CD4+ and CD8+ T Cell Count in Colonic mucosa of patients with IBD and IBS

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Abstract: Background: Ulcerative colitis (UC) and Crohn's disease (CD) are of bowel immune-mediated disorders. Irritable bowel syndrome (IBS) is a multi-factorial functional disease. The aim of study was evaluating of T cells infiltration and counting of two important phenotypes (CD4+,CD8+) between IBS and IBD (Inflammatory Bowel Disease)subtype of Ulcerative colitis patients. **Methods:** In this analytical-descriptive study, 83 patients including 35 patients with ulcerative colitis and 48 patients with IBS were compared with 37 healthy volunteers. Tissue samples were obtained by colonic biopsy during colonoscopy and evaluated by histological and Immunohistochemistry (IHC) techniques. **Results:** The study compared 37 healthy volunteers, 35 patients with ulcerative colitis, and 48 patients with IBS. Statistically, there was not any significant difference between these groups and they were matched considering age and gender. There was similarity between IBS patients and healthy volunteers considering $CD4^+$ and $CD8^+$ T cells count and their ratio. However, $CD4^+$ T cells count and $CD4^+/CD8^+$ ratio were significantly higher in ulcerative colitis than healthy volunteers ($p<0.001$) while $CD8^+$ count was lower in comparison with IBS patients and healthy volunteers ($p<0.001$). **Conclusion:** $CD4^+$ and $CD8^+$ T cells infiltrations do not play an important role in mucosal immunity disorder of IBS patients. Further studies are required to evaluate others T cells phenotypes or immune mediators in future.
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1. Introduction

Irritable bowel syndrome (IBS) is a multi-factorial functional disease and is one of the most prevalent diseases of digestive system among patients referring to gastroentrology clinics (Thompson, 1999). There was little medical development for the disease due to its complexity and availability of less pathological information. Variations in bowel movements and increase of gut sensivity perception of the patients have been introduced as mechanisms of the disease. It seems that the mentioned physiological factors are not significantly involved in the disease pathogenesis. Therefore, mucosal immune factors and genetic background are concerned in the disease pathogenesis, according to modern infection theories (Camilleri and Ford, 1998; Barbra, 2004). Irritable bowel syndrome manifested by stomach pain and excretion changes and is experienced by 9-13% of normal population during their life. The disease begins acutely in 20-30% of IBS patients associated with bowel disorders and acute bowel infections (Heaton, 1992; Harvey, 1987). According to modern theories, serotonin metabolism and genetic factors involved in the disease pathogenesis result in functional bowel variations. Other researchers introduced infection and immune factors as other causes affecting the disease pathophysiology (Barbra, 2004).

Inflammatory bowel diseases (IBD) are complex and chronic diseases and include ulcerative colitis and Crohn's diseases. They are regarded as bowel immune-mediated disorders. However, its etiology is not exactly known and the primary studies refer to increase of inflammatory cell infiltration in bowel mucusa of the patients (Heaton, 1992; Harvey, 1987). Several studies were conducted about probable role of antibodies and humoral immune system (Dotan, 2007). There was few published study about role of cellular immune status in gastrological functional diseases. Considering role of status of $CD4^+$ and $CD8^+$ T cells lymphocytes in colonic mucus, it seems that responses related to $CD4^+$ and $CD8^+$ T cells have two opposite effects in these patients: the response is mainly necessary in order to protect tissues against organisms through helping B cell lymphocytes to produce neutralizing antibodies, on one hand, and it may damage the bowel if not contrlled through developing inflammatory responses, on the other hand (Holmen and Isaksson, 2007). It is required to study status of $CD4^+$ and

$CD8^+$ T cells lymphocytes as a main representative of cellular immunity in colonic mucosa of IBS and IBD patients. Therefore, the present study aims at quantitatively evaluating $CD4^+$ and $CD8^+$ T cells lymphocytes as well as their ratio as a marker of cell-type special immune system in colonic mucusa of IBS and IBD patients. The findings were compared with that of the healthy subjects.

