement (C3 and C4) level by single radial immune diffusion method (Biomaghrib-Tunis). Fecal and rectal swabs were taken from those groups, cultured on different bacteriological media, and isolated using different bacteriological methods for enteric bacteria isolation according to type of bacteria.

Results: Male more than female are affected. About 53.3 % had bleeding per rectum. Most of their diseased location was in rectum then sigmoid and ascending colon. Bacteriological results showed a significant decrease in the existence of Bacteroids fragilis (anaerobic bacteria) in patients group compared with control. Studying humoral immune response demonstrated a significant higher level in IgG, IgA, C3 and C4 in patients group and significant decrease in IgM level in patients group.

Conclusions: Reduction in anaerobic bacteria might be a cause in initiation colitis with stimulation of immunoglobulins production and complement activation to overcome this inflammation.

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic inflammatory disorder of the gastrointestinal tract that include (ulcerative colitis (UC), Crohn’s disease (CD), indeterminate colitis (ID) and other types of colitis) (1). While the etiology of IBD remains obscure, it is thought to be the result of a combination of interaction between genetic, the immune system and environmental factors(2).

There was an evidence indicates that IC is the result of dysregulated immunogenetic parameters that depends on impaired coordination between luminal microorganisms, gut epithelium, and the host immune system in genetically susceptible individual(3). The luminal microorganisms in the distal ileum and colon contain high concentrations of bacteria (> 10^12 organisms/g), these may include pathogens that could be directly responsible for initiating and promoting IBD in the context of an underlying genetic mucosal or immune defect. Studies have shown that there are differences in the microbiota between healthy and IBD subjects , one of the differences is that there is a decrease biodiversity in IBD compared to healthy subjects by 30%-50%(4). There are pathogens that are found in increasing frequency in IBD and have been implicated to associate with its development. These pathogens include Pectinatus, Sutterella, Fusobacterium, Verrucomicrobiun, various Clostridium, Mycobacterium paratuberculosis, M. paramyxovirus, Listeria monocyogenes, and Helicobacter hapaticus (5,6). Despite the differences in the microbiota, one must keep in mind that the dysbiosis seen in IBD patients may not be causal, but simply reflect the different ecological conditions of the inflamed gut such as changes in pH redox potential, substrate availability, etc.

The importance of the microflora in the induction and maintenance of disease has been demonstrated in murine model of colitis that it is genetically predispose to disease( for example mice deficient in IL-10 or IL-2 cytokines (7). Different normal bacteria may lead to different types of colitis in the same genetic host. In (IL-10/-/- mice), E. coli induced proximal colitis whereas Enterococcus faecalis lead to distal colitis (8).
Subsequently, several studies showed serum responses to various bacterial antigens and loss of tolerance to pathogenic as well as commensal bacteria in clones derived from peripheral and lamina propria T-cells(9,10). This indicates that disordered features of T-cell microbial recognition and effector function are likely to be important to IC disease biology (11).

This study tried to investigate the type of bacteria that colonize the colon of IC patients and compare it with control group. Secondly, we tried to demonstrate the humoral immune response in those patients with complement activity in their serum and evaluate it with control group. Lastly to find if there was any correlation between immunoglobulins and complement level and result of colonscope.

PATIENTS AND METHODS

PATIENT GROUP: Consisted of 75 patients with IC, median age was 35 years, 50 of them were male and the rest were female. Diagnosed as IC by clinical physician according to the clinical presentations and examinations, endoscope examinations, radiological results and histological results. They were admitted to Al-Kindi Teaching Hospital – Colonoscopic department from Jun -2008 to May- 2009. All enrolled patients were not under any antibiotic treatments.

ENDOSCOP EXAMINATION: Sigmoidoscope and colonoscope examination were done for the patients group. The location of disease was determined. They were divided according to the results of colonoscope examination into two groups (normal and abnormal colonoscope examinations).

