

The Role Of T Helper Cytokines In Indeterminate Colitis

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Abstract : Background: Indeterminate colitis is one of the inflammatory bowel diseases that cannot be classified over time as having ulcerative colitis or Crohn's disease. Dysregulated immunity has been implicated in indeterminate colitis. Aim: to measure serum levels of pro-inflammatory cytokines (tumor necrosis factor (TNF- α), interferon-gamma (IFN- γ) and IL-6 and anti-inflammatory cytokines IL-10 in indeterminate colitis patients. Methods: A cross-sectional study carried out in Al-Kindy Teaching Hospital, Baghdad city from 2007 - 2010. Sixty patients with indeterminate colitis participated in the study. Serum levels of cytokines (IL-6, IL-10, TNF-alpha and IFN-gamma) were done by Enzyme Linked Immuno Sorbent Assay (ELISA) and compared with age and sex matched thirty healthy controls. Results: There is a significant increase in IL-10 ($p=0.003$) and IFN-gamma ($p=0.0001$) in indeterminate colitis patients. TNF-alpha was significantly decreased ($p=0.0001$) while IL-6 showed no significance different with control group ($p=0.735$). Conclusions: There is increase in IL-10 and IFN-gamma levels and decrease in TNF-alpha levels in patients with IC.

Key words: Cytokines, inflammatory bowel diseases, Indeterminate colitis

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INTRODUCTION: Kent et al ¹ first used the term indeterminate colitis (IC) when evaluation rendered a diagnosis of UC or Crohn's indefinite. Thus, some pathologists suggest that IC is a temporary diagnosis ². Burakoff supported the premise that IC may be a separate entity in 2004 ³. This favors the broader uses of IC, which is based on endoscopic, histologic, and radiologic tools. The pathologist Geboes K *et al* 2009 ⁴. used the term inflammatory bowel disease unclassified and the surgeons used the term indeterminate colitis for the same condition.

The etiology of this disease is unknown and believed to be an immunologically mediated disease in a genetically susceptible host. It results from an aberrant immune response and loss of tolerance to many self-antigens, leading to chronic inflammation of the gut. This idea is supported by the occurrence of antibodies directed to microbial and self-antigens and by the identification of

NOD2/CARD15 as a gene conferring susceptibility to some diseases ⁵. This inflammation is triggered by cytokines secreted by helper T cells that are important in the regulation of immune responses. T cells have been divided into two functionally important types based on the cytokines they produce ⁶. It provides a clinically useful framework to understand T cell responses in the normal mucosal immune system and in IBD. T-helper-1 cells (TH1) produce the inflammatory cytokines IFN-gamma, TNF-alpha and IL-2. The important regulatory T cell populations function to keep mucosal TH1 immune responses in check are T-helper-2 (TH2) cells that produce the cytokines IL-4, IL-5, IL-6, IL-10, and IL-13. These cytokines provide efficient help for B cell antibody production and are involved in host defense ⁷. TH1 and TH2 cells may reciprocally down regulate each other. Regulatory T cell subsets have a primary role in inhibiting

inflammatory responses by production large amounts of IL-10 and IFN-gamma⁸.

This study was carried out to determine the level of cytokines in the serum of IC patients.

MATERIAL AND METHODS: This cross – sectional study included 60 patients with indeterminate colitis (IC). They were defined and diagnosed as IC by their specialist physicians according to the clinical, endoscopic, radiological and histopathological examinations. The inclusion criterion was biopsy findings that were inadequate to make a definitive diagnosis of either UC or CD. They were consulted Al-Kindi Teaching Hospital from 2007 - 2010 at Baghdad city.

Criteria for Diagnosis of indeterminate colitis:

Patients with clinical features of chronic IBD, without small bowel involvement, in whom endoscopy was inconclusive and -histopathology revealed active and patchy transmucosal chronic inflammation with minimal or moderate architectural distortion and absence of diagnostic features for either CD or UC. Small bowel involvement was excluded by macroscopic and microscopic evaluation of the terminal ileum at ileocolonoscopy and by small bowel x-ray barium follow-through studies.

The histopathological study were done in triplicate, the slides read by more than one observer who were blind about it. When all pathologists agreed about the diagnosis of IC, the slides considered as a case of IC.

Patients with polyp , hamartomatous, hyperplastic and inflammatory , diverticular disease, hemorrhoids and drug -induced colitis were excluded from the study. Infectious colitis and other causes of colitis were excluded by stool cultures and parasitic examinations.

The second set was control group, consisted of 30 healthy volunteer's age- and sex-matched with first group from staff .

