Humoral Immune Response and Luminal Microorganisms in Patients with Indeterminate Colitis

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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.

Objective: Determination of humoral immune response and luminal microorganisms in patients with indeterminate colitis.

Patients & Methods: The study consisted of two groups: 75 patients groups with indeterminate colitis and control group consisted of 30 healthy volunteers. Sigmoidoscope and colonoscope examination were done for the patients group and biopsy were also taken from ulcer 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.

Results: Males more than females are affected. The majority of them were complaining from rectal bleeding. 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.

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

Key wards: colitis, Immunoglobulins, anaerobic bacteria.

Introduction:

Inflammatory bowel disease (IBD) is a chronic inflammatory disorder of the gastrointestinal tract that includes (ulcerative colitis (UC), Crohn's disease (CD), indeterminate colitis (IC) 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 is an evidence indicates that colitis 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 (> 0^{12} organisms/g); these may include pathogens that could be directly responsible for initiating and promoting colitis in the context of an underlying genetic mucosal or immune defect. Studies have shown that there are differences in the microbiota between healthy and colitis subjects, one of the differences are that there is a decrease biodiversity in colitis compared to healthy subjects by 30%-50% [4]. There are pathogens that are found in increasing frequency in colitis and have been implicated to associate with its development.

These pathogens include *Pectinatus*, *Sutterella*, *Fusobacterium*, *Verrucomicrobium*, various *Clostrodia*, *Mycobacterium paratuberculosis*, *M. paramyxovirus*, *Listeria monocytogenes*, and *Helicobacter hapaticus* [5,6].

Despite the differences in the microbiota, one must keep in mind that the dysbiosis seen in colitis patients may not be causal, but simply reflect the different ecological conditions of the inflamed gut such as changes in p_H 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 predisposed 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 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 colitis disease biology [11].

So this study tried to investigate the type of bacteria that colonize the colon of IC patients and compare it with control group. In addition to that, to demonstrate the humoral immune response in those patients with complements activity in their serum and evaluates it with control group.

Patients & Methods:

Patients Group: Consisted of 75 patients with IC, median age was 35 years, 50 of them were male and the rest were female. They were diagnosed as IC by clinical physician according to the clinical presentations, endoscopic, radiological and histopathological 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.

Endoscopic Examination: Sigmoid scope and colonoscope examination were done for the patients group. The location of disease was determined.

Histopathological Examination: Biopsy was taken from the ulcer 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^0 till examination was done in the Microbiology and Immunology laboratory in Al-Kindi- College of Medicine – Baghdad University, Estimation of immunoglobulin's level (IgG, IgM and IgA) and complement (C3 and C4) were done by single radial immune diffusion method (Biomaghrib-Tunis).

Fecal and rectal swabs were taken from these groups, cultured on different bacteriological media (Tetrathionate broth and alkaline peptone water for enrichment). Other bacteriological media (MacConkey, *Salmonella-Shigella*, Thiosulfate citrate bile salts sucrose, blood, Chocolate, thioglycollate or cooked meat) (Oxoid) and bacteria were isolated using biochemical and analytical profile index (API) (BioMerieux-France) for its isolation [12], this was done in Laboratory of Microbiology in Al-Kindi Teaching Hospital. Statistical analysis: Student's t-test used in analysis the data statistically. Results were expressed as mean±SEM. [13].P-value less than 0.05 was considered significant.

Results:

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

Colonoscopy were done, they 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** here 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 Immunoglobulin and complement levels. **Table-3-** demonstrated significant higher level in IgG, IgA, C3 and C4 in IC patients group in comparison with control and significant decrease in IgM level.

Table-1- Demographic data of indeterminate colitis compared with healthy control.

	Indeterminate colitis patients		**Healthy control**	
	No.	**%**	**NO.**	**%**
Age	35 median	-	33 median	-
Sex ratio M/F	50/25	66.6/33.3	15/15	50/50
Smoking	42	56	19	31.6
Duration of disease	1-6 ms.	-	-	-
Bleeding per rectum	40	53.3	-	-
Location of disease				
Rectum	25	33.3	-	-
Sigmoid colon	19	25.3	-	-
Ascending colon	17	22.6	-	-
Transverse colon	9	12	-	-
Descending colon	5	6.6	-	-