2. Material and Methods

This analytical-descriptive study was conducted at internal medicine and gastroentrology wards of Imam Reza hospital (affiliated by Tabriz University of Medical Sciences) in 2010. The study was conducted on endoscopic biopsy sample of colonic mucusa of a case group consisting of IBS and IBD patients during necessary endoscopy in order to exclude detectable factors. The control group, i.e. healthy subjects without any gastroentrological complaints, underwent colonoscopy to be evaluated considering non-gastroentrological factors (screening of malignancies and evaluating anemia origin). Finally, 37 healthy subjects were selected as members of the control group. They were studied and compared with patients of two other groups. Case and control groups were matched considering age, gender, and non-consumption of medicines affecting immune system. Patients with records of infectious disease within the last two months as well as patients dissatisfied to participate in the study were excluded.
In IBS and IBD patients with whole abdominal and normal pelvis sonography as well as normal endoscopy without any special microorganism in their biopsy sample, the biopsy samples were obtained in order to count $CD4^+$ and $CD8^+$ T cells and were pathologically evaluated by a pathologist. Slides required to pathological and Immunohistochemical (IHC) evaluations were provided through preparing four-micron cuts, dehydration via putting them in a 37°-oven for one night, putting them at 120° buffer-citrate, adding peroxide hydrogen solution (3%), adding primary $CD8^+$ and $CD4^+$ antibodies of Envision solution, using chromogen, haematoxylin, and slides hydration. About 30 healthy subjects (without any gastrological complaint) who underwent colonoscopy for different reasons were histologically evaluated by the same pathologist. The case and control groups were matched considering age, gender, and non-consumption of medicines stimulating or suppressing immune system. Once qualitative status of the mentioned cells was determined, the resulted information was compared between two groups following statistical processing. Then, the final result was obtained. The present study was supervised and confirmed by ethics Committee of Tabriz University of Medical Sciences.

Statistical analysis

The data was analyzed using descriptive statistical methods (frequency, percentage, mean± standard deviation). Statistical test of variance analysis (ANOVA) was used to descriptively evaluate the data and compare lymphocyte count among three understudy groups. Then, appropriate follow-up statistical test (tukeys-b) was used to compare the groups two and two. To qualitatively evaluate the variable, frequencies test as well as chi-square test was applied using SPSS.17 software. In this study, $P<0.05$ was regarded meaningful.

3. Results

The study was conducted on 120 subjects with the mean age of 40.6±17.5 years. The youngest and oldest subjects were 15 and 89 years old, respectively. The sample was consisted of 53 (44.2%) males and 67 (55.7%) females. In this study, the control group (n=37) was compared with 48 IBS and 35 IBD patients.

According to Table 1, there was statistically meaningful difference among three groups considering $CD4^+$ cell count lymphocytes ($P<0.001$). The follow-up test indicates to the statistical difference found among ulcerative colitis and IBS and the healthy subjects ($P=0.001$). However, there was not any difference between the healthy subjects and IBS patients considering $CD4^+$ cell count lymphocytes ($P<0.1$). Statistically, a meaningful difference was also observed among three groups considering $CD8^+$ cell count lymphocytes ($P<0.001$). Intergroup evaluations referred to a statistically meaningful difference among ulcerative colitis and IBS patients, and healthy subjects ($P=0.001$). However, there was not any significant difference between the healthy subjects and IBS patients considering $CD8^+$ cell count lymphocytes. Statistically, a meaningful difference was observed among three groups considering $CD8^+$ and $CD4^+$ cell count lymphocytes ($P<0.001$). There was not any difference between the healthy subjects and IBS patients in this regard.

Table 1: Comparing lymphocyte count among groups

Group	CD4	CD8	CD4/CD8	P
Healthy subjects	3.7±2.3%	3.2±2.2%	1.4±1.3%	<0.001
IBS	3.3±2.2%	3.1±2.2%	1.3±0.6%	<0.001
Ulcerative colitis	6.5±2.8%	2.3±1.6%	6.2±2.3%	<0.001

4. Discussions

Inflammatory bowel disease (IBD) including Crohn's disease (CD) and ulcerative colitis (UC) is one of chronic idiopathic inflammatory diseases of bowel and the patients often suffer from rectal hemorrhage, severe diarrhea, abdominal pain, fever, and weight loss (Koboziev, 2010). According to clinical evidences and experimental tests, several factors including genetic, mucosa immune system and environmental factors are involved in IBD pathogenesis, although its etiology is not exactly known. According to the studies on IBD patients, the more the class II antigen offering factors in entrocytes and epithelial cells, the more the accumulation of T cells lymphocytes in these patients (Damle and Engleman, 1983; Scott, 1986). According to the study conducted by Mayer et al, there was high accumulation of CD4+ T cell lymphocytes in 100% of Crohn patients and 35 ulcerative colitis patients, out of 38 ones. However, there was not any difference between the patients and healthy subjects considering accumulation rate of $CD8^+$ phenotype (Mayer, 1990). Similar to other researches about IBD patients, Caballero et al indicated to meaningful difference between the case and control groups considering CD4+ phenotype accumulation in lamina properia and bowel mucosa. Nevertheless, there was not any difference between the patients and healthy subjects considering $CD8^+$ accumulation. It was even less in some cases (Caballero, 1995).

In their study, Caballero et al suggested that there is a difference between colon sigmoid and rectum considering lymphocyte accumulation. It may occur due to closeness of rectum to the external environment and more antigen irritation of the area (Caballero, 1995).