HISTOPATHOLOGICAL EXAMINATION: Biopsy were taken from the suspicious lesion of colonic mucosa of patients group and send for histopathological examination.

CONTROL GROUP: Consist of 30 healthy volunteers, median age was 33 years, 15 of them were male and other 15 were female.

At the beginning blood samples were collected from two groups and serum were collected and stored at -10C till examination was done in the microbiology and immunology laboratory in Al-Kindi- College of Medicine – Department of microbiology/ Baghdad University, for immunoglobulins (IgG, IgM and IgA) levels and complement (C3 and C4) level by single radial immune diffusion method (Biomaghrib-Tunis).

Fecal and rectal swabs were taken from these groups, cultured on different bacteriological media, and isolated using different methods for enteric bacteria isolation according to type of bacteria (12), this was done in microbiology and immunology laboratory in Al-Kindi- College of Medicine – Department of microbiology/ Baghdad University and in the microbiology and immunology laboratory in Al-Kindi Teaching Hospital.

STATISTICAL ANALYSIS

Student's t-test used in analysis the data statistically. Results were expressed as mean ± SEM. and correlation coefficient was calculated (13).

RESULTS

The demographic data of IC patients are demonstrated in Table-1-. Median age was 35. Most of them were smoker and Male more than female. About 53.3 % were complaining from bleeding per rectum. The commonest site of lesions was in the rectum, then sigmoid and ascending colon.

Colonoscopy was done and 42 of them (56%) had normal examinations and the rest 33 (34%) had abnormal colonoscopy (edema, lose of normal vascular pattern, ulceration and bleeding) (figure-1-2-). Biopsy was taken from suspicious lesions and histopathological examination was done to confirm the diagnosis of IC.

The type of luminal bacteria were detected using fecal material and rectal swab. As shown in table-2-, There was a significant decrease in the existence of anaerobic spp. of bacteria in patients group compared with normal control set. Humoral immune response was assessed by measurement Immunoglobulins and complement levels. Table-3 – demonstrated significant higher level in IgG, IgA, C3 and C4 in normal colonoscopy in comparison with other groups and significant decrease in IgM level in normal colonoscopy.

There was no significant correlation (r =+ 0.434) between the level of immunoglobulins and complement with the results of colonoscopy exam (normal and abnormal) table-4-.
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Figure 1
Table-1- Demographic data of IC compared with healthy control.

<table>
<thead>
<tr>
<th></th>
<th>IC patients</th>
<th>Healthy control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Age</td>
<td>55</td>
<td>Median</td>
</tr>
<tr>
<td>Sex ratio M:F</td>
<td>59:25</td>
<td>66.6:33.3</td>
</tr>
<tr>
<td>Duration of disease</td>
<td>1.6 m.</td>
<td>-</td>
</tr>
<tr>
<td>Bleeding per rectum</td>
<td>40</td>
<td>53.3</td>
</tr>
<tr>
<td>Location of disease</td>
<td>Rectum</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Sigmoid colon</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Ascending colon</td>
<td>17</td>
</tr>
<tr>
<td>Transverse colon</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>Descending colon</td>
<td>5</td>
<td>6.6</td>
</tr>
</tbody>
</table>

Figure 2
Table-2- Type of luminal bacteria that could be isolated from both groups.

<table>
<thead>
<tr>
<th>Type of bacteria</th>
<th>Patients group (NO=75)</th>
<th>Control group (NO=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteroides fragilis</td>
<td>11</td>
<td>15.5</td>
</tr>
<tr>
<td>Enterobacter spp.</td>
<td>8</td>
<td>11.8</td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td>3</td>
<td>4.7</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>14</td>
<td>20.0</td>
</tr>
</tbody>
</table>

Figure 3
Table-3- Mean± SEM of Immunoglobulins and complement levels in both groups.