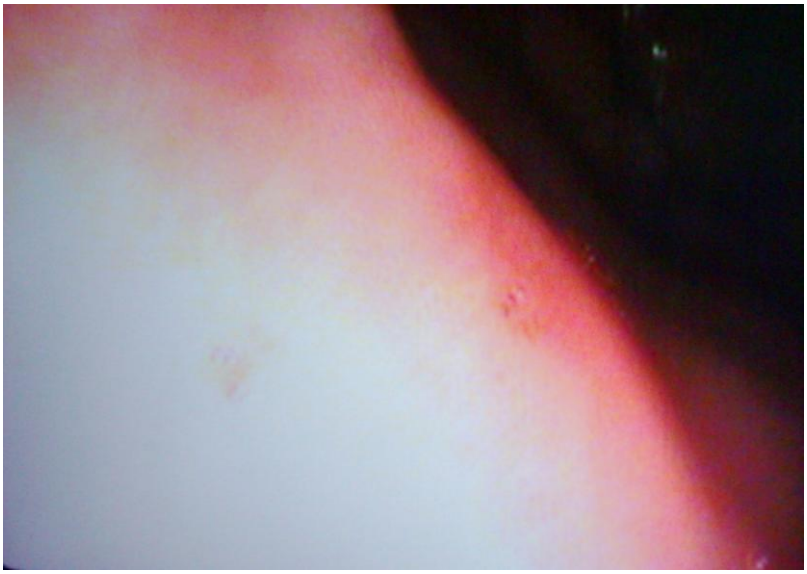

Figure-1- Edematous mucosa and loss of vascular pattern of colonic mucosa.

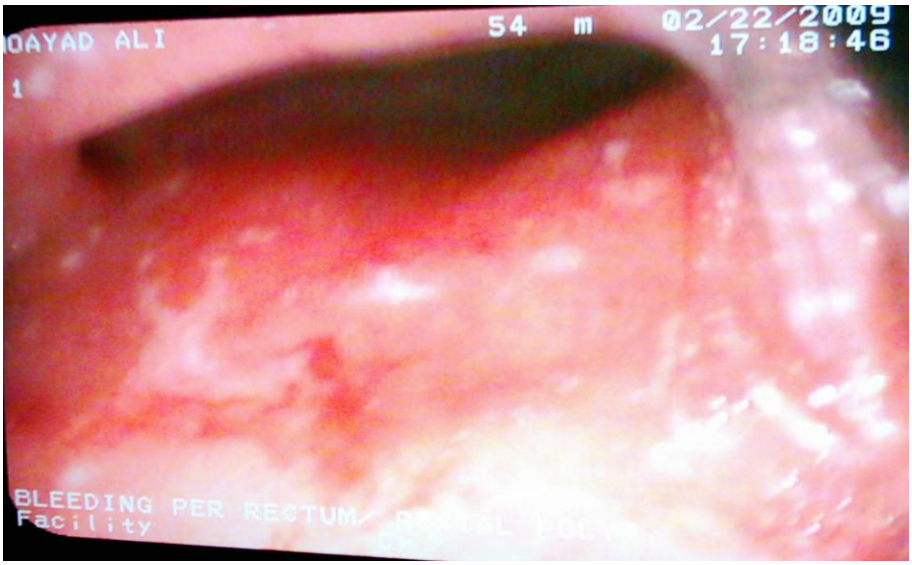

Figure-2- Edematous colonic mucosa with ulceration.

Table-2- Type of luminal bacteria that isolated from patients with indeterminate colitis and control group.

Type of bacteria	Indeterminate colitis group N=75		Control group N=30		P-value
	No.	%	No.	%	
Bacteroids fragilis	27	36	27	90	p<0.001
Escherichia coli	18	24	10	33.3	NS
Klebsiella spp.	29	38.6	10	33.3	NS
Proteus spp.	5	6. 6	1	3.3	
Enterobacter spp.	27	36	7	23.3	p<0.001
Streptococci spp.	5	6.6	0	0	NS
Staphylococcus aureus	7	9.3	0	0	NS
Staphylococcus epidermidis	14	18.6	1	3.3	p<0.001

NS= not significant

Table-3- Serum immunoglobulin (IgG, IgM and IgA) and complement (C3 and C4) levels of patients with indeterminate colitis and control group.

Types of Ig and Complement	Patients group N=75 Mean±SEM	Control group N=30 Mean±SEM
IgG	8183.08±12.27, P<0.001	1273±3.18
IgM	150.5±0.86, P<0.001	249±2.08
IgA	2049.2±6.71, P<0.001	114±1.45
C3	1152.3±4.76, P<0.001	139±1.24
C4	356.5±2.65, P<0.001	30±0.43

Discussion:

The 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 nutrition [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 Indeterminate colitis. This was in agreement with Ott *et al*, 2009 [18] who showed mucosal inflammation is associated with loss of 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 P_H redox potential inside the lumen of the intestine.

The intestinal microflora have been analyzed 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* remain high in colitis [20]. Other studies showed higher numbers of *E. coli* in patients group than control [21] which is disagree with this study. It may be due to lesion site of the colon that was affected because *E coli* are 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*, *Streptococci* and *Staphylococcus aureus*) did not significantly different from control group. One can concluded that *Bacteroid fragilis* (anaerobic bacteria) might be an important cause in induction indeterminate 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].

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. 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].

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].

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