Most lymphocyte cells such as $CD8^+$ phenotype may serve as suppressing lymphocytes and high $CD8^+$ phenotype count found in tissue samples of Crohn patients' rectum confirms the claim because mucosa lesions is less prevalent in this area in these patients while high involvement of the area is seen among ulcerative colitis patients. In addition, $CD8^+$ lymphocyte accumulation of the patients is less than that of the healthy subjects of the control group. In this study, there were 23.9±7.4 $CD8^+$ cells per every mm^2 in rectum of Crohn patients while it was 12.5±2.5 in colon and $CD4^+$ was introduced as the dominant phenotype (Caballero, 1995).

Similar to other studies, lymphocyte accumulation especially $CD4^+$ was high in IBD patients. In this regard, there was a statistical difference indicating to significant role of mucosa immune system disorder in pathogenesis of the disease. According to Carballero et al (Caballero, 1995), accumulation rate of $CD4^+$ phenotype in ulcerative colitis patients was less than that of the healthy subjects. The meaningful difference indicates to contradictory role of these two phenotypes. In our study, there was a statistical difference between ulcerative colitis patients and healthy subjects considering lymphocytes accumulation rate. The high rate may be attributed to high $CD4^+$ lymphocyte count in comparison with $CD8^+$ phenotype. The same findings are reported by the previously conducted studies and there is a full correspondence between our findings and that of other studies in this regard (Mayer, 1990;Caballero, 1995).

Several lines of evidence support the hypothesis that a low-grade mucosal inflammatory process may play a significant role in IBS pathogenesis. High prevalence of IBS-like complications following recovery of IBD patients (Isgar, 1983; Simren, 2002), progress and incidence of IBS symptoms after an acute infection of the digestive system(post-infection IBS) (Gwee, 1996; Spiller, 2003), increase of inflammatory cells such as mast cells, T lymphocytes, and macrophages in colon and ileum mucusa of IBS patients were observed (Spiller, 2000; Dunlop, 2003; Chadwick, 2002; Dunlop, 2003). Inflammatory cells are activated in bowel mucus of IBS patients and lead to excretion of high quantity of mediators including interleukins, nitric oxide, histamines, and proteases (Barbara, 2004).

The mediators affect neural function of bowel enteric and lead to abnormal activation of secretory-motor response in bowel and its functional variations (Barbara, 2004). In their study, Cornialdesi et al (2009) used Mezalazin to treat IBS patients. They suggested that the medicine reduces mucosal immunity but does not meaningfully affect $CD8^+$ and $CD4^+$ cell count lymphocytes. However, it significantly affects total lymphocyte count and may affect other lymphocyte phenotypes. The study demonstrated that the Mezalazin leads to meaningful

decrease of mast cells in comparison with the placebo group (Corinaldesi, 2009). Sun Kim et al studied IBS patients (2010) and concluded that there is not any difference between the patients and control group considering $CD8^+$ cell count lymphocytes. However, there was a difference between CD^{4+} and mast cells (Kim, 2010).

Similar to some previously conducted studies and considering few researches conducted on IBS patients about the relationship between the disease and mucosal immunity, the studies were used to compare the results. In our study, there was not any difference between IBS patients and healthy subjects considering $CD8^+$ and $CD4^+$ cell count lymphocytes. Contrary to IBD patients, it seems that other T cells lymphocytes are involved in IBS patients and humoral immunity plays a significant role in this regard because inflammatory solution mediators are more produced in comparison with inflammatory bowel diseases. However, further histobiochemical studies are needed to clarify exact functional role of immune system in future.

Conclusion

According to the our study, there is not any difference between IBS patients and healthy subjects considering $CD8^+$ and $CD4^+$ count phenotypes. It seems that other T lymphocytes are involved in these patients. Similar to other medical reports, $CD4^+$ phenotypes accumulation is dominant in IBD patients and there are less $CD8^+$ lymphocytes in these patients in comparison with the healthy subjects.

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References

1-Thompson WG, Longstreth GF, Drossman DA, Heaton KW, Irvine EJ, Muller-Lissner SA(1999). Functional bowel disorders and functional abdominal pain. Gut; 45(4) : 43–7.

2-Camilleri M, Ford MJ(1998). Colonic sensorimotor physiology in health, and its alteration in constipation and diarrhoeal disorders (Review article). Aliment Pharmacol Ther;12(3):287–302.

3-Barbra G, De Giorgio R, Stanghellini V, et al (2004). New pathophysiological mechanisms in irritable bowel syndrome. Aliment Pharmacol Ther; 20(2): 1–9.

4-Heaton KW, O'Donnell LD, Braddon FM, et al (1992). Symptoms of irritable bowel syndrome in a British urban community: Consulters and nonconsulters. Gastroenterology;102(6):1962–7.