Cytokines study: Blood samples (5 mL) were drawn into plain vacutainers from the antecubital vein. The blood was allowed to clot for 30 minutes and centrifuged at 2000g for 15 minutes for clear separation of serum. Separated serums were stored at -20°C until analyzed. Estimation of cytokines (IL-6, IL-10, TNF-α and IFN-γ) were done by ELISA method using US Biological Kit - USA.

The Ethical Committee of the Al-Kindi College of Medicine, Baghdad University and Al-Kindi Teaching Hospital approved the study, and all samples were obtained with informed consent in accordance with the Al-Kindi Teaching Hospital Declaration.

Statistical analysis: Data were analyzed statistically using descriptive statistics (Frequencies for tables, mean and standard deviation, inferential statistics (Chi- square test). Considering the scattering of the collected data, the non-parameter Mann-Whitney test is used for measuring the differences between the groups. All of these were done using MiniTab statistical software program 13.20. A P- value ≤ 0.05 was considered significant.

RESULTS: 66.6% were men in both groups. Detail characteristic of patients is shown in table-1. Age and smoking had no significant effect on indeterminate colitis . Mean age of patients group was 45.67±SD15.54 that was not significant difference (p=0.629) from mean control 44± SD 15.54. The percentage of positive smoking patients were 41.6% which was not significant difference (p=0.648) with control group 63.3%.

Cytokines profile showed significant increase in IL-10 (p=0.003), IFN-γ (p=0.0001) and significant decrease in TNF- alpha (0.000) in IC patients compared –to controls. In addition to that, there

was no significant difference in IL-6 between two groups as shown in table-2.

Sex disparity between indeterminate colitis patients and control group (Table-1-).

Sex	IC patients (n=60){%}		Control group (n=30){%}		P-value
Male	40	66.6	15	50	0.126
Female	20	33.3	15	50	

The difference in the median levels of serum IL-6 ,IL-10, TNF- α and IFN- γ (pg/ml) concentration between healthy and IC patients group (Table-2-).

values	IC patients (n=60){%} Median	Control group (n=30){%} Media	Mann – Whitey P -value
IL-6	5.67 (2.21-48.1)	5.5 (2.5-6.5)	0.735
IL-10	20.0 (3.8-59.76)	6.7 (1.0-45.1)	0.003
TNF α	13.23 (2.0-27.2)	25.0 (10.0-33.7)	0.000
IFN- γ	39.25 (15.9-12.0)	17.0 (12.0-25.0)	0.000

DISCUSSION: There is no effect of sex and age on IC. In addition to that, smoking had no effect on the development of this disease. Colitis represents a disease characterized by an inflammation of the intestinal mucosa associated with a dysregulation of the immune system ⁹. Under normal conditions, there is in a state of “controlled” inflammation regulated by a balance of proinfl ammatory and anti-inflammatory cytokines. In this study, there is a significant increase in the IL-10 (p=0.003) that down regulate the IL-6 that is not significant difference with control group. IL-10 inhibits the production of IL-6, and TNF- alpha that there is significant decrease in TNF- alpha (p=0.000) production in our

results. In animal, IL-10 preserves immune regulation in the gut and may play a role in the treatment of enterocolitis ¹⁰. IL-6 is a cytokine with a central role in immunoregulation and inflammation ¹¹. Increased serum concentrations of IL-6 and soluble IL-6 receptor (IL-6R) have been correlated to clinical activity of colitis, and animal models have recommended in the therapeutic possibility of anti-IL-6R monoclonal antibody in colitis ^{12,13}.

TNF-alpha had a multiple proinflammatory properties that secreted by monocytes and macrophages upon activation, and encourage intestinal macrophages, neutrophils and fibroblasts to secrete prostaglandins, proteases and other mediators of inflammation. TNF confirmed in the genesis of colitis ¹⁴. In our study, we found significant decrease in serum level of TNF in IC patients that help in healing the inflamed mucosa and predict a good prognosis of IC patients

IFN-gamma . is anti-inflammatory cytokine that secreted by Th1 cells and elevated in animal models of colitis but ablation of IFN- γ gene in T cells does not prevent the disease ¹⁵ . Our study showed significant increased (p=0.000) in IFN-gamma level. Furthermore, the significantly higher IFN-gamma serum levels in patients with IC suggest the use of anti- IFN-gamma in such patients.

Thus, the use of biologic therapy that neutralize the pro-inflammatory cytokines and use of anti-inflammatory cytokines instead of corticosteroid and immunosuppressive drugs ¹⁶.

CONCLUSIONS: Increased IL-10 that down regulate TNF-alpha and IL-6 and increased in IFN-gamma. Therefore, we may use interleukins and TNF as biological therapies however; this needs to be confirmed by further research.

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