<table>
<thead>
<tr>
<th>Types of Ig and Complement</th>
<th>Patients group (NO=75)</th>
<th>Control group (NO=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>8183±2,137</td>
<td>1189±2,98</td>
</tr>
<tr>
<td>IgM</td>
<td>150±0,86</td>
<td>116±1,62</td>
</tr>
<tr>
<td>IgA</td>
<td>204±7,11±2,3±1,2±3</td>
<td>203±1,15</td>
</tr>
<tr>
<td>C3</td>
<td>1152±4,76</td>
<td>85,9±0,79</td>
</tr>
<tr>
<td>C4</td>
<td>356±2,65</td>
<td>30,9±0,36</td>
</tr>
</tbody>
</table>

Figure 4
Table-4- correlation between the level of immunoglobulins and complement with the results of colonoscopy
DISCUSSION

The data and studies have incriminated intestinal bacteria in the initiation of colitis(14). The intestinal microbiota play a crucial role in perpetuating this inflammation in both animal and patients models (15). Thus, exclusion of the faecal stream from the diseased bowel has been successfully used in the treatment of many patients. This can be achieved medically by total parenteral nutrition1(16) or elemental diets (17). Thus in our study, there were a significant decrease in Bacteroids fragilis (anaerobic bacteria) and significant increase in Enterobacter spp in patients with abnormal colonoscopy. This was in agreement with Ott etal ,2009(18) who showed mucosal inflammation is associated with loss of a normal anaerobic bacteria .This may be due to a breakdown in the balance between putative species of “protective” versus “harmful” intestinal bacteria. This concept has been termed “dysbiosis”(19). The other reason might be due to change in luminal PH redox potential inside the lumon of the intestine. The intestinal microflora have been analysed repeatedly by different methods like (culture and DNA based methods) .Culture remained the gold standard for identifying bacteria and the sensitivity of the culture is high for most rods under optimal conditions. In a complex bacterial population, however, rapid growing bacteria overgrow the culture plate, making the identification of slow growing bacteria impossible so selective media can help to overcome this problem. Accordingly, biodiversity of the microflora especially Enterobacteria remains high in colitis (20). Other studies showed higher numbers of E.coli in patients group than control (21). In this study, E coli did not significantly difference in patients group which is the main cause of proximal colitis (8). Other type of bacteria was Staph.epidermidis, showed significantly increased which might be due to contamination from perinea .Other types of bacteria that could be isolated (Klebsiella, Proteus, Enterobacter, Streptococci and Staphylococcus aureus) did not significantly different from the control group. One can concluded that Bacteroid fragilis (anaerobic bacteria) might be an important cause in induction colitis. Certain probiotic microorganisms (normal intestinal microflora) such as Lactobacillus, Bifidobacterium, and Saccharomyces have been shown to be effective in remission of colitis, suggesting anti-inflammatory effects of these bacteria (19).Other types of pathogenic bacteria that leads to colitis could not be isolated in this study.

Studying humoral immune response in IC patients showed significant increased in IgG, IgA, C3 and C4 in order to overcome the inflammation. In case of IgM , showed decreased its level in comparison with control , this may be due to secondary immune response initiation and IgM level return to its level because its only raised in primary immune response and the duration of disease in those patients were (6-12 months). In case of IgA , it was increased in intestinal secretions and other types of secretions. At the same time, the higher level of complement indicated its activation due to the presence of antigen-antibody immune complex leading to complement activations or activation by alternative and lectin pathways. Other studies showed significantly higher systemic antibody responses in patients group, in parallel with higher recovery rates(22). Others found, there was a significant rise in the number of IgG cells at the expense of IgM (23). Lastly , there was no correlation between humoral immune response and the degree of colonic lesion because we studied the systemic immune response and not localized humoral immune response in the colonic mucosa . So studying humoral immune response in
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Thus changes in bacterial flora in IC are not secondary to inflammation, but it may be a cause for induction colitis as a result of a specific host immune response derangement. Therefore, healthy mucosa is capable of holding back fecal bacteria and this function is profoundly disturbed in patients with IC (24).

References

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