5-Harvey RF, Maudad EC, Brown AM(1987). Prognosis in the irritable bowel syndrome: a 5 year prospective study. Lancet; 1:963–5.

6- Dotan I, Allez M, Nakazawa A, Brimnes J, Schulder-Katz M, Mayer L(2007). Intestinal epithelial cells from inflammatory bowel disease patients preferentially stimulate CD4+T cells toproliferate and secrete interferon-gamma. Am J Physiol Gastrointest Liver Physiol;292(6):G1630-40. Epub 2007 Mar 8.

7-Holmen N, Isaksson M(2007). CD4+ CD25+ regulatory T Cell in irritable bowel syndrome patients. Neurogastroenterol Motil; 19(5): 119-125.

8- Koboziev I, Karlsson F, Grisham MB(2010). Grisham. Gut-associated lymphoid tissue, T cell trafficking, and chronic intestinal inflammation. Ann N Y Acad Sci; 120(7):86–93.

9-Damle N K, Engleman EG(1983). Immunoregulatory T cell circuits in man. Alloantigen-primed inducer T cells activate alloantigen-specific suppressor T cells in the absence of the initial antigenic signal. J Exp Med; 158(6):159-173.

10-Scott M G, Nahm M H, Macke K, Nash GS, Bertovich M, MacDermott R P(1986). Spontaneous secretion ofIgG subclasses by intestinal mononuclear cells: differences between ulcerative colitis, Crohn's disease, and controls. Clin Exp Immunol;66(4):209-215.

11-Mayer L, Eisenhardt D, Salom Pon, Bauer W, Plous R, Piccininni L(1990). Expression of class II molecules on intestinal epithelial cells in man: differences between normal and inflammatory bowel disease. J Clin Invest;86(7):1255-1260.

12- Caballero T, Nogueras F, Medina MT, Caracuel MD, de Sola C, Martínez-Salmerón FJ, et al (2005). Intraepithelial and lamina propria leucocyte subsets in inflammatory bowel disease: an an immunohistochemical study of colon and rectal biopsy specimens. J Clin Pathol;48(8):743-8.

13-Isgar B, Harman M, Kaye MD, Whorwell PJ (1983). Symptoms of irritable bowel syndrome in ulcerative colitis in remission. Gut; 24(3): 190–2.

14-Simren M, Axelsson J, Gillberg R, Abrahamsson H, Svedlund J, Bjornsson ES(2002). Quality of life in inflammatory bowel disease in transmission: the impact of IBS-like symptoms and associated psychological factors. Am J Gastroenterol; 97(8): 389–96.

15- Gwee KA, Graham JC, McKendrick MW, Collins SM, Marshall JS, Walters SJ, Read NW (1996). Psychometric scores and persistence of

irritable bowel after infectious diarrhoea. Lancet; 347: 150–3.

16-Spiller RC(2003). Postinfectious irritable bowel syndrome. Gastroenterology; 124: 1662–71.

17- Spiller RC, Jenkins D, Thornley JP, Hebden JM, Wright T, Skinner M, Neal KR(2000). Increased rectal mucosal enteroendocrine cells, T lymphocytes, and increased gut permeability following acute Campylobacter enteritis and in post-dysenteric irritable bowel syndrome. Gut; 47:804–11.

18-Dunlop SP, Jenkins D, Neal KR, Spiller RC (2003). Relative importance of enterochromaffin cell hyperplasia, anxiety, and depression in postinfectious IBS. Gastroenterology; 125: 1651–9.

19- Chadwick VS, Chen W, Shu D, Paulus B, Bethwaite P, Tie A, Wilson I(2002). Activation of the mucosal immune system in irritable bowel syndrome. Gastroenterology; 122: 1778–83.

20-Dunlop SP, Jenkins D, Spiller RC(2003). Distinctive clinical, psychological, and histological features of postinfective irritable bowel syndrome. Am J Gastroenterol; 98: 1578–83.

21- Barbara G, Stanghellini V, De Giorgio R, Cremon C, Cottrell GS, Santini D, Pasquinelli G, Morselli-Labate AM, Grady EF, Bunnett NW, Collins SM, Corinaldesi R(2004). Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome. Gastroenterology; 126: 693–702.

22-Corinaldesi R, Stanghellini V, Cremon C, Gargano L(2009). Effect of mesalazine on mucosal immune biomarkers in irritable bowel syndrome: a randomized controlled proof-of-concept study. Aliment Pharmacol The; 30: 245–252.

23- Kim HS, Lim JH, Park H, Lee SI(2010). Increased Immunoendocrine Cells in Intestinal Mucosa of Post-infectious Irritable Bowel Syndrome Patients 3 Years after Acute Shigella Infection An Observation in a Small Case Control Study. Yonsei Med J; 51(1): 45-51

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